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Presenting author names **are bold** in the contributor lists.

P01

REPRODUCTIVE GENETICS

P01.001A An unusual number of high mutations expand in the male germline in tyrosine kinase receptors

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Background/Objectives: The higher risk of older fathers having an affected offspring with early or late-onset rare disorders has been quite unsettling; but unfortunately, the methods have been limited to better characterize this phenomenon. So far, studies have focused on well-characterized mutations mainly identified in the receptor tyrosine kinase receptor (RTK) signalling pathway [1-3].

Methods: The establishment of duplex sequencing opened exciting new possibilities in ultra-rare variant detection with a very high accuracy for a sequencing-based method [4, 5]. This is the first study that has used this sequencing approach to explore this type of mutagenesis directly in sperm in the FGFR3 gene.

Results: We found mutations associated with congenital disorders at increased frequencies and identified new unreported selfish mutations expanding with age [6]. We further characterized the expansion of these in the male germline with droplet digital PCR and analysed the change in receptor signalling [7, 8].

Conclusion: Our work sheds light into different mutational mechanisms potentially affecting the receptor kinase activity.

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Conflict of Interest: Irene Tiemann-Boege Johannes Kepler University Linz, principal investigator, Ingrid Hartl Johannes Kepler University Linz, Sofia Moura: None declared, Renato Salazar: None declared.

P01.002.B Using accurate duplex sequencing to explore the connection between elevated germline mutation rates, sperm selection, and male (sub)fertility

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Background/Objectives: Elevated germline de novo mutation rates can impact health and fertility, especially in the context of male subfertility. In 2020, we associated elevated paternal germline mutation rates with reduced lifespans, mirroring the somatic theory of aging. Similarly, studies of subfertile men report elevated individual and familial cancer risks compared to

age-matched fertile controls. We will examine the mechanistic connection between germline instability, somatic mutation burden, subfertility, and poor health.

Methods: Analysis of germline mutation rates frequently involves the use of whole-genome sequencing from pedigrees. However, this strategy suffers from high sequencing error rates (10^-3) and the indirect determination of mutations in sperm. To account for this, we use TwinStrand Duplex Sequencing (TDS) on bulk sperm and blood samples to accurately detect true mutations in individual gametes as those occurring on complementary DNA strands, reducing our per-nucleotide error rate to <10^-9.

Results: Studying a normozoospermic individual's bulk sperm and blood, we obtained high (≤50,000X) coverage across our sequencing panel. We estimate sperm and blood mutation rates of ~4e-7 (95% CI: 3.2-4.8e-7) and ~2.3e-7 (95% CI: 2.1-2.6e-7), respectively.

Conclusion: We find mutation rates in sperm to be elevated compared to those inferred from pedigree studies, and in line with expectations based on per-cell-division error rates. This supports a hypothesis that sperm with elevated mutation rates are selected against during capacitation and/or fertilization which has significant implications for IVF and ICSI procedures that bypass these processes. We will share results from additional samples to explore the link between mutation rates, male fertility, and health.

References:.

Grants:.

Conflict of Interest: Jason Kunisaki: None declared, Suchita Lulla: None declared, Xichen Nie: None declared, Joemy Ramsay: None declared, Yixuan Guo: None declared, Joshua Horns: None declared, Jim Hotaling NIH R01 Grant - Fertility Status as a Marker for Overall Health, Kenneth Aston NIH R01 Grant - Fertility Status as a Marker for Overall Health, Aaron Quinlan NIH R01 Grant - Fertility Status as a Marker for Overall Health.

P01.003.C Bi-allelic variants in INSL3 and RXFP2 cause bilateral cryptorchidism and male infertility

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Background/Objectives: Impaired testicular descent is a common birth defect, leads to cryptorchidism and predisposes to infertility. In mice, the hormone insulin-like factor 3 (INSL3) and its receptor relaxin family peptide receptor 2 (RXFP2) are essential for inguinal canal opening and gubernaculum dilatation pulling the testes into the scrotum. Heterozygous variants in *INSL3* and *RXFP2* were also proposed as associated with cryptorchidism but those variants are frequently found in unaffected controls.

Methods: We screened exome sequencing data of >1600 infertile men for variants in *INSL3* and *RXFP2*. Clinical, semen, and testicular phenotypes were evaluated and INSL3 serum level measurements in patients with identified rare (MAF <0.01) LoF variants are ongoing.

Results: Two patients with homozygous LoF variants in either *INSL3* or *RXFP2* were identified. The *INSL3* variant c.143dup p.(Arg50Profs*33) is located in the first exon and the *RXFP2* variant c.1406del p.(Phe469Serfs*8) affects the transmembrane domain, thus encoding a non-functional protein if not degraded. Both patients showed bilateral cryptorchidism, azoospermia, elevated FSH and variably impaired spermatogenesis. In a multiply affected family, segregation indicates no association of heterozygous LoF variants with cryptorchidism.

Conclusion: Aside from a family in which a homozygous missense variant in *RXFP2* segregated with cryptorchidism in boys, this is the first report of homozygous LoF variants in *INSL3* and *RXFP2* in adults. Our findings show that for both genes, bi-allelic variants are a more convincing cause of bilateral cryptorchidism than a previously assumed autosomal dominant inheritance.

References:.

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Conflict of Interest: None declared.

P01.004.D Associations between epigenetic biomarkers of aging and infertility

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Background/Objectives: As more individuals in developed countries postpone parenthood, more experience infertility and use assisted reproductive technologies (ART). While the effect of increased chronological age on the risk of infertility is well known, the relationships between epigenetic biomarkers of aging and male or female infertility are understudied.

Methods: Using 1,945 mother-father pairs from the Norwegian Mother, Father and Child Cohort Study, we compared five epigenetic biomarkers of aging between 1,000 couples who conceived by coitus and 894 couples who conceived by ART – in vitro-fertilization (IVF, n = 525) and intracytoplasmic sperm injection (ICSI, n = 369).

Results: We found a significant difference in one epigenetic biomarker of a pace of aging, the standardized Dunedin Pace of Aging methylation (DunedinPoAm), between non-ART and IVF mothers (0.208, P-value = 0.0004) after adjustment for chronological age and potential confounders. Further, we detected elevated DunedinPoAm in mothers with tubal factor infertility (0.326, P-value = 0.0001), ovulation factor (0.248, P-value = 0.0075) and unexplained infertility (0.245, P-value 0.0014) compared to non-ART mothers. No differences in epigenetic biomarkers of aging between non-ART and ICSI fathers were found. DunedinPoAm also showed stronger associations with smoking, education, and parity than other epigenetic biomarkers of aging.

Conclusion: In conclusion, DunedinPoAm captured a difference in the pace of epigenetic aging between non-ART and IVF mothers, indicating an association between female epigenetic aging and infertility.

References:.

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Jugessur: None declared, Maria Christine Magnus European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement number 947684)., Siri Håberg Research Council of Norway. Women's fertility (project no. 320656) and Centres of Excellence Funding Scheme (project no. 262700)., Hans Ivar Hanevik Research Council of Norway. Women's fertility (project no. 320656).

P01.005.A Aftermath of long-term cigarette smoking on telomere length and mitochondrial DNA copy number in human cumulus cells prior to In Vitro Fertilization

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Background/Objectives: The female reproductive system represents a sensitive target of the harmful effects of cigarette smoke, with folliculogenesis as mostly affected by this exposure. Some of the main causes of ovarian injury can be represented by cigarette smoke-induced oxidative stress (OS), abnormal crosstalk between oocyte and granulosa-cells and increased cell death [1]. Studies have shown that smoking can also result in telomere shortening and mitochondrial dysfunction in human reproduction [2]. Based on these premises, we estimated the effect of tobacco smoking on telomere length and mitochondrial DNA (mtDNA) copy number in cumulus cells (CCs) of healthy smoking and non-smoking women (<35 years of age) enrolled from Assisted Reproductive Technology protocols.

Methods: DNA was manually extracted from CCs of all subjects and subsequently quantified. 96-well plates were prepared in order to perform dual quantification qPCR assays using telomere, mtDNA and a single copy reference primer sets, as well as a reference genomic DNA sample. Results were deemed significant when showing a fold change >1.4 or <0.7 and a P-value < 0.05 (two-tailed T-test).

Results: Statistical analysis indicated a significantly lower relative telomere length and mtDNA copy number (p-value ~ 0,014/0,012) of the target sample to the reference sample in CCs of smokers compared to corresponding controls.

Conclusion: A significant correlation between smoke exposure and telomere length, as well as mtDNA copy number has been evidenced in cumulus cells of smokers. Further epigenetic and proteomic investigations could be useful to better elucidate the underlying mechanisms.

References: [1] Konstantinidou, F et al. (2021).
[2] Thilagavathi, J et al. (2013).

Grants:.

Conflict of Interest: None declared.

P01.006.B BMP15 and FSHR genetic polymorphisms in Bulgarian patients with premature ovarian insufficiency

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Background/Objectives: Premature ovarian insufficiency (POI) is the early depletion of the ovarian reserve affecting 1–2% of women < 40 years of age and 0.1% of women < 30 years. Genetic factors have been identified in approximately 20–25% of cases. Among the genes associated with POI, *BMP15* and *FSHR* have been proposed to be incorporated as diagnostic biomarkers. The aim of our study was to determine the frequency of genetic polymorphisms in both genes in Bulgarian women with POI in comparison to non-selected Bulgarian individuals (controls), in order to find an association of these genetic factors with the disease.

Methods: Eighteen patients with POI (median age of 30.6 years) were subjected to genetic sequencing of all *BMP15* exons and *FSHR* exon 10, containing SNPs rs6165 (c.919G>A, p.Ala307Thr) and rs6166 (c.2039G>A, p.Ser680Asn). The sequencing data were compared to data from clinical exome sequencing in 485 controls.

Results: We didn't find any difference for the analysed *FSHR* variants between groups. Statistically significant difference was discovered for *BMP15* rs41308602 (c.308A>G, p.Asn103Ser) heterozygotes frequency, as it was 4.8 times higher in POI patients compared to controls.

	Allele frequency		*Heterozygous frequency*		*Homozygous frequency*	
	POI	**Controls**	**POI**	**Controls**	**POI**	**Controls**
BMP15 rs3810682	33.3%	23.2%	22.2%	19.6%	22.2%	13.4%
BMP15 rs41308602	**13.9%** **p < 0.06**	**6.2%**	**27.8%** **p < 0.0002**	**5.8%**	0%	3.3%
FSHR rs6165	47.2%	49.5%	38.9%	45.8%	27.8%	26.6%
FSHR rs6166	52.8%	49.4%	61.1%	46.4%	22.2%	26.2%

Conclusion: We discovered an association between *BMP15* rs41308602 polymorphism and POI in Bulgarian patients and lack of association for *FSHR* polymorphisms with this condition.

References:.
Grants:.

Conflict of Interest: None declared.

P01.007.C A GWAS of parental allelic interactions associated with subfertility

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Background/Objectives: Subfertility is a heterogeneous phenotype, the causes of which are often unclear. One hypothesis is that the joint contribution of risk alleles in the parents may partly explain subfertility. We, therefore, performed a GWAS to study parental allelic interactions.

Methods: In the 29,199 pregnancies available for analysis in the Norwegian Mother, Father and Child Cohort Study (MoBa), 609 newborns were conceived using assisted reproductive technology (ART). We used ART as proxy for subfertility and analyzed interaction effects using logistic regression and a multiplicative

dose-response model, adjusting for principal components, age, and BMI.

Results: Our results revealed interaction effects between maternal and paternal SNPs and use of ART. Entries in various public gene databases indicated that several of the identified genes are highly expressed in tissues relevant for the phenotype studied here. For example, the gene for coiled-coil domain containing 171 (*CCDC171*, 9p22.3) is expressed in both testis and endometrium. Two other genes, DENN domain containing 6B (*DENND6B*, 22q13.33) and karyopherin subunit beta 1 (*KPNB1*, 17q21.32), are also expressed in testis.

Conclusion: Our results pointed to several significant interaction effects between maternal and paternal SNPs in genes that are highly relevant to subfertility. Further replications are necessary to confirm these findings.

References: Magnus et al. Cohort profile update: the Norwegian Mother and Child Cohort study (MoBa). Int J Epidemiol. 2016.

Gjessing & Lie. Case-parent Triads: Estimating Single-and Double-dose Effects of Fetal and Maternal Disease Gene Haplotypes. Annals of human genetics. 2006.

Grants: Norwegian Research Council (grant 262700).

Conflict of Interest: None declared.

P01.008.D Establishment of the niPGT-A programme in two IVF centres

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Background/Objectives: Preimplantation genetic testing for aneuploidy (PGT-A) selects euploid embryos, improving the results in in vitro fertilisation (IVF) cycles. The evolution of next-generation sequencing (NGS) has made it possible to reduce the amount of genetic material needed to obtain reliable results. NGS allows analysis of spent culture medium (SMC) to determine embryo ploidy, with even better concordance with embryo ploidy than trophectoderm (TE) biopsy (1). Non-invasive PGT-A (niPGT-A) promises to be one of the main tools for embryo selection, as it provides insight into embryo ploidy without altering embryo development at all. Our objective is to determine the best protocol for niPGT-A.

Methods: We established a niPGT-A programme in 2 IVF centres. We determined concordance, FPR and FNR.

Centre A stablish standard protocol for remove granulosa cells from oocyte and Centre B stablish a strict protocol for remove granulosa cells.

Results: Control: 47 SMC from vitrified embryos. Concordance 95.74% SMC vs TE, FNR of 2.13% and FPR 2.13%.

Centre A: 34 fresh SMC, 52,94% concordance, 38,24% FNR and 8,82% FPR.

Centre B: 16 fresh SMC. 100% concordance and 0% FPR and FNR.

Conclusion: niPGT-A is one of the best techniques for embryo selection, but it is necessary to establish a strict protocol for the removal of granulosa cells from the oocyte and/or embryo to ensure consistent results.

References: 1. Huang L, Bogale B, Tang Y, Lu S, Sunney X, Racowsky C. Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy. 2019:1-8. https://doi.org/10.1073/pnas.1907472116.

Grants: UMH-Citolab1.19A.

Conflict of Interest: None declared.

P01.009.A Experimental validation of PRD-like homeobox genes expressed in bovine oocytes and early IVF embryos

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Background/Objectives: Identification of genes involved in embryonic genome activation (EGA) in humans revealed a role of PRD-like homeobox genes during early embryo development. In this study we investigate bovine (*Bos taurus*) oocytes and preimplantation embryos in order to clarify the possible roles of PRD-like homeobox genes in EGA.

Methods: Bovine oocytes/embryos were used to prepare cDNA by STRT-N which was followed by PCR for subsequent TOPO cloning and Sanger sequencing.

Results: We analysed RNA-seq data derived from a bovine model system to investigate the transcriptome in germinal vesicle and metaphase II oocytes, and in embryos at the four-, eight-, 16-cell, and blastocyst stages. This revealed the expression of a similar set of early embryo PRD-like homeodomain transcription factors previously identified in humans. In addition, evolutionary comparisons of oocyte and early embryo transcription factors helped predict gene structures and the genomic location of these PRD-like homeobox genes. cDNA cloning allowed the validation of these genes in bovine as candidate igniters of EGA.

Conclusion: Orthologues of human PRD-like homeobox genes, *ARGFX*, *DUXA*, *NOBOX*, *LEUTX*, *TPRX1*, and *TPRX2* were experimentally identified in bovine at the transcript level. A TPRX duplicate, *TPRX3*, which was earlier predicted but not confirmed, was also shown to be expressed in bovine.

References: Töhönen, V. et al. Novel PRD-like homeodomain transcription factors and retrotransposon elements in early human development. Nat Commun 6, 8207 (2015).

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Conflict of Interest: None declared.

P01.010.B SizeMatters: a novel algorithm for fetal-fraction estimation based on fragment-size distributions

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Background/Objectives: Non-invasive prenatal testing (NIPT) for trisomy 13,18, and 21 is performed on cell free DNA (cfDNA) extracted from peripheral blood from the pregnant woman. The

cfDNA contains both foetal and maternal DNA and the foetal fraction (FF) is an important factor for NIPT test accuracy. In general, NIPT on cfDNA with an FF < 3% is considered unreliable.

In a male fetus, the FF is estimated from chromosome Y signals (FFY), however, no such patterns are available for female fetuses. Instead, the FFY is complemented by machine learning approaches, requiring large training sets and homogenous data. As such, there is a need to develop robust computational tools for the estimation of FF in both male and female fetuses. Herein, we present a novel algorithm (SM) based on a machine-learning consensus approach for FF estimation from DNA fragment size distribution in shallow genome sequencing data (sGS).

Methods: We develop the SM-algorithm, combining multilayer perceptron regression, support-vector machine, and multiple linear regression in a machine-learning framework.

Results: We analysed 4000 pregnancies using the SM-algorithm for FF estimation based on sGS data and fragment size distribution and compared it to current leading methods for sGS FF estimation. The SM-algorithm was able to successfully estimate FF, providing high concordance with FFY (median absolute error (MAE) =0.9), significant correlation with trisomy 21 signal (r = 0.75), and final NIPT quality (fail < 1%).

Conclusion: Our novel algorithm for FF estimation provides a FF estimation in both male and female fetuses.

References: None.

Grants: None.

Conflict of Interest: None declared.

P01.011.C Shedding light on Endometriosis (EM) disease: Whole Exome Sequencing (WES) and new genes discovery in a fully clinical characterized Italian cohort

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Background/Objectives: EM is a multifactorial disease, involving the proliferation of endometrial epithelium and stromal tissue outside the uterine cavity. Despite its prevalence, little is known about the genetic factors involved.

Methods: To date, 50 EM adult women with a deep clinical evaluation (i.e., second-level ultrasound echography) were recruited and WES was performed. WES focused on a first list of 285 known EM genes and later on new candidates. Likely pathogenic variants have been selected and their frequency checked in 1000 already sequenced healthy controls.

Results: Preliminary results allow the identification of 47 variants within 23 genes. In particular, two approaches have been conducted: a) the identification of recurrent genes shared among many patients (e.g., LAMA5, CSMD1, NEB), b) private variants within specific genes (e.g., IL18). In group a) 4/50 patients carry different heterozygous missense variants within LAMA5 gene, already known to be associated with EM and EM-related infertility; 3/50 patients carry pathogenic variants (i.e., two heterozygous missense/one intronic deletion) within CSMD1 gene, a negative complement regulator previously associated with infertility. Furthermore, 7/50 patients carry heterozygous missense variants within NEB gene, a new candidate encoding a structural sarcomere protein. In group b) one patient carries a heterozygous missense variant in IL18 gene, known to be involved in EM pathogenesis and in embryo implant regulation.

Conclusion: This study allows the identification of novel pathogenic variants in EM known genes as well as new candidates which can guide clinicians in defining diagnostic/prognostic markers and tailoring targeted therapies for patients and their families.

References:.

Grants:.

Conflict of Interest: None declared.

P01.012.D Design and validation of a next-generation sequencing workflow for simultaneous detection of aneuploidy, ploidy, and common pathogenic microdeletions within Preimplantation Genetic Testing (PGT)

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Background/Objectives: Standard methodologies employed in preimplantation genetic testing allow to identify chromosomal aneuploidies but not to determine embryo ploidy status nor the presence of microdeletions (MD). Transferring embryos with these abnormalities can result in miscarriage, molar pregnancy, or multiple congenital anomalies, requiring later-stage invasive prenatal diagnosis. Our objective is the development of a novel NGS-based framework to resolve current limitations.

Methods: PGT-A products were reamplified and sequenced using a custom AmpliSeq panel targeting 357 genomic regions harbouring high frequency SNPs and/or associated to 8 pathogenic microdeletions (-4p = Wolf-Hirschhorn, -8q = Langer-Giedion, -1p = 1p36 deletion, -22q = DiGeorge, -5p = Cri-du-Chat, -15q = Prader-Willi/Angelman, -11q = Jacobsen, -17p = Smith-Magenis). Sequencing data were processed by a bioinformatic algorithm which accounts for sequencing noise through gaussian-mixture modeling of B-allelic ratios measured at each SNP locus and estimates the likelihood of different ploidy levels and of the presence of MD.

Results: Ploidy was correctly determined in 233/234 cases, with only one diploid sample misclassified as triploid (PPV = 94.1%, NPV = 100%). Microdeletions could be consistently detected with high reliability (PPV = 98.5%, NPV = 99.5%) in 6 out of 8 microdeletion regions. Detection in -1p and -4p was less reliable due to the presence of recurrent haplotype blocks in the population, as confirmed by the analysis of a dataset of 2504 whole genome sequencing from 1KGP database.

Conclusion: This study provides, for the first time, detection of common pathogenic microdeletions and ploidy status from a single trophectoderm biopsy, expanding PGT clinical validity. This new assay will also help elucidate fundamental biological and clinical questions related to the genetics of implantation failure and pregnancy loss of apparently euploid embryos.

References:.

Grants:.

Conflict of Interest: Matteo Figliuzzi Igenomix Italy, Silvia Caroselli Igenomix Italy, Francesco Cogo Igenomix Italy, Paola Zambon Igenomix Italy, Cristina Patassini Igenomix Italy, Dany Bakalova Igenomix UK, Federico Favero Arc-Ster, Attilio Anastasi: None declared, Francesco Capodanno: None declared, Andrea

Gallinelli: None declared, Danilo Cimadomo GeneraLife, Laura Rienzi GeneraLife, Filippo Maria Ubaldi GeneraLife, Carmen Rubio Igenomix Spain, Jose Miravet-Valenciano Igenomix Spain, Jorge Jimenez Almazan Igenomix Spain, David Blesa Igenomix Spain, Carlos Simon Igenomix Spain, Antonio Capalbo Igenomix Italy.

P01.013.A Actionable secondary findings in infertile men and their clinical significance

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Background/Objectives: Whole exome sequencing (WES) has been increasingly used in medicine for patients with suspected genetic disorders. WES also enables the identification of secondary findings (SFs) – genetic variants in disease-causing genes that are unrelated to the patient's primary condition but are still medically actionable. The prevalence of SFs in the general population is 1–3%; data of SFs in specific clinical conditions is limited. This study aimed to describe SFs in non-obstructive azoospermia (NOA) patients.

Methods: GEMINI[1] represents a multi-center study aiming to map the genetic composition of NOA. We evaluated SFs across 85 genes in a cohort of 836 GEMINI participants from six countries. Recommendations from the American College of Genetics and Genomics (ACMG), the Geisinger Health System's MyCode and the Clinical Genome Resource were used to compile the gene list.

Results: Based on the ACMG guidelines for the interpretation of genetic variants, we identified 27 SFs in 21 genes in 30 patients (3.6%, 30/836). The majority of these patients carried SFs in genes linked to familial cancer syndromes, followed by cardiac conditions. Moreover, 11 patients with SFs (37%, 11/30) carried variants in genes linked to spermatogenic failure in knockout mice. Clinico-molecular diagnoses were confirmed in multiple cases during a retrospective evaluation of patients with SFs.

Conclusion: Every 28th analyzed NOA patient carried a medically actionable SF. Disclosure of SFs is valuable in genetic disease prevention and intervention, and long-term health management of andrology patients.

References: 1. Nagirnaja et al. (2021) N Engl J Med; 385:707-719.

Grants: Estonian Research Council IUT34-12, PRG1021; NIH R01HD078641, P50HD096723.

Conflict of Interest: None declared.

P01.014.B Asymptomatic male carriers of long copy number variants are predisposed to spermatogenic failure

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Background/Objectives: Infertility affects 7-10% of all men. About 60% of these cases remain idiopathic. Among other genetic factors, a burden of copy number variants (CNVs) has been suggested to increase the risk for spermatogenetic failure. Only a limited number of genome-wide studies have been conducted on the subject.

Methods: All study participants were recruited at the Andrology Center of Tartu University Hospital (2000-2015). The study group consists of azoospermia (no sperms, n = 73), severe (sperm count per ejaculate <10 mln, n = 83) or moderate oligozoospermia (10-39 mln, n = 58) and normozoospermia (>39 mln, n = 63) cases. All individuals were genotyped using Illumina HumanOmniExpress-24-v1.0/v1.1 BeadChips. Calling of autosomal CNVs (>100bp) from the genotyping data was performed with three algorithms (GADA, QuantiSNP, and CNstream) (1). CNVs detected by at least two programs were included in the analysis.

Results: No difference was detected between the groups for the mean number (7.2-7.4) and length (101-120kb) of CNVs per subject. CNVs longer than >1Mb (n = 33) were located in pericentromeric regions and near telomeres. Three loci overlapped with known pathogenic CNV regions (1q21.1, 7p22.2, 14q32.33). A recurrent CNV within the CSMD1 gene (n = 4) was detected only in azoospermia and severe oligozoospermia cases, supporting its reported link to spermatogenic failure (2).

Conclusion: Large pericentromeric and subtelomeres CNVs may affect spermatogenesis through meiotic and mitotic chromosomal missegregation. CNVs located within genes showing enhanced testicular expression may compromise normal spermatogenesis.

References: 1. Kasak et al (2015) Sci Rep 5:8342.
2. Lee et al (2019) Nat Commun 10:4626.

Grants: PRG1021 (Estonian Research Council).

Conflict of Interest: None declared.

P01.015.C Novel STARD8 and STARD9 mutations identified in 46,XY gonadal dysgenesis patients lend support to these genes as DSD candidates

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Background/Objectives: Investigating mutation in genes, affecting gonad development is essential for understanding the genetic mechanisms causing Disorders/Differences in Sex Development. The aim of the research was to identify novel DSD genetic variants using whole exome sequencing (WES).

Methods: The WES was performed for two unrelated 46,XY SRY positive patients with gonadal dysgenesis.

Results: In first patient the hemizygous missense mutation NM_001142503.2 c.2659C>T (p.Arg887Cys) (rs766188656) in *STARD8* gene (MAF = 0.0000251) was identified and confirmed as pathogenic using bioinformatic tools. After analysis of second patient two deferent mutations in compound heterozygous state were identified in *STARD9* gene: NM_020759.3 c.5585_5590del (p.Ser1862_Thr1863del) (rs528276071) – inframe deletion (MAF = 0.0019) combined with NM_020759.3 c.3514C>T (p.Arg1172Cys) (rs12594837) – missense mutation (MAF = 0.00837). The analysis of genetic background which were performed for both patients did not reveal any pathogenic variants implicated in DSD phenotype. All detected mutant variants were inherited from healthy parents – heterozygous carriers and were not previously implicated in the pathogenesis of any disease. Bioinformatic analysis revealed that

mutant variant in *STARD8* and both mutations in *STARD9* genes located in positions that are conserved in primates.

Conclusion: Based on the results obtained in current study, previous reports of STARD gene family mutations in DSD patients, expression patterns of *STADR8* and *STARD9* genes and steroidogenic properties of those protein products we conclude that *STADR8* and *STARD9* considered as 46,XY DSD causing genes.

References:.

Grants: Study was funded by the Swiss National Science Foundation, grant number SCOPES IZ73Z0_152347/1 and National Academy of Sciences of Ukraine [0121U110054].

Conflict of Interest: None declared.

P01.016.D Using marginal analysis to optimise the number of tested genes for reproductive genetic carrier screening

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Background/Objectives: Genomic technologies now permit carrier testing using very large panels, or even the entire exome. Including more genes increases diagnostic sensitivity, but at higher cost. We have explored if there is an optimum number of genes for clinical utility and cost effectiveness.

Methods: We used a comprehensive list of 1,300 genes causative of autosomal recessive and X-linked recessive conditions relevant to carrier screening. From data in the Genome Aggregation Database (gnomAD), for both total population as well as major population subgroups, we extracted the allele frequency (AF) in these genes for all known single nucleotide variants (SNV) and indel variants that have been reported as Pathogenic or Likely Pathogenic in ClinVar. We also considered a notional population subgroup, in which the AF was set equal to the highest encountered AF in any other population subgroup. Using marginal analysis techniques, we calculated the number of genes needing to be incorporated into a carrier screening panel to achieve 90%, 95% or 99% of theoretical maximal diagnostic sensitivity.

Results: To achieve the stated fraction of theoretical maximal diagnostic sensitivity, the number of genes needing to be included in a screening panel was found to be:

Diagnostic sensitivity	90%	95%	99%
Couples	84	160	381
Individuals	383	546	842

Conclusion: To achieve generally accepted levels of diagnostic sensitivity, the number of genes needing inclusion in a screening panel is much smaller than the total number of genes potentially available for testing. This has significant policy implications for both cost and public health benefits of carrier screening.

References:.

Grants:.

Conflict of Interest: Leslie Burnett Garvan Institute of Medical Research, Takeda Pharmaceuticals USA Inc, Invitae Asia-Pacific, Honorary academic positions at University of NSW and University of Sydney, Matthew Hobbs Garvan Institute of Medical Research, Heather Gordon Garvan Institute of Medical Research.

P01.017.A Cytogenetic investigation of infertile patients in Hungary: a 10-year retrospective study

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Background/Objectives: Infertility is a very common health problem that can caused by numerical or structural abnormalities of sex chromosomes or autosomes. The main purpose of this retrospective study was to determine the types and frequency of chromosomal aberrations detected in infertile patients and to compare the frequency of structural aberrations with control group.

Methods: Constitutional karyotyping was performed in 1489 men and 780 women diagnosed with infertility between 2010 and 2020. The control group was represented by 869 male and 1160 female patients having cytogenetic investigation with other referral reason then infertility. Karyotyping was performed on peripheral blood lymphocytes as standard protocol by using G-banding method.

Results: Thirty three (2,22%) of 1489 infertile men showed sex chromosomal aberrations [47,XXY (n = 28); 47,XXY/46,XY (n = 3); 47,XXY/48,XXXY (n = 1); 46,XX (n = 1)] and 89 (5,98%) had structural abnormalities [inversion (n = 21); balanced translocation (n = 16); single cell translocation (n = 52)]. Three (0,38%) of the 780 infertile women had sex chromosomal aberrations [X numerical mosaicism (n = 2); 46,XY (n = 1)] and 58 (7,44%) had structural abnormalities [inversion (n = 21); balanced translocation (n = 10); single cell translocation (n = 27)]. Structural chromosomal abnormalities were detected in 27/869 (3,11%) control male cases [inv(9) (n = 23); single cell translocations (n = 4)] and 39/1160 (3,36%) control female samples [inversions (n = 31); single cell translocations (n = 8)]. The prevalence of single cell translocations was higher in infertile patients (3,5% vs. 0,46% in men and 3,46% vs. 0,7% in women).

Conclusion: The type and frequency of chromosomal abnormalities of infertile patients was comparable to literature data. The frequency of less-studied single cell translocations was significantly higher in infertile patients.

References:.

Grants:.

Conflict of Interest: None declared.

P01.019.C Familial Mayer-Rokitansky-Küster-Hauser syndrome

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Background/Objectives: Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is characterized by congenital agenesis/aplasia of the uterus and part of the vagina in women with a normal 46,XX karyotype. Most cases are sporadic and do not have a molecular diagnosis, although individual case reports have been described in association with copy number variants or candidate genes (*WNT9B, LHX1, GREB1L*, 1q21.1 deletion, 22q11.21 duplication etc). We describe a dominantly inherited case of MRKH, in which a

mother with MRKH had a biological daughter with the same syndrome through a surrogate pregnancy.

Methods: We used G-banded chromosomal analysis in order to confirm a 46,XX karyotype, and exome sequencing in order to investigate single nucleotide variants (SNV). Copy number variants were inferred from read depth analysis of the exome data.

Results: Chromosome analysis on the mother and daughter with MRKH revealed a female karyotype with a deletion of chr2q37 in both. The deletion was found to be de novo in the mother. Exome sequencing, undertaken in the daughter, confirmed the ~4Mbp deletion and did not demonstrate pathogenic or likely pathogenic variants. Similar deletions have been described in the literature in association with a spectrum of neurophysical findings but never with MRKH.

Conclusion: Our data suggest that deletion of 2q37 may be associated with MRKH. The developing options of surrogacy and uterine transplantation stress the importance of genetic investigation for MRKH patients.

References:.

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Conflict of Interest: Hagit Daum Mentoring Grant of the Hebrew university and Hadassah Medical Center. PI: Hagit Daum, Ayala Frumkin: None declared, Vardiella Meiner: None declared, Iris Harel: None declared, Dvora Bauman: None declared.

P01.020.D A Novel Cause of Male Infertility and Hypergonadotropic Hypogonadism: Inhibin-A Deficiency Due to Loss of Function Variations of INHA

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Background/Objectives: The human *INHA* gene encodes the inhibin subunit alpha protein, which is common to both inhibin A and B. The functional importance of inhibins in male sex development, sexual function, and reproduction remain largely unknown. Todate no clinical manifestation were related to INHA gene variations. Here we report for the first time two male siblings sharing the same clinical features associated with homozygous *INHA* pathogenic variations.

Methods: The medical documents were examined for clinical, biochemical, and imaging data. Genetic analysis was performed using next-generation and Sanger sequencing methods.

Results: Two brothers were referred to us because of gynecomastia, testicular pain, and having a history of hypospadias. They were born to a consanguineous parents and no similar cases were reported in the family. Biochemistry revealed low serum testosterone, high gonadotropin and anti-Mullerian hormone, and very low/undetectable inhibin concentrations, where available. Both patients had azoospermia in spermiogram. We have identified a homozygous 2bp deletion (c.208_209delAG, R70Gfs*3), which leads to a truncated *INHA* protein in both patients, and confirmed heterozygosity in the parents. The external genital development, pubertal onset and progression, reproductive functions, serum gonadotropins, and sex hormones of mother and father, who were heterozygous carriers of the identified mutation were normal.

Conclusion: Homozygosity for pathogenic *INHA* variations causes decreased prenatal and postnatal testosterone production and infertility in males, while the heterozygous female and male carriers of *INHA* variations do not have any abnormality in sex development and reproduction.

References:.

Grants:.

Conflict of Interest: None declared.

P01.023.C Copy number variant (CNV) analysis in couples with recurrent miscarriages

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Background/Objectives: Recurrent miscarriage (RM) is an important global health issue and carries psychological and financial burden for affected couples. Anatomy factors, antiphospholipid syndrome, endocrinological and chromosomal abnormalities are the most commonly recognized aetiologies for RM. Aetiology of RM remains idiopathic in 40-60 % of couples. The aim of this study was to evaluate copy-number variation (CNV) characteristics in couples with RM and compare to the Lithuanian population cohort.

Methods: Microarray analysis was performed for couples with unexplained RM (anatomy factors, antiphospholipid syndrome, chromosomal and endocrinological abnormalities were excluded) to detect CNVs. Genotyping was performed with Illumina HumanCytoSNP-12 v2.1 arrays using the standard Illumina Infinium HD Ultra Assay Protocol Guide.

Results: In total, 68 RM patients were tested and 72 CNVs were detected. The average number of CNVs per patient was 1,1 (1,67 in Lithuanian population cohort). No CNVs were identified in approximately 40 % of patients (30-40 % in Lithuanian population cohort). CNV regions (CNVRs) presented >1 individual (criteria met 13 CNVRs) were selected for further analysis. Three of 13 CNVRs – at 3q29, 5p15.33 and 19p12-q11 – were not reported in Lithuanian population cohort or in DGV database.

Conclusion: The number of CNV is similar in the RM group and Lithuanian population cohort. For more detailed results, the study continues to involve more patients.

References:.

Grants:.

Conflict of Interest: None declared.

P01.024.D DNA methylation in newborns conceived by assisted reproductive technology

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Background/Objectives: Assisted reproductive technology (ART) may affect fetal development through epigenetic mechanisms as

the timing of ART procedures coincides with the extensive epigenetic remodeling occurring between fertilization and embryo implantation. However, it is unknown to what extent ART procedures alter the fetal epigenome. Underlying parental characteristics and subfertility may also play a role.

Methods: Here we identify differences in cord blood DNA methylation, measured using the Illumina EPIC platform, between 962 ART conceived and 983 naturally conceived singleton newborns.

Results: We show that ART conceived newborns display widespread differences in DNA methylation, and overall less methylation across the genome. There were 607 genome-wide differentially methylated CpGs. We find differences in 176 known genes, including genes related to growth, neurodevelopment, and other health outcomes that have been associated with ART. Both fresh and frozen embryo transfer show DNA methylation differences. Associations persist after controlling for parents' DNA methylation, and are not explained by parental subfertility.

Conclusion: Our results support the hypothesis that ART procedures influence DNA methylation in fetal life. The epigenetic differences between ART and naturally conceived children were not explained by parental DNA methylation or parental subfertility. Longitudinal assessments of DNA methylation are necessary to establish whether ART-induced differences in DNA methylation persist and influence later health outcomes.

References:.

Grants:.

Conflict of Interest: None declared.

P01.025.A Genome sequencing reveals causative CHH structural and single nucleotide variants in consanguineous families

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Background/Objectives: Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder that causes incomplete or absent puberty leading to infertility due to defects in secretion or action of gonadotropin-releasing hormone. CHH is very heterogeneous phenotypically, and its genetics are complex. More than 25 genes have been associated with it explaining up to 50% of cases. Discovering new causative genes, or new variants in known genes may aid explaining the intricacies of CHH improving diagnosis and treatment. Here, we present 6 consanguineous families from Pakistan, each with at least one member affected by CHH. Using different approaches, we determined in each case the genetic origin for the disorder.

Methods: 18 genomes from 6 families were sequenced. They were analysed using Sentieon. Then single nucleotide variants (SNVs) were filtered based on frequency in gnomAD and/or potential for pathogenicity. Copy number variations (CNVs) were extracted using CoverageMaster.

Results: A genetic cause for CHH was identified in all families. 4 SNVs following an autosomal recessive mode of inheritance: GNRHR:p.P320Q, GNRHR:p.F309del, KISS1R:p.S262X, KISS1R:p.W108X in 4 of them and 2 CNVs following an X-linked mode of inheritance ANOS1:NC_000023.11:g.8,593,145-8,733,4053del, ANOS1:NC_000023.11:g.8,471,269-8,568,633del in the last two. Except for GNRHR:p.P320Q, all the variants were not known before.

Conclusion: Systematic analysis of CNVs on top of SNVs can heavily increase the success rate of finding a genetic cause for CHH in patients. Performing such analysis in consanguineous families can uncover new causative variants leading to better understanding the function of genes involved in CHH.

References: Boehm, U., Bouloux, PM., Dattani, M. et al. Nat Rev Endocrinol 2015.

Grants:.

Conflict of Interest: None declared.

P01.026.B Autosomal recessive disorders - A common cause of early pregnancy losses?

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Background/Objectives: Miscarriage or early pregnancy loss (EPL) represents one of the most common pathologies in the reproductive medicine occurring in about 15% of couples trying to conceive. About half of the miscarriages are caused by numerical chromosomal abnormalities. Up to 5% of couples experience recurrent miscarriage, defined as two or more consecutive pregnancy losses. Increasing number of case reports suggest Mendelian causes of recurrent miscarriages. Whole exome sequencing (WES) may help uncover the genetic etiology, but so far it has been rarely used to study EPL.

Methods: We have performed WES on seven euploid miscarried tissues from couples with recurrent EPL.

Results: Compound heterozygous pathogenic variants in *CPLANE1* (c.1819delT and c.5820+3_5820+6delAAGT) and *DHCR7* (c.964-1G>C and c.452G>A) genes were detected in two miscarriages. The variants were confirmed with Sanger sequencing and the partners in both families were heterozygous carriers. The compound heterozigosity in *CPLANE1* was also confirmed in a second miscarriage from the same couple. Pathogenic variants in *CPLANE1* and *DHCR7* genes cause Joubert syndrome 17 and Smith-Lemli-Opitz syndrome, respectively. Both are multi-systemic disorders associated with variable clinical severity and, besides in patients, have been confirmed in pregnancy losses and/or fetuses with ultrasound abnormalities.

Conclusion: In conclusion, our study shows a high percentage of autosomal recessive disorders (2/7 or 28.5%) as a cause of EPL. Increased number of WES analysis in recurrent euploid miscarriages could improve our understanding of the etiology of EPL and relevant biological processes. The knowledge of genetic causes responsible for EPL allows for genetic counselling and gives hope to the families.

References:.

Grants:.

Conflict of Interest: None declared.

P01.028.D Genetic variation in genes linked to telomere length in Estonian non-obstructive azoospermia patients

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United States; [3]University of Utah School of Medicine, Andrology and IVF Laboratory, Department of Surgery (Urology), Salt Lake City, United States; [4]Tartu University Hospital, Andrology Center, Tartu, Estonia; [5]University of Tartu, Institute of Clinical Medicine, Tartu, Estonia.

Background/Objectives: Intact telomeric structures are important in protecting chromosomes from damage. Association between telomere length and risk to chronic diseases has become an engrossing topic. Sperm quality has been correlated with shorter telomeres[1] and rare variants in genes implicated in meiotic telomere complex have been linked to non-obstructive azoospermia (NOA)[2]. We investigated the suggested potential causative link between rare variants in genes linked to telomere length and spermatogenic failure.

Methods: Exomes of 85 idiopathic NOA cases from Estonia were sequenced in collaboration with the GEMINI (GEnetics of Male INfertility Initiative) project. A list of 66 genes associated with telomere length in the scientific literature was compiled. Rare variants were identified and their possible pathogenicity was evaluated based on the ACMG guidelines.

Results: In our study group, no likely pathogenic/pathogenic rare variants in 66 candidate genes were identified. However, two autosomal dominant genes (pLI > 0.8) *PELI2* and *TERF1* carried two heterozygous missense variants of uncertain significance (VUS). Among these *TERF1* is also implicated in the male reproductive system.

Conclusion: None of 85 Estonian NOA cases could be explained by causative variants in the analyzed genes modulating telomere length. The study may have been limited by an insufficient number of patients given a high heterogeneity of monogenic NOA and uncertainties in evaluating the effect of variants.

References: 1. Darmishonnejad *et al Andrologia* **52**, e13546 (2020).

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Grants: IUT34-12, PRG1021; NIH R01HD078641, P50HD096723.

Conflict of Interest: None declared.

P01.029.A Role of progesterone receptor genetic variants rs4754732 and rs1942836 in spontaneous premature birth

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Background/Objectives: To evaluate the roles of two selected genetic variations in fetal and maternal progesterone receptor gene (PGR) and to identify women who may have higher or lower odds for spontaneous premature birth compared to the general population.

Methods: A preliminary case–control study with two groups of pregnant women (with term and premature delivery, 219 in total) and two groups of newborns (term and preterm, 219 in total) was performed. Two single nucleotide polymorphisms (SNPs) of the progesterone receptor gene (rs4754732 and rs1932836) were genotyped.

Results: Our results suggest that women who gave birth to male newborns with CC or CT rs4745732 genotype have 2.5 higher chance for preterm birth (OR = 2.5; 95%CI 1.09 – 5.6; P = 0.03; Fisher's Exact Test). We also found that women older than 35 whose newborns have CC or CT rs1942836 genotype have 5.3 higher chance for preterm birth (OR = 5.3; 95%CI 1.05 – 26.2; P = 0.04; Fisher's Exact Test).

Conclusion: Our study suggests that patients with selected genetic variants of the progesterone receptor gene could have greater odds for premature birth compared to general population. Replication studies with a larger population of different ethnicity are needed in order to confirm these findings.

References:.

Grants: 'Role of PROGINS Mutations in Progesterone Receptors as Modulators of Risk for Premature Birth'(VIF2017-MEFOS-3), Faculty of Medicine in Osijek, Croatia.

Conflict of Interest: None declared.

P01.030.B Does aneuploidy discordance between trophectoderm and spent culture medium suggest embryo mechanism of self-correction?

Anna Strychalska[1], *Wojciech Sierka*[1], *Joanna Smolen-Dzirba*[1], *Anna Kokot*[1], *Natalia Jodłowiec-Lubańska*[1], *Klaudia Simka-Lampa*[1], *Urszula Wroblewska*[1], *Patrycja Piotrowska*[1], *Adam Pudełko*[1], *Emilia Morawiec*[1], *Anna Bednarska-Czerwińska*[1], *Simone Palini*[1;2]

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Background/Objectives: Investigating the utility of spent culture media (SCM) and mixture of SCM and blastocoel fluid (BF) in non invasive preimplantation genetic testing for aneuploidy (PGT-A).

Methods: Embryo was cultured until day 4, then washed and transferred to fresh medium. Trophectoderm (TE) biopsied from embryo day 6 has been tested for PGT-A in our routine procedure. SCM and mix of SCM + BF were collected prior to TE biopsy. Testing was performed with the use of Illumina VeriSeq NGS platform and MiSeq system.

Results: Comparison of chromosome 8 changes is presented in table.

Material	Start cyto	End cyto	Type of change	Copy#
Trophectoderm	pter	q24.13	Gain	2.66
Spent coulter medium	q24.13	qter	Gain	6
SCM+BF	pter	q24.13	Loss	1
	q24.13	qter	Gain	4

TE analysis showed partial gain of chromosome 8 (pter->q24.13). In SCM we found gain of remaining part of chr8 (q24.13->qter). Sample of mix (SCM+BF) showed DNA gain of chr8 (q24.13->qter) and loss of chr8 (pter->q24.13).

Conclusion: Human embryo ability of self-correction has been discussed widely. Repairing mechanisms may result from increased aneuploid cells death and release of fragmented DNA into culture media. We tested cell-free embryo DNA released from the blastocyst to SCM and mix of SCM+BF during cell cleavage (day 4 to day 6). We have observed chromosomal status after breakage occurred in mitotic cell division of chromosome 8 in embryo. Discordance between results in collected samples may be evidence for embryo undergoing self-correction mechanism.

References:.

Grants: WND-RPSL.01.02.00-24-0477/19-007.

Conflict of Interest: Anna Strychalska Gyncentrum Genetic Laboratory, Wojciech Sierka Gyncentrum Genetic Laboratory, Joanna Smolen-Dzirba Gyncentrum Genetic Laboratory, Anna Kokot Gyncentrum Genetic Laboratory, Natalia Jodłowiec-Lubańska Gyncentrum Genetic Laboratory, Klaudia Simka-Lampa Gyncentrum Genetic Laboratory, Urszula Wroblewska Gyncentrum Genetic Laboratory, Patrycja Piotrowska Gyncentrum Genetic Laboratory,

Adam Pudełko Gyncentrum Genetic Laboratory, Emilia Morawiec Gyncentrum Genetic Laboratory, Anna Bednarska-Czerwińska Gyncentrum Genetic Laboratory, Simone Palini Gyncentrum Genetic Laboratory.

P01.032.D Novel variant curation strategies and preclinical technology to improve diagnosis of differences of sex development

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Background/Objectives: Despite being collectively among the most frequent congenital developmental conditions, differences of sex development (DSD) lack recognition and research funding. As a result, what constitutes optimal management remains uncertain. Identification of the individual conditions is challenging and molecular diagnosis frequently not achieved, which may have psychosocial and health-related repercussions for individuals and their families. While research genome sequencing of samples from the DSD-TRN repository contributed to the description of the new GUBS syndrome (OMIM 618820) and diagnosed previously unsolved cases, network-wide diagnosis rate remains under 50%.

Methods: New pre-clinical genomic approaches have the potential to increase diagnostic yield through ascertainment of under-recognized etiology such as mosaic, structural, non-coding, or epigenetic variants. We undertook a study to determine the usefulness of Optical Genome Mapping for clinical DSD diagnosis and etiology discovery. As interpretation of now widespread exome sequencing requires expert interpretation rarely available in clinical settings, we also examined variants in ClinVar for 32 DSD genes.

Results: OGM identified 7 common DSD so far. We showed that curation of DSD gene variants in clinical databases used by algorithms to classify variants is currently insufficient even for the best validated DSD genes.

Conclusion: To address this gap, a special-interest group on the Franklin.genoox.com platform, opened to interested curators worldwide, integrates curation of DSD-specific variants into the genetic data analysis platform, with variant classification available through the free Franklin interface. Analysis of DSD-TRN registry data also shows that practice needs to evolve to prioritize early genetic testing.

References: PMID33513338.

Grants: NICHD RO1HD093450.

Conflict of Interest: Emmanuèle Délot Estranged spouse has stock options in Bionano Genomics, current value $12,000 USD., Surajit Bhattacharya: None declared, Hayk Barseghyan Bionano Genomics Inc, part-time employee, Stock/stock options, Eric Vilain Stock options, Bionano Genomics. These are current theoritical value $13,000, acquired over 4 years, they are non vested (non-sellable)., Scientific consultancy, Bionano Genomics.

P01.033.A Polygenic risk score for recurrent pregnancy loss in LUCAR study, Ukraine

Yevheniya Sharhorodska[1;2], *Zhanna Balkhiyarova*[2;3], *Anna Ulrich*[2;3], *Vincent Pascat*[4], *Liliia Chorna*[1], *Ivanna Shymanska*[1], *Harmen M Draisma*[3], *Danuta Zastawna*[1], *Oleg-Roman Gnateyko*[1], *Halyna Makukh*[1], *Marika Kaakinen*[2;3], *Inga Prokopenko*[2;4]

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Background/Objectives: The loss of 2+ sequential pregnancies before 24 weeks of gestation is defined as recurrent pregnancy loss (RPL). In RPL etiology, endocrine, immunological, anatomical and other factors could drive individual susceptibility, still ~50% of RPL cases remain idiopathic. We assessed the predictive ability of a polygenic risk score (PRS) for RPL in the LUCAR study (Lviv Ukrainian Cohort for Advancing Reproductive Health) from the Western Ukraine.

Methods: LUCAR includes 350 idiopathic RPL cases/458 controls with at least one healthy child, all having the genome-wide association study (GWAS) data imputed to TopMED and RPL association analysis performed using log-additive model. We used summary statistics from recurrent miscarriage (RM) GWAS meta-analysis (Laisk et al., PMID:33239672) and PRS software PRSice-2.

Results: In LUCAR, the unweighted common variant $PRS_{RM,}$ with independent SNP sets after LD clumping, did not increase the RPL risk (Table). The lead variants from the Laisk et al., rs146350366 and rs7859844, did not affect RPL susceptibility.

Laisk et al.	PRS_{RM} in LUCAR			
Association test Significance Threshold	**Total SNPs**	**R^2**	**Beta(SE)**	**P-value**
5×10^{-8}	50	0.0021	0.082(0.075)	0.27
10^{-3}	336	0.0054	0.13(0.076)	0.075
0.01	767	0.0013	0.065(0.075)	0.39
0.5	9889	0.0022	0.084(0.075)	0.26
0.99	21013	0.015	0.23(0.076)	0.0028

Conclusion: Despite the previous meta-analysis showing good predictive ability of the PRS for RPL, we were unable to replicate this in dataset from Western Ukraine. We believe this highlights the commonly observed problem of low portability of PRS derived in one population to another, and an urgent need for well-powered GWAS for RPL in world-wide populations.

References:.

Grants:.

Conflict of Interest: None declared.

P02

PRENTAL GENETICS

P02.001.B Exome sequencing for structurally normal fetuses - yields and dilemmas

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Background/Objectives: There is a growing body of literature regarding the yield of chromosomal microarray analysis (CMA) in structurally normal fetuses (1-3). We aimed to assess the empirical data as to the yield of exome sequencing (ES) in this population.

Methods: From February 2017 to January 2022, 1,298 fetuses were subjected to ES; 374 of them were structurally normal (28.8%). Only pathogenic and likely pathogenic (P/LP) variants, per the American College of Medical Genetics and Genomics (ACMG) classification, were reported. Additionally, ACMG secondary findings relevant to childhood were reported.

Results: Five fetuses (5/374; ~1.33%) had a P/LP variants indicating a moderate to severe disease: Wilson disease, Enhanced S-cone syndrome, Legius and Muenke syndromes (upon affected genes ATP7B, NR2E3, SPRED1 and FGFR3 respectively). One fetus had a paternally inherited pathogenic variant in RET.

Conclusion: Our data suggest that offering only CMA for structurally normal fetuses may provide false reassurance. Prenatal ES mandates restrictive analysis, and careful management combined with pre and post-test genetic counselling.

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Grants:.

Conflict of Interest: None declared.

P02.002.C DES-NIPT study: Global approach to detect inherited paternal variants in NIPT samples using deep exome sequencing

Ieva Miceikaite[1], *Charlotte Brasch-Andersen*[1;2], *Qin Hao*[2], *Christina Fagerberg*[1;2], *Britta Schlott Kristiansen*[2], *Pernille Torring*[2], *Geske Sidsel Bak*[3], *Lene Sperling*[3], *Martin Larsen*[1;2]

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Background/Objectives: Deep sequencing has now enabled detection of sequence variants and diagnosis of specific monogenic diseases by non-invasive prenatal testing (NIPT), using tailor-made assays for each pregnancy. However, a comprehensive non-invasive genome-wide diagnostic approach suitable for all cases and all known monogenic disorders is needed.

The aim of this study was to utilize high-throughput deep exome sequencing (DES) on cfDNA as a global approach to detect inherited paternal sequence variants, allowing the detection of known dominant monogenic disease variants and enabling paternal variant exclusion in recessive disease diagnostics.

Methods: For our pilot study, we included 16 pregnancies with high-risk criteria for genetic testing. The gestational age ranged 14-21 weeks. We obtained plasma, parental blood and invasive fetal samples (trio), and applied DES. We performed bioinformatic evaluation of the results from trio and DES-NIPT analysis. To estimate DES-NIPT sensitivity and precision, we compared paternal variants found in the overlapping exonic regions in both the NIPT and the invasive sample, where variants from invasive samples were the reference.

Results: We achieved a mean alignment target coverage of 537x. Using the paternal allele frequency in NIPT samples, we estimated fetal fraction, which ranged 5.13-18.77%. The sensitivity and precision of our approach to detect all inherited paternal variants from plasma samples were 98.19% and 99.41%, respectively.

Conclusion: Using DES-NIPT approach, we can detect and exclude inherited paternal variants from NIPT samples with high sensitivity and precision. This potentially allows the detection of the majority known inherited monogenic disorders early in the pregnancy and non-invasively.

References:.

Grants: RegionSyddanmarksForskningspulje(20/14085);SyddanskUniversitet;FondentilLægevidenskabensFremme(19-L-0281);AaseogEjnarDanielsensFond(19-10-0259).

Conflict of Interest: None declared.

P02.004.A Valuable insights after one year whole exome sequencing in a fetal/prenatal setting

Sarah Delbaere[1], *Machteld Baetens*[1], *Candy Kumps*[1], *Ellen Roets*[2], *Noortje Van Oostrum*[2], *Bert Callewaert*[1], *Sandra Janssens*[1], *Olivier Vanakker*[1], *Björn Menten*[1]

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Background/Objectives: The aim of this study is to evaluate the implementation of whole exome sequencing (WES) for fetuses with congenital anomalies in a diagnostic workflow.

Methods: A fast-WES track for prenatal testing was implemented, with a maximal turn-around-time of 8 weeks. Depending on the fetus's abnormalities, targeted gene panel or Mendeliome analysis was performed in-silico, the latter comprising all human disease-linked genes. The variant analysis strategy was optimized to efficiently identify the causal variant, by focusing on de novo, X-linked or biallelic inheritance. National Belgian guidelines have been established to standardize the testing and reporting strategy.

Results: Over the period of one year, 59 structurally abnormal fetuses with normal copy number variant (CNV) analysis were investigated via exome sequencing. The overall diagnostic yield was 16.9%; in 10 out of 59 fetuses, a class 4 or 5 variant could be identified. When comparing analysis in on-going versus terminated pregnancies or miscarriages, diagnostic yield was 17.9% (5/28) and 15.6% (5/32) respectively. Diagnostic yield for targeted gene panels was 13% (3/23), which is less compared to 18.4% (7/38) for Mendeliome analysis. Additional CNV analysis on the WES data using the ExomeDepth algorithm revealed a small intragenic ASCC1 homozygous deletion in a fetus with heterozygous carrier parents. Furthermore, two inherited pathogenic incidental findings were reported (APOB and BRIP1).

Conclusion: These results proof an important contribution of WES within prenatal and fetal context to obtain a higher number of molecular diagnoses and can broaden the understanding of aberrant fetal phenotypes and their underlying genetic cause.

References:.

Grants:.

Conflict of Interest: None declared.

P02.005.B C-terminal cFLIP disruption in humans causes intrauterine multisystem anomalies due to aberrant cell death response switch

Ivana Lessel[1], *Leonie Piehl*[1], *Kira Kirchner*[1], *Thilo Diehl*[2], *Christian Kubisch*[1], ***Davor Lessel***[1]

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Background/Objectives: Pregnancy losses are mostly attributable to chromosomal abnormalities although recent studies also identified selected Mendelian disorders responsible for stillbirth. However, in the majority of cases the underlying cause of foetal death remains elusive. Here, we studied a consanguineous family with history of recurrent early miscarriages and two foetuses who presented with severe intrauterine multi-system anomalies, resulting in early demise.

Methods: Next-generation sequencing and segregation analyses were used to identify candidate variants. Functional characterization was performed in a dual-cellular model utilizing cell viability analyses, immunoblotting, immunoprecipitation, caspase activity assays, RNA-sequencing and methylation profiling.

Results: We identified a homozygous frameshift variant in CFLAR resulting in C-terminally truncated cFLIP, a master regulator of multiple cell death pathways. In-vitro analyses of the identified variant revealed enhanced binding to unprocessed procaspase-8, which correlated with abrogated caspase-8 activity, thus disclosing the inability to execute CASP8-mediated extrinsic apoptosis. Furthermore, we observed enhanced binding to RIPK1 and phosphorylated-RIPK1, which correlated with an enhanced aberrant execution of the RIPK1-RIPK3-MLKL phosphorylation cascade upon apoptotic stimuli. Thus, aberrant necroptosis induction concomitant with impaired ability to execute apoptosis during embryonic development underlies the cFLIP-deficiency. Moreover, integrative transcriptome and epigenome analysis of patient-derived fibroblasts revealed compensatory, pro-survival silencing of RIPK3 and CASP10.

Conclusion: Above identifying a novel Mendelian cause for foetal death and linking for the first time a pathogenic CFLAR variant to a human disease, we further expand the knowledge underlying the molecular switch between apoptosis and necroptosis, and provide the first direct evidence for the importance of necroptosis inhibition for proper human embryonic development.

References:.

Grants:.

Conflict of Interest: Ivana Lessel Full time, Leonie Piehl Full time, Kira Kirchner Full time, Thilo Diehl Full time, Christian Kubisch Full time, Davor Lessel Full time, 1. Deciphering molecular mechanisms of CHAMP1 deficiency-associated developmental delay, German Research Foundation (Deutsche Forschungsgemeinschaft, DFG). 2. The role of RNA interference in human neurodevelopment, Werner-Otto Foundation. 3. Identification and characterization of highly penetrant risk genes in testicular germ cell tumors, German Cancer Aid (Deutsche Krebshilfe). 4. Elucidating the role of stress granules associated translational control in aging and aging-associated diseases, Fritz Thyssen Foundation. 5. The RNA helicase DHX30: Physiological function and role in a neurodevelopmental disorder, German Research Foundation (Deutsche Forschungsgemeinschaft, DFG). 6. Genomic context analysis of clinically-relevant disease-associated variants in zinc finger proteins (mis-ZFs), German Academic Exchange Service (Deutscher Akademischer Austauschdienst, DAAD), The Champ1 Research Foundation, Member of the Scientific Advisory Board, https://champ1foundation.org/scientific-advisory-board/ AGO2 Association, Member of the Scientific Advisory Board, https://ago2.org/en.

P02.006.C Increased nuchal translucency (NT). Can we do more? Prenatal trio exome sequencing of >150 cases revealed unexpected findings in fetuses with increased NT

Heinz Gabriel[1], *Björn Schulte*[1], *Martin Ritthaler*[1], *Saskia Biskup*[1]

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Background/Objectives: Ultrasound diagnosis allows for the detection of half of all major structural anomalies. As a soft marker, nuchal translucency (NT) is associated with a spectrum of structural anomalies. Mostly, NT measurement is offered as part of screening for chromosomal abnormalities. About 20% of fetuses with increased NT will have a chromosomal abnormality. Noonan syndrome is considered the most frequently reported syndrome associated with increased NT. Prenatal panel testing for Noonan syndrome genes is nowadays widely offered in cases with increased NT. Aside from these Noonan syndrome genes, there are only a limited number of gene for which increased NT is documented.

Methods: In this study, we performed prenatal trio exome sequencing in cases with increased NT.

A cohort of 158 fetuses with increased NT were analysed by trio exome sequencing. The mean turn-around-time (TAT) was 12 days.

Results: We detected (likely) pathogenic variants in 46 cases (29%). Noonan syndrome genes were found in 11 cases. In 22 cases pathogenic variants were found in genes which are not to be known to be associated with increased NT so far.

Conclusion: Our data demonstrate that prenatal testing should not be limited to chromosomal aberrations, or RASopathies/Noonan syndrome, in cases with increased NT. Increased NT can be found in a wide range of syndromic conditions and might be one of the earliest detectable phenotypic features in the first trimester. We have shown the value of trio exome sequencing for the prenatal genetic diagnosis of fetuses with increased NT.

References:.

Grants:.

Conflict of Interest: Heinz Gabriel full time, Björn Schulte full-time, Martin Ritthaler full-time, Saskia Biskup full-time.

P02.007.D Prenatal WES for rapid detection of copy number variants and single gene disorders in uncultured samples

Vincenzo Cirigliano[1], *Elisabet Lloveras*[2], *Elena Ordonez*[1], *Isabel Castilla*[1], *Mireia Lechuga*[1], *Mafalda Almeida*[1], *Cristina Perez*[2], *Miriam Leon*[1;1;1]

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Background/Objectives: aCGH is currently the first line test in prenatal diagnosis. However, most cases with normal results require additional tests targeting specific indications or whole exome sequencing. We evaluated an optimized WES capture assay to detect both CNVs and SNVs in a single assay aiming to assess its performance for genomewide detection of CNVs in uncultured prenatal samples.

Methods: DNA extracted from uncultured amniotic fluids (17) and CVSs (8) were selected with pathogenic CNVs of different sizes reported by aCGH. Samples were sequenced using an improved whole exome capture of 51 Mb designed to allow genomewide CNV detection and optimised for coverage of a wide subset of genes related with fetal structural abnormalities and developmental delay.

Results: All samples passed sequencing QC metrics, full concordance between WES and aCGH results was observed with an expectable slight difference in CNVs breakpoints locations. CNVs ranged between 13.8Kb and 52.9Mb in size: 17 cases with a unique event, 4 cases with 1 gain and 1 loss, two more samples had 2 and 3 losses. One rare case of chromothripsis, with 8 duplications along different regions of the same chromosome was also correctly classified.

Conclusion: WES can be used to identify pathogenic CNVs in uncultured prenatal samples. CNVs testing as first analysis step on sequencing data has the advantage of allowing rapid inclusion of SNVs/indels detection in multiple genes if required. While reducing time/costs for the analyses, this single test approach has the potential to increase the overall diagnostic yield in prenatal diagnosis.

References:.

Grants:.

Conflict of Interest: None declared.

P02.008.A A positive nonivasive prenatal testing result for sex chromosome aneuploidies. What could we expect?

Radostina Raynova[1], *Stoyan Bichev*[2], *Katerina Vladimirova*[2], *Kameliya Kercheva*[1], *Violeta Dimitrova*[3], *Irena Bradinova*[2], *Ivanka Dimova*[4], *Alexey Savov*[1]

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Background/Objectives: Non-invasive prenatal testing (NIPT) for detection of fetal aneuploidy risk has been widely adopted in clinical practice due to its high positive predictive value (PPV) for common aneuploidies, involving chromosomes 21, 18, 13. However, PPV for sex chromosome aneuploidies (SCA) is as lower as 57.6 %. NIPT remains classified as a screening, non-diagnostic test with standard recommendations that any positive NIPT result be followed by confirmatory diagnostic testing.

Methods: We present three cases referred to our Laboratory for confirmation of positive results for SCA from NIPT: 1) for Jacobs syndrome (47, XYY), 2) Turner syndrome (45, X) and 3) a deletion of 9.71 Mb on Xp11.4-Xp11.23. Amniocenteses were performed and DNA was extracted from uncultured amniocytes. Commercial QF-PCR kit and MLPA probe mix were used and samples were analyzed on ABI 3130. Karyotyping was performed on cultured amniocytes using standard protocol.

Results: For the first case, QF-PCR showed a XXY profile instead of a XYY, and the karyotype was 46, XY[12]/47,XXY[18]. Monosomy X was excluded for the second case, karyotype was 46, X, del(Y)(q12). The Xp deletion of the third case wasn't confirmed by MLPA probe mix P034; the QF-PCR result was consistent with Turner syndrome.

Conclusion: The confirmation of a positive NIPT result for SCA demands an integral approach for the elucidation of the case and thus facilitating the counseling and patients' decision making.

References: None.

Grants: None.

Conflict of Interest: None declared.

P02.009.B Exome sequencing in prenatal diagnosis: results from 156 cases

Emmanuelle Ranza[1], *Xavier Blanc*[1], *Federico Santoni*[1], *Katayoun Afshar*[2], *Guerry Frederic*[3], *Bernard Conrad*[4], *Isabelle Eperon*[5], *Cécile Tissot*[6], *Cecile Deluen*[7], *Tanguy Araud*[7], *Loïc Baerlocher*[7], *Yasmine Sayegh-Martin*[8], *Anne-Claude Müller-Brochut*[9], *Christian Bisch*[5], *Wawrzyniec Rieder*[5], *Joel Fluss*[10], *Jean-Marie Pellegrinelli*[11], *Marta Carrasco*[12], *Thibaud Quibel*[13], *Sylvie Lacroix*[13], *Philippe Extermann*[5], *Graziano Pescia*[4], *Romaine Robyr Susini*[13], *Stylianos Antonarakis*[2]

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Background/Objectives: Exome sequencing (ES) has become a standard test for undiagnosed developmental disorders, now increasingly used in prenatal diagnosis.

Methods: We report here our experience with 156 consecutive prenatal cases through ES. The most common fetal signs were: increased nuchal translucency (25 %), congenital heart defect (21.8 %), skeletal malformations (18.6 %) and brain abnormalities (9.6 %). After performing a-CGH, 28 cases had solo and 128 trio ES.

Results: In 21.8 % of cases, we have identified a pathogenic or likely pathogenic variant (as per the ACMG guidelines) likely causative of the fetal phenotype. The diagnostic yield was 34.5 % for skeletal malformations, 15.4 % for nuchal translucency, 11.8 % for congenital heart defect, and 33.3 % for brain anomalies. In 29 of the solved cases, the pathogenic variants were SNVs, while 2 were pathogenic structural variants (SV). Furthermore, the trio ES has identified in three cases complete uniparental disomies UPD6, UPD16 and UPD17, all including isodisomic segments with likely causative recessive genes.

Conclusion: ES is a powerful method for the identification of causative variants in prenatal; trio sequencing reduces the turnaround time and increases the diagnostic yield. The VUS remain a challenge and guidelines are needed to assist in the interpretation and disclosure of the results.

The discovery of novel mendelian genes and the introduction of additional laboratory and computational methods such as long-read sequencing and improvement of SV detection may further increase the diagnostic yield.

References:.

Grants:.

Conflict of Interest: Emmanuelle Ranza Medigenome, Medigenome, Xavier Blanc Medigenome, Federico Santoni Medigenome, Katayoun Afshar Medigenome, Guerry Frederic Genesupport, Bernard Conrad Genesupport, Genesupport, Isabelle Eperon Dianecho, Cécile Tissot Clinique des Grangettes, Cecile Deluen Genesupport, Tanguy Araud Genesupport, Loïc Baerlocher Genesupport, Yasmine Sayegh-Martin Centre Jean-Violette, Centre Jean-Violette, Anne-Claude Müller-Brochut GynEcho, Christian Bisch Dianecho, Wawrzyniec Rieder Dianecho, Joel Fluss HUG, Jean-Marie Pellegrinelli HUG, Marta Carrasco: None declared, Thibaud Quibel Echofemme, Sylvie Lacroix Echofemme, Philippe Extermann Dianecho, Dianecho, Graziano Pescia Genesupport, Genesupport, Romaine Robyr Susini Echofemme, Echofemme, Stylianos Antonarakis Medigenome, Medigenome.

P02.010.C Frequency of cystic fibrosis in fetuses with echogenic bowel in Bulgarian population

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Background/Objectives: Cystic fibrosis (CF) is the most common life-limiting condition with autosomal recessive inheritance. The at birth prevalence of CF in Bulgaria was estimated to be 1:3,600 live

births. According to prenatal ultrasound evaluations 0,5-9,9% of cases with fetal echogenic bowel (FEB) are affected with CF. Recent studies revealed that detected echogenic bowel is associated with CF, chromosome abnormalities, congenital CMV infections, intrauterine growth retardation, and even fetal or neonatal death. However, the relation between the presence of FEB and cystic fibrosis is still scarcely studied. The purpose of this study was to assess the frequency of occurrence of cystic fibrosis in cases with fetal echogenic bowel.

Methods: 101 cases with FEB, detected during a routine ultrasound examination were screened for transmembrane conductance regulator (CFTR) mutations with Sanger sequencing at National Genetic Laboratory.

Results: Of the 101 cases studied, in 9 cases only one parent was carrier for CFTR mutation. Additionally, in 7 cases both parents were carriers of CFTR mutation. In five of these cases the fetuses had CF. Only one pregnancy was terminated. Other four was confirmed postnatally. In the other two cases the fetuses were negative.

Conclusion: We observed relatively high frequency of occurrence of CF in fetuses with echogenic bowel (5%, 5/101). However, the presence of CFTR mutation in the parents is not mandatory associated with the presence of CFTR mutation and CF in their fetus. For this reason after screening for carrier status of the parents, we recommend prenatal diagnosis for fetal CF when FEB is detected.

References: None.

Grants: None.

Conflict of Interest: None declared.

P02.011.D Genetic variants identified in Slovenian families with apparent non-syndromic orofacial cleft phenotypes

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Background/Objectives: Orofacial clefts (OFCs) are common congenital anomalies that manifest as cleft lip and/or cleft palate. Most OFCs are non-syndromic (nsOFCs), but can also occur in various syndromes (syOFCs). The majority of syOFCs have a known, monogenic cause, while nsOFCs usually have a complex aetiology. In some syOFCs, the presence of additional anomalies is not obvious, thus they often remain undiagnosed as such. Examples of such syOFCs are Van der Woude syndrome (VWS; *IRF6*, *GRHL3* mutations), and X-linked cleft palate (CPX; *TBX22* mutations). Moreover, there is increasing evidence that mutations in single genes may be implicated in some families with nsOFCs.

Methods: We recruited 35 Slovenian multiplex families. Using whole-exome sequencing (WES) and Sanger sequencing, we firstly screened the proband of each family for causal variants in *IRF6*, *GRHL3*, and *TBX22*. In probands without any relevant variants in these three genes, we filtered WES data for 72 additional genes. Family segregation was checked for each identified variant.

Results: We genetically confirmed the diagnosis of VWS in five families. We identified four (likely) pathogenic variants in *IRF6*: two stop-gain, one frameshift, and one missense variant. In one family, we identified the deletion of *TBX22* and confirmed CPX. We have also found one nsOFC family with deletions in coding regions of *ARHGAP29*.

Conclusion: With a two-step approach in gene selection and analysis, we identified families with syOFCs, and found a monogenic mutation in a family with nsOFCs. We present the first genetic study performed on Slovenian patients with apparent nsOFCs.

References:.

Grants: Slovenian Research Agency (J3-8207, MR51882).

Conflict of Interest: None declared.

P02.012.A Is nuchal translucency of 3.0- 3.49 mm an indication for NIPT or microarray-still needs a debate

Magda Rybak-Krzyszkowska[1;2], *Anna Madetko-Talowska*[3], *Katarzyna Szewczyk*[3], *Miroslaw Bik-Multanowski*[3], *Agata Sakowicz*[4], *David Stejskal*[5], *Marie Trková*[5], *Dagmar Smetanova*[5], *Silvia Serafim*[6], *Hildeberto Correia*[6], *Julián Nevado*[7;8], *Fe Amalia García Santiago*[7;8], *Maria Angeles Mori*[7;8], *Lena Rutkowska*[9], *Agata Kucińska*[9], *Agnieszka Gach*[9], *Hubert Huras*[1], *Magdalena Kołak*[1], *Malgorzata Srebniak*[10]

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Background/Objectives: Nowadays, fetal nuchal translucency (NT) values of >3.5 mm constitute an indication for invasive testing, optimally followed by chromosomal microarray. In our analysis, we wanted to evaluate the occurrence of clinically relevant sub-microscopic chromosomal aberrations in fetuses with the NT range from 3.0 to 3.49 mm, which would be missed by currently available non-invasive prenatal tests (NIPT).

Methods: A retrospective analysis of 271 fetuses with NT between 3.0 and 3.49 mm and increased risk after combined test in five cohorts of pregnant women referred for invasive testing and chromosomal microarray was performed: Czech Republic (n = 152), Poland (Cracow n = 48, Lodz n = 7) Portugal (n = 46) and Spain (n = 18).

Results: A chromosomal aberration was identified in 1:5 fetuses (19.19%; 52/271). In 15.13% (41/271) of cases trisomy 21, 18 or 13 was found. In 0.74% (2/271) sex chromosome aneuploidy was found. In 1.1% (3/271) of cases copy number variant (CNV) >10Mb was detected, which would potentially also be detected by NIPT. The residual risk for missing a clinically relevant (sub)microscopic chromosome aberration in the presented cohorts is 1:45 (2.21%; 6/271).

Conclusion: Our results indicate that a significant number of fetuses with increased risk after combined test and presenting NT of 3.0-3.49 mm carry a clinically relevant chromosomal abnormality other than common trisomy. Invasive testing should be offered and counselling on NIPT should include the resolution limitation that may result in NIPT false negative results in a substantial percentage of fetuses.

References:.

Grants:.

Conflict of Interest: None declared.

P02.013.B Sodium channel gene variants in fetuses with abnormal sonographic findings

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Background/Objectives: Voltage-gated sodium channels (VGSCs) are responsible for the initiation and propagation of action potentials in the brain and muscle. Pathogenic variants in genes encoding VGSC may therefore cause epileptic encephalopathies and congenital myopathies resulting in severe phenotypes. This study presents two cases of sodium channelopathies in pregnancies with abnormal ultrasound findings; identified as part of a larger cohort study.

Methods: Fetal samples with abnormal sonographic findings accompanied by normal karyotype and array-CGH results were included in the study. Trio-based whole-exome sequencing was performed, followed by Sanger sequencing for the confirmation of pathogenic variants.

Results: Two unrelated fetuses presented pathogenic variants in genes encoding the alpha subunit of VGSCs. A known de novo heterozygous missense variant was identified in the gene *SCN2A* (c.751G>A; p.Val251Ile) in a fetus with intrauterine growth retardation, hand clenching, ventriculomegaly and other structural findings that neonatally also exhibited refractory epilepsy, spasms, and MRI abnormalities. The second fetus was compound heterozygous for two parentally inherited novel missense variants in the gene *SCN4A* (c.4340T>C; p.Phe1447Ser), (c.3798G>C; p.Glu1266Asp). The fetus presented a severe prenatal phenotype including talipes, fetal hypokinesia, hypoplastic lungs, polyhydramnios, ear abnormalities and other. Both probands died soon after their birth.

Conclusion: Our results suggest a potentially crucial role of the sodium channel gene family in fetal development since non-functional VGSCs have been associated with severe fetal phenotypes and early lethality. New genotype-in utero phenotype associations related to this gene family may ultimately result in new additions to preconception and prenatal diagnostic panels.

References:.

Grants:.

Conflict of Interest: None declared.

P02.014.C Diagnostic yield and implications of exome sequencing analysis, including the use of copy number variant analysis pipeline, for pregnancy management in a series of 46 fetuses with structural anomalies

Sebastien Moutton[1;2], *Ines Harzallah*[3], ***Laure Raymond***[1], *Jérémie Mortreux*[1], *Nada Houcinat*[1], *Marine Dancer*[1], *Vanna Geromel*[1], *Radoslava Saraeva-Lamri*[1], *Angeline Preto*[1], *Luc Druart*[1], *Marc Nouchy*[1], *Fabienne Prieur*[3], *Marine Lebrun*[3], *Francis Ramond*[3], *Rodolphe Dard*[4], *Aude Tessier*[4], *Benjamin Dauriat*[5], *Valentin Marquet*[5], *Constance Wells*[6], *Caroline Deiller*[6], *Elise Schaefer*[7], *Leila Frigere*[8], *Patrice Bouvagnet*[1;8], *Renaud Touraine*[3], *Marie-Emmanuelle Naud-Barreyre*[1]

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Background/Objectives: During the past years, exome sequencing has been used more and more widely and contributed to increased diagnostic yield in many fields of human genetics including developmental disorders (25-40% according to the studies, the design, the limitation to diagnosis or extension to research). Its use has been progressively implemented in a prenatal setting, first to assess the yield in these particular patients, and second to assess its usefulness in pregnancy management.

Methods: Between June 2020 and November 2021, 46 exome analyses were asked for fetuses with structural anomalies (at the same time or after normal chromosomal microarray result), questioning the prognosis. Prescriptions came from several French multidisciplinary centers for prenatal diagnosis. Exome data were producted by Eurofins Biomnis laboratory and analyzed by 2 or 3 independant molecular geneticists, medical geneticists and/or scientists followed by meetings before reporting the results.

Results: In 11/46 = 24% of cases, a conclusive diagnosis could be made, with implications in pregnancy management in 5/11 = 45%, leading to opt for termination in 3 cases and to reassure in 2 cases (and 3 additional cases where the molecular diagnosis confirmed the suspicion by imaging and helped decision-making of termination). One diagnosis was an intragenic *GPC3* duplication not seen by chromosomal microarray. In at least 16 until 29/35 unconclusive results, the negativity contributed to the continuation of the pregnancy.

Conclusion: Although the cohort remains quite small, this study suggests that exome sequencing could be useful in selected prenatal situations, as reported in still not so numerous other studies.

References: -.

Grants: -.

Conflict of Interest: None declared.

P02.015.D Designing a quantitative PCR assay for fetal RhD genotyping from maternal plasma derived cell-free fetal DNA (cffDNA)

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Background/Objectives: Hemolytic disease of the fetus and newborn (HDFN) is typically caused by maternal alloimmunization against antigens of RhD positive red blood cells. During pregnancy, these anti-RhD antibodies can induce the destruction of fetal RhD positive red blood cells and lead to HDFN. Maternal alloimmunization can be prevented with antenatal and postnatal anti-D prophylaxis treatment, which is often routinely performed for all RhD negative mothers. However, fetal RhD genotyping allows anti-D prophylaxis to be targeted only for women carrying RhD positive fetus. Therefore, unnecessary anti-D prophylaxis treatments can be avoided. The aim of the study was to develop a multiplexed, quantitative PCR (qPCR) assay for fetal RhD genotyping using maternal plasma derived cell-free fetal DNA (cffDNA) as sample material.

Methods: The fetal RhD genotyping assay was designed for simultaneous detection of *RHD* gene exons 5, 7 and 10, in addition to an internal control gene. Dry qPCR chemistry was utilized to enable simplified qPCR workflow. The assay was confirmed to

be compatible with cfDNA samples extracted with PerkinElmer Vanadis Extract® system.

Results: The assay was able to distinguish RhD positive and negative cfDNA samples and showed similar performance with both liquid and dried qPCR chemistry. The presence of fetal DNA in the maternal cfDNA samples was demonstrated by amplification of male-specific sex-determining region Y (*SRY*) gene.

Conclusion: Dried qPCR chemistry enables a simple and time-saving workflow, which can be easily combined with other prenatal cffDNA screening. Further clinical testing is required to determine clinical sensitivity and specificity.

References:.

Grants: In collaboration with PerkinElmer Wallac Oy.

Conflict of Interest: Suvi Parviainen This Master's Thesis study was done as collaboration with PerkinElmer Wallac Oy and University of Turku. I am currently working full-time at PerkinElmer Wallac Oy as Subject Matter Expert (customer support)., Ville Veikkolainen Employee at PerkinElmer (Senior R&D Scientist).

P02.016.A LARS1-related disorder presenting as recurrent severe early onset fetal growth restriction attributed to placental and immune disease

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Background/Objectives: While the yield of chromosomal microarray in isolated fetal growth restriction (FGR) has been established, the added value of whole exome sequencing (WES) in such cases is unknown. We report recurrent severe early onset FGR in three of the five pregnancies in one family related to Infantile liver failure syndrome type 1 (OMIM 615438).

Methods:.

Results: Fetal autopsy showing hepatocyte hemosiderosis and placental pathology showing >50% necrosis of villi, multiple cysts and synechia, raised suspicion of both gestational alloimmune liver disease and thrombophilia. However, FGR fully reoccurred albeit IVIG and antithrombotic treatment. Fetal chromosomal microarray analysis was normal. Quatro WES revealed that two affected fetuses were compound heterozygotes for paternal truncating (c.1144G>T;p.Glu382Ter) and maternal missense (c.1511C>T;p.Ala504Val) variants in the *LARS1* gene (NM_020117.11) which is related to autosomal recessive infantile liver failure syndrome type 1. The variants co-segregated in a third affected fetus but not in a healthy daughter.

Conclusion: Aminoacyl-tRNA synthetase deficiencies, including *LARS1*-related condition, cause multisystemic disorders mostly affecting growth and nervous system. Inefficient translation and consequential decreased cell proliferation may explain the FGR phenotype. The family's genetic diagnosis will allow prenatal diagnosis or preimplantation genetic testing in future pregnancies and will help to avoid unnecessary treatments. This report highlights the importance of incorporating WES in the evaluation of early severe isolated FGR, as genetic diagnosis may completely change medical interventions and shed light on the expected outcome. Further studies are needed to evaluate the prevalence of single gene disorders in isolated FGR.

References:.

Grants:.

Conflict of Interest: None declared.

P02.017.B The role of a multidisciplinary team in managing variants of uncertain clinical significance in prenatal genetic diagnosis

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Background/Objectives: The aim of this study was to evaluate the role of the multidisciplinary team (MDT), the elements that facilitate rapid management of variants of unknown clinical significance (VUS) in prenatal diagnosis, and which types of VUS were considered for reporting.

Methods: We reviewed the frequency of MDT meetings and factors contributing towards decision making on reporting VUS after prenatal exome sequencing.

Results: The crucial elements that facilitate rapid VUS management were regular meetings, appropriate expertise, professional connections with other experts (ad hoc present) and psychological team safety. The following VUS were considered for reporting: possibly matching the fetal phenotype, associated with severe disorders when a functional test is available (e.g. enzymatic test), possibly associated with a disorder where early post-partum diagnosis and treatment are crucial for a better prognosis.

Conclusion: In order to protect prospective parents from the burden of VUS, the professionals should limit reporting them. Apart from using software filters and building professional guidelines, this can be achieved by sharing professional experience and responsibility in a MDT setting. Moreover, we noted that emotional distress caused by communicating uncertain information to the prospective parents can only be borne when supported by psychological team safety.

References:.

Grants:.

Conflict of Interest: None declared.

P02.018.C CHRAB – a tool for identification and visualization of chromosomal abnormalities in preimplantation embryos using next-generation sequencing data

Timing Liu[1], *Zainul Arifin*[1], *Vivienne Tan*[1], *Beatrice Pak*[1], *Arnold Tan*[1], *Simin Wong*[1], *Henry Yang*[1], *Caroline Lee*[1], *Mahesh Choolani*[1], *Peng-Cheang Wong*[1], ***Samuel Chong***[1]

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Background/Objectives: Current commercial solutions for preimplantation genetic testing of aneuploidies (PGT-A) using next generation sequencing (NGS) data entail the use of specific proprietary analysis software, which preclude analysis of NGS data generated from other sources. We have developed and evaluated CHRAB, a tool for identifying CHRomosomal ABnormalities in preimplantation embryos using NGS data.

Methods: Single-cell and five-cell replicates from aneuploid and segmentally unbalanced cell lines were whole genome amplified using SurePlex and subjected to Nanopore single-molecule sequencing, followed by CHRAB analysis and visualization. An aliquot of each Sureplex-amplified sample was also sequenced using a leading commercial NGS-based PGT-A solution (Illumina VeriSeq PGS) and analyzed using its proprietary analysis software (BlueFuse Multi).

Results: Using Nanopore sequencing and CHRAB analysis and visualization, the expected cell-line specific aneuploidy was detected in 84 of 88 (95.45%) single- and multi-cells tested, and the expected segmental imbalance (≥10 Mb) was detected in 88 of 90 (97.78%) single- and multi-cells tested. The corresponding percentages obtained using the commercial PGT-A solution were 95.45% (84 of 88 single- and multi-cells with an aneuploidy) and 98.89% (89 of 90 single- and multi-cells with a segmental imbalance of ≥10 Mb). Detection sensitivity for smaller segmental imbalances of <10 Mb was significantly lower on both platforms.

Conclusion: We have developed a bioinformatics tool for aneuploidy and segmental imbalance detection from single- and multi-cell NGS data, with accuracy comparable to commercial platforms.

References:.

Grants: NMRC OF-IRG 20Nov-0060 (Ministry of Health, Singapore) and NUHSRO/2019/055/RO5+5/Seed-Mar/08 (National University Health System, Singapore).

Conflict of Interest: None declared.

P02.019.D Perlman Syndrome: A prenatal and genetic diagnostic challenge

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Background/Objectives: Perlman syndrome (PS) is a very rare congenital overgrowth syndrome, with a prevalence estimated to be less than 1/1.000.000. Generally the diagnosis is based on neonatal clinical appearance and histologic findings. For a long time the genetic basis of PS was unknown with an assumed autosomal recessive inheritance. Since 2012 mutations in *DIS3L2* gene have been found to be associated to PS. Some cases have been described prenatally, but precise diagnosis in a prenatal setting remains difficult to obtain due to overlap with other overgrowth syndromes. Both prenatal imaging and genetic diagnostic technologies have enormously evolved over the past decade and are being implemented in prenatal diagnosis today, enhancing diagnostic yield.

Methods: Here, we a present prenatal case of PS, with phenotyping by subsequent ultrasound and MRI examinations and molecular diagnosis by NGS, with an overview of existing literature. Accurate prenatal phenotyping of PS and comparison with Beckwith-Wiedemann and Simpson-Golabi-Behmel sydrome was made.

Results: Confirmed prenatal diagnosis of PS was obtained by subsequent high resolution imaging with next generation sequencing (NGS) on amniotic fluid sample, showing 2 pathogenic variants in the *DIS3L2* gene, not yet described in existing literature.

Conclusion: Since knowledge of the concerned gene and use of new technologies, for imaging as well as genetic analysis, the diagnosis of PS can be made prenatally. This is of great value during prenatal counseling of pregnant couples, as PS is known to have a poor prognosis.

References: Astuti D et al Nature Genet. 2012.

Grants:.

Conflict of Interest: None declared.

P02.020.A Prenatal Trio-Exome-Analysis reveals not yet published prenatal manifestations of rare diseases

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Background/Objectives: Between 7-2021 and 12-2021 we performed 79 prenatal trio exome analyses with 23 positive results (29.5%) with a turnaround time of 12.05 days on average. In all cases, indication has been ultrasound abnormalities. Most frequent leading symptoms were increased nuchal translucency (n = 19), skeletal (n = 14), brain (n = 14), and heart (n = 13) abnormalities. Most pathogenic variants found in association with skeletal abnormalities were de novo and located in known disease genes. In some cases, pathogenic variants were associated with prenatal manifestations of rare diseases not published so far. Here we present two case reports.

Methods: Prenatal trio exome analysis.

Results: Case 1: Fetus with cerebella vermis hypoplasia, choroid plexus cyst and micrognathia in 19th week of pregnancy. Trio exome analysis demonstrated a frameshift variant in *CNOT3* (disease IDDSADF; OMIM #P: 618672), already published as pathogenic. Postnatal manifestations of IDDSADF are small chin, short stature, hypotonia, cerebellar abnormalities. Prenatal manifestations are not published yet.

Case2: Fetus with increased nuchal translucency and cystic hygroma in 13th week of pregnancy. Trio exome analysis provided a probably pathogenic missense variant in *KCNT2* (disease DEE57, OMIM #P: 617771). Postnatal manifestations of DEE57 are global developmental delay, hypotonia, intellectual disability and seizures. Hitherto only fetal hiccup has been mentioned in one case report.

Conclusion: Notwithstanding the possibility of spurious correlations, the two presented cases reveal so far unreported prenatal manifestations of rare diseases. More to be found. In addition prenatal trio exome analysis increases the diagnostic yield of genetic prenatal investigation, especially if increased nuchal translucency is present.

References:.

Grants:.

Conflict of Interest: None declared.

P02.021.B Expanded NIPS does not significantly increase the detection rate of abnormal genetic findings in pregnancies with normal ultrasound

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Background/Objectives: Non-invasive prenatal screening (NIPS) is currently widely used in many countries. The common NIPS technique is focused on trisomies 13, 18, 21 and sex-chromosomal aberrations (5-NIPS), while many laboratories offer expansion to include common microdeletions as well as genome-wide aberrations sized over 7Mb. We aimed to evaluate the theoretical yield of NIPS expansions compared to the commonly used 5-NIPS, based on chromosomal microarray analysis (CMA) results of pregnancies with normal ultrasound.

Methods: CMA results of pregnancies with normal ultrasound (including soft markers and abnormal maternal serum screening) were recorded. We have calculated the detection rate of 5-NIPS detectable aberrations and compared those to rates of findings detectable by 5-NIPS plus common microdeletions (1p36.3-1p36.2, 4p16.3-4p16.2, 5p15.3-5p15.1, 15q11.2-15q13.1, and 22q11.2), and genome-wide NIPS-detectable findings (including variants >7Mb).

Results: Of the 8,605 pregnancies, 44 (0.51%) 5-NIPS detectable findings were noted, ranging from 1.56% in 642 pregnancies with abnormal maternal serum screening, 0.63% in 318 pregnancies with soft markers, 0.62% in 4,378 women aged over 35 years, to 0.15% in younger women. Three cases of common microdeletions were detected in the overall cohort (0.03%), as well as six genome-wide-NIPS detectable findings (0.07%), yielding a non-significant difference compared to 5-NIPS.

Conclusion: NIPS expansion to common microdeletions as well as to genome wide-findings does not significantly increase the detection rate compared to 5-NIPS. These results facilitate informed decisions of couples regarding prenatal genetic screening using NIPS technology in comparison to CMA from invasive fetal sample.

References:.

Grants:.

Conflict of Interest: None declared.

P02.022.C A rare case of fetoplacental discrepancy: a normal result in chorionic villi in contrast to de novo unbalanced translocation 46,XX,der(6)t(6;17) in amniotic fluid

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Background/Objectives: We present a gravidity with different results from invasive prenatal testing. Firstly, a normal analysis of chorionic villi and subsequently an abnormal karyotype of cultured amniocytes were detected. Combined first trimester screening was positive and the fetal ultrasound scan revealed hygroma colli cysticum.

Methods: Prenatal ultrasound, aCGH, karyotyping and FISH.

Results: Microarray analysis of native chorionic villi showed a normal female profile of the fetus. The amniocentesis (at 16 WG) was performed due to other fetal ultrasound findings: ascites, hyperdense lung and hyperechogenic fetal bowel. Cytological analysis of cultured amniocytes revealed an abnormal karyotype: 46,XX,der(6) which was confirmed by microarray: arr[GRCh37] 6q26q27(162799322_170884575)x1,17q21.32q25.3(47109888_81044553)x3. De novo unbalanced translocation was determinated in the fetus: 46,XX,der(6)t(6;17)(q26;q27.3), where the 8 Mb terminal deletion of long arm of chromosome 6 and 34 Mb terminal duplication of chromosome 17 were determined. Karyotypes of both parents were normal. The gravidity was terminated and the samples of placenta, amniotic fluid, cord blood and skin were analyzed. Cytological analysis of placenta confirmed a normal karyotype, whereas in the other types of tissues the normal cell line was detected together with abnormal cell line with der(6) in different levels of mosaicism. The mosaicism of cell line with der(6) was defined by FISH analysis: amniotic fluid, fetus tissue, cord blood; 90%, 66%, 17.5% of interphase nuclei, respectively.

Conclusion: The mosaicism of normal cell line 46,XX together with abnormal cell line with de novo unbalanced autosomal translocation is extremelly rare and needn´t be detected in CVS.

References:.

Grants:.

Conflict of Interest: None declared.

P02.023.D False positivity and false negativity as a standard part of noninvasive prenatal testing

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Background/Objectives: Nonivasive prenatal testing (NIPT) is becoming highly accepted as a routine prenatal screening tool in most of developed countries. With advanced versions of NIPT tests covering aneuploidies of all chromosomes and subchromosomal aberrations false positives and false negatives appear more frequently.

Methods: To get comprehensive information about the reason of NIPT result falseness methods as qPCR, aCGH, FISH, karyotyping in diagnostic settings were used additionally to original low coverage whole genome sequencing based NIPT.

Results: After 19159 NIPT performed in routine clinical laboratory 9 analyses were reported as false negative (2) and false positive (7), in statistics focused solely on chromosomes 21, 18 and 13 trisomies, representing less than 0.05% of cases. When focusing on all chromosomes and also subchromosomal aberrations the frequency of such false results is even higher. From the portfolio of potential biological reasons we detected and confirmed by molecular methods cases related to confined placental mosaicism, true fetal mosaicism, maternal aberration in full and mosaic state, precancerous maternal aberration, incorrect previous anamnestic data. As technical reasons grey zone result, insufficient coverage and syndrome specific information were detected.

Conclusion: False negative and false positive NIPT results are reported every day all over the world and need to be addressed with comprehensive supplementary diagnostic testing. Consensus about its routine usage is needed and international guidelines should reflect this need too.

References:.

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Conflict of Interest: Gabriel Minarik part-time employee of Medirex Group Academy n.p.o and Trisomy test Ltd., principal investigator of running grants, Michaela Hyblova part-time employee of Medirex Group Academy n.p.o and Trisomy test Ltd., collaborator on several grants, Martina Sekelska part-time employee of Medirex Group Academy n.p.o and Trisomy test Ltd., collaborator on several running grants, Erika Tomkova full-time employee of Medirex Inc., Katarina Tothova full-time employee of Medirex Inc., Dagmar Landlova full-time employee of Medirex Inc., Peter Krizan part-time employee of Medirex Inc. and Medirex Group Academy n.p.o.

P02.024.A Clinical implementation of a custom oligonucleotide array-CGH. Experience in the largest cohort of Spanish prenatal samples (>5300 samples)

Olaya Villa Marcos[1], *Marina Viñas-Jornet*[1], *Noemí Sousa*[1], *Nerea Alvarez*[1], *Neus Fornés*[1], *Manel García-Aragonés*[1], *Luis Pérez-Jurado*[2], ***LLuís Armengol***[1]

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Background/Objectives: The implementation of genomic array in prenatal routines, when accompanied by pre- and post-test genetic counseling, has demonstrated its utility by fulfilling the longstanding need for a diagnostic test with a higher resolution and higher diagnostic yield than its predecessor, the conventional karyotype.

Methods: Array CGH was performed in 5388 prenatal samples (3972 amniotic fluids, 1131 corions and 285 fetal samples), using a custom 60K oligonucleotide-based microarray (qChip® CM) designed to maximize the detection of clinically relevant copy-number alterations, and minimize the detection of variants of unknown significance (VOUS). As a general rule, VOUS with unclear phenotypic effect according to current knowledge, and some susceptibility variants are not reported.

Results: We identified a total of 363 pathogenic or likely pathogenic alterations (detection rate: 6.73%) and 76 VOUS (1.41%). As expected, the greatest pathogenic detection rate (7.28%, 326/4477) was among fetuses with ultrasound anomalies, while detection rate was 4.06% (37/911) in normal ultrasound gestations. When both parental samples were available, the vast majority of the VOUS were inherited from a non-affected parent (90.24%) and could be reclassified as likely benign.

Conclusion: Our series reinforces the clinical utility of prenatal microarray testing: it nearly doubles the diagnostic yield of conventional karyotype (153/363 with variants <10Mb), with no significant increase in the frequency of VOUS that could interfere in decision making. In our experience, we highlight the importance of implementing aCGH in prenatal routines, for all gestations with an indication of invasive fetal sampling.

References:.

Grants:.

Conflict of Interest: Olaya Villa Marcos qGenomics, Marina Viñas-Jornet qGenomics, Noemí Sousa qGenomics, Nerea Alvarez qGenomics, Neus Fornés qGenomics, Manel García-Aragonés qGenomics, Luis Pérez-Jurado qGenomics, LLuís Armengol qGenomics.

P02.026.C The diagnostic yield of prenatal exome sequencing in fetuses with ultrasound anomalies

Karin Diderich[1], *Florentine Thurik*[1], *Martina Wilke*[1], *Marieke Joosten*[1], *Myrthe van den Born*[1], *Robert-Jan Galjaard*[1], *Marjolein Weerts*[1], *Marjon van Slegtenhorst*[1], *Frank Sleutels*[1], *Sander Galjaard*[2], *Maarten Knapen*[2], *Attie Go*[2], *Lies Hoefsloot*[1], *Malgorzata Srebniak*[1], *Hennie Brüggenwirth*[1]

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Background/Objectives: The aim of the study was to evaluate the diagnostic yield of routine exome sequencing (ES) in fetuses with ultrasound anomalies detected in the first two trimesters that had a normal molecular karyotype by chromosomal microarray.

Methods: A retrospective analysis of 138 fetuses with multiple or isolated ultrasound anomalies that were referred for ES in May 2019- March 2021. Some specific isolated abnormalities were excluded from ES: e.g. neural tube defect, ventricular septal defect and echogenic bowel. Variant analysis was limited to a broad gene panel consisting of genes involved in multiple congenital anomalies and/or intellectual disability as published before. We used trio analysis and filtering for de novo variants, compound heterozygous variants, homozygous variants, X-linked variants, variants in imprinted genes and for known pathogenic variants.

Results: Pathogenic and likely pathogenic variants (class 5 and 4 respectively) that explained the fetal phenotype were identified in 13% (18/138) fetuses. Potentially actionable class 3 variants (matching the phenotype) were reported in few cases after extensive discussion in a multidisciplinary team. The risk for a single gene disorder in the presented cohort was ~ 1:8. The time between the invasive procedure and genetic diagnosis was 13-18 days.

Conclusion: Our results indicate that a significant number of fetuses with ultrasound anomalies and normal molecular karyotype carry a clinically relevant (likely) pathogenic variant that can be diagnosed through prenatal exome sequencing. In our opinion not only microarray testing, but also exome sequencing should be offered in case of a fetus with ultrasound anomalies.

References:.

Grants:.

Conflict of Interest: None declared.

P02.027.D Use of prenatal exome sequencing in fetuses with ultrasound anomalies

Maria Segura-Puimedon[1], *Marta Carreño*[1], *Raquel Garcia*[1], *Lidia Carreño*[1], *Hector San Nicolás*[1], *César Arjona*[1], *Cèlia Sintas*[1], *Olaya Villa Marcos*[1], *Marina Viñas-Jornet*[1], *Mònica Vall*[1], ***Nina Bosch***[1], *LLuís Armengol*[1]

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Background/Objectives: Whole exome sequencing (WES) is an established diagnostic tool in postnatal settings for individuals with a suspected genetic condition. Recently, it is increasingly used as a diagnostic tool in prenatal settings as well. We present here our experience using this technology in fetuses with ultrasound anomalies.

Methods: WES was performed in 350 fetal samples with ultrasound anomalies. 248 samples were from evolutive pregnancies and 102 were from legal interruptions or stillbirths. Segregation studies were performed in cases with a candidate variant when possible.

Results: Common reasons for referral were skeletal anomalies, polymalformated fetuses, cerebral anomalies or specific syndrome suspicion. Pathogenic or likely pathogenic variants were identified in n = 56 (16%) of samples. In n = 64 (18.2%) cases, variants of unknown significance were identified. In more than half of the cases (58%) no candidate variant was identified. Diagnostic yield was higher in fetuses with skeletal anomalies, where pathogenic or likely pathogenic variants were identified in (n = 19) 33% of cases, and in fetuses with increased nuchal translucency or hydrops (Noonan syndrome suspicion), where a pathogenic variant was found in 23.5% (n = 13) of the samples.

Conclusion: Exome sequencing is a valuable diagnostic tool in fetuses with ultrasound anomalies, especially when skeletal anomalies are present or when Noonan syndrome is suspected.

References:.

Grants:.

Conflict of Interest: Maria Segura-Puimedon qGenomics, Marta Carreño qGenomics, Raquel Garcia qGenomics, Lidia Carreño qGenomics, Hector San Nicolás qGenomics, César Arjona qGenomics, Cèlia Sintas qGenomics, Olaya Villa Marcos qGenomics, Marina Viñas-Jornet qGenomics, Mònica Vall qGenomics, Nina Bosch: None declared, LLuís Armengol qGenomics.

P02.028.A Two novel variants in the POR gene causing Antley-Bixler syndrome type 2 detected prenatally in a foetus with abnormal ultrasound findings

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Charles University in Prague and Motol University Hospital, Department of Biology and Medical Genetics, Prague, Czech Republic.

Background/Objectives: Antley-Bixler syndrome (ABS) is a rare severe form of syndromic craniostenosis.

Type 1 ABS is autosomal dominant due to heterozygous pathogenic variants in the *FGFR2* gene, type 2 ABS is autosomal recessive due to biallelic pathogenic variants in the *POR* gene. Phenotypic features include craniostenosis, characteristic facial features, proptosis, skeletal anomalies and wide range of organ malformations.

We present a case of ABS type 2 detected on the basis of prenatal ultrasound examination.

Methods: Prenatal ultrasound, aCGH, targeted NGS panel of 89 genes associated with craniostenosis.

Results: Atypical head shape of foetus was seen on ultrasound in the 21st week of pregnancy. Combined screening of the 1st trimester was negative but level of PAPP-A was extremely high - 41.112 MoM. Specialized ultrasound examination confirmed turicephaly, deformity of the cerebral hemispheres, hypertelorism, low set ears and talipes varus. Craniostenosis was suspected. Parents decided to terminate pregnancy.

Autopsy of the foetus showed turicephaly, frontal bones agenesis, hypertelorism, proptosis, low set ears, lack of thumbs and arachnodactyly forefingers on both feet. Examination of the targeted NGS gene panel revealed 2 novel heterozygous probably pathogenic variants in the POR gene - c.1898 + 1del/c.450>A p.(Asp150Glu). Each variant is present in one of the parents.

Conclusion: This case demonstrates the importance of collaboration between precision ultrasound diagnostics and modern laboratory methods (NGS) in elucidating the causes of rare serious foetal disorders. We believe this is the first case of prenatal detection of ABS type 2 in the Czech Republic.

References:.

Grants:.

Conflict of Interest: None declared.

P03

SENSORY DISORDERS (EYE, EAR, PAIN)

P03.001.B Accurate clinical evaluation and high throughput technologies for the molecular characterization of hereditary hearing loss in a large cohort of Italian patients

Anna Morgan[1], *Stefania Lenarduzzi*[1], *Beatrice Spedicati*[2], *Paola Tesolin*[2], *Aurora Santin*[2], *Elisa Rubinato*[1], *Giorgia Girotto*[1;2]

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Background/Objectives: HL is the most prevalent sensory disorder, affecting ~6% of the world's population. From a genetic perspective, HL is a challenging disease because of its genetic heterogeneity and clinical variability with different onset of symptoms complicating the molecular diagnosis.

Methods: We applied a multi-step approach for testing 290 syndromic and non-syndromic HL families (negative to GJB2 mutation), which included:

1. an accurate clinical evaluation,.
2. the assessment of STRC-CATSPER2 and OTOA Copy Number Variants (CNV) via Multiplex Ligation-dependent Probe Amplification (MLPA),.
3. Whole Exome Sequencing (WES) in patients negative to steps 2-3.
4. Whole Genome Sequencing (WGS) in a subset of families negative to 2-3.

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Results: MLPA and WES led to the characterization of ~41% of cases. In particular, data analysis allowed to 1) confirm the relevant role of CNVs in the STRC gene, with ~5.9% of patients carrying a pathogenic deletion, 2) discover six new disease genes (e.g. PSIP1, TBL1Y, SPATC1L, PLS1, SLC12A2, MYO5C), and 3) shed the light on unexpected scenarios, such as the presence of dual molecular diagnosis, further exploring the complexity of HL. In negative cases to steps 2-3 we proceeded with WGS, searching for new possible causative alleles, including deep intronic and structural variants, starting from patients carriers of one pathogenic variant in recessive HL genes (e.g. SLC26A4).

Conclusion: Our approach led to an overall detection rate of ~41% in the Italian population. Furthermore, we expect WGS to unveil other possible disease mechanisms, deepening the knowledge of the biological mechanisms of HL.

References: NA.

Grants: NA.

Conflict of Interest: None declared.

P03.002.C A comprehensive WGS-based pipeline for the identification of new candidate genes in inherited retinal dystrophies

Elena Fernández-Suárez[1;2], *María González-del Pozo*[1;2], *Nereida Bravo-Gil*[1;2], *Cristina Méndez-Vidal*[1;2], *Marta Martín-Sánchez*[1], *Marcela Mena*[1;2], *Enrique Rodríguez-de la Rúa*[3;4], *Manuel Ramos-Jiménez*[5], *Maria Jose Morillo Sanchez*[3], *Salud Borrego*[1;2], *Guillermo Antiñolo*[1;2]

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Background/Objectives: To enhance the use of Whole Genome Sequencing (WGS) in clinical practice, it is still necessary to standardize data analysis pipelines. Herein, we aimed to define a WGS-based algorithm for the accurate variant interpretation in inherited retinal dystrophies (IRD).

Methods: This study comprised 429 phenotyped individuals divided into three cohorts: i) the training cohort with 209 genetically diagnosed IRD individuals was used to perform a statistical comparison of 14 pathogenicity predictors, redefine cutoffs and design the algorithm; ii) the validation cohort, consisting of 50 additional IRD individuals, 109 hereditary cancer patients and 47 with neurological diseases, allowed to select the optimal combination of predictors and to evaluate its translational value and iii) the discovery cohort including WGS data of 14 individuals from 7 genetically undiagnosed IRD families that were analysed applying our optimised workflow.

Results: The statistical analysis disclosed the most effective combination of predictors for non-splicing (CADDv1.6, MAPP, Grantham, and SIFT) and splicing (SpliceAI and NNS) variants. The pipeline validation showed high sensitivity, detecting 90% of causal variants in all the pathologies tested. This approach allowed identifying variants in candidate genes for IRD, such as CFAP20, in the discovery cohort families. Moreover, our results showed a 90% reduction of variants to be manually reviewed applying the customized cutoffs instead of the literature values.

Conclusion: Given the lack of consensus on the use of prediction tools, we offer a translational strategy for accurate WGS data prioritization in the clinical setting.

References: PMIDs: 24618965; 20238084.

Grants: ISCIII-ERDF/ESF (PI18/00612; PI21/00244; FI19/00091), Andalusian Government (PEER-0501-2019), F.Isabel Gemio/F.Cajasol [FGEMIO-2019-01].

Conflict of Interest: None declared.

P03.003.D Evaluation of CFAP20 as a candidate gene for autosomal recessive non-syndromic retinitis pigmentosa

María González-del Pozo[1;2], *Elena Fernández-Suárez*[1;2], *Nereida Bravo-Gil*[1;2], *Cristina Méndez-Vidal*[1;2], *Marta Martín-Sánchez*[1], *Marcela Mena*[1;2], *Enrique Rodríguez-de la Rúa*[3;4], *Manuel Ramos-Jiménez*[5], *Maria Jose Morillo Sanchez*[3], *Salud Borrego*[1;2], *Guillermo Antiñolo*[1;2]

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Background/Objectives: The cilia and flagella-associated protein 20, CFAP20, encodes for a highly conserved protein that plays a critical role in cilia formation and morphology. Previous studies have demonstrated that CFAP20 (Bug22) depletion causes a ciliary phenotype in both in-vitro and in-vivo models, supporting its likely implication in human ciliopathies. On the other hand, retinitis pigmentosa (RP) is a highly penetrant phenotype among a vast majority of ciliopathies. Here, we aimed to evaluate the association between CFAP20 variants and autosomal recessive RP.

Methods: Two members of one consanguineous non-syndromic RP family underwent WGS using Illumina's HiSeqX platform. Segregation analysis was conducted by Sanger sequencing. The retinal expression and localization of human CFAP20 were evaluated by real-time-qPCR and immunohistochemistry using retinal sections from healthy donors. The CFAP20 clinical impact was further investigated using 3D-modeling and protein-protein interaction networks.

Results: An in-house data analysis pipeline allowed the identification of one homozygous variant in CFAP20 (c.337C>T; p.Arg113Trp) segregating with the disease. Comparison of the relative mRNA levels between different tissues showed that CFAP20 mRNA was highest in the retina. Specific immunolabeling was observed in the photoreceptors layer. Our in-silico mutagenesis experiments also predicted conformational changes secondary to hydrogen-bonds loss. Additionally, CFAP20 is known to interact with the RP-associated protein, ARL2BP.

Conclusion: Although additional studies are needed, this work led us to propose CFAP20 as a candidate gene for non-syndromic RP and is expectedly to expand the mutational landscape of ciliary genes associated with human diseases.

References: PMIDs:24414207;20118210;24574454;31763178; 23849777.

Grants: ISCIII-ERDF/ESF (PI18-00612;PI21-00244;FI19/00091), Andalusian Government (PEER _0501_2019), F.Isabel Gemio/F.Cajasol (2019-01).

Conflict of Interest: None declared.

P03.005.B Genetic heterogeneity of prelingual severe to profound hearing impairment in patients from North African countries and Jordan

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Background/Objectives: Prelingual hearing impairment (HI), one of the most frequent monogenic disorders, has a very high genetic heterogeneity. For genetic counseling as in the perspective of gene therapy, it is essential that all patients can benefit from molecular diagnosis.

Methods: We explored the variants responsible for deafness in a total of 450 unrelated patients affected by severe to profound early onset HI from North African (Algeria, Mauritania, Morocco, Tunisia) and Middle East (Jordan) countries. To this purpose, we developed a targeted exome high throughput sequencing of 156 known deafness genes (HearPanel-IdA), followed by qPCR analysis and only retained predicted pathogenic variants.

Results: This allowed us to ascertain molecular diagnosis for a total of 391/450 patients (86.9%). Remarkably, we found 63/450 patients (14%) with variants in Usher syndrome (USH) genes of whom 40 had been clinically diagnosed as Usher. In the other 23 patients, subsequent clinical exams established the existence of a retinitis pigmentosa. Moreover, a total of 213 predicted pathogenic variants were identified in 49 known deafness genes of which 84 (39.4%) never reported. This makes the HearPanel-IdA a powerful and cost-effective tool to identify rare mutations causing HI.

Conclusion: The use of HearPanel-IdA as a routine investigation should not only facilitate the detection of rare mutations in uncommon HI genes, but also contribute significantly to the early diagnosis of specific forms of syndromic HI such as Usher syndrome, for which early cochlear implantation is of utmost importance because of the secondary sight loss.

References:.

Grants: FPA-IDA05, ANR-10-LABX-65, ANR-11-IDEX-0004-02, ANR-11-BSV5-0011, ANR-16-CE13-0015-02.

Conflict of Interest: None declared.

P03.006.C Deciphering the genetic architecture of inherited retinal diseases in the Iranian population by integrated exome sequencing

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Background/Objectives: To uncover the underlying molecular causes of inherited retinal disease (IRD) in 105 unrelated families of Iranian descent, an integrated approach consisting of whole exome sequencing (WES) and autozygosity mapping was used.

Methods: WES was performed in 105 Iranian IRD families, predominantly originating from a consanguineous background (77%). Data-analysis was performed using the in-house Seqplorer tool. Copy number variants (CNVs) were assessed via ExomeDepth. Variants were validated, classified (ACMG/ACGS guidelines) and segregation analysis was performed. AutoMap was used to determine runs of homozygosity (ROHs).

Results: By interrogating 290 known IRD genes using a WES-based analysis, we obtained a molecular diagnosis for 85% of the IRD cohort. In total, 103 (likely) disease-associated variants were identified in 42 genes, 58 of which are novel variants. *ABCA4*, *EYS, AIPL1* and *CRB1* were the four most implicated genes. In addition, the importance of structural variation (SV) in IRD was demonstrated, with CNVs identified in 8% of the cohort, including novel CNVs in *CDHR1, CHM* and *RD3*. Homozygous nonsense and missense deleterious variants were found in novel retina-expressed candidate IRD genes, including *PFKFB2* and *QRFPR*.

Conclusion: This integrated study using WES and an in-depth analysis of the variants provides insight into the genetic architecture of IRD in Iran. We provided 85% of patients with a molecular diagnosis and expanded the molecular spectrum of IRD in Iran by the identification of novel variants in the majority of patients. Finally, autozygome-guided exome sequencing revealed several novel candidate genes for IRD in unsolved cases.

References:.

Grants: FWO1802220N.

Conflict of Interest: None declared.

P03.007.D Transient receptor potential ankyrin 1 (TRPA1) channel: rare variants in chronic neuropathic pain patients

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Background/Objectives: Chronic neuropathic pain is amongst the most common non-communicable disorders, with a significant impact on patients' quality of life. The aim of this study is to assess the incidence of *rare* mutations in patients with neuropathic pain (NeuP).

Methods: We collected 480 patients complaining NeuP, and 216 healthy controls (HC) aged over 50. Subjects were classified according to symptoms distribution as "length-dependent", "non-length-dependent" and "atypical" and analysed by target NGS of 107 genes involved in pain signalling. Rare genetic variants were selected and classified following ACGS recommendations.

Results: *TRPA1* showed a significant overrepresentation of rare variants with functional or disrupting impact on the protein in NeuP patients compared to HC. We found that mutated samples with rare functional or disruptive variants were 2.92 time ($p = 0.017$) as prevalent in NeuP (7.7%) compared with HC (2.85%). *TRPA1* variants are mainly clustered in the N-terminal domain containing the ankyrin repeats, involved in channel function and regulation. Among patients harboring *TRPA1* mutations, 18 (69%) has non-length-dependent NeuP, 10 (38%) reported cold-induced or cold-accentuated pain, 12 (46%) neuropathic itch and 14 (48%) electric shock-like pain.

Conclusion: *TRPA1* encodes for a polymodal Ca2+ channel, involved in pain and inflammation, activated by potentially noxious stimuli, chemicals, and cold. The only genetic disease associated with *TRPA1* is Familial Episodic Pain Syndrome. High-throughput studies on SNPs frequency found no association with NeuP susceptibility. Focusing on rare high-impact mutations, our study suggests that *TRPA1* could increase the risk for complex painful conditions.

References:.

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P03.008.A Molecular characterization of inherited retinal degenerations in a Swiss patient cohort

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Background/Objectives: Inherited retinal degenerations (IRDs) are a group of disorders known for their genetic and clinical heterogeneity, which make molecular diagnosis a challenging process.

Methods: We collected approximately 200 DNA samples from more than 170 families with IRDs and other ocular disorders, ascertained by the Department of Ophthalmology at the University of Basel, and we developed a robust workflow based on the next generation sequencing (NGS) to identify the genetic determinants for these conditions. All patients underwent an ophthalmic examination performed by an IRD specialist, and their DNA was extracted from whole blood or saliva samples. Whole Exome Sequencing (WES) was performed for at least one affected individual from each family.

Results: Thus far, NGS analysis of 109 probands enabled the identification of causative variants in 37 known disease genes for 80 individuals, leading to an overall molecular characterization rate of 73%. Most identified mutations were single nucleotide variants (SNVs) and small deletion or insertions, whereas 6% of mutations were copy number variants (CNVs). Interestingly, mutations in intronic regions contributed to the diagnosis of 17 patients (>20% of all solved cases), with 3 deep intronic variants identified by an extensive analysis.

Conclusion: Integration of a research approach into analytical pipeline, together with the precise description of the clinical phenotype, lead to a high molecular diagnostic rate for patients with IRDs. Molecular diagnosis is crucial for the development of future gene therapies and proper genetic counseling of all patients.

References:.

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Conflict of Interest: None declared.

P03.010.C Visual acuity GWAS in children identify novel genes and pathways

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Background/Objectives: Visual acuity (VA) is a measure of the eyes' ability to distinguish between shapes and overall detail of objects. Reduction in VA is associated with myopia, reading disability (RD) and lower quality of life. Genetics of VA are not well understood due to most of the genome wide association studies (GWAS) related to vision, focusing on myopia or age-related macular degeneration, predominantly in adults.

Methods: We performed GWAS for VA on children aged 11,5 years old, from the ALSPAC data (N = 6,807), calculated polygenic risk scores and applied novel, non-standard post GWAS analyses tools - FINDOR and Downstreamer. FINDOR uses functional annotations to reweight GWAS summary statistics p-values and Downstreamer prioritises genes and pathways that would not normally be analysed for GWAS loci. Neither tool uses any phenotypic information.

Results: We report the first GWAS for VA in children. The top hit (*NPLOC4* locus) was previously associated in GWAS for vision outcomes in adults and we find that having a higher polygenic risk for ADHD and RD is correlated with worse VA. Post-hoc analysis showed significant enrichments of genes and pathways related to vision. FINDOR re-weighted p-values resulted in six additional SNPs reaching statistical significance. Downstreamer prioritised two genes, namely *PDE6G* and *RFSN*, both important in sensory functions.

Conclusion: Our GWAS and post-GWAS analyses for VA resulted in significant associations despite a small sample size. This indicates the potential of VA as a useful phenotype for dissecting the genetics of vision.

References:.

Grants:.

Conflict of Interest: None declared.

P03.012.A Spectrum of ABCA4 variants in Slovenian patients with Stargardt disease

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Background/Objectives: There are >1290 known pathogenic variants in *ABCA4* causing heterogeneous phenotypes. This study aims to report the genetic and clinical characteristics of the Slovenian ABCA4 cohort.

Methods: Study included 75 patients (28 male; median age at the last exam 32 years, range 8-75). Genetic analysis was done with next-generation sequencing, deep intronic regions were included in 65/75. Phenotype analysis included age at disease onset, Snellen decimal best corrected visual acuity (VA), pattern and full-field electroretinography (ERG), and fundus autofluorescence (FAF) appearance.

Results: 63 (84%) patients had ≥2 identified variants, whereas 12 (16%) had 1 identified variant. Most frequent variants were p.(Asn1868Ile) (11% alleles), p.(Gly1961Glu) (11%), p.[(Leu541-Pro;Ala1038Val)] (10%), p.(Arg681*) (7%) and c.5714+5G>A (7%). The latter was more frequent than in a multicentric study of 279 patients (2%) (1). 7/52 different variants were novel. Median age at onset was 15 years (range 5-60). Late-onset (>40 years) was present in 28%. Median VA was 0.16 (range counting fingers-1.0). According to ERG, generalized retinal dysfunction (ERG group 2 or 3) was present in 52%. Abnormalities on FAF extended beyond the vascular arcades in 60%. Patients with late-onset had a higher frequency of foveal sparing (44%) than other patients (9%). However, frequency of missense variants was similar between the two groups (72% vs 67%).

Conclusion: Slovenian ABCA4 cohort has a specific spectrum of *ABCA4* variants. 13% of variants were novel, while c.5714+5G>A was relatively more frequent than in other cohorts. Around one-third of patients had late-onset disease, which may be misdiagnosed as age-related macular degeneration.

References: (1). PMID:29925512.

Grants:.

Conflict of Interest: Jana Sajovic Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia, Andrej Meglič Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia, Martina Jarc-Vidmar Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia, Vlasta Hadalin: None declared, Zelia

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P03.013.B Biallelic variants in KITLG cause Waardenburg syndrome type 2, albinism-deafness syndrome and oculocutaneous albinism

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Background/Objectives: Pathogenic variants in *KITLG*, a key player in melanocyte proliferation and pigment production, cause autosomal dominant non-syndromic hearing loss, Waardenburg syndrome type 2 and familial progressive hyperpigmentation and familial progressive hyper- and hypopigmentation. We expand this spectrum to include a recessive disorder presenting with a hypomelanosis spectrum with or without hearing impairment.

Methods: We characterized the largest case series with biallelic KITLG variants identified through precision phenotyping, multiple sequencing approaches and extensive networking.

Results: We expand the KITLG-related hypomelanosis spectrum to include different patterns of distal depigmentation, partial depigmentation resembling Tietz albinism-deafness syndrome and complete depigmentation reminiscent of oculocutaneous albinism. We speculate that loss-of-function KITLG variants cause oculocutaneous albinism while those with possible residual function cause Waardenburg syndrome type 2 or albinism-deafness syndrome.

Conclusion: We expand the understanding of the mode of inheritance of Waardenburg syndrome type 2 to include autosomal recessive transmission. This work defines KITLG as a new molecular cause of autosomal recessive albinism-deafness syndrome and oculocutaneous albinism.

References:.

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P03.014.C Dysregulated microRNA in skin epidermis contributes to small fiber neuropathy pathophysiology

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Background/Objectives: Small fiber neuropathy (SFN) is a multifactorial condition defined by impairment of peripheral nerve fibres often accompanied by pain. Lately, miRNAs are emerging as key fine-tuning regulators in complex neurodegenerative diseases, particularly associated with regulation of axon guidance and cross-talk. Our study aimed to investigate miRNA profiles of painful SFN patients and their possible correlation with underlying disease mechanisms.

Methods: Total RNA was isolated from skin epidermis. Unbiased miRNA expression quantification was performed using microfluidic array containing 754 miRNAs. Discovery and validation profiling involved 23 painful SFN patients and 15 healthy controls. We applied the relative threshold (Crt) method and NormFinder software was used to determine the optimal normalization gene. Results were expressed as relative quantification with healthy control tissue as reference group. Moreover, target and functional enrichment analyses were performed to explore possibly affected biological processes and molecular functions.

Results: We identified a miRNA as significantly downregulated in SFN patients respect to controls. The validation revealed an optimal diagnostic accuracy (AUC = 96.4%). Functional enrichment analysis showed involvement in biological processes and pathways that are closely related to nociception signaling cascade triggering various cellular responses and pain-related channel trafficking. Specifically, miRNA's effect on target genes such as *TRPV1, MAPK14, NTRK1* dysregulates inflammatory mediator regulation of TRP channels, cAMP and MAPK signaling pathways.

Conclusion: Our proof-of-concept study of miRNA profiling of human epidermis provided novel hints on epigenetic regulation of epidermal innervation and chronic neuropathic pain, while adding value to the dysfunction of axon-cell network in the contest of SFN.

References: None.

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P03.016.A Achromatopsia: The curious case of triallelic inheritance

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Background/Objectives: Achromatopsia is a retinal disorder characterized by reduced or complete loss of color vision, reduced visual acuity, pendular nystagmus, and congenital photophobia (1). Affected patients show limited or complete lack of cone photoreceptor activity, caused in the majority of cases by biallelic variants in the *CNGA3* or *CNGB3* genes (2, 3), encoding CNG channel subunits that heterodimerize to mediate membrane hyperpolarization in response to light. A recent study by Burkard et al (2018) looked into the controversial p.R403Q hypomorphic variant in *CNGB3*, which in a biallelic state shows high variability, reduced penetrance, with high frequency in unaffected individuals. The authors showed that a trialleic co-occurrence of heterozygous pathogenic *CNGA3* variant, in individuals with the homozygous *CNGB3* p.R403Q variant, contributes to an exacerbated retinopathy (3).

Methods: We present a four-year-old boy with visual defects since age of six months, preferring dark or dimly lit rooms, showing nystagmus, and defective color discrimination. We performed whole exome sequencing to identify a possible genetic cause.

Results: The analysis lead to the identification of a new triallelic combination of the homozygous hypomorphic *CNGB3* p.R403Q variant with the monoallelic known pathogenic *CNGA3* variant p.A323P, which can explain the phenotype of the boy.

Conclusion: Along with broadening the list of triallelic variant combinations for achromatopsia, with this case, we aim to raise awareness in the clinical genetics community of the presence and importance of digenic-triallelic inheritance, which has implications for diagnosis, prognosis, and genetic counseling.

References: PMID: (1) 20301591, (2) 9662398, (3) 10958649, (4) 30418171.

Grants:.

Conflict of Interest: None declared.

P03.017.B Retinitis pigmentosa caused by mutations in the ush2a gene: clinical trend comparison and genotype-phenotype correlations

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Background/Objectives: Mutations in the USH2A gene are responsible for both isolated and syndromic (Usher syndrome type IIa) retinitis pigmentosa. We studied genotype-phenotype correlations and compared clinical prognosis between Usher syndrome type IIa and non-syndromic RP.

Methods: Clinical data, visual acuity (VA), visual field (VF), retinal imaging and electrophysiologic features were extracted from medical records of patients affected, with non-syndromic RP (n 6) and Usher syndrome type IIa (n 25). The patients were carriers of at least one pathogenetic USH2A mutation and they underwent to counseling in the medical genetics unit of University of Bologna (2003-2021). Statistical analysis has been performed (Student's t-test, Chi-squared test, Fisher's exact test).

Results: Participant groups had similar distributions of gender and similar ethnicity. Usher syndrome type IIa patients demonstrated earlier symptoms than non-syndromic RP (14 vs 27 years, P = 0,04), earlier clinical diagnosis (24 vs 43 years, P = 0,01), earlier molecular diagnosis (37 vs 58 years, P = 0,04) and earlier visual impairment (40 vs 56 years for VF, 45 vs 68 years for VA, P = 0,02). Truncating mutations are associated with earlier symptoms (13 vs 20) and with syndromic phenotype. We found novel variants (4 missense, 2 frameshift, 1 splicing) in both groups and suspected pathogenicity has been raised for other (n 3).

Conclusion: Patients with Usher syndrome type IIa have symptoms and severe visual impairment earlier than non-syndromic RP. One truncating mutation seems to predispose to worse clinical trend, two truncating mutations in USH2A is associated with syndromic phenotype.

References:.

Grants:.

Conflict of Interest: None declared.

P03.019.D Functional characterization of potential spliceogenics PAX6 variants in aniridia combining minigenes and long-reads sequencing

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Background/Objectives: *PAX6* is a highly-conserved transcription factor involved in ocular development. Several aniridia-associated exonic and intronic variants located in the hotspot exon 6 of *PAX6* generate aberrant splicing patterns involving 5 cryptic donors. We aim to functionally study the potential spliceogenic effect of other variants affecting this exon.

Methods: Potential spliceogenic variants located in exon 6 were selected from our aniridia cohort or mutational databases based on in silico splicing analysis and further characterized in vitro. Then, a minigene was generated from the pSPL3 vector containing the full sequences between exons 5 to 7 of the *PAX6* gene. Directed mutagenesis was used to introduce the selected variants in the PAX6_exon 5-7 minigene. Mutated and wild-type minigenes were transfected in HEK293 cells and total RNA was extracted. Finally, the splicing isoforms were analysed by RT-PCR using Oxford Nanopore long-read and Sanger sequencing.

Results: After an in silico analysis of 120 *PAX6* variants, 6 of them showing potential splicing effects were analysed using in vitro minigenes. Four showed aberrant splicing patterns, including intronic retentions and partial or total exon skipping. Two of them, initially considered variants of uncertain significance, were reclassified as probably pathogenic.

Conclusion: In silico and in vitro minigenes assays are a good approach to test the involvement of different genetic variants in the splicing process. In addition, this functional characterization is useful to deepen into the pathogenicity of aniridia-causing variants.

References:.

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Conflict of Interest: None declared.

P03.020.A Rare missense variants and deletions in α-tectorin may drive a change in tectorial membrane stability in familial Meniere's disease

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Background/Objectives: Meniere's disease (MD) is an inner ear disease characterized by low frequency sensorineural hearing loss associated with vertigo and aural fullness. Familial aggregation has been found in 9-10% of MD patients showing, mostly, an autosomal dominant inheritance with incomplete penetrance. Nevertheless, other inheritance patterns have been proposed, such as recessive and digenic inheritance involving rare variants in *OTOG* and *MYO7A* genes. This study aimed to search for relevant genes not previously linked to MD.

Methods: Exome sequencing was performed in 99 individuals diagnosed with MD. Candidate variants were classified according to the ACMG/AMP guidelines and their effects were evaluated by protein modeling. Audiometric evaluations were retrieved, and a case report was made of each family to assess genotype-phenotype correlations.

Results: *TECTA* gene was highlighted as candidate for four multicase MD families and two additional families with one MD patient and relatives with partial syndromes. Four rare missense variants and two frameshift deletions were found in heterozygous state segregating the MD phenotype. These variants could affect the stability of α-tectorin based on the predicted protein model.

Conclusion: Several MD families were identified carrying rare variants and deletions in the *TECTA* gene, which encodes one of the main proteins of the tectorial membrane (TM). The TM is an extracellular matrix localized over the sensory epithelium mediating the mechanical stimulation of cochlear hair cells. Modifications on the TM stability and the micromechanics involved in the sound-evoked motion of stereocilia may drive MD.

References:.

Grants: H2020-SC1-2019-848261 (UNITI), CECEU PY20-00303 (EPIMEN), European Regional Funds B-CTS-68-UGR20 (M3N-OMIC) and the Schmieder-Bohrisch Foundation.

Conflict of Interest: None declared.

P03.021.B Investigation of gene variations in inherited retinal dystrophies via next-generation sequencing

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Background/Objectives: Inherited Retinal dystrophies(IRD) are a group of progressive degenerative disorders of the retina with clinical and genetic heterogeneity. Common presentations include night blindness and peripheral vision abnormalities. Among retinal dystrophies most common sub-type is retinitis pigmentosa. In addition Leber congenital amaurosis, Usher syndrome, Stargardt disease are the other rare subtypes. This study aims to determine the effectiveness of the multigene panel testing in retinal dystrophies and to reveal genetic etiology of the IRD patients.

Methods: Thirty probands having retinal dystrophy with specific examination findings were included in this study. After evaluation of clinical and family histories, probands were screened using a custom designed retinal disorders panel including 140 genes via next-generation sequencing (NGS). The family members

of the probands carrying pathogenic variations were screened via Sanger Sequencing.

Results: Among 30 cases, 14 were born to consanguineous parents and IRD family history was reported in 8 cases. Onset of the ages of the probands ranged between 3 months-48 years.We detected in 21 (70%) probands biallelic pathogenic variations, which of 5 were novel, in CYP4V2(n = 4), ABCA4(n = 4), USH2A (n = 3), RDH12 (n = 2), PROM1, ABHD12,TTC8, BBS1, RP1, CRB1, MAK, GRK1 genes.

Conclusion: The detection of 5 novel pathogenic variations has contributed to the expansion of the mutation spectrum. Also, the diagnosis of 70% of the patients supports that the NGS panel is an effective diagnostic method in IRD. Defining molecular etiology of IRD is important in terms of screening at risk family members and giving appropriate genetic counseling for preimplantation genetic diagnosis opportunities.

References:.

Grants:.

Conflict of Interest: None declared.

P03.022.C Cryptic chromosomal rearrangements disrupting the PAX6 locus identified in aniridia cases based on long-read whole-genome sequencing

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Background/Objectives: Haploinsufficiency of the transcription factor *PAX6* is the main responsible for aniridia, a disorder mainly characterized by iris and foveal hypoplasia. 11p13 microdeletions altering *PAX6* or its 3′ regulatory regions were found in ~25% of aniridia patients. However, only few complex chromosomal rearrangements have been involved in aniridia which can be attributed to the inherent difficulty of detecting structural variants (SVs) involving repetitive/low-complexity regions. Long-read sequencing (LRS) techniques may overcome these limitations. Our objective was to assess the presence of cryptic SVs in our "PAX6-negative" cases suffering from aniridia using LRS approaches.

Methods: Long-read genome sequencing was conducted in two previously unsolved sporadic patients of our aniridia cohort after short-reads sequencing and CGH arrays. Libraries were prepared using high-weight molecular DNA following Oxford Nanopore Technologies protocols and sequenced at 30x on a PromethION. Detected SVs were validated using Sanger sequencing and/or FISH analysis.

Results: LRS analysis revealed a 4.9 Mb inversion in 11p13 involving the intron 7 of the canonical *PAX6* isoform disrupting its linear structure. In a second patient, LRS revealed breakpoints for an apparently balanced translocation t(6;11) that affects the centromeric region on 6p11.1 and the downstream regulatory region (DRR) of *PAX6* on the 11p13 region.

Conclusion: We first report two cases of cryptic balanced chromosomal SVs disrupting directly *PAX6* or its regulatory elements. We demonstrate here how LRS can provide insight into hidden sources of variation.

References:.

Grants: H2020 EasiGenomics program (PID10291), PI20_00851 and Spanish National Organization of the Blind (ONCE).

Conflict of Interest: None declared.

P03.023.D Burden of rare coding variants in connexin genes involving Meniere Disease

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Background/Objectives: Gap junctions consist of connexin proteins forming hemichannels in the sensory epithelia. Mutations in GJB2, GJB6 and GJB3 cause genetic deafness and hearing loss. Meniere Disease (MD) is an inner ear disorder characterized by episodic vertigo and associated with sensorineural hearing loss, tinnitus and/or aural fullness.

Methods: Whole Exome Sequencing (WES) was performed in 313 patients with MD. Gene Burden Analysis was done filtering the variants by a Minor Allele Frequency<0.05 and using gnomAD database as reference population. Connexin genes expressed in the mammalian inner ear were retrieved for further analyses. The pathogenicity of each variant was estimated and the protein stability changes were studied using PremPS and DynaMut2.

Results: An enrichment of rare missense variants was found in 2 genes expressed in the inner ear: *GJB5* (OR = 29.53) and *GJD2* (OR = 13.77). *GJA10* presents an enrichment in rare stop-gain variants (OR = 2.28). *GJB5* (OR = 103.28) and *GJC2* are enriched in rare frameshift, inframe insertion and deletion variants (OR = 22.73). Functional analysis revealed biological processes involved in gap junctions, cell communication or transmembrane transport.

Conclusion: We have found a burden of rare missense variants, stop-gain variants and indels in patients with MD in 4 genes: *GJB5 (CX31.1), GJD2 (CX36), GJA10 (CX62)* and *GJC2 (CX47)*. Further studies, including segregation analysis, are needed to confirm the role of these variants in MD.

References:.

Grants: European Union's Horizon 2020 Research and Innovation Programme, Grant Agreement Number 848261.

Conflict of Interest: None declared.

P03.024.A PACS1-related syndrome: three cases with colobomas further delineating the phenotype

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Background/Objectives: *PACS1* gene (MIM #607492) is related to Schuurs-Hoeijmakers syndrome (MIM #615009), a condition characterized by the association of intellectual disability, recognizable facial appearance, and a wide range of congenital abnormalities. Here we report 3 patients with coloboma and developmental delay due to the recurrent *PACS1* missense variant.

Methods: After clinical examination, NGS sequencing was performed (WGS for patient 1, gene panels for patients 2 and 3).

Results: The first proband is a 3 years old male presenting with congenital bilateral iris, chorioretinal and papillary coloboma, ectopia lentis, hypotonia, cryptorchidism. The second proband is a 9 years old female presenting with congenital bilateral chorioretinal coloboma, microphthalmia, right iris coloboma, hypotonia. The third proband is a 6 years old male presenting with congenital iris and chorioretinal coloboma, hypotonia, cryptorchidism. Cerebral MRI showed diffuse cortical atrophy, dysplastic corpus callosum, vermis hypoplasia. All 3 patients evolved towards global developmental delay. The same pathogenic de novo heterozygous variant in *PACS1* (c.607C>G, p.(Arg203Trp)) has been identified in all 3 patients.

Conclusion: *PACS1* encodes the Phosphofurin Acid Cluster Sorting Protein 1, a trans-golgi-membrane traffic regulator. So far, 61 patients with Schuurs-Hoeijmakers syndrome have been published, carrying the p.(Arg203Trp) heterozygous missense or rarely the p.(Arg203Gln) missense in *PACS1*. Intellectual disability is constant but congenital malformations are variable. Iris and chorioretinal colobomas are rare (9% and 13% respectively). Our patients present with coloboma at the forefront of the clinical description, further delineating the phenotype and highlighting the variable expressivity of this condition. *PACS1* implication should be considered in patients with congenital coloboma.

References:.

Grants:.

Conflict of Interest: None declared.

P03.025.B Longitudinal follow-up of Slovenian patients with RPGR associated retinitis pigmentosa

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Background/Objectives: To report the variant spectrum and longitudinal follow-up data of Slovenian RPGR-retinitis pigmentosa (RP) patients.

Methods: The study included 13 subjects (7 males) from 6 RPGR-RP families. Age at onset, logMAR best corrected visual acuity (BCVA), visual fields and fundus autofluorescence (FAF) were analysed. Eyes with milder disease were taken for the analysis to best reflect the functional impairment.

Results: Genetic analysis revealed two reported (c.1217dupT(p.Ser407fs), c.2236_2237delGA(p.Glu746ArgfsTer23)) and four novel variants (c.457G>A(p.Ala153Thr), c.1245+704_1415-2286del, c.G1978G>A(p.Glu660Ter), c.2340_2341delAG(p.Arg780SerfsTer54)). Median age at onset in males was 3 years (0-18). At first exam at median age of 16 years (0-61), median BCVA was 0.3 logMAR (0.15-2.0). All had constricted visual fields and hyperautofluorescent rings on FAF. At the last follow-up at median age of 39 years (4-71), median BCVA was 0.48 logMAR (0.0-2.30). FAF showed ring constriction, transitioning to patch in 2/7 cases. Median age at onset in females was 22.5 years (5-66). At first exam at median age of 40 years (6-66), median BCVA was 0.0 logMAR (0.0-0.30). One had normal/near-normal autofluorescence, 2 radial pattern, 1 focal pigmentary retinopathy and 2 male phenotype with partial AF ring. At the last follow-up at median age of 45 years (10-66), median BCVA was 0.10 logMAR (0.0-0.20). In 2 cases with male phenotype partial AF ring constricted.

Conclusion: RPGR-RP-causing variants in Slovenian cohort include 4/6 (67 %) novel variants, suggesting a distinct genetic background of the *RPGR* alleles in Slovenian population. Longitudinal follow-up of RPGR-RP patients showed disease progression in all males and 5/6 (83%) females.

References:.

Grants:.

Conflict of Interest: None declared.

P03.026.C Impaired bestrophin channel activity in a iPSC-RPE model of Best Vitelliform Macular dystrophy (BVMD) from an early onset patient carrying the P77S dominant mutation

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Background/Objectives: Best Vitelliform Macular dystrophy (BVMD) is the most prevalent of the distinctive retinal dystrophies caused by mutations in *BEST1* gene. This gene, which encodes for a homopentameric calcium-activated ion channel, is crucial for the homeostasis and function of the retinal pigment epithelia (RPE), the cell type responsible for recycling the visual pigments generated by photoreceptor cells. In BVMD patients, mutations in this gene induce functional problems in the RPE cell layer with an accumulation of lipofucsin that evolves into cell death and loss of sight.

Methods: In this work, we employ iPSC-RPE cells derived from a patient with the p.Pro77Ser dominant mutation to determine the correlation between this mutation and the ocular phenotype. To this purpose, gene and protein expression and localization are evaluated in iPSC-RPE cells along with functional assays like phagocytosis and chloride ion entrance. The apoptotic profile is determined through several markers as well as TUNEL assay.

Results: Our cell model shows no differences in gene expression, protein expression/localization or phagocytosis capacity. There are no signs of apoptosis but the iPSC-RPE presents an increased chloride entrance that would explain the patient's phenotype.

Conclusion: The molecular mechanisms underlying this dominant mutation from a BVMD patient widen the understanding of this pathology and open the door for a better diagnosis and prognosis of the disease.

References: Domingo-Prim et al., Generation of Best disease-derived induced pluripotent stem cell line (FRIMOi006-A) carrying a novel dominant mutation in BEST1 gene.

Grants:.

Conflict of Interest: None declared.

P03.030.C Targeted retinal dystrophy panel as a reliable tool for genetic diagnostics of retinal disorders in Polish patients

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Background/Objectives: A developed targeted panel approach for genetic diagnosis of inherited retinal dystrophies (IRD) was

successfully implemented in the routine diagnostics of Polish IRD patients. Here we present the results for the second part of Polish patients group subjected to IRD genetic diagnosis using the established method.

Methods: A group of Polish patients with clinical symptoms of retinal dystrophies (N = 182) were subjected to an NGS analysis using a custom panel capturing coding regions of 270 IRD genes (RetNet), developed with the Roche NimbleGen SeqCap EZ. Bioinformatic analysis was performed with the GATKv4 and CoNVaDING (for CNV analysis), using the GRCh38 reference genome. Variant analysis and interpretation were completed according to ACMG guidelines using the in-house variant interpretation tool - BroVar.

Results: The NGS data presented a satisfactory level of QC data metrics with mean target coverage in a range of 200-250x, heterozygote SNP calling sensitivity level above 0.997, and fraction of target bases covered >20x between 0.996 and 0.997). An IRD diagnosis was confirmed in 109 cases (59.8%). In several cases, the disease confirmation was enabled by CNVs or deep intronic variants calls. In further 29 patients only one pathogenic/likely pathogenic variant was identified in the context of AR disease.

Conclusion: The results of the application of the existing targeted retinal dystrophy panel are promising. Further increase in the method sensitivity is expected in the second version of the approach, which would broaden the targeted gene list, include more deep intronic clinvar variants and target the mtDNA using additional spike-in.

References:.

Grants:.

Conflict of Interest: Ewa Matczyńska Employed in Genomed S.A., Anna Wąsowska Employed in Genomed S.A., Marta Beć Employed in Genomed S.A., Przemysław Łyszkiewicz Employed in Genomed S.A., Robert Szymańczak Employed in Genomed S.A., Ewa Suchecka Employed in Genomed S.A., Monika Jurkowska Employed in Genomed S.A., Maria Jędrzejowska Employed in Genomed S.A., Sławomir Teper: None declared, Anna Boguszewska-Chachulska Genomed S.A. CEO.

P04

INTERNAL ORGANS & ENDOCRINOLOGY (LUNG, KIDNEY, LIVER, GASTROINTESTINAL)

P04.001.D Genetic polymorphisms and susceptibility to urinary tract infection among paediatric and adult populations: a systematic review and meta-analysis

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Background/Objectives: The lifetime risk of UTI is known to be highly heritable. Associations have also been observed across the life course from paediatric UTI to recurrent UTI in adulthood, suggesting lifelong susceptibility factors. We conducted a systematic review to identify all genetic polymorphisms tested for an association with UTI in children and adults, and to assess their strength, consistency, and risk of bias.

Methods: PubMed, HuGE Navigator and Embase were searched from 01/Jan/2005 to 01/Sep/2021, using a combination of genetic and phenotype key words. Fixed and random effects meta-analyses were conducted using co-dominant models of inheritance in metan. The interim Venice criteria were used to assess their credibility.

Results: 1283 studies were screened, with 42 included in the analysis (18 adult papers and 24 paediatric papers). All possible meta-analyses summarised in Table 1 (paediatric samples) and Table 2 (adult samples). These meta-analyses demonstrated significant pooled associations for paediatric UTI with variation in ACE, CXCR1, IL8, TGF, TLR4, VDR and VEGF. These meta-analyses also demonstrated a significant pooled association for adult UTI with variation in ACE and CXCR1. All significant pooled associations were graded as providing at most weak epidemiological credibility.

Gene	SNP identifier	n studies	n participants	Pooled OR	95% CI	p	I^2 (%)	Venice Grade
ACE	rs4646994	3	860	15.98	4.1-62.3	0.001	98.1	weak
CXCR1	rs2234671	3	1247	0.69	0.49-0.98	0.04	14.2	weak
IL6	rs1800795	2	543	0.90	0.66-1.25	0.53	0	-
IL8	rs4073	2	925	0.74	0.61-0.89	0.002	93.9	weak
TGF	rs1800468	2	572	1.45	1.01-2.08	0.04	0	weak
TGF	rs1800469	2	572	1.18	0.89-2.53	0.24	93.4	-
TGF	rs1982073	2	572	1.14	0.88-1.48	0.31	0	-
TLR4	rs4986790	4	1283	0.56	0.37-0.83	0.005	23.2	weak
TLR4	rs4986791	3	1008	0.97	0.17-5.62	0.97	81.5	-
VDR	rs2228570	2	317	0.70	0.50-0.97	0.032	86.3	weak
VDR	rs731236	2	317	0.86	0.62-1.18	0.346	0	-
VDR	rs1544410	2	317	0.72	0.53-0.99	0.042	81.7	weak
VDR	rs7975232	2	317	1.34	0.97-1.84	0.076	96.0	weak
VEGF	rs833061	3	676	8.98	2.58-31.2	0.001	97.0	weak
VEGF	rs2010963	2	453	11.53	5.0-26.6	0.001	91.2	weak

Table 1: Pooled associations for paediatric UTI

Gene	SNP identifier	n studies	n participants	Pooled OR	95% CI	p	I^2 (%)	Venice Grade
ACE	rs5744168	2	1962	0.14	0.09-0.20	0.001	70.6	weak
CXCR1	rs2234671	2	566	0.51	0.30-0.86	0.01	89.7	weak
IL8	rs4073	2	1389	1.09	0.93-1.27	0.28	84.9	-
TIRAP	rs8177374	3	2838	1.01	0.85-1.22	0.85	0	-
TLR5	rs5744168	2	1992	1.08	0.83-1.40	0.55	88.4	-
TLR4	rs4986790	4	2006	0.95	0.58-1.54	0.82	65.5	-
TLR4	rs4986791	3	1642	1.25	0.95-1.64	0.11	0	-
TLR2	rs5743708	2	2002	1.12	0.77-1.65	0.53	0	-
TLR1	rs5743618	2	1900	1.10	0.96-1.26	0.15	0	-

Table 2: Pooled associations for adult UTI

Conclusion: This systematic review provides a current synthesis of the known genetic architecture of UTI in childhood and adulthood, and should provide important information for researchers planning or analysing future genetic association studies. Although, overall, the credibility of pooled associations was weak, the consistency of findings for rs2234671(CXCR1) in both populations suggests a key role in UTI pathogenesis.

References:.

Grants:.

Conflict of Interest: None declared.

P04.002.A KIF12 variants and disturbed hepatocyte polarity in children with a phenotypic spectrum of cholestatic liver disease

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Background/Objectives: Recently, KIF12 has been identified as a cholestasis-associated candidate gene(1,2). As KIF12 is a member of a microtubule-associated motor protein family involved in intracellular transport, KIF12-associated cholestasis is assumed to be a result of disturbed cell polarity. We describe six cases with likely pathogenic KIF12 variants from four unrelated families, their different phenotypes and our investigations to study hepatocyte polarity.

Methods: Children with familial cholestasis and a likely pathogenic variant in the KIF12 gene were identified by exome sequencing. Segregation was analysed by testing parents and siblings. Immunofluorescence imaging of apical markers MRP2 und BSEP, basolateral marker OATP1B1, tight junction protein ZO-1 and KIF12 itself was performed on patient's liver tissue sections.

Results: We detected two homozygous KIF12 variants in five patients ((NM_138424.1) 4 patients: c.655C>T p.(Arg219*); 1 patient: c.482-4_500del p.?). Segregation analyses confirmed autosomal recessive inheritance. The patients' clinical manifestation ranged from neonatal cholestasis with complete clinical remission, or absent clinical symptoms to a progressive course ending in liver transplantation. Immunofluorescence imaging of liver sections of KIF12 patients revealed an ectopic cytoplasmic MRP2 staining. BSEP staining appeared in thickened long clustered structures, the latter was detected partly also for ZO-1. KIF12 and OATP1B1 staining was widely unremarkable.

Conclusion: Our results strongly support pathogenic KIF12 variants as cause for familial cholestatic liver disease and suggest that these variants result in functional cell polarity disturbance. Due to its wide clinical presentation with even asymptomatic cases, KIF12-associated cholestatic liver diseases are potentially underdiagnosed.

References: 1.PMID: 30250217; 2.PMID:30976738.

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Conflict of Interest: None declared.

P04.003.B Elevated polygenic burden for brain haemorrhage related traits in kidney donors

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Background/Objectives: Brain haemorrhage (BH) is a common cause of death among kidney donors, but little research has been done to investigate polygenic risk scores (PRSs) for BH and related traits in kidney donors. A PRS estimates the cumulative effect of common genetic variation on an individual's disease status.

Methods: We had 2,122 genotyped donor-recipient pairs from across the UK and Ireland and 5,519 ancestry matched controls. We calculated PRSs for stroke, Intracranial Aneurysm (IA) and hypertension. We compared PRSs between the donors who died of intracranial haemorrhage (DDICH) (1,303 individuals) and controls. We then used PRS to predict case/control status.

Results: PRS for stroke explained the greatest amount of variance in case/control status between DDICH and controls (6.7%, p: 8.1×10^{-63}), with still significant values for the other PRSs (IA: 5.3%, hypertension: 0.24%). A null model using just sex and principal components to predict case/control status achieved an AUC of 0.63, while models using just hypertension, IA and stroke PRSs achieved AUCs of 0.54, 0.61 and 0.67 respectively. A combined model with all the PRSs and covariates achieved an AUC of 0.74.

Conclusion: These observations support the hypothesis that DDICH carry an increased burden for traits related to BH. PRSs can play a part in being able to distinguish DDICH from the general population. These findings could have utility in testing relatives of DDICH to determine if they share the same risk for ICH.

References:.

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Conflict of Interest: Kane Collins Full time, Science Foundation Ireland Grant number 18/CRT/6214.

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P04.005.D Genetic complexity of congenital hypopituitarism: oligogenic inheritance may explain the variable expression and incomplete penetrance of deleterious GLI2 variants in congenital hypopituitarism

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Background/Objectives: Pathogenic GLI2 variants are implicated in the etiology of the broad clinical spectrum of congenital hypopituitarism (CHYP), showing a highly variable expressivity and incomplete penetrance.

Aims: Genotype-phenotype correlations in a cohort of patients with CHYP and deleterious GLI2 variants.

Methods: Subjects: 114 patients with CHYP (n = 72), septo-optic dysplasia (SOD; n = 33) or isolated GH deficiency (n = 9).

Molecular analysis: Targeted NGS (HYPOPIT_V3 panel) of 310 genes implicated in the etiology of CHYP and hypothalamic-pituitary development.

Results: Deleterious GLI2 variants were identified in 11/114 (9.6%), all of them with CHYP. These included 4 truncating variants [p.(Arg1226*), p.(Leu709Trpfs*15), p.(Ser267*) and p.(Ser859-Profs*53)] (ACMG: pathogenic). All had pituitary hypoplasia, 75%

had ectopic neurohypophysis, and those with truncating variants had both conditions. 10/11 were GH and TSH deficient and 8/11 were also ACTH deficient. Other associated traits observed included postaxial polydactyly, ogival palate, bulbous nose, nephrourological dyspasia, and holoprosencephaly, with midfacial hypoplasia and hypotelorism being the most pervasive among the studied relatives ($n = 25$) of the 11 probands. All of them (11/11) also presented with a burden of additional deleterious variants in multiple genes involved in the regulation of pituitary development signaling pathways (SHH, BMP/TGFb, FGF, WNT, NOTCH, NODAL).

Conclusion: All probands with GLI2 variants presented deleterious variants in other genes involved in pituitary development and function, suggesting the possibility of a complex multifactorial genetic component.

This may explain the highly variable expressivity and incomplete penetrance observed, which seems determined by the burden of additional inherited variants in other relevant genes.

References:.

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Conflict of Interest: Francisco Javier Rodríguez-Contreras: None declared, Purificación Ros Pérez: None declared, Fe Amalia García Santiago: None declared, Elena Vallespín: None declared, Angela Del Pozo Maté: None declared, Mario Solís: None declared, Isabel Gonzalez-Casado: None declared, Karen E. Heath: None declared, Angel Campos-Barros Research grant as PI received from ISCIII (PI18/00402).

P04.006.A Clinical and genetic features in two patients carrying PAX2 variants

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Background/Objectives: Pathogenic variants in *PAX2* have been associated with a spectrum of kidney and eye disorders, including isolated renal hypoplasia, papillorenal syndrome (renal coloboma syndrome) and focal segmental glomerulosclerosis.

Methods: We report clinical, pathology and genetic data of 2 unrelated adult male patients carrying previously reported heterozygous mutations in *PAX2* gene.

Results: 27-years-old patient 1 referred to clinical geneticist with suspicion of hereditary cystic kidney disease. At 11 years, he was diagnosed with bilateral multicystic kidney dysplasia, now presenting with proteinuria and chronic kidney disease (CKD 3 stage). Patient's father, grandmother had unspecified kidney disease. A kidney-focused NGS panel analysis identified heterozygous nonsense pathogenic c.685C>T, p.(Arg229*) variant in *PAX2*. Later ocular evaluation revealed bilateral optic disc dysplasia.

36-years-old patient 2 referred to clinical geneticist after his daughter was diagnosed with bilateral kidney hypoplasia, caused by heterozygous missense pathogenic c.250G>A, p.(Gly84Ser) variant in *PAX2*. Patient presented with persistent proteinuria, observed since 20 years of age, CKD 3 stage. Two kidney biopsies were performed since that time: the first one showing signs of IgA nephropathy, the second one – focal segmental glomerulosclerosis. Sanger sequencing analysis confirmed known familial mutation in *PAX2* gene, ocular evaluation revealed optic disc pits.

Conclusion: Hereditary kidney diseases are rare and still underdiagnosed due to variable expressivity and wide range of clinical manifestations even within single families. Reported patients demonstrate this variability and expands knowledge about disorders, caused by *PAX2* mutations, reported in literature. It also suggests that ocular evaluation should be performed for a better clinical diagnosis, when kidney pathology is present.

References:.

Grants:.

Conflict of Interest: None declared.

P04.007.B CD55-deficiency in Jews of Bukharan descent is caused by the Cromer Dr(a-) blood type variant

Alina Kurolap[1], *David Hagin*[2;3], *Tal Freund*[2], *Sigal Fishman*[3;4], *Noa Henig*[1], *Eli Brazowski*[5], *Josepha Yeshaya*[6], *Tova Naiman*[1], *elon pras*[3;7], *Jacob N. Ablin*[3;8], *Hagit Baris Feldman*[1;3]

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Background/Objectives: The complement system regulator CD55 was initially found to carry the Cromer blood group antigens, and its complete loss-of-function was recently revealed to cause a severe monogenic syndrome characterized by protein-losing enteropathy and susceptibility to venous thrombosis.

Methods: Patient 1 was diagnosed using flow cytometry and targeted Sanger sequencing. Patient 2 was diagnosed by unrelated exome sequencing. Additional testing on Patient 1 samples included RNA analysis and B-cell immunophenotyping. A population screen was performed using enzymatic restriction method.

Results: Both patients are Bukharan Jewish (BJ) and presented with CD55-deficiency of variable severity; testing revealed homozygosity for the CD55:c.596C>T;p.Ser199Leu variant. RNA analysis confirmed that this missense variant causes aberrant splicing and deletion of 44bp in exon 5, leading to premature termination and low CD55 protein levels. Furthermore, Patient 1 exhibited a mildly abnormal B-cell immunophenotyping profile. The variant is highly prevalent in BJs (carrier frequency: 1:17).

Conclusion: The CD55 p.Ser199Leu variant is known as the Cromer Dr(a-) genotype, yet this is the first report in terms of CD55-deficiency. The phenotypic variability, ranging from abdominal pain on a high-fat diet to the full CD55-deficiency phenotype, is likely related to modifiers affecting the proportion of the variant that is able to escape aberrant splicing. The high frequency in the BJ population suggests that many similar patients are un- or mis-diagnosed. Establishing the diagnosis of CD55-deficiency in a timely manner may have critical effects on patient management and quality-of-life, since treatment with the complement inhibitor eculizumab is highly effective in ameliorating disease manifestations.

References:.

Grants:.

Conflict of Interest: Alina Kurolap Patent with Alexion Pharmaceuticals on eculizumab treatment protocol for CD55-deficiency (WO/2018/217638), which does not include any royalties., David Hagin: None declared, Tal Freund: None declared, Sigal

Fishman: None declared, Noa Henig: None declared, Eli Brazowski: None declared, Josepha Yeshaya: None declared, Tova Naiman: None declared, elon pras: None declared, Jacob N. Ablin: None declared, Hagit Baris Feldman Patent with Alexion Pharmaceuticals on eculizumab treatment protocol for CD55-deficiency (WO/2018/217638), which does not include any royalties, Advisory board member for the C5-inhibitor Pozelimab clinical trial by Regeneron Pharmaceuticals.

P04.008.C Asthma exacerbations in the UK Biobank: a Genome-Wide Association Study

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Background/Objectives: Asthma exacerbations affect morbidity and mortality of the disease, and increase the burden to both patient and healthcare system. We aimed to assess the effects of genetic variants on asthma exacerbation risk in UK Biobank participants with asthma.

Methods: Within the UK Biobank study, individuals with asthma were identified as those self-reporting doctor-diagnosed asthma, or with a code for asthma in hospital inpatient and/or General Practitioners (GP) records. Exacerbations were identified as either asthma–related hospitalization, GP record of asthma exacerbation, or an Oral Corticosteroid (OCS) burst prescription. A logistic regression model adjusted for age, sex, and smoking status was used to assess the association between genome-wide imputed SNPs and risk of an asthma exacerbation.

Results: We identified 11,604 individuals with asthma with at least one exacerbation (cases), and 37,890 individuals with asthma with no recorded exacerbations (controls). While no variants reached genome wide significance ($p < 5x10^{-8}$), two SNPs (rs1449836 (chr14, *YWHAQP1/TUBBP3*) and rs34643691 (chr15; intergenic variant)) were suggestively associated with asthma exacerbation risk ($P < 1x10^{-7}$). Rs1449836 has been previously associated with response to metformin, and is located in a genetic region previously associated with inflammatory autoimmune diseases.

Conclusion: We identified two SNPs suggestively associated with risk of asthma exacerbations. Further research and replication of these findings may be needed to ascertain genetic variants' role in the severity of asthma and its clinical presentation.

References:.

Grants:.

Conflict of Interest: Ahmed Edris Mohamed: None declared, Katherine Fawcett: None declared, Ian P. Hall Research collaborations with GSK, Boehringer Ingelheim and Orion outside of submitted work., Martin D Tobin GSK and Orion for collaborative research projects outside of the submitted work., Lies Lahousse: None declared.

P04.009.D A screening of the genetic causes of Familial Pulmonary Fibrosis in patients from the Canary Islands (Spain)

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Background/Objectives: Pulmonary Fibrosis (PF) is a rare progressive scarring lung disease with poor prognosis. A significant role of rare and common genetic variation underlying its etiology is known. We relied on whole-exome sequencing (WES) to screen the genetic causes of familial PF (FPF) from Canary Islands patients.

Methods: We recruited 54 subjects from 11 families. WES was obtained using a HiSeq4000 Illumina system and small germline variant identified with BWA-GATK v3.8 against the GRCh37/hg19 reference. Relative telomere length (TL) was measured by quantitative PCR and severe reduction denoted when length was $<10^{th}$ percentile compared to age-matched controls. Rare (AF<1%) non-synonymous exonic and splicing variants from known PF or interstitial lung diseases genes were considered for manual review.

Results: Initial analysis excluded common genetic variants among affected individuals. A total of 22 rare variants from 15 genes were prioritized and classified for pathogenicity according to ACMG recommendations. Pathogenicity was supported only for a variant in *NAF1* gene (NM_138386.3:c.1104T>G) found in one family and the TL of the index case was in the 1^{st} percentile. The variant was absent from gnomAD, local population controls ($n = 920$), and from healthy relatives ($n = 2$).

Conclusion: Our results evidence the complexity of this disease and the challenges of variant interpretation. Further studies are ongoing examining the remaining of the WES data and additional families.

References:.

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Conflict of Interest: None declared.

P04.010.A The value of genomic testing for the kidney patient and for pre-transplantation donor evaluation

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Background/Objectives: The spectrum of kidney genetic disease is extremely broad and phenotypes are variable and overlapping, involving extra-kidney manifestations in some disease groups. Elucidating potential genetic causes is challenging due to the number of genes involved and unknown disease mechanisms. Exome sequencing (ES) may unveil the genetic background in patients, allowing definitive diagnosis and/or timely interventions while it may provide valuable information for kidney-donor candidates.

Methods: Thirty-three individuals (kidney-patients/donor-candidates) were referred to our lab, by a single reference clinic for ES,

performed on DNA extracted from peripheral blood: libraries preparation was performed with Clinical Exome (Sophia Genetics) or Whole Exome (Twist Bioscience) and sequenced on Illumina NextSeq-550 (Illumina). Bioinformatics analyses were conducted by SOPHiA DDM® bioinformatics pipelines.

Results: Pathogenic/likely pathogenic variants were detected in 19 patients: 10 in collagen-genes, 5 PKD1 and 1 INF2 confirming suspected diagnosis i.e., Alport syndrome, autosomal dominant polycystic kidney disease and focal segmental glomerulosclerosis, respectively. All cases were consistent with biopsy, imaging and urine/blood tests. Copy number variation analysis revealed a NPHP1 homozygous deletion (nephronophthisis) and a COL4A5 heterozygous deletion (Alport syndrome). In 9 cases results were negative either failing to elucidate genetic disease or clearing genetic burden for 5 kidney-donor candidates. In 5 cases, variants of unknown significance were detected giving eligibility to 1 donor, rejecting 2.

Conclusion: ES permitted timely, accurate diagnosis confirmation for kidney-patients allowing appropriate intervention, maintenance treatment or transplantation programming. Further, it provided valuable information for donor-candidates regarding their and the recipient's best interest. Incorporating exome sequencing in Nephrology may lead to higher-quality healthcare.

References:.

Grants:.

Conflict of Interest: Georgia Christopoulou Full time employee: Genotypos M.S.A., Stavroula Samara Full time employee: Genotypos M.S.A., Aikaterini Oikonomaki Full time employee: Genotypos M.S.A., Vassilis Filiopoulos: None declared, Stathis Tsiakas: None declared, Christina Melexopoulou: None declared, Ioannis Boletis: None declared, Pantelis Constantoulakis Full time employee: Genotypos M.S.A.

P04.011.B Novel pathogenic ALG8 variants and evidence for somatic loss of heterozygosity in 542 autosomal dominant polycystic liver disease patients

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Background/Objectives: Autosomal dominant polycystic liver disease (ADPLD) is caused by pathogenic variants in at least 6 different genes. *ALG8* has been linked to ADPLD in 5 isolated cases. We aim to identify novel pathogenic variants in *ALG8* by different next-generation sequencing techniques and to confirm loss of heterozygosity in liver cyst tissue in ADPLD patients.

Methods: We performed whole exome sequencing in a cohort of 60 genetically undiagnosed ADPLD patients and targeted sequencing of all *ALG8* exons using MiniSeq in 482 genetically undiagnosed ADPLD patients. We performed expression analysis by immunohistochemistry on liver cyst tissue with a hepatocyte-specific, a cholangiocyte-specific, and an ALG8 antibody.

Results: We screened 542 ADPLD patients and identified 8 heterozygous variants in *ALG8* (nonsense n = 4, frameshift n = 2, splice site n = 1, missense n = 1) in 16 ADPLD patients. A 19-member family included 4 patients with severe ADPLD who shared the novel *ALG8* variant c.160C>T p.(Gln54*). This variant was also present in 7 asymptomatic family members with 0-2 liver cysts. The 7 other variants were found in 1 small family and 10 singletons. All patients had mild to severe ADPLD and 0-6 kidney cyst that did not affect renal functioning. We found evidence for somatic loss of heterozygosity, as ALG8 immunoreactivity was absent in the cyst lining and cyst bordering hepatocytes of a patient carrying the c.1501delG variant.

Conclusion: Our results are consistent with a pathogenic role of ALG8 in ADPLD. Loss of heterozygosity of *ALG8* in liver cyst lining is a key event in the pathogenesis of ADPLD.

References: -.

Grants: -.

Conflict of Interest: None declared.

P04.012.C Clinical exome sequencing as a valuable diagnostic tool for glomerulopathies: a cohort study

Angelo Corso Faini[1], *Valeria Bracciamà*[1], *Silvia Kalantari*[1], *Giulia Margherita Brach del Prever*[1], *Martina Callegari*[1], *Licia Peruzzi*[2], *Roberta Fenoglio*[3], *Dario Roccatello*[3;4], *Tiziana Vaisitti*[1], *Antonio Amoroso*[1], *Silvia Deaglio*[1]

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Background/Objectives: The diagnosis of glomerulopathies remains a hard task, as different diseases can present with similar characteristics and bioptic evidence is often non-specific. In this complex scenario, genetic analyses may play an important role.

Methods: Adult and pediatric patients were recruited from multiple centers of the Piedmont region (Italy), exploiting a pre-existing transplantation network. When referred to our center, patients had already undergone nephrological counselling and suspicion of glomerulopathy was raised. Clinical exome sequencing (CES) was performed, and analysis was restricted to a panel of genes associated with glomerulopathies.

Results: CES was performed on a diagnostic cohort of 166 patients. Alport syndrome scored the highest percentage of solved cases (59%), followed by glomerulonephritis (35%), non-nephrotic proteinuria (29%), FSGS (12%) and nephrotic proteinuria (19%). Of note, 54% of clinically undiagnosed glomerulopathies were solved. Overall, results show a diagnostic rate of 36% which is in line with published data[1].

Conclusion: Our data confirm genetic analysis is a valuable diagnostic resource when a precise glomerulopathy is suspected and – unexpectedly – also when the clinical suspicion is less defined. Analysis by CES i) shows a high diagnostic rate; ii) allows a precise diagnosis avoiding invasive procedures (biopsy); iii) prevents ineffective treatment; iv) speeds up the enrollment in transplantation awaiting lists and v) allows to determine recurrence risk, post-transplantation prognosis and living-donor eligibility. Taken together, our results strongly support genetic analysis as a first-line diagnostic tool in kidney disease.

References: 1. Connaughton et. al, Kidney Int., 2019,95(4):914-928.

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Conflict of Interest: None declared.

P04.013.D Clinical exome sequencing (CES) as a tool for genetic diagnosis of Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Valeria Bracciamà[1], *Angelo Corso Faini*[1], *Silvia Kalantari*[1], *Giulia Margherita Brach del Prever*[1], *Martina Callegari*[1], *Licia Peruzzi*[2],

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Background/Objectives: Autosomal dominant polycystic kidney disease (ADPKD #173900, #613095, #600666) is the most common inherited nephropathy and is predominantly caused by mutations in *PKD1* and *PKD2*. Genetic testing of *PKD1* is challenging for the presence of homologous pseudogenes with genetic and allelic heterogeneity. Due to phenocopies, diagnostic criteria are unable to provide a definitive diagnosis. We propose NGS-based strategy for genetic diagnosis of patients with ADPKD.

Methods: CES was performed in 121 subjects with clinical suspicion of PKD. Patients were recruited by the hospitals of the Piedmont region (Italy). *PKD1* and *PKD2* were analyzed and for negative cases additional cystogenes were investigated. Identified variants were segregated and validated by Sanger sequencing.

Results: Causative variants in *PKD1* and *PKD2* were identified in 78 patients, 56% of them presenting family history. 31 cases resulted negative or unconclusive. Most of the diagnostic genetic variants affected *PKD1* (78%) and *PKD2* (9%). 20 patients had more than one variant in the same gene or the 2 different cystogenes. Furthermore, we identified 12 possible alternative diagnoses among which 5 patients with biallelic mutations in *PKHD1* and two patients with variants in *TSC1* and *DSTYK*, respectively.

Conclusion: Identification of the specific molecular basis of a disease is essential to avoid genetic misdiagnosis, unnecessary and invasive therapies and for early detection of renal and extrarenal comorbidities. The broad phenotypic and genetic heterogeneity of cystic kidney diseases make CES a time- and cost-efficient diagnostic approach for these indications.

References: Cornec-Le Gall, et al., Lancet. 2019.

Grants: Ministero dell'Istruzione, Progetto strategico di Eccellenza Dipartimentale #D15D18000410001.

Conflict of Interest: None declared.

P04.014.A Transcriptomic landscape of pancreatic neuroendocrine tumours

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Background/Objectives: Pancreatic neuroendocrine tumours (PanNETs) arise from the neuroendocrine cells in pancreatic islets and account for approximately 12% of digestive system NETs, with an overall 5-year survival rate of 85%. PanNETs are heterogeneous lesions, classified according to cell proliferation rate and differentiation grade. In this study, we aimed to create a well-defined cohort of PanNETs and characterize the transcriptomic landscape on a molecular level to discover novel markers and therapeutic strategies for better tumour management options.

Methods: PanNET FFPE samples used in this study were obtained from different consortium members from Greece, Slovakia, and Spain. RNA was then extracted from FFPE samples and libraries prepared. Transcriptome sequencing was then carried out on DNBSEQ-G400 platform (MGI) to identify differentially expressed genes (DEGs) in tumour and non-tumour tissues.

Results: We have sequenced transcriptomes of 72 PanNET FFPE samples and detected different transcriptome profiles that distinguish tumour and non-tumour tissues of the pancreas. In total, we were able to find 265 DEGs. Among these were insulin regulation pathway genes and other markers related to tumour pathogenesis pathways, cell proliferation, and invasion, for example, ISL1, SYCN, KLK1, CUZD1 and others.

Conclusion: The results of the PanNET transcriptomic landscape highlight the heterogeneity of PanNETs, which is dependent on various tumour characteristics. Transcriptome analysis is valuable for understanding PanNET tumour biology, which will help to discover new markers and develop new therapeutic approaches for PanNETs.

References:.

Grants: European Regional Development Fund project "Establishing an algorithm for the early diagnosis and follow-up of patients with pancreatic neuroendocrine tumors (NExT)", grant number 1.1.1.5/ERANET/20/03.

Conflict of Interest: None declared.

P04.015.B Four years' experience in cystic fibrosis neonatal screening in Luxembourg: 2018-2021

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Background/Objectives: Cystic fibrosis (CF) newborn screening (NBS) was introduced in Luxembourg in January 2018. Biochemical and genetic testing is centralized at the Laboratoire national de santé (LNS) and we present the results of the first 4 years.

Methods: Immunoreactive Trypsinogen (IRT) is assessed for all newborns (app 7000/year) on day 3 of life (D3). In case of high IRT-D3 (≥60ng/mL), CFTR genetic testing using the CF-EU2v1 (Yourgene Health) kit to detect 50 frequent CFTR pathogenic variants is performed, and a second IRT test on day 21 (D21). Patients screened positive (one or two pathogenic variant detected and/or IRT-D21 ≥40ng/mL) are referred to the Paediatric National CF centre for sweat testing and further multi-disciplinary clinical follow up. In case of positive sweat test without confirmed molecular diagnosis, complete CFTR gene sequencing is performed.

Results: IRT-D3 level was above the threshold in 0.6% newborns in 2018, 0.97% in 2019, 1.38% in 2020 and 1.32% in 2021. CF was diagnosed in 10 infants ($n = 3$; $n = 4$; $n = 2$; $n = 1$ respectively). The positive predictive value (PPV) was 15%. All patients had at least one CFTR pathogenic variant detected by the kit (55% of them were F508del). In four cases, the second rare CF-causing

variant was detected by sequencing (4382delA, E664X, I502T, S489L). The incidence of CF was 1/2454 live births.

Conclusion: CF NBS in Luxembourg is in line with current recommendations for standards of CF care. To improve the PPV, we will adapt the protocol and consider cases with no mutation and IRT-D3<100ng/ml as screened negative.

References:.

Grants:.

Conflict of Interest: None declared.

P04.017.D Genetic revision of the Hungarian cystic fibrosis registry

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Background/Objectives:.

In order to develop a diagnostic strategy for cystic fibrosis and to facilitate mutation-specific treatments, the genetic revision of the Hungarian cystic fibrosis Registry was performed.

Methods: 528 patients' data and samples were used for the revision. First we reviewed the patients' existing genetic findings. Wherever necessary, a comprehensive three-level genetic analysis of the CFTR gene was done.

Results: According to our study, of the 528 patients present in the Registry 395 (74.8%) had two pathogenic CFTR mutation. We completed and corrected 94 patients' previously incomplete genetic status. 73 different pathogenic variants were detected, in which 6 aberrations were not previously reported. The five most common mutations were: F508del (68.3%); CFTRdele2,3 (3.7%); G542X (3.2%); 2184insA (2.7%); W1282X (2.3%). Based on genotype and age, 192 patients (36.4%) are eligible for the available lumacaftor-ivacaftor combination therapy. Soon ivacaftor-tezacafor-elexacaftor combination will be an option for 364 (68.9%) patients.

Conclusion: Due to the revision, we could identify the patients who can benefit from mutation-specific drugs instead of symptomatic therapy. In addition, the obtained data have been used to map the Hungarian distribution of mutations in the CFTR gene, which will help to develop a diagnostic strategy.

References:.

Grants:.

Conflict of Interest: None declared.

P04.018.A Gene expression profiling through Whole Transcriptome Sequencing predicts novel mechanisms in Hirschsprung Associated Enterocolitis

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Background/Objectives: Hirschsprung (HSCR) associated Enterocolitis (HAEC) is a common life-threatening complication in HSCR, likely due to a gut immune system impairment. We have recently identified a HAEC susceptibility variant in the Oncostatin-M receptor gene, also implied in Inflammatory Bowel Diseases (IBDs), through Whole-Exome Sequencing on HSCR and HAEC patients. This study also confirmed the immune system impairment in HAEC, however no data is available on possibly different gene expression in HAEC versus HSCR patients.

Methods: We have carried out a transcriptome analysis on Intraepithelial lymphocytes (IEL) derived from biopsies of the gut of 6 HAEC patients, 6 HSCR-only patients and 4 paediatric patients affected by neither Hirschsprung nor inflammatory related diseases. For the sequencing, the Ion AmpliSeq Transcriptome Human Gene Expression Kit was used.

Results: We found a clear clustering between the three different groups of patients. Several transcripts were nominally significantly over- and under-expressed in HAEC vs HSCR-only patients, among which several transcripts also involved in IBDs pathogenesis. Very preliminary results showed an enrichment in immune and inflammatory pathways in the HAEC group, although further analyses are still in progress.

Conclusion: We have identified several differentially expressed genes between HSCR patients that had developed HAEC vs those without HAEC occurrence, including genes or pathways already affected in several intestinal pathological conditions. This could lead to the identification of key factors in the HAEC pathogenesis, possibly shared with other intestinal inflammatory diseases.

References:.

Grants:.

Conflict of Interest: None declared.

P04.019.B Association between arterial hypertension and liver-related outcomes using polygenic risk scores – a population-based study

Fredrik Åberg[1], ***Katri Kantojärvi***[2], *Ville Tapio Männistö*[3], *Anna But*[4], *Veikko Salomaa*[2], *Teemu Niiranen*[2;5], *Martti Färkkilä*[6], *Panu Luukkonen*[7;8;9], *Satu Männistö*[2], *Annamari Lundqvist*[2], *Markus Perola*[2], *Antti Jula*[2]

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Background/Objectives: Arterial hypertension (HTA) is associated with liver disease, but the causality remains unclear. We investigated whether genetic predisposition to HTA is associated with incident liver disease in the general population, and if antihypertensive medication modifies this association.

Methods: Participants of the Finnish health-examination surveys, FINRISK 1992-2012 and Health 2000, were followed for incident liver-related outcomes (ICD-10 codes: K70-K77, C22.0) and initiation of antihypertensive medication through the linkage with national registers. Polygenic risk scores (PRS) for systolic (SBP) and diastolic (DBP) blood pressure were derived from GWAS where over 1 million people were studied (Evangelou et al. 2018). PRSs

were calculated with PRS-CS method. Cox regression analyses were adjusted for multiple confounders.

Results: In the fully-adjusted Cox regression models, both measured systolic blood pressure and clinically defined HTA were associated with increased risk of liver-related outcomes. Similarly, the PRSs for systolic and diastolic blood pressure were associated with liver-related outcomes. Recent initiation of antihypertensive medication was associated with reduced rates of liver-related outcomes in persons with high genetic HTA risk.

Conclusion: HTA and a genetic predisposition for HTA are associated with liver-related outcomes in Finnish population. New initiation of antihypertensive medication attenuates this association in persons with a high genetic risk for HTA.

References: Evangelou E. et al. 2018. https://doi.org/10.1038/s41588-018-0205-x.

Grants: Mary and Georg Ehrnrooth Foundation, Medicinska Understödsföreningen Liv och Hälsa, Finska Läkaresällskapet, Academy of Finland (#338544) and Sigrid Jusélius Foundation.

Conflict of Interest: None declared.

P04.020.C Whole Exome Analysis to identify related genes that predispose to the progression of Non-Alcoholic Fatty Liver (NAFL) to Non-Alcoholic Steatohepatitis (NASH)

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Background/Objectives: Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease that includes a wide range of liver damage ranging from simple steatosis (non-alcoholic fatty liver; NAFL) to non-alcoholic steatohepatitis (NASH)(1). The genetic factors underlying the progression from NAFL to NASH remain partially explored. We performed Whole Exome Sequencing (WES) analysis to better characterize the genetic landscape of NAFL vs NASH.

Methods: The study includes patients with biopsy-proven NAFLD enrolled at the Liver Unit of the Department of Medical Sciences, University of Turin. DNA samples from 157 subjects (111 NASH; 46 NAFL) were used for WES analysis (Illumina Novaseq6000).

Results: For variant analysis, we filtered based on the pathogenic/likely-pathogenic status according to ClinVar's clinical annotation and Allele Frequency (Max AF) less than 5%. Preliminary results on a subset of 112 patients (76 NASH; 36 NAFL) revealed that NASH subjects have on average fewer mutations than NAFL subjects (Wilcox test p-value = 0.014). We identified 113 genes exclusively mutated in NASH patients. The majority of them is involved in pathways such as DNA repair, primary cilium formation and lipid metabolism, which are all linked to the pathophysiology of the progressive form of the disease.

Conclusion: WES analysis allows the detection of novel genetic alterations that could be useful to identify genes and pathways that may help in risk stratification in patients with NAFLD.

References: 1)Armandi, et al. Insulin Resistance across the Spectrum of Nonalcoholic Fatty Liver Disease. Metabolites 2021.

Grants: Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) Project "Dipartimenti di Eccellenza 2018–2022".

Conflict of Interest: None declared.

P04.021.D Genetics of transient congenital hypothyroidism

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Background/Objectives: The etiology of transient congenital hypothyroidism (TCH) is heterogeneous and can result from maternal thyroid autoimmune disease, antithyroid medications, iodine deficiency or iodine excess iodine in the perinatal period or due to a genetic cause usually related to mild thyroid dyshormonogenesis.

Methods: The study cohort included 11 patients with TCH from unrelated families including 9 cases with normal sized eutopic thyroid gland and 2 cases with goiter. All patients had CH without extrathyroidal manifestations. Genomic DNA was extracted from peripheral blood leukocytes using standard techniques, and Sanger sequencing was used to screen for *TSHR, DUOX2*, and *DUOXA2* genes were screened for mutations in all coding exons and exon/intron boundaries amplified by PCR specific primers. In cases for whom pertechnetate scan data were lacking, *SLC5A5* (*NIS*) was also screened.

Results: Heterozygous *DUOX2* variants were identified in 18% of the TCH cases. The p.E1546G mutation was previously reported to be associated with transient CH. An additional novel heterozygous *DUOX2* variant p.D1440N was detected affecting a conserved amino acid within the NADPH-oxidase domain in which other pathogenic mutations have been identified, supporting the phenotype characteristics of mild, goitrous CH. Two novel monogenic *TSHR* mutations were identified p.L452P and p.E107D. *TSHR* variants were associated with mild CH and normal sized thyroid gland. In both cases TSH normalization was readily achieved after levothyroxine initiation without requiring supraphysiological FT4 levels.

Conclusion: Identification of genetic causes of TCH is important for delineation form permanent forms and possible earlier re-evaluation, as infants with TCH usually receive thyroid hormone replacement.

References:.

Grants:.

Conflict of Interest: None declared.

P04.022.A A comprehensive stepwise approach to study a large group of Egyptian referral patients with disorders of sex development (DSD)

Mona Mekkawy[1], *Inas Mazen*[2], *Alaa Kamel*[1], *Heba Hassan*[3], *Mona Essawi*[3], *Mohamad Abdelhamid*[3], *Ola Eid*[1], *Amal Mahmoud*[1], *Aya Elaidy*[2], *Shereen Adel*[1], *Hala Soliman*[3], *Ken McElreavey*[4], *anu bashamboo*[5]

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Background/Objectives: Disorders of sexual development (DSD) include conditions affecting urogenital development, associated with atypical chromosomal, gonadal, or phenotypic sex. The phenotype variability result from a wide range of genetic alterations that may be chromosomal, CNVs, monogenic, digenic or polygenic (Mazen et al., 2021). The study aimed to improve the diagnostic strategy of DSD patients by applying a stepwise approach.

Methods: The study included 599 Egyptian DSD patients, over a period of 6 years. They underwent clinical examination, hormonal and imaging studies, cytogenetic and FISH analysis and

Sanger sequencing. Detection of copy number variations (CNV) using MLPA was applied on 35 patients. Whole exome sequencing (WES) was applied on 18 patients and chromosomal microarray (CMA) was conducted for five patients with associated anomalies.

This study was approved by NRC Ethics Committee.

Results: Sex chromosomal abnormalities were found in 41%, while autosomal abnormalities were detected in 2.3%.

Sanger sequencing identified pathogenic variants in 33.7%. MLPA identified deletions of SOX9 in two patients. The detection rate of WES reached 66.7%, while CMA analysis revealed pathogenic copy number variations in two patients.

Conclusion: The study reports a large number of DSD patients from the same ethnic group with a wide cytogenetic spectrum and characteristic mutational profile with novel and rare variants.

References: Mazen I, Mekkawy M, Kamel A, et al. (2021) Advances in genomic diagnosis of a large cohort of Egyptian patients with disorders of sex development. Am J Med Genet A. 185:1666-1677.

Grants: STDF–IRD Joint Innovative Projects Fund, Grant/Award Number: 4632.

In house NRC projects: 2013-2019.

Conflict of Interest: None declared.

P04.024.C NEK8-associated nephropathies: do autosomal dominant forms exist?

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Background/Objectives: Nephronophthisis (NPHP) is a group of autosomal recessive renal diseases characterized by a reduced ability of the kidneys to concentrate solutes, chronic tubulointerstitial nephritis and cystic kidney disease. It represents the most common genetic cause of childhood renal failure. To date, around 20 different genes, encoding primary cilia proteins, have been linked to NPHP. These contribute to one third of cases with NPHP while the majority of patients remain molecularly undiagnosed.

Methods: Whole exome sequencing (WES) was carried out on a two-year-old Lebanese boy with infantile NPHP characterized by multicystic kidney dysplasia, kidney insufficiency and enlarged kidneys in addition to chronic anemia. The candidate variant, detected by WES, was then tested in the patient and his parents by Sanger sequencing. Copy number variations analysis (CNV) was subsequently performed in the proband.

Results: Our studies enabled the detection of a heterozygous *de-novo* variant in *NEK8* (NM_178170: pArg45Trp) in the proband. CNV analysis excluded the presence of big deletions or insertions in this gene.

Conclusion: In conclusion, here we report a *de-novo* heterozygous variant in the *NEK*8 gene in infantile NPHP. This variant was detected at a *de-novo* state in a patient presenting with the same clinical features as the proband (and reported in VarSome). This suggests that autosomal dominant forms of NEK8-linked nephropathies may exist. Reporting further patients is essential to confirm these findings and assess whether dominant forms of the disease are restricted to a specific mutational spot or are linked to variants scattered throughout the NEK8 gene.

References: NA.

Grants: NA.

Conflict of Interest: None declared.

P04.025.D Lipid metabolism is altered in alpha-1 antitrypsin deficiency derived-patients organoids

Sara Perez-Luz[1], *Jaanam Lalchandani*[1], *Nerea Matamala*[1], *Iago Justo*[2], *Alberto Marcacuzco*[2], *Cristina Garfia*[2], *Loreto Hierro Llanillo*[3], *Gema Gomez-Mariano*[1], *Beatriz Martinez-Delgado*[1]

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Background/Objectives: Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder due to mutations in SERPINA1 gene presenting clinical manifestations in liver and lungs. More than 90% of severe deficiency patients are homozygous for PiZ (Glu342Lys) mutation located in exon 5, which produces an altered protein (AAT-Z) prone to accumulate in hepatocytes. The aggregated polymers avoid protein secretion into the circulation, resulting in plasma levels 10% to 15% of the levels of normal M homozygous while they related to different hepatic alterations such as fibrosis, hepatocarcinome or lipid alterations.

Methods: We have used hepatic organoids derived from patient homozygous for the Z mutation and HepG2 cells overexpressing Z-AAT to verify the relationship among AAT-Z protein and lipid accumulation by detecting neutral lipids using oil red O staining. In addition, we performed a lipidomic analysis to determine the lipidic species accumulated in our model as well as a transcriptomic study to reveal differential expressed genes, which could explain the aforementioned results.

Results: Our results show how Z-AAT protein accumulation triggered an increase in lipid content in hepatocytes and identified specifically three species presenting a more notably increment as measured by mass spectrography. Furthemore, the transcriptomic analysis expose several genes somehow related to lipid metabolism whose variation suggest involvement in such lipid deposits.

Conclusion: AAT polymers are associated to intracellular lipid accumulation mainly because the altered expression of lipogenic genes without ruling out the possibility that lipid accumulation could be cause, as well as consequence, and hence responsible of some clinical features associated with DAAT.

References:.

Grants: AESI/PI/307/20.

Conflict of Interest: None declared.

P04.027.B Benefits of whole exome sequencing to advance the genetic diagnosis in patients with differences (disorders) of sex development

Hannes Syryn[1], *Hannah Verdin*[1], *Julie Van De Velde*[1], *Marianne Becker*[2], *Cécile Brachet*[3], *Marieke den Brinker*[4], *Sylvia Depoorter*[5], *Julie Fudvoye*[6], *Daniel Klink*[7], *Philippe Lysy*[8;8], *Guy Massa*[9], *Nele Reynaert*[10], *Anne Rochtus*[10], *Willem Staels*[11], *Marlies Van Loocke*[10], *Martine Cools*[12], *Elfride De Baere*[1]

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Background/Objectives: Differences of sex development (DSD) are heterogeneous conditions affecting the development of chromosomal, gonadal or anatomical sex. Although over 75 genes have been associated with DSD, the diagnostic yield of whole exome sequencing (WES) studies is typically not higher than 35% in a clinical setting. Here, we investigated the benefits of WES for the genetic diagnosis in patients with DSD.

Methods: Between 2016 and 2022, 144 unrelated index patients with a clinical diagnosis of DSD or the broader DSD umbrella underwent WES-based panel testing interrogating the coding regions of 130 genes implicated in DSD, primary ovarian insufficiency and hypogonadotropic hypogonadism. Variants were extracted and classified according to the ACMG guidelines. Copy number variant (CNV) analysis was performed using the ExomeDepth algorithm.

Results: In 13% of patients, we identified a likely pathogenic (LP) or pathogenic (P) rare variant in 12 distinct DSD genes, including *AR* (6), *NR5A1* (2), *WT1* (2), *ATRX*, *CYP21A2*, *DHX37*, *HSD3B2*, *HSD17B3*, *RXFP2*, *SRD5A2*, *SRY*, and *TXNRD2*. The majority are sequence variants; four defects are CNVs identified using ExomeDepth. Interestingly, in two brothers displaying bilateral cryptorchidism and infertility an intragenic *RXFP2* deletion was found to occur in *trans* with a heterozygous missense variant, corroborating its role in familial bilateral cryptorchidism.

Conclusion: We demonstrate the benefit of WES-based genetic testing of DSD in a clinical context. The low detection rate emphasizes the need for more stringent inclusion criteria on the one hand and for advanced genome analysis to solve missing heritability in this condition.

References:.

Grants: BESPEED, FWO1802220N, FWO1801018N.

Conflict of Interest: None declared.

P04.028.C Unique pipeline for the assessment of novel genetic variants leads to confirmation of PCD diagnosis

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Background/Objectives: Primary ciliary dyskinesia (PCD) is a disease caused by impaired ciliary motility and mainly affects the lungs and reproductive organs. Inheritance is autosomal recessive and X-linked with more than 40 disease-causing genes, wherefore PCD patients have diverse clinical manifestations, thus making diagnosis difficult. The utility of next-generation sequencing (NGS) technology for diagnostic purposes allows a better understanding of the PCD genetic background. However, the identification of specific disease-causing variants is challenging. The objective of this study was to create a unique guideline that will enable the standardization of the assessment of novel variants within PCD associated genes.

Methods: The study included designing a pipeline for the classification of the rare genetic variants detected using NGS. The pipeline included in silico (translation, 3D-model, protein-protein interactions, sequence conservation, posttranslational modifications) and functional analysis (expressional analysis, Western Blot) of the variants.

Results: The designed pipeline consists of three steps: sequencing, detection, and identification of genes/variants; classification of variants according to their effect; and variant characterization using in silico structural and functional analysis. The pipeline was validated by the analysis of the variants detected in a disease-causing gene (*DNAI1*) and the novel candidate gene (*SPAG16*).

Conclusion: The application of the pipeline resulted in the identification of disease-causing variants, as well as pathogenicity validation, through the analysis on transcriptional, translational, and posttranslational levels.The application of created pipeline leads to the confirmation of PCD diagnosis and enables a shift from candidate to disease-causing gene.

References:.

Grants: This work was funded by MESTD, Republic of Serbia(451-03-68/2022-14/200042).

Conflict of Interest: None declared.

P04.029.D TSHB R75G is a founder variant and prevalent cause of low or undetectable TSH in Indian Jews

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Background/Objectives: Bi-allelic loss-of-function mutations in *TSHB*, encoding the beta-subunit of TSH, cause congenital hypothyroidism. Homozygosity for the TSHB p.R75G variant, previously described in South Asian individuals, does not alter TSH function, but abrogates its detection by some immune-detection-based platforms, leading to erroneous diagnosis of hyperthyroidism. We set out to identify and determine carrier rate of the p.R75G variant among clinically euthyroid Bene Israel Indian Jews, to examine possible founder origin of this variant worldwide and to determine phenotypic effects of its heterozygosity.

Methods: Molecular genetic studies of Bene Israel Jews and comparative studies with South Asian cohort were performed. TSHB p.R75G variant was tested by Sanger sequencing and RFLP. Haplotype analysis in the vicinity of the *TSHB* gene was performed using SNP arrays.

Results: Clinically euthyroid individuals with low or undetectable TSH levels from three apparently unrelated Israeli Jewish families of Bene Israel ethnicity, originating from the Mumbai region of India, were found heterozygous or homozygous for the p.R75G TSHB variant. Extremely high carrier rate of p.R75G TSHB in Bene Israel Indian Jews (~4%) was observed. A haplotype block of 239.7kB in the vicinity of *TSHB* shared by Bene Israel and individuals of South Asian origin was detected.

Conclusion: Our findings highlight the high prevalence of the R75G TSHB variant in euthyroid Bene Israel Indian Jews, demonstrate that heterozygosity of this variant can cause erroneous detection of subnormal TSH levels, and show that R75G TSHB is an ancient founder variant, delineating shared ancestry of its carriers.

References:.

Grants:.

Conflict of Interest: None declared.

P04.030.A Genotype-phenotype correlation in patients with PHEX-related hypophosphatemia: identification of novel variants and a case of mosaicism

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Background/Objectives: X-linked hypophosphatemia (XLH) is an X-linked dominant disorder caused by inactivating mutations in the *PHEX* gene. The clinical phenotype ranges from isolated hypophosphatemia to severe manifestations of rickets, such as bowing of the lower extremities, bone pain, low stature, and dental anomalies.

Methods: Medical records from 2008 to 2021 were retrospectively reviewed to collect clinical, biochemical and molecular findings for patients carrying pathogenic *PHEX* variants, detected through Sanger or Next Generation Sequencing on DNA extracted from peripheral blood lymphocytes.

Results: We report a series of seven unrelated patients from our clinical center carrying pathogenic *PHEX* variants. In particular, we identified one intragenic deletion and five distinct nonsense or frameshift variants (one of which was present in two unrelated patients); three of the variants we identified were novel and one was present as a somatic mosaicism in a male patient. To our knowledge, only 12 cases of somatic mosaicism for a *PHEX* mutation in hemizygous males have been reported in the literature; we reviewed their clinical and biochemical features, confirming the wide phenotypic variability among mosaic male patients with XLH.

Conclusion: While some reports suggested a dosage effect, with a milder clinical phenotype in mosaic males, our case and a review of the literature show a broad range of severity in these patients, overlapping that of male and female patients with germline mutations.

References:.

Grants:.

Conflict of Interest: None declared.

P04.031.B Exome sequencing in adult nephropathy of unknown origin: a population study

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Background/Objectives: Despite the importance of an etiological diagnosis, up to 20% of nephropathies remain of undetermined origin. Increased availability of genome-wide testing, such as exome sequencing (ES), may allow many cases of undetermined kidney disease to be reclassified. Here, we report the largest French cohort of ES in adult nephrology.

Methods: Between September 2018 and February 2021, 538 unrelated patients underwent an ES (Twist Human Core Exome kit, 100 base pair-end sequenced on NextSeq500- Illumina). CNV research was done from the same assay, calling was based on model built by GATK4. Variant co-segregation was investigated by Sanger sequencing in relatives when needed.

Results: Of 538 unrelated patients, ES resulted in a molecular diagnosis in 135 patients (diagnostic yield of 25%). Seven patients had a conclusive result with identification of a pathogenic CNV (5% of positive diagnoses). The mean age of the patients was 43 years [± 13]. 80% of the patients were analysed in solo exome. The genetic diagnosis had various consequences: allowing for a pre-symptomatic diagnosis (63%), assisting in the selection of a potential related living donor (7.5%), not considering immunosuppressive therapy (11%), ruling-out potential recurrence on the graft (20%), prescribing further investigations in the context of retrophenotyping (36%).

Conclusion: With a high diagnostic yield, major clinical and therapeutic consequences and a measured cost, our study shows the interest of ES with analysis integrating CNV detection in indeterminate adult nephropathies. The diagnoses made were often unexpected, validating the interest of a genome-wide approach.

References:.

Grants: None.

Conflict of Interest: None declared.

P04.032.C Exome sequencing in isolated population: new insights into nephropathies of unknown origin in New Caledonia

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Background/Objectives: Recently, exome sequencing (ES) has shown a good diagnostic yield in adult nephrology. New Caledonia has a very high incidence of Chronic Kidney Disease (CKD) with a clear predominance of indeterminate nephropathies (40%) and a young population (60% of dialyzed patients < 65 y/o). Given its insularity, genetics offer very interesting perspectives for New Caledonian patients with nephropathy of unknown origin.

Methods: Since November 2019, ES is offered to adult patients with CKD of unclear origin with early-onset (< 45 y/o) or with a family history of kidney disease, regardless of age.

Results: From November 2019 to January 2022, 84 unrelated patients received a solo ES as a first-line exploration. The sequenced population was predominantly of Melanesian (60%) and Polynesian (30%) origin. ES identified pathogenic diagnostic variants in 15 patients (19%). Twenty-two patients (26%) presented a variant of unknown significance (VUS). None of the 84 patients carried APOL1 risk alleles. This exome-wide approach had major consequences (allow a new look on the whole clinical picture, description of new pathogenic variants, selection of a related living donor for 15 patients, a renal biopsy could be avoided in one relative).

Conclusion: With a diagnostic yield of 19% and major clinical impact, nephropathies of unknown origin in New Caledonia appear as an interesting indication of ES. As the Oceanian populations are absent from Gnomad most variants required co-segregation and retrophenotyping studies to reach ACMG 4/5 rank. Thus an important number of variants remained VUS.

References:.

Grants:.

Conflict of Interest: None declared.

P04.033.D Stringent variant assessment in pulmonary fibrosis reveals a stronger genetic predisposition in female than in male patients

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Background/Objectives: Pulmonary fibrosis (PF) is a severely progressive and fatal fibrotic interstitial lung disease, with a late age of onset (55-75 years) and an average survival rate of 3 years post diagnosis. The phenotype is strongly associated with a reduction in telomere length and pathogenic variants in telomere-associated genes, have been found in approximately 8-15% of familial PF cases. Genetic testing provides diagnostic accuracy in symptomatic patients, the possibility of predictive testing in family members and can aid in disease course prognostication and risk stratification when considering lung transplantation.

Methods: 180 adult patients with PF (mean age 59 years, 102 female and 78 male) were referred for genetic testing and sequenced on our custom "Respigene" panel for inherited respiratory conditions. Analysis was bioinformatically targeted to 25 genes associated with familial pulmonary fibrosis (FPF).

Results: Sixteen patients (9%, 11 female and 5 male) were found to have a pathogenic or likely pathogenic variant in the *RTEL1*, *TERT, PARN or SFTPC* genes; 48 patients (27%, 27 female, 21 male) harboured at least one variant of uncertain significance (VUS). The *MUC5B* rs3570590 risk-associated variant was detected in 23.69% of PF patients, compared to 18.94% in patients with a non-ILD respiratory condition.

Conclusion: Female patients appear more likely to have a genetic cause to their PF than male patients. Functional studies are required to refine the pathogenicity of the many VUSs detected. The presence of the *MUC5B* risk allele at a significantly higher frequency in PF patients than in patients with other respiratory conditions, was confirmed.

References:.

Grants:.

Conflict of Interest: None declared.

P02

SKELETAL, CONNECTIVE TISSUE, ECTODERMAL AND SKIN DISORDERS

P05.001.B Disruption of zfhx4 leads to defects in zebrafish craniofacial development matching human characteristics of nonsyndromic cleft lip with cleft palate

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Background/Objectives: Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is a frequent congenital anomaly with high heritability estimates.[1,2] Despite the discovery of several common nsCL/P risk loci, major fractions of its complex genetic background yet remain unrevealed.[3].

Methods: In order to identify copy number variants that might be causal for nsCL/P we reanalyzed an exome sequencing dataset comprising 50 Central European nsCL/P trios. Here, we identified a heterozygous 86 kb de novo deletion that affects exons 3-11 of 11 of the *ZFHX4* gene. *ZFHX4* encodes the transcription factor Zinc Finger Homeobox 4 which recently has been shown to be enriched for de novo mutations in patients with nonsyndromic orofacial clefting.[4] To functionally characterize the role of *ZFHX4* in craniofacial development, we knocked down the zebrafish orthologue *zfhx4* in wild type zebrafish larvae (zfl) by a translational blocking morpholino (TB MO).

Results: Cartilage staining of TB MO injected zfl at 4 days post-fertilization showed underdeveloped and abnormally shaped cartilaginous jaw and ethmoid plate structures, and therefore matched human characteristics of nsCL/P. Moreover, preliminary data of transient CRISPR/Cas9 zfhx4 F_o-knockout in zfl replicated these observations.

Conclusion: In conclusion, our human genetic findings and subsequent functional studies in zfl indicate the importance of *ZFHX4* in the process of craniofacial development and confirm its role as susceptibility gene for nsCL/P.

References: [1]Mangold, et al., 2011. Trends Mol Med0., [2]Grosen, et al., 2011. Epidemiology., [3]Welzenbach, et al., 2021. HGG Advances., [4]Bishop, et al., 2020. Am J Hum Genet.

Grants: S.H.: BonnNI Q-614.1254.; E.M.: DFG, MA 2546/6-1.; K.U.L.: DFG, LU 1944/3-1.

Conflict of Interest: None declared.

P05.002.C LRP4 intracellular variant may lead to high bone mass with bone fragility: a functional analysis

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Background/Objectives: The LRP4 receptor is associated to bone and musculoskeletal diseases[1]. We identified a novel

missense variant located in the intracellular domain in *LRP4* in a 6-years-old patient, with an atypical bone phenotype characterized by osteocondensation and fractures. Another patient presented a rare *LRP4* variant in extracellular domain with high bone mass, without fractures. Our goal was to confirm the pathogenicity of the variants and to unravel the dysregulates pathways.

Methods: A CRISPR-Cas9 approach was used to generate mutated osteosarcoma cells (U2OS) for functional analysis.

Results: We generated two heterozygous mutant cell lines, one with the mutation in intracellular domain c.5317_5320del p.(Thr1773Argfs*3) and other in the extracellular domain c.4699_4727del p.(Arg1567Glnfs*7). Both mutant cells presented a slightly decrease in apoptosis compared to control. An increased mineralization was observed in the intracellular mutant, as well a higher RUNX2 and ALPL expression by RT-qPCR. The extracellular mutant had a lower mineralization/differentiation and an increase in BMP2 expression. RNAseq analysis in both mutant cells revealed 1700 and 1400 under and over expressed genes for intracellular mutant and 100 and 40 under and over expressed genes for extracellular mutant.

Conclusion: Mineralization and expression of target genes differ according to the variant localization, showing that the pathways and the pathogenicity are not the same. This approach will allow to study new functions of *LRP4* in bone.

References: 1. Shen, et al. "LRP4 in neuromuscular junction and bone development and diseases." *Bone* (2015). https://doi.org/10.1016/j.bone.2015.05.012.

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Conflict of Interest: None declared.

P05.003.D Developmental genomics on split-hand/foot malformation, further indication of allelic series and gene dosage effects

Ruizhi Duan[1], *Hadia Hijazi*[1;2], *Haowei Du*[1], *Jawid Fatih*[1], *Christopher Grochowski*[1], *Shalnini Jhangiani*[3], *Jennifer Posey*[1], *Zeynep Coban Akdemir*[1;4], *V. Reid Sutton*[1], *Claudia Carvalho*[1;5], *Davut Pehlivan*[1;6], *James Lupski*[1;3;6;7]

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Background/Objectives: Split-hand/foot malformation (SHFM) is a rare form of congenital limb anomaly with unclear molecular etiology, presumably because of a failure to maintain the central portion of apical ectodermal ridge (AER) during limb bud proximal-distal (P/D) axial development.

Methods: Family-based genomics and rare variant analyses were implemented using exome sequencing (ES) combined with whole-genome array-based comparative genomic hybridization (aCGH) to investigate 5 SHFM cases that originated from diverse world geographical populations.

Results: We identified one novel pathogenic variant of *WNT10B* in two unrelated Turkish families; one family with a de novo 7Mb large chromosomal deletion encompassing the entire *HOXD* territory; two families involving copy number gain of Chr17p13.3 including *BHLHA9*. Notably, breakpoint junction analyses for all three CNV alleles provided evidence for microhomology-mediated break-induced replication (MMBIR) as the putative molecular mutational mechanism facilitated by *Alu/Alu*-mediated rearrangement (AAMR). In contrast to heterozygous duplication of *BHLHA9* associated with SHFLD3, homozygous duplication CNV was observed in association with the Gollop-Wolfgang Complex, implicating semi-dominant inheritance.

Conclusion: Genes acting on limb patterning are sensitive to a gene dosage effect and often associated with an allelic series. Novel variant identifications involving these genes/loci provide potential insights that further elucidate the developmental genomics of SHFM. We extend a gene dosage model which could potentially, in an adjuvant way, assist interpreting the interconnections among allelic series, clinical phenotypical severity, and reduced penetrance of the *BHLHA9*-related CLM spectrum.

References: da Rocha *et al. Clinical Genetics*. 2021; 100(5):615-623.

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Conflict of Interest: Ruizhi Duan: None declared, Hadia Hijazi: None declared, Haowei Du: None declared, Jawid Fatih: None declared, Christopher Grochowski: None declared, Shalnini Jhangiani: None declared, Jennifer Posey J.E.P. was supported by NHGRI K08 HG008986, and is the principal investigator of Baylor College of Medicine Genomics Research Elucidates Genetics of Rare (BCM-GREGoR; U01 HG011758), Zeynep Coban Akdemir: None declared, V. Reid Sutton: None declared, Claudia Carvalho: None declared, Davut Pehlivan D.P. is supported by the International Rett Syndrome Foundation (IRSF grant #3701-1), James Lupski United States (U.S.) National Human Genome Research Institute (NHGRI) and National Heart Lung and Blood Institute (NHBLI) to the Baylor-Hopkins Center for Mendelian Genomics (BHCMG, UM1 HG006542) and Baylor College of Medicine Genomics Research Elucidates Genetics of Rare (BCM-GREGoR; U01 HG011758), U.S. National Institute of Neurological Disorders and Stroke (NINDS) (R35NS105078 to J.R.L.); and Muscular Dystrophy Association (MDA), (512848 to J.R.L.) and Spastic Paraplegia Foundation (SPF) (to J.R.L.), J.R.L. has stock ownership in 23andMe, J.R.L. is a paid consultant for Regeneron Genetics Center; The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted at Baylor Genetics (BG); J.R.L. serves on the Scientific Advisory Board (SAB) of BG., J.R.L. is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, genomic disorders and bacterial genomic fingerprinting.

P05.004.A Weill-Marchesani syndrome: natural history and genotype-phenotype correlation from 18 cases

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Background/Objectives: Weill-Marchesani syndrome (WMS) belongs to the acromelic dysplasia group, defined by short stature, brachydactyly and progressive joint stiffness. WMS is characterized by ophthalmological abnormalities such as microspherophakia, severe myopia secondary to lens shape anomaly, and lens ectopia. Cardiovascular defects have been reported. Monoallelic variations in FBN1 are associated with a dominant WMS, while biallelic variations in ADAMTS10, ADAMTS17 and LTBP2 are responsible for recessive WMS. These four genes code for components of the extracellular matrix.

Objectives: Natural history description of WMS and genotype-phenotype correlation establishment.

Methods: Retrospective multicenter study. Inclusion criteria: clinical diagnosis of WMS with identified mutations.

Results: 18 individuals, 11 females and 7 males, with a mean age of 20 years (from 1.5 to 59 years) were included. Eight have a mutation in FBN1 without specific localization, 6 in ADAMTS10, 4 in ADAMTS17, none in LTBP2. All individuals presented with eye anomalies including lens ectopia (10/18), high myopia (9/18), microspherophakia (8/18), glaucoma (5/18), and cataract (4/18). 10/18 have a short stature (-2 to -4 DS), 12/18 joints limitation, 12/18 brachydactyly. 7/18 individuals have cardiac defect such as valvulopathy (pulmonary stenosis 2/7, aortic stenosis 1/7, mitral insufficiency 3/7, mitral thickening 1/7). Other manifestations included: recurrent laryngitis (1), hepatomegaly (1), carpal tunnel syndrome detected at 22 years of age (1).

Conclusion: Apart from ophthalmological findings which are mandatory for diagnosis, phenotype of WMS seems to be more variable than initially described, notably, only half of patients presented with short stature. No genotype-phenotype correlation emerges from this cohort.

References:.

Grants:.

Conflict of Interest: None declared.

P05.005.B Potential therapeutic targets for hypermobile Ehlers-Danlos syndrome from proteome and secretome analyses of patients' dermal fibroblasts

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Background/Objectives: Hypermobile Ehlers-Danlos syndrome (hEDS) is a connective tissue disorder without a known genetic etiology and specific therapies, which is mainly characterized by generalized joint hypermobility and musculoskeletal complaints. Patients' dermal fibroblasts exhibit a widespread extracellular matrix (ECM) disarray and myofibroblast-like characteristics including α-SMA cytoskeleton organization. Control fibroblasts treated with hEDS cells-derived conditioned media (hEDS-CM) acquire this pathological phenotype.

Methods: A comprehensive proteomic study based on top-down and bottom-up approaches was performed by comparing the intra- and extracellular proteome of patients' and controls' fibroblasts. We also performed in vitro studies to define the effect of the matrix metalloproteinases (MMPs) inhibitor doxycycline on ECM organization and fibroblast-to-myofibroblast transition (FMT).

Results: Cellular proteome analyses unveiled protein changes essential to actin cytoskeleton dynamics, energy metabolism and redox balance, proteostasis, intracellular trafficking and secretion. Secretome profiling of hEDS-CM mainly revealed an MMPs dysfunction as a possible disease driver by causing a detrimental feedback loop of excessive ECM degradation coupled with myofibroblast differentiation. Doxycycline-mediated MMPs inhibition rescued in patients' cells a control-like ECM organization and induced a partial reversal of their myofibroblast-like phenotype. Furthermore, the addition of doxycycline to hEDS-CM abolishes its capability to induce in control cells ECM disarray and FMT.

Conclusion: Altogether, these data provide evidence on several putative disease targets for the development of therapeutic strategies with a potential benefit for patients' management.

References:.

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P05.006.C Mutation spectrum in Bulgarian patients with skeletal tissue disorders, revealed by clinical exome sequencing

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Background/Objectives: Identifying a molecular diagnosis for an individual with a skeletal dysplasia can lead to improved clinical care, guide future medical management and treatment, and inform assessment of risk for familial recurrence. We aimed in revealing the type and frequency of genetic mutations in Bulgarian patients with skeletal tissue disorders.

Methods: We performed next generation sequencing (NGS) by using TruSight One kit (clinical exome) and MiSeq platform of Illumina in 26 patients with clinical diagnosis, suspicious for skeletal tissue disorder.

Results: We found pathogenic and likely pathogenic variants in 10 and 2 patients, respectively. Six were missense mutation, 3 – nonsense, 2 - frameshift and 1 splicing variant. The highest frequency of mutations was detected for Osteogenesis imperfecta. The Table below represents our findings.

Gene	Gene variant	Genetic diagnosis
Pathogenic variants		
COL1A1	c.252dupC (p.Glu85Argfs*84) c.581G>A (p.Gly194Asp) c.1251delC (p.Ser418-Leufs*123) c.2644C>T (p. p.Arg882Ter)	Osteogenesis imperfecta
FBN1	c.8051+2T>A c.1546C>T (p.Arg516Ter)	Marfan and associated syndromes
COL2A1	c.1510G>A (p.Gly504Ser)	
FGFR2	c.314A>G (p. Tyr105Cys)	Crouzon syndrome
FGFR3	c.1626C>G (p.Asn542Lys)	Hypochondroplasia-like phenotype
NSD1	c.1831C>T (p.Arg611Ter)	Sotos syndrome
Probably pathogenic variants		
COL5A1	c.4819G>A (p.Gly1607Ser)	Ehlers-Danlos syndrome, classical type
SKI	c.1877A>T (p.Lys626Met)	Shprintzen-Goldberg syndrome

Conclusion: We put genetic diagnosis in 46% of the patients with clinical diagnosis of skeletal tissue disorder by using clinical exome sequencing. These findings demonstrate the utility of NGS testing for individuals with a suspected skeletal dysplasia or growth disorder.

References: Scocchia, A., et al. Diagnostic utility of next-generation sequencing-based panel testing in 543 patients with suspected skeletal dysplasia. Orphanet J Rare Dis 16, 412 (2021).

Grants: Д01-285-17.12.2019.
Д01-395-18.12.2020.
Д01-302-17.12.2021.

Conflict of Interest: None declared.

P05.007.D The familiar and the sporadic form of Trichorhinophalangeal syndrome- case reports

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Background/Objectives: Trichorhinophalangeal syndrome type I (TRPS I) is a rare autosomal dominant disorder caused by mutation in the TRPS1 gene coding a transcriptional repressor of GATA-regulated genes. TRPS1 haploinsufficiency reduces activity of genes regulating the growth of bone and cartilage with clinical picture of TRPS I: abnormal bones in the fingers and toes, joint abnormalities, distinctive facial features and other symptoms (fine, sparse hair, pear-shaped nose, prominent ears, brachydactyly, cone shaped epiphyses of the phalanges, dystrophic nails, short stature, small breasts, hip dysplasia). We report 2 cases. The first case is the 16 years old girl with growth retardation and typical facial features. The proband´s mother manifested the same facial signs and. The 2nd sporadic case is 12 years old girl with similar facial signs, disproportion between leg length and scoliosis.

Methods: In the first case we carried out a NGS amplicon analysis of TRPS1 gene. In the second case SNP array (OmniExpress-24 v1.3 chip, Illumina) was performed and result was confirmed by MLPA (Salsa MLPA KIT Illumina P-228 TRPS1-LGS).

Results: In the first case a missense mutation c.2761C>T (p. Arg921Ter) in exon 6 of TRPS1 gene of maternal origin and in the second case de novo 155kb microdeletion involving exons 3-5 of TRPS1 gene were found.

Conclusion: Correct diagnosis based on characteristic facial features in combination with targeted genetic testing is essential for confirmation of diagnosis with adequate follow up and therapeutic care and is very important for possibility to provide appropriate genetic counselling.

References:.

Grants:.

Conflict of Interest: None declared.

P05.008.A Molecular characterization and investigation of the role of genetic variation in phenotypic variability and response to treatment in a large pediatric Marfan syndrome cohort

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Background/Objectives: In a large cohort of 373 paediatric patients with Marfan syndrome (MFS) with a severe cardiovascular phenotype, we explored the proportion of patients with MFS with a pathogenic FBN1 variant and analysed whether the type/location of FBN1 variants was associated with specific clinical characteristics and response to treatment. Patients were recruited on the basis of the following criteria: aortic root z-score > 3, age 6 months to 25 years, no prior or planned surgery, and aortic root diameter < 5 cm.

Methods: Targeted resequencing and deletion/duplication testing of FBN1 and related genes were performed.

Results: We identified (likely) pathogenic FBN1 variants in 91% of patients. Ectopia lentis was more frequent in patients with dominant-negative (DN) variants (61%) than in those with haploinsufficient variants (27%). For DN FBN1 variants, the prevalence of ectopia lentis was highest in the N-terminal region (84%) and lowest in the C-terminal region (17%). The association with a more severe cardiovascular phenotype was not restricted to DN variants in the neonatal FBN1 region (exon 25-33) but was also seen in the variants in exons 26 to 49. No difference in the therapeutic response was detected between genotypes.

Conclusion: Important novel genotype-phenotype associations involving both cardiovascular and extra-cardiovascular manifestations were identified, and existing ones were confirmed. These findings have implications for prognostic counselling of families with MFS.

References: PMID: 35058154.

Grants: FWO Flanders: 12X8520N and G044720N.

Conflict of Interest: None declared.

P05.009.B Serum calcification propensity T50 associates with disease severity in patients with pseudoxanthoma elasticum

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Background/Objectives: Pseudoxanthoma elasticum (PXE) is a currently intractable Mendelian disorder characterized by progressive ectopic calcification in the skin, eyes and arteries. Therapeutic trials in PXE are severely hampered by the lack of reliable biomarkers. Serum calcification propensity T50 is a novel blood test measuring the functional anti-calcifying buffer capacity of serum. Here, we evaluated T50 in PXE patients aiming to investigate its determinants and suitability as a potential biomarker for disease severity.

Methods: Fifty-seven PXE patients were included and demographic, clinical, imaging and biochemical data were collected from medical health records. PXE severity was assessed using Phenodex scores. T50 was measured using a validated, nephelometry-based assay. Multivariate models were then created to investigate T50 determinants and associations with disease severity.

Results: Mean age of patients was 45.2 years, 68.4% was female and mean serum T50 was 347 minutes. Multivariate regression analysis identified serum fetuin-A ($p < 0.001$), phosphorus ($p = 0.007$) and magnesium levels ($p = 0.034$) as significant determinants of T50, while no correlations were identified with serum calcium, eGFR, plasma PPi levels or ABCC6 genotype. After correction for covariates, T50 was found to be an independent predictor of ocular ($p = 0.013$), vascular ($p = 0.013$) and overall disease severity ($p = 0.016$) in PXE.

Conclusion: In this cross-sectional cohort study, shorter serum T50 – indicative of higher calcification propensity – associated with a more severe phenotype in PXE patients. This study indicates for the first time that serum T50 might be a reliable and clinically relevant biomarker in PXE and may act as a surrogate endpoint in future therapeutic trials.

References:.

Grants:.

Conflict of Interest: Lukas Nollet: None declared, Matthias Van Gils: None declared, Suzanne Fischer: None declared, Laurence Campens: None declared, Swapna Karthik Swapna Karthik is an employee of Calciscon AG., Swapna Karthik is a stockholder of Calciscon AG., Andreas Pasch Andreas Pasch is an employee of Calciscon AG., Andreas Pasch is a stockholder of Calciscon AG., Julie De Zaeytijd: None declared, Bart Leroy: None declared, Daniel Devos: None declared, Tine De Backer: None declared, Paul Coucke: None declared, Olivier Vanakker: None declared.

P05.010.C Genetic and functional studies of novel candidate genes for Psoriatic Arthritis Mutilans

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Background/Objectives: Psoriatic arthritis mutilans (PAM) is the most severe and rare clinical subtype of psoriatic arthritis (PsA) characterized by severe destruction of the distal joints. The prevalence of PAM in the Nordic countries is estimated to 3.69 cases per million inhabitants.

Methods: In this project, we aim to study the genetic basis of PAM in a unique Nordic PAM cohort consisting of 61 well-characterized patients[1] by the whole genome sequence (WGS) and whole exome sequence (WES).

Results: Using next generation sequencing we found three very rare variants in the same gene in four unrelated patients. The gene found suggest involvement of the Reactive oxygen species (ROS) in development of PAM. To measure ROS generation in patients' blood we are using the very sensitive Electron Paramagnetic Resonance (EPR) method. In addition, we are creating cell models to study the effects of the mutations in global expression, and we plan to use zebrafish models to study the consequences of these variants in vivo.

Conclusion: In summary, identifying the genetic cause of PAM is very valuable as it gives affected individuals the opportunity for diagnosis, important information about prognosis and treatment.

References: 1.Gudbjornsson, B et al. "Psoriatic arthritis mutilans (PAM) in the Nordic countries: demographics and disease status. The Nordic PAM study." *Scandinavian journal of rheumatology* vol. 42,5 (2013): 373-8. https://doi.org/10.3109/03009742.2013.771211.

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Conflict of Interest: None declared.

P05.011.D Expanding the allelic and locus heterogeneity of Multiple osteochondromas

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Background/Objectives: Multiple osteochondromas (MO) is a skeletal dysplasia characterized by the growth of two or more osteochondromas, caused by pathogenic nucleotide variants in *EXT1* or *EXT2* genes in 70%-90% of patients. Another rarer disease characterized by multiple osteochondromas but with additional enchondromas is metachondromatosis, associated with the loss-of-function variants in the *PTPN11* gene. The clinical similarity of the two diseases suggests that atypical forms of metachondromatosis can be misdiagnosed as MO. This study aims to characterize the genotype profile of the Russian population and find additional molecular causes in patients with MO.

Methods: DNA sequencing (Sanger Method and NGS panel) and MLPA analysis were performed to identify SNV and CNV in the sample of 348 patients from 153 families. Patients were divided into groups according to the severity of the disease, using a three-degree clinical scale with two subgroups.

Results: Germline pathogenic or likely pathogenic variants were identified in 137 (89%) families. 122 unique disease-causative variants (53 novel and 69 known) and 15 recurrent (4 novel) were identified including frameshift (34,4%), nonsense (26,2%), missense (12,3%), splicing (15,5%) variants, genomic rearrangements (5,7%) and in-frame deletion (0,8%). In 16 families with the clinical picture of MO with no significant findings in *EXT1* and *EXT2* genes, five loss-of-function variants in the *PTPN11* gene were found.

Conclusion: Our work expanded the allelic and locus heterogeneity of MO and showed that some of the undiagnosed patients with MO could have pathogenic variants in the *PTPN11* gene.

References:.

Grants:.

Conflict of Interest: None declared.

P05.013.B A biallelic gain-of-function variant in MSGN1 causes a new skeletal dysplasia syndrome

Asuman Koparir[1], *Caroline Lekszas*[1], *Kemal Keseroglu*[2], *Aboulfazl Rad*[3], *Atefeh Hasanzadeh*[4], *Ehsan Ghayoor Karimiani*[5], *Nakul Narendran*[6], *Ertugrul Ozbudak*[7;8], *Barbara Vona*[1;3;9;10], *Thomas Haaf*[1]

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Background/Objectives: Mesogenin1 (MSGN1, OMIM: *612209), a basic-Helix–Loop–Helix transcription factor, is expressed in the presomitic mesoderm (PSM) and plays a crucial role in formation of PSM progenitor cells during somitogenesis. We aimed at identifying the molecular basis of a patient with severe skeletal dysplasia in a single family through utilization of whole-exome sequencing and subsequent Sanger sequencing.

Methods: The proband displays short stature and multiple skeletal problems, including mesomelic dysplasia of the arms with complete humero-radio-ulna synostosis, arched clavicles, pelvic dysplasia, short and thin fibulae, proportionally short vertebrae, hyperlordosis and mild kyphosis. To test the pathogenicity of the detected mutation, we overexpressed either wild-type (WT) or the mutant msgn1 in zebrafish via mRNA injection and analyzed tbxta (i.e., T/brachyury/ntl) expression in the tailbud.

Results: A novel homozygous c.374G>T (p. Arg125Leu) missense variant was detected in MSGN1 (NM_001105569.2) in a patient. MSGN1 resides in a ~13.2 Mb homozygous interval on chromosome 2. We found overexpression of WT or the mutant msgn1 significantly reduces tbxta expression in the tailbud compared to uninjected WT embryos ($p < 0.0001$). In addition, we also found the mutant msgn1 has a more severe effect than WT msgn1 ($p = 0.0186$, Kruskal-Wallis), causing a gain-of-function.

Conclusion: In contrast to loss-of-function effect, gain-of-function of MSGN1 explains mild affected vertebrae of the proband. Furthermore, as somite segments from 9 to 13 are known to contribute to the forelimb muscle, these might potentially be related with mesomelic shortening in our case. Our findings highlight a new skeletal dysplasia syndrome caused by a gain-of-function mutation in MSGN1.

References:.

Grants:.

Conflict of Interest: None declared.

P05.014.C MN1-related disorders in two Bulgarian patients

Mila Sleptsova[1], *Tihomir Todorov*[1], *Slavena Atemin*[1;2], *Mihaela Mladenova*[1;2], *Daniela Avdjieva-Tzavella*[3], *Albena Todorova*[1;2]

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Background/Objectives: Pathogenic variants in the MN1 gene are a known cause of CEBALID syndrome, which is characterised mainly by craniofacial and structural brain abnormalities, and global developmental and variable intellectual delay. Predominantly nonsense and frameshift variants have been reported as pathogenic so far. The syndrome is with autosomal dominant inheritance (Mak et al., 2019). We report two patients which presented with similar clinical characteristics and were found to carry heterozygous variants in the MN1 gene.

Methods: Whole Exome Sequencing was performed for both patients and a panel of genes was analysed. Variants flagged through the analysis were confirmed and segregated in the patients' families via Sanger sequencing.

Results: The first case is a patient with craniofacial dysostosis, low-set ears, polycystic kidney dysplasia and congenital intellectual delay. The following variant in MN1 was found in a heterozygous state: c.3743G>A, p.Trp1248Ter. The segregation analysis confirmed the variant occurs de novo.

The second case is a patient which presents with craniofacial malformations. The c.2486C>A, p.Ser829Tyr variant was found in a heterozygous state. The segregation analysis confirmed the variant occurs de novo.

Conclusion: Those cases provide insight into the diversity of MN1 variants that could be disease-causing. Although no missense variants have been reported as pathogenic so far, functional studies might be worth looking into.

References: Mak, C., Doherty, D., Lin, A., et al., 2019. MN1 C-terminal truncation syndrome is a novel neurodevelopmental and craniofacial disorder with partial rhombencephalosynapsis. Brain, 143(1), pp.55-68.

Grants:.

Conflict of Interest: None declared.

P05.015.D Functional analyses of pathogenic variants in SNRPE associated with the rare hair loss disorder hypotrichosis simplex

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Background/Objectives: Pathogenic variants in SNRPE cause autosomal-dominant hypotrichosis simplex. The extent of scalp and body hair involvement shows great interindividual variability, even within the same family. In particular, all of the affected individuals present with scanty or no eyebrows, while some of them show also hypotrichosis of scalp and body hair.1 SNRPE encodes a core protein of the U snRNPs, key factors of the minor and major spliceosomes. It is yet not clear how pathogenic variants in this gene cause the hair loss phenotype and which specific signaling pathways are involved. We aim at understanding the function of SNRPE in relevance to hair biology.

Methods: We transfected HEK293T and/oror HaCaT cells with wild type and mutant SNRPE constructs and performed immunofluorescence, co-immunoprecipitation and WB. We silenced the endogenous SNRPE mRNA, performed RNAseq.

Results: SNRPE mutants are less expressed than the wild type and are partially degraded via the proteasome; they are also incorporated in the minor spliceosome. They show

mislocalization in the cytosol, seeming to fail to enter the nucleus. RNAseq data on HaCaT cells downregulated for the endogenous SNRPE show an alteration of many physiological processes and a reduction in KRT81 expression levels, previously linked to monilethrix.

Conclusion: The mechanism by which mutations in SNRPE cause hypotrichosis is still unclear. However, mutant forms of SNRPE are probably unable to exert their function. Down-regulation of SNRPE in HaCaT affects the expression of KRT81, known to cause monilethrix.

References: 1 Pasternack et al., 2013.

Grants: I-1443-422.13/2017 (German-Israeli Foundation).

Conflict of Interest: None declared.

P05.016.A Age-specific effects of body size on fracture risk in later life: A lifecourse Mendelian randomization study

Grace Marion Power[1], *Jonathan Tobias*[1;2], *Timothy M. Frayling*[3], *Jessica Tyrrell*[3], *April Hartley*[2], *Jon Heron*[1], *George Davey Smith*[1], *Tom G. Richardson*[1]

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Background/Objectives: Musculoskeletal conditions, including fractures, can have severe and long-lasting consequences. Higher body mass index in adulthood is widely acknowledged to be protective for most fracture sites. However, the association between weight and bone health is complex and confounding bias may have distorted earlier findings. Employing a lifecourse Mendelian randomization (MR) approach, this investigation explores how prepubertal and adult body size independently influence fracture risk in later life.

Methods: Using data from a large UK-based prospective cohort, univariable and multivariable MR were conducted to simultaneously estimate the effects of age-specific genetic proxies for body size (n = 453,169) on the odds of fracture in later life (n = 416,795). A two-step MR framework was additionally applied to elucidate potential mediators.

Results: Univariable and multivariable MR indicated strong evidence that higher body size in childhood reduced fractures in later life (OR, 95% CI: 0.89, 0.82 to 0.96, P = 0.005 and OR, 95% CI: 0.76, 0.69 to 0.85, P = 1x10-6, respectively). Conversely, higher body size in adulthood increased fracture risk (OR, 95% CI: 1.08, 1.01 to 1.16, P = 0.023 and OR, 95% CI: 1.26, 1.14 to 1.38, P = 2x10-6, respectively). Two-step MR analyses suggested that the effect of higher body size in childhood on reduced fracture risk was mediated by its influence on higher estimated bone mineral density in adulthood.

Conclusion: This investigation provides novel evidence that higher body size in childhood reduces fractures in later life and higher body size in adulthood is a risk factor, opposing findings from earlier research. Protective effect estimates previously observed are likely attributed to childhood effects.

References:.

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Conflict of Interest: Grace Marion Power GMP is employed part-time by Clifton Insight outside of this work., Jonathan Tobias: None declared, Timothy M. Frayling: None declared, Jessica Tyrrell: None declared, April Hartley: None declared, Jon Heron: None declared, George Davey Smith: None declared, Tom G. Richardson TGR is employed part-time by Novo Nordisk outside of this work.

P05.017.B Genetic risk factors for developmental dysplasia of the hip in the Trøndelag Health Study

Kaya Kvarme Jacobsen[1], *Sigrid Børte*[2;3], *Lene Bjerke Laborie*[4;5], *Hege Kristiansen*[6;7], *Annette Schäfer*[6], *Trude Gundersen*[8;9], *Tetyana Zayats*[10;11;12], *Bendik Kristoffer Slagsvold Winsvold*[2;3;13], *Karen Rosendahl*[14;15]

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Background/Objectives: Developmental dysplasia of the hip (DDH) is a congenital condition affecting 2-3% of all infants[1]. DDH increases the risk of osteoarthritis, is the cause of 30% of all total hip arthroplasties (THAs) in adults <40 years of age and can result in loss of life quality[2]. We aim to explore the genetic background of DDH in order to improve diagnosis, management and longterm outcome.

Methods: We used the large, ongoing, longitudinal Trøndelag Health Study (HUNT) database[3], Case definition was based on ICD-9/-10 diagnoses of DDH, or osteoarthritis secondary to DDH. Analyses were performed using SAIGE software, with covariates including sex, batch, birth year and principal components. We included only SNPs with MAF ≥ 0.01, R^2 ≥ 0.8 and HWE ≥ 0.0001. Significance level was set at $p < 5x10^{-8}$. The regional ethical committee approved the study.

Results: Analysis included 69,500 individuals, of which 408 cases, and 8,531,386 SNPs. Two SNPs near *COL11A1* were significantly associated with DDH; rs713162 (β = -0.43, SE = 0.07, $p = 8.4x10^{-9}$) and rs6577334 (β = -0.43, SE = 0.08, $p = 8.9x10^{-9}$). COL11A1 has previously been associated with acetabular dysplasia and osteoarthritis[4,5].

Conclusion: This large, genome-wide case-control study indicates an association between COL11A1 and DDH and is an important contribution to investigating the etiology of DDH, with further research needed.

References: [1]Rosendahl et al, 1996, Pediatric Radiology.
[2]Engesæter et al, 2011, Acta Orthopaedica.
[3]Ferreira et al, 2017, Nat Genet.
[4]Raine et al, 2013, BMC Musculoskeletal Disorders.
[5]Versteeg et al, 2016, Osteoarthrits and Cartilage.

Grants: Helse Vest (F-12550-D11544).

Conflict of Interest: None declared.

P05.018.C Satisfaction with resilient denture liner versus acrylic resin telescopic prostheses for patients with ectodermal dysplasia: A non randomized crossover clinical trial

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Background/Objectives: Ectodermal dysplasia is a rare genetic disorder that manifests dry skin, sparse hair, xerostomia, complete or partial anodontia of deciduous and/or permanent teeth and malformed teeth. The crowns are usually conical anteriorly. The roots are stunted having a huge pulp chamber. Enamel usually forms a delicate layer, decreasing in thickness towards cervical line. Xerostomia is mostly present due to absence of salivary glands or reduction in their number. Alveolar ridges are commonly flat due lack of tooth formation, it is the main reason for the low vertical dimension of the face and the senile appearance of the patient. These oral features can hinder proper restoration of teeth.

Methods: In this non-randomized clinical trial, ectodermal dysplasia patients with partial anodontia were recruited. Overdentures were constructed for the patients, that were later relined opposing to the teeth with a soft liner. Follow up was done 1 week and 3 months following denture delivery and denture relining. After each stage, patient satisfaction, retention, and periodontal health parameters were assessed. Patient satisfaction was assessed with a validated, reliable questionnaire.

Results: A statistical significant difference was found between the groups regarding retention ($P = 0.025$), probing depth ($P < 0.001$), and gingival index ($P = 0.011$) favouring the acrylic resin coping-retained overdentures 3 months after overdenture delivery.

Conclusion: The resilient denture liner-retained maxillary complete overdenture improved patient satisfaction and tooth mobility of anterior teeth, while minimally jeopardizing the periodontal condition of the abutment teeth in children with ectodermal dysplasia.

References:.

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Conflict of Interest: Yasmine H. Mohsen National research centre, Mohamed Abdel Kader: None declared, Nouran Abdel Nabi: None declared, Iman A. W. Radi: None declared.

P05.019.D Identification of a novel deep intronic variant in CRTAP by genome sequencing as the cause of osteogenesis imperfecta

Gandham SriLakshmi Bhavani[1], *Prajna Udupa*[1], *Shalini Nayak*[1], *Neethukrishna Kausthubham*[1], *Ashwin Dalal*[2], *Katta Girisha*[1]

[1]Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Department of Medical Genetics, Manipal, India; [2]Centre for DNA Fingerprinting and Diagnostics, Diagnostics Division, HYDERABAD, India.

Background/Objectives: CRTAP is one of the 21 genes known to be associated with Osteogenesis Imperfecta (OI). Biallelic variants in CRTAP (cartilage-associated protein) cause autosomal recessive OI type VII. CRTAP forms a complex with P3H1 in the endoplasmic reticulum and binds to pro-alpha collagen chains to mediate post-translational modification of collagen triple helix.

Methods: Detailed clinical evaluation and a complete skeletal survey were performed. We did exome sequencing followed by genome sequencing to identify the genetic aetiology. RNA was extracted from the fetal fibroblasts for transcript analysis.

Results: Antenatal sonography at 19 weeks of gestation showed shortening of long bones and rocker bottom feet. Perinatal pathological evaluation showed protruding thoracic cage, depressed nasal bridge with anteverted nares, and long philtrum. Radiographic findings were short and bowed legs with multiple fractures at different stages of healing, and generalized osteopenia. Exome sequencing did not reveal any significant pathogenic variants. Genome sequencing identified a deep intronic homozygous variant in intron 3, c.794-1403A>G. RNA studies revealed that it affects splicing and results in two different abnormal transcripts. The detected variant generates the novel splice donor site and utilizes upstream acceptor sites resulting in the formation of two different transcripts of 68 bp and 75 bp cryptic exons in CRTAP mRNA.

Conclusion: The deep intronic variant is associated with alteration of splicing, leading to the generation of two abnormal transcripts. This novel splicing effect in CRTAP is the likely cause of lethal form of OI in the fetus.

References:.

Grants:.

Conflict of Interest: None declared.

P05.020.A Pathogenic LEF1 variants disrupt WNT signaling to cause ectodermal dysplasia associated with limb malformations

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Background/Objectives: Ectrodactyly ectodermal dysplasia without cleft lip/palate (MIM 129810) is reported with clinical manifestation of split-hand/foot malformation accompanied by ectodermal anomalies like hypotrichosis and abnormal dentition. This disorder has received insufficient attention in the past. We aim to delineate the underlying genetic determinants of this disorder.

Methods: Whole exome and transcriptome sequencing, Pull down assay, Immunoblotting, Immunofluorescence.

Results: We recruited 12 individuals from 5 unrelated families manifesting a syndrome with variable expression of limb malformations and/or ectodermal dysplasia. The phenotypic spectrum includes various limb malformations, such as radial ray defects, polydactyly or split hand/foot, and ectodermal dysplasia in some individuals. We identified four novel *LEF1* variants — monoallelic in 11 affected individuals and biallelic in one. We have shown that out of four, only the p.Met23dup impaired interaction with β-catenin due to its location in a highly conserved β-catenin binding domain of LEF-1. Whole transcriptomic profiling further confirmed that Wnt/ β-catenin signaling pathway is impaired. We have seen significant differential expression of transcripts already known as downstream targets of the Wnt/ β-catenin signaling

pathway and HOX family — both Wnt and HOX are crucial for embryonic developmental events.

Conclusion: Our functional data show that two molecular mechanisms are at play: haploinsufficiency or loss of DNA-binding are responsible for a mild to moderate phenotype, while loss of β-Catenin binding due to biallelic variants is associated with a severe phenotype. Our findings establish mono- and biallelic variants in *LEF1* as a cause for a syndrome comprising limb malformations and ectodermal dysplasia.

References:.

Grants: CHU Lille, CMMC, DAAD, NHGRI, NHLBI.

Conflict of Interest: None declared.

P05.021.B Contribution of SHOX gene sequencing in the etiological diagnosis of short stature

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Background/Objectives: Analysis of the *SHOX* gene is common in clinical practice, as an abnormality justifies a treatment by growth hormone. Our study aims to determine the interest of *SHOX* sequencing when large rearrangements analyses are inconclusive, and in relation with the precise clinical context[1].

Methods: We analyzed the clinical and genetic data of 163 probands referred to our laboratory for analysis of the *SHOX* locus for Idiopathic Short Stature (ISS) or Léri-Weill dyschondrosteosis (LWD), after Turner syndrome was excluded by karyotyping. All patients underwent CNV analysis by MLPA followed by gene sequencing.

Results: Out of the 163 patients analyzed, 15 had a CNV affecting *SHOX* or its regulatory regions: 11 pathogenic deletions and 4 duplications (2 likely benign and 2 VUS). In the 152 patients without pathogenic CNV, 5 had SNVs or indels. The only patient carrying a variant classified as pathogenic also exhibited major clinical signs of LWD. The remaining variants were classified as benign for one rare polymorphism, likely benign because of inheritance.

Conclusion: In our cohort, the diagnostic yield of *SHOX* sequencing was 33% (1/3) for patients with signs of LWD, whereas it was zero in patients with ISS. Thus this analysis seems questionable in the context of ISS and could be limited to patients with significant signs of LWD (i.e. a positive "Rappold revisited" score[1]).

References: [1]Hirschfeldova et *al*. *Journal of Human Genetics*, 2016.

Grants:.

Conflict of Interest: None declared.

P05.022.C Body mass index and the risk of rheumatic disease: linear and nonlinear Mendelian randomization analyses

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Background/Objectives: While the association between obesity and risk of rheumatic disease is well established, the causal relation is not fully established. Here we estimate the causal effect of body mass index (BMI) on the risk of developing five types of rheumatic disease.

Methods: Linear and nonlinear mendelian randomization (MR) was used to estimate the effect of BMI on the risk of developing of rheumatic disease and to identify sex-specific effects. Primary MR analyses were performed in 361,952 participants from the UK Biobank cohort.

Results: We found that a one standard deviation increase in BMI increases the risk for rheumatoid arthritis (IRR = 1.52; 95% CI = 1.36-1.69), osteoarthritis (IRR = 1.49; 1.43-1.55), psoriatic arthropathy (IRR = 1.80; 1.31-2.48), gout (IRR = 1.73; 1.56-1.92), and inflammatory spondylitis (IRR = 1.34; 1.14-1.57). BMI was also found to be a stronger risk factor for psoriatic arthropathy (sex-interaction $P = 3.3 \times 10\text{-}4$) and gout ($P = 4.3 \times 10\text{-}3$) in females compared to males. Nonlinear effects of BMI were identified for osteoarthritis and gout in males, and for gout in females. The nonlinearity for gout was more extreme in males compared to females, and was similar to what was observed for urate levels.

Conclusion: Higher BMI causes an increased risk for rheumatic disease, an effect that is more pronounced in females compared to males for both gout and psoriatic arthropathy. These results give further insight into etiology and pathology of rheumatic disease.

References:.

Grants: This work was supported by the Swedish Research Council, the Marcus Borgströms, the Åke Wibergs, A and M Rudbergs foundations, Vleugels foundation and Hedströms foundation.

Conflict of Interest: None declared.

P05.023.D MED12 somatic mutation in Paget's disease of bone

Nerea Gestoso-Uzal[1;2;3], *Luis Antonio Corchete*[2;3], *Ana-Belén Herrero*[1;2;3], *Javier del Pino-Montes*[2;4], *Juan Francisco Blanco-Blanco*[2;5], *Rogelio González-Sarmiento*[1;2;3]

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Background/Objectives: Paget's disease of bone (PDB) is a metabolic bone disease characterized by an increase in bone turnover in a disorganized way. Germinal mutation in *SQSTM1* gene constitutes the most important known genetic factor predisposing to PDB, but it can not explain the etiopathogenesis of all the patients. Moreover, PDB is a focal disorder that does not affect to all the bone tissue. The aim of this study was to identify somatic mutations that can determine the development of PDB.

Methods: DNA was extracted from both pagetic and normal bone tissue from one PDB patient following a standard phenol/chloroform procedure. Whole exome sequencing was performed to identify pagetic bone-exclusive mutations.

Results: We identified 40 somatic variants in the pagetic bone that did not appear in the normal tissue, including 11 exonic variants. We found just one nonsense mutation, c.103C>T; p.E35*, in *MED12* gene. Somatic mutation of *MED12* has been associated with uterine myomas, characterized by uncontrolled but benign cell proliferation. Moreover, it has been reported that *MED12* mutation causes a blockade of autophagy, as it occurs with reported mutations in *SQSTM1*(*1*).

Conclusion: Our results suggest that somatic alteration of *MED12* gene could be responsible of PDB development.

References: 1. A. El Andaloussi, A. Al-Hendy, N. Ismail, T. G. Boyer, S. K. Halder, *Reprod. Sci*. 27, 823 (2020).

Grants: This study was funded by FIS-FEDER: PI18/01476.

Conflict of Interest: None declared.

P05.024.A Syntaxin-18 defects in human and zebrafish cause traffic jams and unravel key roles in early bone development

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Background/Objectives: Membrane fusion is a key process in all living organisms. SNAREs are required for fusogenic action, where they zipper up at the juncture of membranes, enabling fusion and cargo transfer. Syntaxin-18 (STX18), an endoplasmic reticulum-resident SNARE, is crucial for membrane fusion events, vesicular transport and secretion of collagens. Currently, STX18 is not linked to any human disease.

Methods: Whole exome sequencing (WES) was applied for a fetus with lethal osteogenesis imperfecta, presenting with multiple fractures, abnormal cartilage formation, tibial bowing, irregularly formed mandible, and severe cranial undermineralization. Protein modelling and overexpression studies were performed and stx18 zebrafish crispants (uppercut18) were generated using CRISPR/Cas9.

Results: WES revealed a homozygous missense variant p.(Arg10Pro) in STX18, affecting a critical residue in the cytoplasmic N-terminal alfa-helical domain of syntaxin-18, leading to stable mutant STX18 protein. Uppercut18 had severe craniofacial malformations, curved backbones and underdeveloped fins, and all died at 11-12dpf. Strikingly, uppercut18 revealed impaired cartilage and skeletal development; and interestingly, displayed (1) altered bone remodelling, (2) general upregulated mRNA and protein levels of components acting within the forming stx18-complex and secretory pathway, and (3) altered behaviour including decreased movement and an increased light-to-dark-transition response.

Conclusion: Our study provides evidence for a link between genetic defects in STX18 and human bone disease, expanding the phenotype of the SNAREopathies, and our in vivo data describes for the first time an essential role for syntaxin-18 during skeletal development and its involvement in neurological pathways.

References: https://doi.org/10.1126/science.1161748, https://doi.org/10.7554/eLife.02784, https://doi.org/10.1016/j.ydbio.2016.11.010.

Grants: Research Foundation Flanders (12Q5920N,1842318N), Ghent University.

Conflict of Interest: None declared.

P05.025.B Comprehensive molecular screening using NGS custom gene panel identifies ultra-rare types of Osteogenesis imperfecta and OI-like disorders

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Background/Objectives: Osteogenesis imperfecta (OI) is a rare connective tissue disorder, presenting genetic and phenotypic heterogeneity. The molecular etiology reaches XXI types, of which types I-IV (90% of patients) are associated with mutations in genes encoding collagen type I. Other cases are caused by aberrations in one out of 17 non-collagen genes influencing collagen biosynthesis or related molecular pathways, which prevalence is below 1 per 1 000 000 births or remains unknown.

Methods: The study included 158 patients suspected with Osteogenesis imperfecta, aged 6 months to 44 years, presenting a broad spectrum of clinical manifestation. NGS custom gene panel, encompassing all known genes for OI types I-XIX and OI-like disorders, was performed.

Results: We have identified 9 families with pathogenic mutations located in six non-collagen genes (*IFITM5, SERPINF1, FKBP10, WNT1, P4HB, LRP5*) associated with an ultra-rare OI type V, VI, XI, XV and OI-like disorders as Cole-Carpenter syndrome, Primary Osteoporosis, Idiopathic Juvenile Osteoporosis. This unique group of patients constituted 7% of the study population with a proven molecular diagnosis of congenital bone fragility. Of 10 non-collagen variants, four have not been reported previously.

Conclusion: Obtained results reflect phenotypic and genetic variety of skeletal disorders, as primary patients with mutations in non-collagen genes were classified as having OI type I (*LRP5, WNT1, P4HB*), type III (*FKBP10, IFITM5*) and type IV (*SERPINF1, IFITM5*). Thus, comprehensive molecular screening is crucial for correct diagnosis, genetic counselling and appropriate treatment.

References: PMID: 24715559; PMID: 20301472; OMIM Database.

Grants: Young Scientist Grant 2016/IV/57-MN.

Conflict of Interest: None declared.

P05.026.C Biallelic loss of function variants in EXOC6B are associated with impaired primary ciliogenesis and cause spondylo-epi-metaphyseal dysplasia with joint laxity type 3

Pelin Simsek-Kiper[1], ***Prince Jacob***[2], *Priyanka Upadhyai*[2], *ekim taskiran*[3], *Vishal Singh Guleria*[2], *beren karaosmanoglu*[3], *gozde imren*[3], *rahşan göçmen*[4], *Gandham SriLakshmi Bhavani*[2], *Neethukrishna Kausthubham*[2], *hitesh shah*[5], *Gulen Eda Utine*[1], *koray boduroglu*[1], *Katta Girisha*[2]

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Background/Objectives: Spondylo-epi-metaphyseal dysplasia with joint laxity type 3 (SEMDJL3) is characterized by multiple joint dislocations and is caused by biallelic pathogenic variants in *EXOC6B*. Only four molecularly-confirmed individuals have been reported hitherto and the underlying mechanism is yet to be elucidated.

Methods: Using exome sequencing, we identified c.2122+15447_2197-59588del and c.401T>G biallelic variants in *EXOC6B* in two individuals from unrelated families with different ethnicities. Immunofluorescence and immunoblotting assays were performed in proband 1 (P1), and osteogenic differentiation assay and total mRNA sequencing in proband 2 (P2). RT-PCR and RT-qPCR were performed for both in patient derived fibroblasts.

Results: P1 at age 3 years demonstrated leptodactyly (slender metacarpals and metatarsals), delayed carpal ossification, lumbar lordosis with bilateral hip, and knee dislocations. P2 at age 13 years had intellectual disability with central nervous system

anomalies including hydrocephalus, hypoplastic mesencephalon, and thin corpus callosum in addition to the known features of SEMDJL3. Increased levels of *EXOC6B* mRNA, absent protein in fibroblast-derived cell lysates, shortening of primary cilia length with minor variability of primary cilia frequency in P1 as compared to control derived fibroblasts were observed. The expression of *EXOC6B* mRNA and osteogenic differentiation potential was reduced in dermal fibroblasts of P2 as compared to that of control fibroblasts. Pathways related to the extracellular matrix were also distrubed in P2.

Conclusion: Our study provides the first evidence of the impairment of exocytosis potentially deregulating primary ciliogenesis in two subjects with SEMDJL3 that might represent yet another ciliopathy with central nervous system involvement and joint dislocations.

References:.

Grants: India Alliance Fellowship (IA/CRC/20/1/600002).

Conflict of Interest: Pelin Simsek-Kiper: None declared, Prince Jacob: None declared, Priyanka Upadhyai: None declared, ekim taskiran: None declared, Vishal Singh Guleria: None declared, beren karaosmanoglu: None declared, gozde imren: None declared, rahşan göçmen: None declared, Gandham SriLakshmi Bhavani: None declared, Neethukrishna Kausthubham: None declared, hitesh shah: None declared, Gulen Eda Utine: None declared, koray boduroglu: None declared, Katta Girisha Founding Director of Suma Genomics Pvt. Ltd. (Incubatee at Manipal Universal Technology Business Incubator).

P05.027.D Genetic anomalies and diagnostic yield in an 11-year birth cohort of craniosynostosis patients

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Background/Objectives: Craniosynostosis is a rare congenital anomaly, defined by premature fusion of one or more cranial sutures. Craniosynostosis can occur in isolation or as part of a clinical syndrome. The genetic cause of craniosynostosis may impact the clinical course and treatment of patients with craniosynostosis. Knowledge on the genetic etiology therefore is key to ensure adequate counseling and to improve clinical management of craniosynostosis patients. In line with this, the Dutch craniosynostosis guideline recommends genetic diagnostic testing in patients with craniosynostosis. This study aims to assess both the prevalence of the different subtypes of craniosynostosis in an 11-year birth cohort of craniosynostosis patients as well as the diagnostic yield of genetic testing.

Methods: We conducted a retrospective cohort study among patients who presented at the outpatient clinic of the Erasmus University Medical Center, The Netherlands. We included all patients, born between 2010-2021, with radiologically confirmed craniosynostosis and assessed diagnostic yield of genetic testing.

Results: We included 993 patients (n = 334 female/659 male), of whom 856 presented with single-suture craniosynostosis (449 sagittal, 268 metopic, 116 unicoronal, 14 unilambdoid, 9 frontosphenoidal) and 136 patients presented with multisutural craniosynostosis, and one unknown (preliminary results).

Conclusion: This study will discuss the prevalence of chromosomal and monogenic (likely) pathogenic variants and provide an update on the diagnostic yield of genetic testing in our 11-year birth cohort craniosynostosis patients. Finally, we will compare the diagnostic yield for different types of craniosynostosis with the long term aim of improving genetic testing strategies and counseling.

References:.

Grants:.

Conflict of Interest: None declared.

P05.028.A The stop-loss variant c.690A>C in RAB33B results in milder phenotype of Smith-McCort dysplasia-2

Ashwin Dalal[1;2], *Prince Jacob*[2], *Katta Girisha*[2], *Prajna Udupa*[2], *debashish ghosh*[2], *Gandham SriLakshmi Bhavani*[2], *hitesh shah*[2]

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Background/Objectives: Smith-McCort dysplasia-2 (SMC-2) is a rare spondylo-epiphyseal-metaphyseal dysplasia, caused by biallelic loss of function variants in *RAB33B*. RAB33B has dual functions in membrane trafficking and in the autophagy process. Studies investigating the dynamics of the autophagy with relation to *RAB33B* in patients with SMC2 have not been reported.

Methods: We identified a biallelic stop-loss variant, c.690A>C in *RAB33B* in two siblings (P1 and P2) by exome sequencing. Functional studies were done using immunofluorescence, immunoblotting, and qPCR in fibroblast cells of P1 and P2.

Results: P1 and P2 at age 13 years and 15 years respectively presented with short trunk, barrel-shaped chest, brachydactyly, plump interphalangeal joints, limited extension of elbow joints, and progressive joint pain. Convex vertebral endplates, reduced hip joint spaces, short metacarpals (4^{th} and 5^{th}), abnormal carpal bone morphology were observed in their radiographs. The mutant RAB33B showed loss of its Golgi body localization which led to aggregation of collagens in cytosol. Abundance of cytoplasmic RAB33B led to increased flux of autophagy, causing accelerated clearance of the aggregated collagen by aggrephagy. Thus, the equilibrium of RAB33B localization to Golgi body and phagophore membrane is modulated by the mutant RAB33B, leading to differential crosstalk of Golgi body-RAB33B-autophaosome in the patient fibroblasts.

Conclusion: Mislocalization of mutant RAB33B at Golgi body with simultaneous increased autophagy probably underlie the milder phenotype of SMC-2. Our work highlights the emerging role of autophagy in collagen degradation and in skeletal dysplasia.

References:.

Grants:.

Conflict of Interest: None declared.

P05.029.B Inversion of LMX1B - a novel cause of nail-patella syndrome in a Swedish family

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Background/Objectives: Nail-patella syndrome (OMIM #161200) is a rare autosomal dominant disorder with an estimated prevalence of 1 in 50 000. The characteristic clinical findings are different nail and skeletal abnormalities including underdeveloped nails, hypoplastic or absent patellae and iliac horns. It is caused by missense or truncating variants in *LMX1B*, resulting in haploinsufficiency. In this case report, we present an unusual cause of

NPS in a family of five affected individuals where Sanger sequencing failed to detect any pathogenic variants in the *LMX1B* gene.

Methods: We describe a large inversion disrupting the *LMX1B* gene in five affected family members with mild but variable clinical features of nail-patella syndrome.

Results: Whole genome sequencing revealed an inversion stretching approximately 4,2 Mb and disrupting *LMX1B* and *ABL1* which was confirmed by Sanger sequencing of the breakpoints.

Conclusion: This expands the molecular spectrum of nail-patella syndrome, indicating that genomic rearrangements should be considered a possible cause in patients where standard genetic investigations fail to detect any pathogenic variants in *LMX1B*.

References: Sweeney, E., Fryer, A., Mountford, R., Green, A., & McIntosh, I. (2003). Nail patella syndrome: a review of the phenotype aided by developmental biology. J Med Genet, 40(3), 153-162.

Grants: The Swedish Rare Diseases Research foundation (Sällsyntafonden), Sällskapet Barnavård and Karolinska Institutet, Swedish Research Council, through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet, Promobilia Foundation and Frimurare Barnhuset foundation in Stockholm.

Conflict of Interest: None declared.

P05.031.D de novo c.784G>A variant in LMNA disturbs nuclear proteostasis and results in progeroid manifestations

Shruti Pande[1], *Debasish Kumar Ghosh*[1], *Jeevan Kumar*[1], *Dhanya Yesodharan*[2], *Sheela Nampoothiri*[2], *Periyasamy Radhakrishnan*[3], *Chilakala Gangi Reddy*[4], *Akash Ranjan*[4], *Katta Girisha*[3;5]

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Background/Objectives: Variants in *LMNA* lead to collapse of nuclear systems network and underlie some disorders of premature aging, including Hutchinson-Gilford progeria syndrome.

Methods: We identified a de novo heterozygous nonsynonymous variant, c.784G>A ($LMNA^{E262K}$) in exon 4 of *LMNA* (NM_170707.4) in a sixteen-years old male by exome sequencing. We performed immunocytochemistry to see expression and localization of the mutant LMNA and immunoblotting to quantify the mutant LMNA in patient fibroblasts. To study the LMNA secondary structure, recombinant wild type and mutant were cloned and circular dichroism spectroscopy, atomic force microscopy, dynamic light scattering and isothermal titration calorimetry were performed. Computation tools and molecular dynamics simulation were used to assess the stability of $LMNA^{E262K}$.

Results: The proband had micrognathia, sparse scalp hair, eyebrows and eyelashes, narrow nasal bridge with broad nasal tip and dental crowding. His fibroblasts showed nuclear aggregates of the mutant LMNA with its mislocalization from the nuclear envelope. Structural destabilization of mutant region results in the clustering of multiple hydrophobic residues, making LMNA unstable and prone to aggregation. $LMNA^{E262K}$ also disrupts the consensus binding site of E2-SUMO ligase UBE2I, further reducing the clearance of mutant LMNA in patient fibroblasts. Aggregates of $LMNA^{E262K}$ temporally sequester HSPA1A1, PSMD8, MRE11 and KU80, resulting in impaired nuclear proteostasis and loss of DNA damage repair response in proband fibroblasts.

Conclusion: $LMNA^{E262K}$ in the rod2 domain causes structure-function destabilization of LMNA, leading to nuclear proteotoxicity in premature aging.

References:.

Grants: India Alliance (IA/CRC/20/1/600002) funded this work.

Conflict of Interest: Shruti Pande: None declared, Debasish Kumar Ghosh: None declared, Jeevan Kumar: None declared, Dhanya Yesodharan: None declared, Sheela Nampoothiri: None declared, Periyasamy Radhakrishnan: None declared, Chilakala Gangi Reddy: None declared, Akash Ranjan: None declared, Katta Girisha Suma Genomics, Private Limited, Manipal (Director).

P05.032.A A novel SLC35D1 variant causing milder phenotype of Schneckenbecken dysplasia in a large pedigree

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Background/Objectives: SLC35D1 gene mutations causes Schneckenbecken dysplasia(SD) which is an rare autosomal recessive disorder and characterized by the snail-like pelvis, flattening of vertebral bodies, short and broad long bones with a dumbbell-like appearance, thoracic hypoplasia. Here we reported a family with novel SLC35D1 variant and milder phenotype of SD (1, 2).

Methods: The clinical features of 5 patients were represented in Table 1. Whole exome sequencing was done using the NextSeq500 Sequencer. The detected variant which was predicted as the causative variant was validated by Sanger sequencing in the proband and her parents. Sanger sequencing were studied for the other affected individuals in the family.

Results: Whole Exome sequencing of the proband revealed a homozygous missense variant of SLC35D1 gene; c.401T>C. A, p.Met134Thr. Affected sibling and her cousins with same phenotype have homozygous missense variant too. The parents and their healthy girl are heterozygous carriers.

Conclusion: Patients with mild phenotypic findings who carry SLC35D1mutation were reported for the first time in this study. This report will have significant consequences since it has the largest SD family with alive patients (ages ranged 4-31 years old) reported to date.

References: 1. Song Z. Roles of the nucleotide sugar transporters (SLC35 family) in health and disease. Molecular Aspects of Medicine. 2013; 34: 590–600.

2. Furuichi T et al. Identification of loss-of-function mutations of SLC35D1 in patients with Schneckenbecken dysplasia, but not with other severe spondylodysplastic dysplasias group diseases. J Med Genet. 2009 ; 46(8): 562–568.

Grants: Table 1. Clinical features of the patients.

Table 1. Clinical features of the patients.

Age	Gender	Specific clinical features
12	F	Short stature, cleft palate, mild scoliosis, hypertrophy of vertebral left transverse process, pectuc carinatum, mild shortening of forearm, shortening of lower extremity, bilaterally genu valgum, puberte precox, narrow thorax, pes planus, the absence of a snail-like pelvis
15	F	Short stature, mild shortening of forearm, shortening of lower extremity, bilaterally genu valgum, puberte precox, narrow thorax,pes planus, the absence of a snail-like pelvis
4	M	Short stature, shortening of lower extremity and mild shortening of forearm
14	F	Short stature, shortening of lower extremity and mild shortening of forearm
31	M	Short stature, shortening of lower extremity and mild shortening of forearm

Conflict of Interest: None declared.

P05.033.B Mapping active regulatory signals at early embryonic stage of face development

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Background/Objectives:.

While craniofacial malformations are among the most common congenital anomalies in humans at frequency 1/1600, their genetic causes remain largely unknown. Previous studies have shown that deletion or mutation of craniofacial enhancers modifies the activity of craniofacial gene(s) and affects face morphology. To reveal the complex regulatory networks that control craniofacial development and gain insights into the effect of non-coding variation, we mapped 3D interactions between craniofacial enhancers and genes.

Methods: We isolated the four facial prominences, i.e., mandible, maxillary, lateral and medial nasal prominences of E11.5 mouse embryo and performed Capture Hi-C to identify chromatin interactions at all gene promoters.

Results: We identified an average of 163'000 interactions per tissue, for a total of 327'459 unique loops. These are chiefly *cis*-interactions, which account for >98% of all detected interactions, with a median distance of interacting fragments of 280kb. Chromatin interactions between a gene and an intergenic space account for 77% of all detected interactions. Importantly, enrichment analyses of matched tissue and developmental stage epigenomics data (e.g. H3K27ac binding sites) show a significant enrichment of craniofacial regulatory sequences in our interacting fragments, supporting the biological significance of these craniofacial interactions' maps. We recapitulate previously reported interactions, reveal new interesting regulatory landscape of craniofacial genes and link GWAS non-coding lead SNPs for craniofacial phenotypes to candidate target gene(s).

Conclusion: Our data provide a starting point to disentangle the gene regulatory signals controlling craniofacial development and point toward new candidate regions for determining the genetic origins of many craniofacial-associated disorders.

References:.

Grants: PRO-Femmes grant (UNIL, Switzerland).

Conflict of Interest: None declared.

P05.034.C Multiple epiphyseal dysplasia: A diagnostic challenge with genetic heterogeneity

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Background/Objectives: Multiple epiphyseal dysplasia (MED) is a rare genetic skeletal disorder with clinical and genetic heterogeneity. MED is caused by mutations in the genes encoding important cartilage extracellular matrix proteins, enzymes, and transporter proteins including COMP, MATN3, COL9A1, COL9A2, COL9A3, CANT1 and SLC26A2. Disproportionate short stature, joint pain, and early-onset osteoarthritis are the main clinical features (1). In this study we aimed to investigate the clinical and molecular findings along with natural course of the disease in a group of patients with MED.

Methods: The molecular etiology was investigated with Sanger sequencing and whole exome sequencing. The clinical findings of mutation positive patients were reviewed.

Results: A total of 36 patients with a clinical diagnosis of MED was evaluated and the genetic etiology was revealed in 20 (55.5%); 11 were male and 9 were female. COMP (n = 12, 60%), MATN3 (n = 6, 30%) and SLC26A2 (n = 2, 10%) mutations were detected in a decreasing order. The most frequent complaints for referral were difficulty in walking, fatigue and joint pain. Proportionate short stature was detected in %15 of patients. Orthopedic follow-up was required in most of the patients. In patients with SLC26A9 mutations characteristic findings of double layered patella and pes equinovarus were not present.

Conclusion: MED is genetically heterogenous yet with unidentified gene mutations in the etiology. The diagnosis should be considered even in the absence of characteristic clinical findings. Multidisciplinary follow-up is mandatory.

References: 1. Dennis EP, Greenhalgh-Maychell PL, Briggs MD. Multiple epiphyseal dysplasia and related disorders: Molecular genetics, disease mechanisms, and therapeutic avenues. Dev Dyn. 2021;250(3):345-359.

Grants:.

Conflict of Interest: None declared.

P05.035.D Further delineating the clinical spectrum of p.Arg444Cys variant in LRP5: a case report and a systematic review of the literature

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Background/Objectives: The LRP5 protein plays a key role in retinal vasculature development and helps regulate bone mineral density (BMD) homeostasis. Loss-of-function variants in *LRP5* were associated with familial exudative vitreoretinopathy (FEVR), osteoporosis-pseudoglioma syndrome and early-onset osteoporosis/osteopenia.

We describe the clinical and genetic features of a family with osteopenia as the presenting feature, displaying the p.Arg444Cys variant in *LRP5*.

Methods: Clinical and familial data were based on physical examination and medical records. Next-generation sequencing of an in-silico panel encompassing 20 genes correlated with bone fragility and detection of index mutation were performed with Twist Custom Panel (clinical exome-twist Bioscience) kit.

A systematic review of the p.Arg444Cys variant in *LRP5*, according to PRISMA guidelines, was performed.

Results: Our proband is a 36-year-old male who suffered from scoliosis and recurrent vertebral fractures even during bisphosphonate treatment. The genetic testing revealed the heterozygous variant c.1330C>T (p.Arg444Cys) in the *LRP5* gene (NM_002335.4), segregating in his father and his 30-year-old sister. Both of them experienced less severe osteopenia and denied visual impairment, supporting the variant's role in reduced BMD.

Ophthalmic examination excluded signs of vitreoretinopathy in our proband and his father.

We systematically review 1384 records and we found four studies reporting the same variant. To our knowledge, the p.Arg444Cys variant was previously identified only in patients with FEVR with or without osteopenia.

Conclusion: We reported a patient displaying the p.Arg444Cys variant in *LRP5* and familial low BMD without eye involvement, expanding the clinical spectrum of this variant. Further clinical and biochemical assessment of the other family carriers are ongoing.

References:.

Grants:.

Conflict of Interest: None declared.

P05.036.A Genetic basis of cleft lip and palate

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Background/Objectives: Cleft lip and/or palate (CL/P) is the most common cranio-facial malformation often divided into syndromic CL/P (syCL/P) and non-syndromic CL/P (nsCL/P). In general, patients with syCL/P follow Mendelian inheritance, whilst those with nsCL/P are thought to have a complex etiology.

Methods: We analyzed 81 a priori non-syndromic index CL/P patients from a continuously growing cohort of 1400 CL/P patients by whole exome sequencing (WES). We looked for Mendelian mutations using Highlander as well as copy number variations using ExomeDepth.

Results: We unraveled pathogenic or likely pathogenic variants in 12 families in *COL2A1*, *CTNND1*, *TP63*, *CHD7*, *PHF8*, *IRF6*, and *GHRL3*. We also identified, and validated by molecular karyotyping, a deletion in *TP63* in 2 siblings.

Conclusion: We identified mutations in 16 % of index cases by WES, providing an accurate diagnosis as well as the possibility of genetic counseling. In some cases, we identified pathogenic variants in syCL/P genes in a priori nsCL/P cases, demonstrating that patients with CL/P without cardinal signs or familial history of a syndrome may still carry a mutation in a gene linked to syCL/P. We also identified a new phenotype in blepharocheilodontic syndrome 2: imperforate anus. These results show that WES is an important tool for identifying the genetic cause of CL/P. For the remaining patients for which we have not identified pathogenic variants in candidate genes, we will enlarge our research towards the rest of the genes in the human genome to identify new genes for clefts.

References:.

Grants: FNRS n°40000521.

Conflict of Interest: None declared.

P05.037.B Novel SDR9C7 mutation causes lamellar ichthyosis in a Spanish patient

Carlos Gutiérrez-Cerrajero[1;2;3], *Nerea Gestoso-Uzal*[1;2;3], *Daniel Salete Granado*[1;2], ***Janet Sotolongo-Ravelo***[1], *David Diez-Castro*[1;2], *Ana-Belén Herrero*[1;2;3], *Ángela Hernández-Martín*[4], *Rogelio González-Sarmiento*[1;2;3]

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Background/Objectives: Ichthyoses are a group of diseases characterized by dry, scaly skin and a disruption of skin barrier function(*1*). Lamellar ichthyosis (LI) is a severe variant (*1*) and *SDR9C7* has recently been identified as one of the causal genes for LI. To date, four mutations in *SDR9C7* have been linked to LI: two in Lebanese families in 2016, one in a Japanese woman in 2016 and one in a Pakistani family in 2017. Here we present a fifth mutation in a Spanish patient.

Methods: The patient was admitted to Hospital Niño Jesús in Madrid. Genomic DNA was extracted from peripheral blood by standard phenol/chloroform protocol, mutations were found using whole-exome sequencing and validated by Sanger sequencing. Candidate mutations were analysed using the predictors SIFT and Polyphen, as well as the databases ClinVar and Varsome.

Results: The patient shows ichthyosis with dark scales and sporadic superficial desquamation, hyperactivity and microcephaly. Whole exome sequencing revealed the patient was homozygous for mutation c.95G>A, p.Gly32Asp in *SDR9C7* and Sanger sequencing validated it. The parents were heterozygous for this mutation. This mutation was not included in ClinVar, but SIFT, Polyphen and Varsome predicted its effect to be pathogenic or likely pathogenic.

Conclusion: We report the discovery of a novel mutation in *SDR9C7* that causes LI. This is the first mutation discovered worldwide and the first in a European patient.

References: 1. V. Oji et al., *J. Am. Acad. Dermatol*. **63**, 607–641 (2010).

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Conflict of Interest: None declared.

P05.038.C Novel RIPK4 variants cause ectodermal dysplasia and alter cell-cell adhesion

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Background/Objectives: Ectodermal Dysplasia Syndactyly Syndrome (EDSS1) is caused by mutations in PVRL4 encoding nectin-4, a component of adherens junctions. EDSS1 shares cutaneous syndactyly (a mild form of pterygia) with other conditions such as Bartsocas-Papas syndrome caused by biallelic mutation in RIPK4, a key player in epidermal development, differentiation, and skin integrity. Here we report two siblings with a phenotype resembling EDSS1, with biallelic variants in RIPK4 and studied their functional effect.

Methods: Exome sequencing. Western blotting, autophosphorylation, proteasome inhibition and immunofluorescence analyses in patient's primary keratinocytes and transfected HEK293 cell line. Immunofluorescence, haematoxylin and eosin staining and transmission electron microscopy on patient's skin.

Results: Our siblings featured ectodermal dysplasia, plantar hyperkeratosis associated to syndactyly of hands and feet suggestive of EDSS1. After excluding PVRL4 variants, exome sequencing revealed biallelic likely pathogenic variants in RIPK4, inherited from heterozygous unaffected parents. Reverse phenotyping revealed that one brother was born with "closed eyes" (ankyloblepharon) and

oral synechiae, surgically treated at birth, typically seen in RIPK4-related disorders. Functional studies showed that RIPK4 deficiency impaired cell adhesion organization downregulating PVRL4/nectin-4 expression through IRF6 transcription factor. Also, PKP1/DSG1/DSP altered expression or localization was detected and desmosomes showed abnormal morphology at electron microscopy.

Conclusion: This work further expands the clinical spectrum seen in RIPK4-pathies highlighting epithelial fusions as diagnostic handles for the disease. In adults, the phenotype coincides with EDSS1. Such clinical overlap is mirrored at functional level, as outlined by this newly uncovered RIPK4-IRF6-nectin-4 axis.

References: Online Mendelian Inheritance in Man, OMIM.

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Conflict of Interest: Chiara De Luca full-time, Rosanna Monetta full-time, Elisabetta Botti full-time, Manuel Belli full-time, Maria Grazia Palmerini full-time, Marco Salvatore full-time, Lucia Militti: None declared, Arianna Di Daniele: None declared, Elena Cicchetti: None declared, Daniele Castiglia full-time, RC2020-2756828, Francesco Brancati full-time, GR2013-02356227, Paola Fortugno full-time.

P05.039.D A unique COL2A1 phenotype as a result of partial gene deletion

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Background/Objectives: Monoallelic *COL2A1* (OMIM *120140) pathogenic variants cause various syndromes associated with several skeletal and ocular disorders such as achondrogenesis, chondrodysplasia, SED congenita, Spondyloperipheral dysplasia, Kniest dysplasia, Stickler syndrome type I, avascular necrosis of the femoral head.

Methods: A 24-year-old male patient referred with the complaint of short stature, skeletal deformities, arthralgia in weight bearing joints, easy fatiguability and high degree myopia. The patient and his skeletal survey were evaluated and multigene panel was performed and followed by multiplex ligation-dependent probe amplification (MLPA) assay.

Results: The patient had acro-mesomelic short stature with short and deformed forearms, legs, hands and feet. X-ray images showed bilateral thickened and short metatarsal and metacarpal bones, bilateral short ulnas, shortened and wide phalanxes. Pelvis MRI showed bilateral avascular necrosis of the femoral heads. Ophthalmologic examination revealed retinal detachment due to high degree myopia. Heterozygous deletion between exons 51-54 was detected in MLPA performed as a result of suspected CNVs detected in exon sequencing.

Conclusion: To date, the majority of variants reported in COL2A1 are single nucleotide changes. Only 2 cases with COL2A1 whole exon deletion have been reported, which have the phenotype of Stickler Syndrome Type 1(1). Our case is unique having a novel phenotype, which possess overlapping features of various COL2A1 related disorders with a monoallelic large deletion.

References: (1) Richards, Allan J et al. "Stickler syndrome and the vitreous phenotype: mutations in COL2A1 and COL11A1." Human mutation vol. 31,6 (2010): E1461-71. https://doi.org/10.1002/humu.21257.

Grants:.

Conflict of Interest: None declared.

P05.040.A Sudden cardiac death - a new phenotypic aspect of PLACK syndrome?

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Background/Objectives: PLACK syndrome (Peeling skin, Leukonychia, Acral punctate keratoses, Cheilitis and Knuckle pads syndrome, OMIM 616295) is an extremely rare genodermatosis caused by biallelic mutations in *CAST*. Here, we report a previously unreported *CAST* variant in a family with PLACK syndrome which may reveal an unexplored aspect of this syndrome for the first time: the sudden cardiac death (SCD).

Methods: Whole exome sequencing (WES) was employed to explore the molecular etiology of a dermatological phenotype comprised of punctate palmoplantar keratoderma, angular cheilitis and hyperkeratosis of the knees in two sisters (4 and 8 years old).

Results: A novel homozygous nonsense mutation (NM_001042440.5: c.1759C>T; p.Gln587Ter) in *CAST* was identified in both sisters, which segregated with the dermatological phenotype in family. During the follow-up, dilated cardiomyopathy became evident in the proband and resulted in SCD similar to her two affected brothers who died suddenly at 3 and 4 years. WES did not reveal any other pathogenic variants that may explain cardiomyopathy/sudden cardiac death.

Conclusion: *CAST* encodes calpastatin, an endogenous inhibitor of calpain. Calpain responds to increased Ca^{2+} in myocardial cells, leading to myocyte death by cleaving structural and functional proteins of myocytes during cardiac ischemia, which is inhibited by calpastatin. Although SCD/cardiomyopathy is not a known feature present in the seven reported families with PLACK syndrome, considering the function of *CAST* in myocardium, SCD/cardiomyopathy may be a hidden phenotypic feature of the syndrome. Whether this phenotype is specific to the variant identified here is yet to be explored.

References: PMID:24333421.

Grants:.

Conflict of Interest: None declared.

P05.041.B Identification of candidate mutations related to adolescent idiopathic scoliosis in the Caucasian population - focusing on post-zygotic alternations

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Background/Objectives: Adolescent idiopathic scoliosis is a multidimensional and multicausal spine deformity with frequency from 1-4 % in general population. Most studies focused on germline mutation analysis, omitting post-zygotic variation leading to pathological effects in organisms. Post-zygotic changes pop up in the human body de novo during a lifetime and usually is not inherited. "Benign" diseases research shows that a combination of germline and somatic variation that explore in the human body early in a lifetime may negatively impact and speed up the manifestation of the disease.

Methods: Whole-exome sequencing were performed to determine scoliosis-related genes in the Caucasian population of 32 adolescent patients with severe scoliosis, with a significant focus on mosaic variations. Types of biological material used: blood and articular processes.

Results: We identified recurrent missense germline and somatic variants located in *OBSCN, NEB* and other genes.

Conclusion: Mentioned genes encode proteins that are fundamental components in the assembly and functioning of vertebrate striated muscles. Germline and somatic variations coexistence across them might cause muscle weakness and correlate with scoliosis manifestation and its advanced level.

References: Cheng, J. C. *et al. Nat. Rev. Dis. Prim*. 1, (2015).
Forsberg, L. A. *et al. Nat. Rev. Genet*. 18, 128–142 (2017).
Mustjoki, S. *et al. N. Engl. J. Med*. 384, 2039–2052 (2021).
Murphy, S. *et al. Expert Rev. Proteomics* 16, 241–256 (2019).

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Conflict of Interest: None declared.

P05.042.C A case with spondyloenchondrodysplasia with immune dysregulation (SPENCDI) caused by a novel missense ACP5 mutation

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Background/Objectives: Spondyloenchondrodysplasia with immune dysregulation (SPENCDI) is a rare autosomal recessive immunoosseous dysplasia, characterized by enchondromatous metaphyseal and vertebral lesions with immune dysfunction and neurologic involvement. SPENCDI is caused by biallelic mutations in the ACP5 gene which encodes tartrate-resistant acid phosphatase, a protein functioning in the type I interferon pathway. Here, we present a case with a novel variant in the ACP5 gene.

Methods: The patient is a 7-year-old male who initially presented with short stature and gait disturbance at the age of 5. He is the first child of consanguineous parents born at term. At the age of 2, he presented to hospital with toe walking, the neurological examination exhibit spasticity and hyperreflexia. Brain-Spinal MRI and EMG were normal. At the age of 5, his height was 95 cm (−3,2 SD), skeletal survey revealed metaphyseal irregularities and platyspondyly. Cranial CT revealed calcifications in the basal ganglia. He was hospitalized for autoimmune hemolytic anemia at age 7. Bone marrow aspiration, autoimmune and immunological screenings were normal.

Results: Whole-exome sequencing analysis displayed a homozygous missense variant of c.389G>T (p.Trp130Leu) in the ACP5 gene, later confirmed by sanger sequencing. It was predicted as a "pathogenic" change conforming ACMG criteria in VARSOME database and wasn't reported in the literature. Parents were found to be heterozygous carriers of the variant.

Conclusion: SPENCDI is a clinically heterogeneous disease with distinctive skeletal findings and pleiotropic extra-osseous phenotype. We aimed to raise awareness of SPENCDI, which has multisystem involvement and a significant risk of morbidity and mortality. **References:**.

https://doi.org/10.1007/s10875-016-0252-y.

Grants: None.

Conflict of Interest: None declared.

P05.043.D Identification of effector cell types for orofacial clefting through integration of murine single-cell expression data and GWAS results

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Background/Objectives: Orofacial clefting (OFC) is among the most common human birth defects. Although about 60 risk loci have been identified to date, the functional consequences of the identified variants are still largely unknown, as are the cell types in which the candidate genes exhibit their effects. We here analyzed the gene expression patterns of OFC candidate genes in recently published single-cell RNA sequencing (scRNA-seq) data from embryonic mice.

Methods: We re-analyzed scRNA-seq data from the mouse face (~8,000 cells at E11.5)[1] and whole embryos (~1.4 million cells E9.5–E13.5)[2] using Seurat v4 and scCATCH. The analyses were reproducibly run on the FASTGenomics platform.

Results: We observed predominant expression of OFC candidate genes in epithelial cells (e.g. Irf6, Grhl3, Tfap2a), and in a combination of chondrocytes, osteoblasts and progenitor cells of connective tissue, jaw and teeth (e.g. Fgfr1, Fgf10, Mmp16). Furthermore, we found that Irf6, Grhl3 and Tfap2a are co-expressed in an epithelial cell sub-population and additional OFC candidate genes are specifically expressed in Irf6+ epithelial cells.

Conclusion: Epithelial cells are involved in processes of proliferation and patterning of the mesenchyme and are therefore one of the crucial cell types during craniofacial development. Our results further suggest a distinct epithelial cell sub-population in which OFC candidate genes are active during this developmental timeframe. We will follow this up by studying a more systematic enrichment of OFC candidate genes on a cell-type and single-cell level using single-cell disease relevance scores[3].

References: [1] Li et. al 2019.
[2] Cao et. al 2019.
[3] Zhang et. al 2021.

Grants:.

Conflict of Interest: Anna Siewert: None declared, Julia Welzenbach: None declared, Benedikt Reiz Comma Soft AG, Elisabeth Mangold: None declared, Henning Dickten Comma Soft AG, Kerstin Ludwig: None declared.

P05.044.A Exploring genotype-phenotype correlations, penetrance and expressivity of HOXD13 associated synpolydactyly in a cohort of 17 families with HOXD13 variants

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Background/Objectives: The homeobox transcription factor HOXD13 is an important regulator of embryonic limb development. Mutations leading to haploinsufficiency of *HOXD13* – typically expansions of a polyalanine repeat in exon 1 – cause synpolydactyly

type 1. How these contribute to genotype-phenotype correlations, penetrance and expressivity of *HOXD13* associated synpolydactyly remains mostly illusive. We present a large cohort of 43 affected individuals from 17 families with *HOXD13* variants.

Methods: 41 patients with synpolydactyly suggestive of *HOXD13* haploinsufficiency were selected for analysis of HOXD13 (NM_000523.4) by Sanger sequencing, microsatellite analysis and next generation sequencing.

Results: We identified 15 causative variants and 2 variants of uncertain significance in *HOXD13*. The most frequent variants (12/17) were repeat expansions of the alanine stretch (5 expansions by 8 and 7 by 7 alanines). Additionally, we identified 3 (likely) pathogenic variants (one frameshift, one stop-gain, one single amino acid deletion) and 2 missense variants of uncertain significance. Pedigree and/or segregation analysis of these index patients revealed a total of 43 affected individuals. The observed phenotypes ranged from unaffected carriers to severe osseous synpolydactyly with phenotypic heterogeneity occurring not only across but also within families and asymmetrically affected individuals. We could also corroborate a previously suggested genotype-phenotype correlation of longer alanine repeat expansions with more severe phenotypes.

Conclusion: In a large cohort of 43 affected individuals from 17 families with *HOXD13*–associated synpolydactyly we could identify variable penetrance and expressivity of *HOXD13* variants and confirm a positive correlation of alanine repeat expansion length and phenotypic severity.

References:.

Grants:.

Conflict of Interest: None declared.

P05.045.B Incontinentia pigmenti female with the NEMOdel4-10 deletion in the IKBKG/NEMO gene in a mosaic form

Ezia Spinosa[1], *Michele Salvia*[1], *Carmela Casale*[1], *Alessandra Pescatore*[1], *Annalaura Torella*[2;3], *Giulio Piluso*[2], *Vincenzo Nigro*[2;3], *Vincenzo Piccolo*[4], *Andrea Diociaiuti*[5], *Maya El Hachem*[5], *Matilde Valeria Ursini*[1], *Francesca Fusco*[1]

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Background/Objectives: Incontinentia pigmenti (IP; OMIM#308300) is an X-linked dominant disease, generally lethal in male, caused by mutations in IKBKG/NEMO gene, essential for NF-κB activation. The IP phenotype is characterized by typical skin lesions and by neuroectodermal defects that contribute to a wide variability of severity of disease.

Here we report the case of IP female with neurological and ocular impairment, carrying the recurrent deletion in NEMO/IKBKG gene as a somatic mutation. Moreover, the patient showed also a de novo pathogenic variant c.1708_1709del, p.Ser570fs*27in MED13L gene.

Methods: An IP female patient with severe form of IP, confirmed by skin biopsy, was analyzed in the IP locus on DNA from blood and the presence of NEMOdel4-10 was detected by long-range PCR and quantified by QPCR.

Results: The IP locus analysis revealed the presence of de novo NEMOdel4-10 deletion and excluded the presence of the risk alleles for IP (MER67dup, NEMOPdel). Somatic mosaicism was strongly suggested by quantitative analysis of the ratio of allele mutated versus wild-type allele in genomic DNA from blood. Consistent with somatic mosaicism, the sample of patient had lower ratios of mutant versus wild-type allele compared to the fully heterozygote IP female control.

Conclusion: Postzygotic genetic mosaics for the IKBKG/NEMO mutation are reported only in IP male, indeed the IP phenotype is almost entirely restricted to females heterozygous for IKBKG/NEMO gene mutation. This is the first report demonstrating mosaicism as a cause of IP in female. To note that the contribute of constitutive alteration in MED13L gene (NM_015335.5: c.1708_1709del) will be discussed.

References:.

Grants:.

Conflict of Interest: None declared.

P05.046.C Two new PRKG1 gene mutations among Polish patients with Marfan syndrome and related disorders

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Background/Objectives: Hereditary connective tissue disorders are a group of over two hundred diseases described so far, with Marfan syndrome (MFS) as the best known. MFS overlaps symptoms with such marfanoid syndromes as Loeys-Dietz and the vascular subtype of Ehlers-Danlos syndrome, making differential diagnosis extremely difficult. MFS and MFS-like syndromes are caused by damage to the connective tissue of different systems. One of the main features of MFS-like syndromes is ascending aorta dissection, and aortic aneurysm. According to current knowledge, *PRKG1* gene is on the list of genes related to the pathogenesis of aneurysms.

Methods: NGS of 12 gene panel sequencing was performed for 105 Polish patients with suspicion of Marfan or a Marfan-like syndrome. Control group consisted of 100 people, healthy at the time of the examination, without family history of MFS or MFS-like syndromes.

Results: As the result of the analysis, two mutations in *PRKG1* gene were detected. Both, c.1040C>G (p.Ser347Cys) and c.1075A>C (p.Lys359Gln) mutations were new, not registered in internet databases or reported in the literature. They also were absent in the control group. According to VarSome, c.1040C>G was classified as VUS, and c.1075A>C as pathogenic mutation. The patient with the first mutation was a 12-year-old boy with physical features of MFS. The 9-year-old girl with the second mutation had only excessive joint mobility. They both had no cardiovascular problems, possibly due to their young age.

Conclusion: The results of the study expands the mutational spectrum of *PRKG1* and may help in prevention, early diagnosis and treatment/management of MFS and MFS-like syndromes.

References:.

Grants:.

Conflict of Interest: None declared.

P05.047.D Internal Skeletal Dysplasia Registry within the electronic database of Department of Clinical Genetics University Children's Hospital in Belgrade - basis for a personalised medicine in the future

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Background/Objectives: Skeletal dysplasia are heterogeneous group of genetic disorders affecting skeletal development. Progress in molecular genetic testing and up-to-date knowledge from pre-clinical trials have been leading to novel therapeutic approaches for genetic skeletal disorders despite the challenges of drug development in rare diseases. Timely diagnosis and patients' information availability is crucial for early and proper application of new treatment options.

Methods: We systematically analyzed data from the internal electronic database of our genetic service in order to create an internal sub-register of patients with genetic skeletal disorders.

Results: In the past six years, 137 patients with suspected genetic skeletal disorders have been referred to the Department of Clinical Genetics, University Children's Hospital in Belgrade. Next generation sequencing was performed for 64 patients with suspected heterogeneous skeletal dysplasia, among them there are confirmed cases of very rare or severe skeletal diseases. Also, single gene sequencing or specific mutation analysis was performed for 27 patients according to clinical suspicion. Other patients are clinically monitored or their proposed genetic testing is in progress. We categorized all patients according to the latest classification of genetic skeletal disorders. The *FGFR3* chondrodysplasia group has 19 pediatric patients, with three familial cases of achondroplasia.

Conclusion: The Internal Skeletal Dysplasia Registry should allow for better monitoring of data and visibility results of genetic testing for these patients so that they are timely included specific treatments in the context of personalised medicine in the future.

References: Non applicable.

Grants: Non applicable.

Conflict of Interest: None declared.

P05.048.A A most unusual phenotype in a patient with a mosaic ASXL1 deletion

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Background/Objectives: Bohring-Opitz syndrome (BOS), caused by loss of function mutation in ASXL1, is characterized by distinctive facial features and posture, growth failure, intellectual disability, and variable anomalies.

Methods: We report a patient carrying a mosaic ASXL1 deletion (arr[hg19]20q11.21(31,004,670-31,147,256)x1) found in approximately 30% of cells from both lesional facial tissue and blood.

Results: She is the first child of unrelated parents, with no familial history. Diagnosis of bilateral cleft of the lip and the palate (BCLP) was made on the 24GW ultrasound. She was born at 37GW with BW:2.680kg, BH:46cm, BHC:32.5cm. A right partial ablepharon and a temporal cutaneous band, 2 scalp cutaneous outgrowths and scalp defect, a left interrupted superior eyelid with irregular arched eyebrows and a pseudo temporal cutaneous band, altogether defining a left Tessier #2, #9 and right Tessier #3, #9 facial cleft, and a right microtia were diagnosed. She had hypoplasia of the right hand with metacarpophalangeal joint hyperextension contrasting with flessum of the proximal and distal interphalangeal joints. On her left-side she had foot hypoplasia, tarsal malformation, instep fatty hypertrophy, IV and V hypoplasia. She had normal psychomotor development. At age 6 years, when last seen, she had normal growth parameters and no intellectual disability. Left upper limb hyperplasia became more evident as she grew.

Conclusion: We consider the deletion as causal and the asymmetry due to the mosaicism. Nethertheless, because of most unusual phenotype, further molecular studies were discussed. No mosaic mutation of *KRAS* or *FGFR1* was found on DNA from lesional tissue, WES studies are ongoing.

References:.

Grants:.

Conflict of Interest: None declared.

P05.049.B A progeroid syndrome with severe osteogenesis imperfecta segregates with an intronic TAPT1 homozygous variant that creates a knockout allele

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Background/Objectives: Exome sequencing has introduced a paradigm shift for the identification of germline variations responsible for Mendelian diseases. However, non-coding regions, which make up 98% of the genome, contain structural, regulatory, and transcribed information that cannot be captured. The lack of functional annotation for intronic and intergenic variants makes RNA sequencing a powerful companion diagnostic.

Methods: Here, we identified five patients with a recessive Osteogenesis Imperfecta (OI) syndrome characterized by bone defects and neonatal progeria. We integrated results obtained from homozygosity mapping, genome and RNA sequencing to find the causative gene.

Results: We delineated a non-coding *TAPT1* mutation (c.1237-52G>A) that segregated with the disease. This private mutation, which is predicted to serve as an alternative splicing branchpoint, results in exon 12 skipping and creates a protein-null allele. Functional studies performed on patients' fibroblasts support the notion that 1) TAPT1 resides in the ER/Golgi, 2) is not an essential receptor for human Cytomegalovirus (HCMV) and 3) controls pathways involved in collagen and extracellular matrix biology.

Conclusion: Overall, our work highlights the power of transcriptomic approaches in identifying genetic defects as well as in illuminating the molecular mechanisms and underlying dysregulated pathways in human diseases.

References:.

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P06

CARDIOVASCULAR DISORDERS

P06.001.C Diagnostic yield of a NGS panel in a Brugada syndrome cohort

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Background/Objectives: Brugada syndrome (BrS) is a rare inherited cardiac arrhythmia disorder affecting 1/2000 individuals. Its diagnosis requires presence of a spontaneous or sodium channel blocker induced ST-segment elevation on an electrocardiogram (ECG). BrS patients are at risk for ventricular fibrillations which could lead to sudden cardiac death. In general, only for 25-30% of the patients a genetic diagnosis can be established in one of the BrS associated genes, of which 20-25% carry a variant in the SCN5A gene.

Methods: We collected clinical history, ECG parameters and genetic results of 294 BrS patients (61% male) screened with a diagnostic panel for inherited primary electrical disorders covering initially 51 and in a later version 60 genes.

Results: In total, 43.5% of patients carried a variant of uncertain significance (VUS, class 3; n = 102) or (likely) pathogenic variant (class 4 and 5; n = 26) following the ACMG guidelines. Most of the class 4/5 variants are found in the SCN5A gene (23/26), whereas the remainder were identified in KCNE1/LMNA/SCN2B. 43.9% of patients had a Shangai score above 3.5 (definite BrS) of which 14.7% carried a class 4/5 variant. Only 4.2% of patients with a Shangai score between 2 and 3 carried a (likely) pathogenic variant. Of the 22.4% of patients with a familial history, 20% carried a class 4/5 variant.

Conclusion: The overall diagnostic yield in our cohort is 8.8%, increasing to 15% in BrS patients with a definite diagnosis, or 20% in clear familial patients, which is slightly lower than reported in literature.

References:.

Grants: Research Foundation Flanders.

Conflict of Interest: None declared.

P06.002.D Clinical characteristics, genetic findings and arrhythmic outcomes of patients with catecholaminergic polymorphic ventricular tachycardia across the globe

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Background/Objectives: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare cardiac ion channelopathy. This study examined the clinical characteristics, genetic basis and arrhythmic outcomes of CPVT patients globally.

Methods: PubMed was systematically searched for case reports or series on CPVT. Clinical characteristics, genetic findings and primary outcome of spontaneous ventricular tachycardia/ventricular fibrillation (VT/VF) were analysed.

Results: A total of 442 patients (mean presentation age: 15±12-years-old, 52% male) were included. On presentation, 368 patients (83%) were initially symptomatic and 214 (48%) had VT. PVCs were present in 356 patients (81%) and VT was present in 352 patients (80%). Genetic tests were performed on 257 (58%) patients with a yield of 98%. RyR2, CASQ2, TERCL and KCNJ2 mutations were found in 232(89%), 27(10%), 2 (0.8%) and 1 (0.4%) patients, respectively. Out of 336 patients, 302 (90%) were prescribed beta-blockers, 88 (29%) were prescribed flecainide, 82 (27%) were prescribed propranolol, 26 (9%) were prescribed atenolol, 24 (8%) were prescribed verapamil, 4 (1%) were prescribed propafenone. Out of 442, implantable-cardioverter defibrillator (ICD) were inserted for 120 (27%) patients and sympathectomy was performed on 32 (7%) of patients. On follow-up, 73 patients (38%) had VT/VF.

Conclusion: This is the first systematic review and meta-analysis of CPVT cases globally. Most patients had symptoms on initial presentation and approximately half had VT as the presenting complaint. RyR2 mutations accounts for the majority (89%) of the CPVT cases, followed by CASQ2 (10%), then TERCL and KCNJ2 mutations (1%). Most patients received beta-blocker therapy. 7% had sympathectomy and 27% had ICDs implanted.

References: None.

Grants: None.

Conflict of Interest: None declared.

P06.003.A Association of the genetic variation in the long non-coding RNA FENDRR with the risk of developing Hypertrophic Cardiomyopathy

Elías Cuesta Llavona[1], *Rebeca Lorca*[1], *Israel David Duarte Herrera*[1], *Belén Alonso*[1], *Sara Iglesias Álvarez*[1], *Jose Julian Rodriguez Reguero*[1], *Eliecer Coto*[1], *Juan Gómez*[1]

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Background/Objectives: Hypertrophic Cardiomyopathy (HCM) is the most common hereditary heart disease. However, in around 40-60% of cases no pathogenic variants are identified in the exome. Studies suggest that there are other genetic factors that

could explain part of the risk of developing HCM. A group of candidate genes to be evaluated are those that encode lncRNAs. The aim of this study was to evaluate the possible association of lncRNAs with the risk of developing HCM.

Methods: We sequence a total of 238 index cases of HCM (58 ± 16 years old, 63% male) and 212 controls (70 ± 7 years old; 45% male) through a panel of 10 lncRNAs coding genes that have been associated with cardiovascular disease (H19, KCNQ1OT1, MHRT, CARMEN, FENDRR, TINCR, ANRIL, MIAT, PVT1, MALAT1) with semiconductor chips and the Ion GeneStudio S5 Sequencer (Ion Torrent). The Chi-square test were used to compare allelic frequencies in the polymorphisms identified.

Results: We observed that FENDRR rs39527 A> G, rs39529 G>C and rs40384 T>C polymorphisms were significantly associated with the risk of developing HCM in our cohort (0.006 patients vs 0.03 controls; p = 0.0274; OR: 0.2381; IC: 0.0660-0.8594).

Conclusion: In summary, this study identified the significant protective effect of the rare allele in the FENDRR rs29527, rs39529 and rs40384 polymorphisms on this disease in a Spanish population. These variants could involve a change in the structure of FENDRR that would modify the regulation of gene expression controlled by this lncRNA. However, the functional relevance of this change requires experimental validation.

References:.

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Conflict of Interest: None declared.

P06.005.C Pathogenic variants are distributed among different genes when comparing adult LQTS patients to infants

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Background/Objectives: Congenital long-QT-syndrome (LQTS) is a rare heart disease characterized by a prolonged ventricular repolarization leading to a QTc of more than 460 ms. Patients are usually between 5 and 18 years old when diagnosed. However, little is known about the genetic background of severe LQTS in the very young (especially neonates/infants). This raises the question which variants in which genes are responsible for the early manifestation.

Methods: Between January 2017 and December 2021, 1391 index patients with suspected LQTS were referred to our laboratory for genetic testing. All the genes for which there was at least limited evidence of association with LQTS were examined. The patients were sorted by age (neonate/infant/toddler/>4years), and it was determined which genes were affected in each age group.

Results: Of the 36 toddlers examined, 8 were confirmed genetically positive. All 8 patients carried variants exclusively in the KCNQ1 gene, indicating that this gene is overrepresented when compared to the 1271 older (>4years) patients (≈45% of positive cases). Additionally, pathogenic variants of one gene were detected in neonates or infants with severe LQTS only: CACNA1C.

Conclusion: In the very young patients (<4 years), the variants are distributed among different genes than in adults. This can be used for variant classification according to ACMG/ACGS guidelines. Criterion PP4, which refers to phenotype specificity, could be assigned to CACNA1C variants if a severe LQTS phenotype is present in a neonate/infant. Moreover, PP4 could be modified to a moderate or strong pathogenic criterion for KCNQ1 variants detected in toddlers.

References:.

Grants:.

Conflict of Interest: None declared.

P06.006.D Heart transcriptome profile of a novel transgenic mouse model for arrhythmogenic cardiomyopathy

Martina Calore[1], *Sofia Compagno*[2], *Sara Vencato*[2], *Robin Colpaert*[1], *Claudia Sacchetto*[1], *Alessandra Lorenzon*[2], *Tommaso Becchi*[2], *Nicola Facchinello*[3], *Chiara Romualdi*[2], *Leon de WIndt*[1], *Paola Braghetta*[3], *Libero Vitiello*[2], *Alessandra Rampazzo*[2]

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Background/Objectives: Arrhythmogenic cardiomyopathy (ACM) is one of the most commonly inherited cardiomyopathies, characterized by the progressive substitution of the myocardium with fibrofatty tissue (1). Clinically, ACM is characterized by ventricular arrhythmias, syncope, and sudden cardiac death and shows wide phenotypic heterogeneity (1). Of the known disease genes, desmosomal proteins plakophilin-2 (PKP2), desmoplakin (DSP), and desmoglein-2 (DSG2) are most commonly mutated (2). To study the pathogenic mechanisms of ACM, we generated a novel mouse model for the disease.

Methods: We generated transgenic mice overexpressing desmoglein-2 carrying the p.G100R mutation (TgG) found in an affected patient. To establish the transcriptomic ACM signature, we performed RNA seq on cardiac samples from 6 month-old TgG and control mice.

Results: TgG mice present several of the clinical features of ACM, such as fibrous replacement, and increased distance between cardiomyocyte membranes. Importantly though, we did not detect the same reductions of the canonical Wnt/b-catenin signalling pathway reported in other ACM models, indicating that additional pathways must be perturbed in TgG. Enrichment analysis and network construction identified upregulation of immune- and extracellular matrix-related processes, as well as epigenetic mechanisms, such as histone acetyltransferase activity. By contrast, downregulated processes included the MAPK and TGF-ß pathways.

Conclusion: Collectively, these findings identified numerous processes potentially altered in TgG mice that could pave the way for further studies focused on ACM pathogenic mechanisms.

References: (1) Thiene et al.,N Engl J Med (1988) 318:129–1331988.

(2) Calore et al.,Cell Tissue Res (2015)360:491–500.

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Conflict of Interest: None declared.

P06.007.A Identification of rare genetic variants associated with stroke outcome

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Mallorca, Spain; [9]Hospital de la Santa Creu i de Sant Pau, Barcelona, Spain.

Background/Objectives: Stroke is a cerebrovascular disease that may lead to an important adult disability. There is a large variability in the functional outcome after a stroke, which may be in part regulated by genetic factors. With the aim to further investigate the genetics of stroke outcome, we performed exome sequencing followed by targeted resequencing in a set of 702 patients.

Methods: A pilot study was performed with 90 exomes of extreme stroke recovery scores (modified Rankin Scale (mRS) at 90 days 0-1 vs 3-5) and target genes involved in functional outcome were selected. 702 samples were sequenced by targeted next-generation sequencing using a capture assay that included these targets along with selected regions based on previous GWAS results. Here, we performed continuous (mRS 0-6) and dichotomic (mRS 0-1 vs 3-5 and 0-1 vs 3-6) analyses with Bayesian-based rare variant association (BATI)[1] adjusting for multiple testing and selecting rare variants with a CADD score >20.

Results: These analyses highlighted coding rare variants in *CNTN5* and *VNN2* genes. Coding variants in VNN2, a protein that may act in cell adhesion and migration of neutrophils, were significantly enriched in cases with better outcome. In contrast, rare variants in CNTN5, a protein involved in synaptogenesis, are associated with a poor outcome.

Conclusion: *CNTN5* and *VNN2* appear to be linked to stroke outcome. Further functional experiments are needed to understand how mutations in these genes lead to differences in recovery.

References: [1]Susak et al., PLoS Comput Biol. 2021 17(2):e1007784.

Grants:.
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Conflict of Interest: None declared.

P06.008.C Penetrance and disease expression of (likely) pathogenic variants associated with inherited cardiomyopathies in the general population

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Background/Objectives: (Likely) pathogenic variants associated with arrhythmogenic cardiomyopathy (ACM), dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) are recommended to be reported as secondary findings in genome sequencing studies. This provides opportunities for early diagnosis, but also fuels uncertainty in variant carriers (G+), since disease penetrance is incomplete. We assessed the prevalence and disease expression of G+ in the general population.

Methods: We calculated the prevalence of (likely) pathogenic variants associated with ACM, DCM and/or HCM extracted from two databases in the UK Biobank. Furthermore, we analysed the frequency of cardiomyopathy/heart failure diagnosis in individuals carrying these variants (G+). In undiagnosed individuals, we analysed early signs of disease expression.

Results: We found a prevalence of 1:578, 1:251 and 1:149 for (likely) pathogenic variants associated with ACM, DCM and HCM respectively. Compared to controls, cardiovascular mortality was higher in DCM G+, but similar in ACM and HCM G+. More specifically, cardiomyopathy or heart failure diagnosis were more frequent in DCM G+ and HCM G+, but comparable in ACM G+. In contrast, ACM G+ had more ventricular arrhythmias. Left ventricular ejection fraction was reduced in undiagnosed DCM G+ individuals.

Conclusion: In the general population, (likely) pathogenic variants associated with ACM, DCM or HCM are not uncommon. Although G+ have increased mortality and morbidity, disease expression in these carriers from the general population remains low (1.2-4%). Decisions on application of cascade screening and frequency of cardiological examination should be based on multiple factors, such as the gene, variant type and family history.

References:.

Grants:.

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P06.009.C Using genotyping and whole-exome sequencing data to improve genetic risk prediction in deep venous thrombosis

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Background/Objectives: Deep venous thrombosis (DVT) is the formation of blood clots in the deep veins. Blood clots traveling to the lung can cause organ damage or sudden death. More than 60% of DVT risk is influenced by genetic factors, such as the Leiden mutation in *F5* (FVL). Characterizing the genetic contribution and stratifying individuals based on their genetic makeup can favourably impact risk prediction.

Methods: We performed a genome-wide association study and constructed a polygenic risk score (PRS) in the 60% (N = 284,591) of the UK Biobank cohort. The remaining 40% (N = 198,362) was employed to evaluate the PRS, and to perform gene-based test on exome-sequencing data to investigate effects by rare variants.

Results: We identified and replicated a new variant (rs11604583) near *TRIM51* gene, and a rare variant (rs187725533), associated with 2.2-fold higher risk of DVT, in *CREB3L1* gene. The top PRS decile was associated with 3.4-fold risk of DVT, an effect that was still 2.3-fold, when excluding FVL carriers. Cumulative risk of DVT at the age of 70 years for FVL carriers in the top PRS decile is of 10%, contraposed to 5% for non-carriers.

Conclusion: We showed that common and rare variants influence DVT risk, and that the PRS improve risk prediction on top of FVL. This suggests that individuals classified with high PRS score could benefit from early genetic screening.

References: Stone et al. "Deep vein thrombosis: pathogenesis, diagnosis, and medical management." Cardiovasc Diagn Ther (2017).

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Conflict of Interest: None declared.

P06.010.D Clinical utility of genetic testing in pediatric patients with polymorphic ventricular tachycardia

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Background/Objectives: Primary electrical disorders affect the myocyte transmembrane ion channels and predispose to malignant arrhythmias, including polymorphic ventricular tachycardia (PVT), which is rare in children. Genetically determined channelopathies, such as catecholaminergic PVT (CPVT), long/short QT (LQTS/SQTS) and Brugada syndromes, may be the cause of these arrhythmias. They present with incomplete penetrance and variable expressivity.

Methods: Next generation sequencing (NGS) analysis of genes associated with inherited arrhythmia conditions was performed in 33 Polish patients with documented PVT.

Results: Sixteen patients had a clinically significant variant in: RYR2, KCNH2, KCNJ2, CALM1, SCN5A or 1p13.2 duplication encompassing KCND3. In one CPVT patient two novel biallelic RYR2 variants co-occurred, and in another with LQTS concomitance of RYR2 and KCNH2 defects implied digenic etiology. Additionally, in two probands a rare variant of unknown significance (VUS), favouring pathogenic, in MYBPC3 was noted. Five known VUS in KCNH2, SCN5A, SCN10A, TRPM4 were denoted possibly benign due to high frequency in an in-house Polish database.

Conclusion: Genetic testing has an essential role in PVT diagnosis, influencing risk stratification, preventive and therapeutic management and genetic counseling. NGS-based testing in PVT patients provides good diagnostic yield (~55%), however variant classification criteria should consider the unique characteristics of primary arrhythmias and population aspects. In PVT patients, concomitance of two or more pathogenic variants (biallelic/digenic) may occur, resulting in exacerbation of the phenotype, and phenotypic overlap may be observed in RYR2 or SCN5A-positive cases. Rarely, PVT may be associated with underlying cardiomyopathy.

References:.

Grants: Partially supported: CMHI-M31/18.

Conflict of Interest: None declared.

P06.012.B Family screening of relatives with vascular Ehlers-Danlos syndrome, an asset in preventing arterial events

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Background/Objectives: Vascular Ehlers-Danlos syndrome (vEDS) is a rare inherited disorder leading to mainly arterial complications, due to pathogenic *COL3A1* variations. Ong et al (Lancet 2010) showed that the introduction of celiprolol significantly reduced arterial events. Screening of relatives allows specific and multidisciplinary management, but the benefit of celiprolol in those without arterial events remains uncertain. We wanted to evaluate the occurrence of arterial events during vEDS relatives follow-up.

Methods: All vEDS relatives diagnosed in our department since 2004 were included. We retrospectively analyzed several criteria (duration of follow-up, presence of arterial events, introduction and dose of celiprolol…).

Results: $n = 72$ relatives had at least one outpatient visit. Arterial events were found in $n = 36$ (50%), symptomatic ($n = 20$) or silent ($n = 16$). Median age was 44.5yrs vs. 23.0yrs for $n = 36$ without any arterial event ($p < 10^{-3}$). During follow-up, $n = 7$ (19%) had a first arterial event at 29yrs and were all treated with celiprolol. Duration of treatment was 3yrs at the onset of the first silent event and 10yrs for the first symptomatic event. Celiprolol was introduced in $n = 27$ (75%) after genetic diagnosis disclosure. Treated relatives had a follow-up duration about 4yrs vs. 2yrs for the untreated ones ($p = 0.01$).

Conclusion: These results confirm the importance of family screening of vEDS relatives. Despite the introduction of celiprolol, some relatives have presented an arterial event during follow-up. However, our data are probably too low to assess the occurrence of events. A study on probands without arterial events at first outpatient visit could confirm the benefit of celiprolol in all vEDS patients.

References:.

Grants:.

Conflict of Interest: None declared.

P06.014.D Cardiovascular and Connective Tissue Disorder features in FLNA-related PVNH patients: progress towards a refined delineation of this syndrome

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Background/Objectives: *FLNA* Loss-of-Function (LoF) causes periventricular nodular heterotopia type 1 (PVNH1), an acknowledged cause of seizures of various types. Neurological symptoms are inconstant, and cardiovascular (CV) defects or connective tissue disorders (CTD) have regularly been associated. We aimed at refining the description of CV and CTD features in patients with *FLNA* LoF and depicting the multisystemic nature of this condition.

Methods: We retrospectively evaluated *FLNA* variants and clinical presentations in *FLNA* LoF patient with at least one CV or CTD feature, from three cohorts: ten patients from the French Reference Center for Rare Vascular Diseases, 23 patients from the national reference diagnostic lab for filaminopathies-A, and 59 patients from literature review.

Results: Half of patients did not present neurological symptoms. Most patients presented a syndromic association combining CV and CTD features. CV anomalies, mostly aortic aneurysm and/or dilation were present in 75% of patients. CTD features were present in 75%. Variants analysis demonstrated an enrichment of coding variants in the CH1 domain of FLNA protein.

Conclusion: In *FLNA* LoF patients, the absence of seizures should not be overlooked. When considering a diagnosis of PVNH1, the assessment for CV and CTD anomalies is of major interest as they represent interlinked features. We recommend systematic study of *FLNA* within CTD genes panels, regardless of the presence of neurological symptoms.

References:.
Grants:.

Conflict of Interest: None declared.

P06.015.A Genome-wide epistasis for cardiovascular severity in Marfan study design: patient organization driven research

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Background/Objectives: Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder with manifestations in the ocular, skeletal and cardiovascular system. Morbidity and mortality are mostly determined by aortic disease. Although mutations in *FBN1* are the well-established genetic cause of MFS, there is a poor correlation with regards to phenotypical outcome, especially cardiovascular. Wide intra- and interfamilial phenotypical variability is observed, but the underlying mechanisms remain largely elusive. Consequently, the identification of genetic variation that modifies these effects will add important novel insights.

Methods: A worldwide collaborative project driven by researchers and a Belgian patient organization, 'Foundation 101 Genomes' (F101G), was established to maximize the number of patients to study the genetic basis of phenotypical variability. RNA-sequencing will be integrated with WGS to reveal MFS aortopathy genetic modifiers, which will be validated using CRISPR/Cas9 in IPSC-VSMC models.

Results: Our research institutions already gathered DNA and PBMCs of 35 patients carrying the most common *FBN1* missense variant (p.Ile2585Thr;c.7754T>C). International collaborations yield at least 200 patients carrying this specific variant. Together with F101G, we created a website to guide patients to participate in our research.

Conclusion: Despite the large number of patients already included, more patients are needed to identify genetic modifiers for MFS aortopathy. Understanding how mother nature by itself modifies the outcome of the primary FBN1 mutation will individualize current treatment protocols to deliver true precision medicine and offer promising new leads to novel therapeutic strategies.

References:.
Grants:.

Conflict of Interest: Lotte Van Den Heuvel Fonds voor wetenschappelijk onderzoek, Josephina (Jeannette) Meester: None declared, Silke Peeters: None declared, romain alderweireldt: None declared, Aline Verstraeten: None declared, Paul Coucke: None declared, Bart Loeys Fonds voor wetenschappelijk onderzoek.

P06.016.B Casq2 deletion: a zebrafish model of CPVT

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Background/Objectives: Autosomal recessive mutations in CASQ2 are the second most common genetic defect identified in catecholaminergic polymorphic ventricular tachycardia (CPVT), one of the most lethal inherited cardiac arrhythmias. Recent findings suggest an autosomal dominant inheritance is also possible for CASQ2.(1) We developed a zebrafish knockout model, which will be used to further explore this hypothesis.

Methods: The casq2 knockout was generated on the background of a transgenic zebrafish line expressing cardiac dual voltage and calcium reporters (Ace2N-mNeon and R-GECO) with CRISPR/Cas9. Loss of expression was confirmed by RT-qPCR. The voltage and calcium signals were measured with a Leica SP8 light sheet microscope at 3 days post-fertilization, after overnight exposure to the adenylate cyclase activator forskolin.

Results: The casq2 knockout line contains a 5 base pair deletion in exon 2. Casq2-/- embryos showed a decreased heart rate at baseline compared to wildtype. Delayed afterdepolarizations (DADs) induced by forskolin were observed significantly more often in casq2-/- embryos (table 1).

Table 1:

	DADs	No DADs	p-value
Casq2-/- 5μM Forskolin	8 (57%)	6 (43%)	0.018 (Fisher's exact test)
Casq2+/+ 5μM Forskolin	2 (12%)	15 (88%)	

Conclusion: The casq2 knockout is the first zebrafish model of the classical CPVT genes. Similar to the human phenotype, we observe bradycardia at rest and a sensitivity to DADs upon stimulation. This model will provide a promising opportunity for further testing of CASQ2 inheritance patterns.

References: (1) Ng K, et al. Circulation, 2020. 142(10):932. PMID:32693635.

Grants: Fund for Scientific Research, Flanders (FWO) and the Antwerp University Research Fund (BOF).

Conflict of Interest: None declared.

P06.017.C A trans-ancestry Mendelian Randomisation study to estimate the causal effects of cardiometabolic factors on coronary artery disease in British Pakistanis and Bangladeshis

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Background/Objectives: British people with South-Asian ancestry have a higher risk of coronary artery disease (CAD) than other ancestry groups. Statistical power can be a limiting factor when extending Mendelian Randomisation (MR) analyses to non-European populations, because ancestry-matched GWAS for risk factors (RFs) of interest might not be sufficiently large.

Methods: We compared different strategies for trans-ancestry MR to assess the effect of cardiometabolic RFs (BMI, triglycerides, HDL-cholesterol, LDL-cholesterol, systolic and diastolic blood pressure) on the risk of CAD in 22,000 British Pakistani and Bangladeshi (BPB) individuals from the Genes&Health cohort. We used an ancestry-matched sample to derive instruments in a two-sample MR of CAD in Genes&Health, with summary statistics for RFs from the BPB group in the UK Biobank. However, insufficient number of genome-wide significant instruments were identified in the UK Biobank BPB population. Therefore, we used a less stringent p-value threshold ($p < 5x10\text{-}5$) for selecting instruments, incorporating results from large European GWASs, and using a subset of loci with evidence of transferability.

Results: We found that most of the associations were not significant in the ancestry-matched MR. We found a risk increasing effect for LDL-cholesterol and risk decreasing effect for HDL-cholesterol when using the variants from the large European GWAS as instruments, and also for the subset of loci that were transferable. The associations for BMI with CAD were significant only for transferable loci.

Conclusion: Incorporating findings from European GWAS can increase power for MR in other ancestry groups. We demonstrated the importance of considering transferability of RF loci to ensure casual inference.

References:.

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Conflict of Interest: None declared.

P06.018.D Whole exome/genome sequencing joint analysis in a family with oligogenic familial hypercholesterolemia

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Background/Objectives: Autosomal Dominant Hypercholesterolemia (ADH) is a genetic disorder caused by pathogenic variants in *LDLR*, *APOB*, *PCSK9* and *APOE* genes. We sought to identify a new candidate gene responsible of the ADH phenotype in patients with no pathogenic variants in known ADH causing genes.

Methods: We performed linkage analysis, whole exome and whole genome sequencing in one French family, with affected and non-affected members presenting a high ADH polygenic risk score (wPRS). We also performed functional studies in HEK293T cells of the four *LRP6* mutants.

Results: Linkage analysis, whole exome and whole genome sequencing in one French family, with affected and non-affected members presenting a high ADH polygenic risk score (wPRS) allowed us to identify p.(Pro398Ala) in *CYP7A1*, p.(Val1382Phe) in *LRP6* and p.(Ser202His) in *LDLRAP1*. Six other variants were identified in 6 from 160 unrelated ADH probands: p.(Ala13Val) and p.(Aps347Asn) in *CYP7A1*, p.(Tyr972Cys), p.(Thr1479Ile) and p.(Ser1612Phe) in *LRP6* and p.(Ser202LeufsTer19) in *LDLRAP1*. All these six probands presented a moderate wPRS. Serum analysis of carriers of p.(Pro398Ala) variant in *CYP7A1* showed no differences in bile acids synthesis when compared to non-carriers serums.

Functional studies in HEK293T cells of the four *LRP6* mutants showed contradictory results. None of the family members heterozygous carriers of the *LDLRAP1* p.(Ser202His) variant alone presented ADH.

Conclusion: Altogether, each variant alone does not seem to contribute sufficiently to the elevation of LDL-C and it is the oligogenic combination of two or three variants that is necessary to reveal the ADH phenotype.

References:.

Grants:.

Conflict of Interest: None declared.

P06.019.A Contribution of SCN5A copy number variations to the genetic diagnosis of Brugada syndrome

Laura Cazón[1], *Luis De la Higuera Romero*[1], *Marlene Perez Barbeito*[1], *Rosalía Peteiro*[1], *Iria Gómez Diaz*[1], *Paula Rebolo*[1], *Paula Velez*[1], *Maria Sanchez*[1], *Anahi Sanluis Verdes*[1], *Guillermo Smith Ramos*[1], *Emilia Maneiro*[1], *Xusto Fernandez*[1], *Almudena Amor*[1], *María Valverde*[1], *Soledad García Hernández*[1;2], *Ivonne Cárdenas Reyes*[1], *Martin Ortiz Genga*[1], *Juan Pablo Ochoa*[3]

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Background/Objectives: Loss-of-function variants in SCN5A are identified in 15-25% of Brugada syndrome (BrS) cases. Most of them are single-nucleotide variants, small insertions / deletions, and splicing errors, whereas information on copy number variations (CNVs) is still limited. The objective of this study is to determine the contribution of CNVs to the genetic diagnosis of BrS.

Methods: A total of 977 consecutive unrelated probands with suspected BrS were genotyped by next-generation-sequencing (NGS) using a panel of genes that included SCN5A. Filtering and classification of NGS data was performed using a custom pipeline; CNVs were detected using a read depth approach and confirmed by orthogonal molecular techniques.

Results: Actionable variants in SCN5A were identified in 128 probands (13%): 83 were pathogenic/ likely pathogenic and 47 variants of unknown significance but favoring pathogenic. CNV analysis could be performed in 888 (90.9%) of the probands and revealed six carriers of CNVs in SCN5A (table 1).

Gender	Age	SCN5A CNV	Confirmation technique
Male	13	630kb deletion (8 whole genes, including SCN5A)	SNP-array
Female	43	143kb deletion (SCN5A exons 1-16 and SCN10A exons 15-27)	SNP-array
Male	Unknown	Deletion exon 4	Sanger sequencing
Male	35	Deletion exons 8-10	MLPA
Male	40	Deletion exons 13-27	Sanger sequencing
Female	61	Deletion exon 22	MLPA

Conclusion: SCN5A CNVs were detected in 0.6% of the BrS probands, but they represented 7.2% of pathogenic / likely pathogenic variations identified in our cohort. This result emphasizes the importance of a complete genetic study including CNVs in the diagnosis of BrS.

References:.

Grants:.

Conflict of Interest: Laura Cazón Full time, Luis De la Higuera Romero Full time, Marlene Perez Barbeito Full time, Rosalía Peteiro Full time, Iria Gómez Diaz Full time, Paula Rebolo Full time, Paula Velez Full time, Maria Sanchez Full time, Anahi Sanluis Verdes Full time, Guillermo Smith Ramos Full time, Emilia Maneiro Full time, Xusto Fernandez Full time, Almudena Amor Full time, María Valverde Full time, Soledad García Hernández Full time, Ivonne Cárdenas Reyes Full time, Martin Ortiz Genga Full time, Juan Pablo Ochoa Full time.

P06.020.B Spontaneous coronary artery dissection: role of the genetic background in pathogenesis and management

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Background/Objectives: Spontaneous coronary artery dissection (SCAD) is a rare, non-atherosclerotic disease of the coronary vascular tunics, frequently starting as an acute coronary syndrome. Recent genetic studies on SCAD have identified a possible correlation with inherited connective tissue disorders and a possible predisposition linked to CGG repeat expansion in FMR1 gene. This study explored rare genetic factors that may contribute to the pathogenetic mechanisms underlying SCAD.

Methods: After the SCAD event between January 2010 and February 2021, fourteen patients referred to the Cardiology Department of IRCCS Policlinico San Martino underwent clinical genetic evaluation, whole-exome sequencing analyses (trio or singleton), and FRAXA analyses. This study was approved by local Ethical Committee.

Results: Although none of the 14 patients enrolled showed clinical features associated with connective tissue disorders, in the 21% of our cohort, we identified variants in genes involved in the formation and in the integrity of connective tissue. We also detected an intermediate allele in the *FMR1* gene in one patient and a de novo variant in a new candidate gene in another one.

Conclusion: Despite the small number of patients analysed, we identified several genetic risk factors for SCAD and a promising novel candidate gene. Although further studies are needed, this work could contribute to give insight into the pathogenetic pathways of this rare condition.

References: Tassanakijpanick N. et al, Cardiovascular problems in the Fragile X premutation; Front Genet. 2020.

Amrani-Midoun A. et al, Recent advances on the genetics of spontaneous coronary artery dissection; CircGenomPrecisMed 2021.

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Conflict of Interest: None declared.

P06.021.C Polygenic risk scores predict overweight and obesity in the Dutch population

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Background/Objectives: Obesity, the fifth leading risk of global deaths, has grown to epidemic proportions. Its etiology is largely unknown but it has a substantial hereditary component. Global GWAS have identified 941 genetic variants influencing body-mass index explaining 6% of heritability. This study combined these variants into a polygenic risk score (PRS) to assess obesity-risk in a Dutch Caucasian population cohort.

Methods: The PRS was tested in 11,209 participants of the Rotterdam Study (mean±SD age = 65.7±10.2 years) to predict BMI as a continuous and categorical outcome (under-weight, normal-weight, overweight, obese, and morbid-obese). We evaluated the risk conveyed by PRS as a linear instrument (per 1 SD) and categorical (highest 10% vs. middle 50% of the PRS distribution).

Results: The PRS was associated with BMI (beta-estimate = 0.9 [95% confidence interval 0.7-1.1];$p < 1 \times 10^{-16}$). One SD increase of the PRS significantly increased the risk of being underweight, over-weight, obese and morbid-obese by 0.8, 1.3, 1.8 and 2.2 fold times, respectively. Similarly, the top 10% of the population with the highest BMI-PRS showed increased risks of 1.1, 1.5, 2.6 and 4.5 for these BMI categories, respectively. The risk increased exponentially in the PRS distribution tails, up to 8.2 of the top 1% PRS for morbid-obese vs. normal-weight.

Conclusion: These results confirm that the PRS significantly impacts BMI in a Dutch Caucasian elderly population. Identifying the biological pathways affected by an individuals' genetic background could aid in targeted and personalized intervention strategies long before the onset of obesity. BMI-PRS utility is being investigated as part of the Genotyping on all patients (GOALL) project.

References:.

Grants:.

Conflict of Interest: None declared.

P06.022.D Novel loss of function KCNA5 pathogenic variants in pulmonary arterial hypertension

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Background/Objectives: Reduced expression and/or activity of Kv1.5 channels (encoded by KCNA5) is a common hallmark in human or experimental pulmonary arterial hypertension (PAH). Genetic variants in KCNA5 have been found in PAH patients. However, their functional consequences and potential impact on the disease is largely unknown. Herein, we aimed to characterize the functional consequences of 7 KCNA5 variants found in a cohort of PAH patients.

Methods: Potassium currents were recorded by patch-clamp technique in HEK293 cells transfected with WT or mutant Kv1.5 cDNA. Flow cytometry, western blot and confocal microscopy techniques were used for measuring protein expression and cell apoptosis in HEK293 and human pulmonary artery smooth muscle cells (hPASMC).

Results: KCNA5 variants (namely, p.Arg184Pro and p.Gly384Arg) resulted in a loss of potassium channel function as assessed by electrophysiological and molecular modelling analysis. The p.Arg184Pro variant also resulted in a pronounced reduction of Kv1.5 expression. Transfection with p.Arg184Pro or p.Gly384Arg variants decreased apoptosis of hPASMCs compared with the WT, demonstrating that KCNA5 dysfunction in both variants affects cell viability. Thus, in addition to affect channel activity, both variants were associated with a clear impairment in a key PASMC process linked to the disease. The estimated prevalence of dysfunctional KCNA5 variants in the PAH population analysed was around 1 %.

Conclusion: Our data indicates that some KCNA5 mutations present in PAH patients have critical consequences for channel function. This encourages the idea that KCNA5 pathogenic variants may be a developing or contributing factor for PAH.

References:.

Grants: PI18/01233, FCHP unrestricted grant.

Conflict of Interest: None declared.

P06.023.A Genetic testing outcomes in a cohort of 21,159 children with heart disease

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Background/Objectives: Pediatric heart conditions affect 1/77 US children. Guidelines recommend cardiogenetic testing in children and at-risk relatives. We investigated outcomes in a large pediatric cohort undergoing genetic testing for a wide range of cardiogenetic conditions.

Methods: Probands <18 years had next generation sequencing and deletion/duplication analysis for cardiogenetic conditions (up to 224 genes). A positive result was defined as a single pathogenic/likely pathogenic (P/LP) variant in a gene associated with an autosomal dominant or X-linked disorder, or two P/LP variants in a gene associated with a recessive disorder. Outcomes were compared to cardiogenetic testing results of 61,368 adults by t-tests with multiple comparison correction.

Results: 21,159 probands were tested (median = 10.03 years, SD = 5.75) and positive result were reported in 16.6%. Cascade testing was pursued in 3.9% of families (mean 3.48 relatives/

family; 59.1% aged <10 years). Positive results were more frequent in children than in adults (16.6% vs 15.3%), and were particularly enriched in genes associated with syndromic disorders relative to non-syndromic ones (41.4% vs 29.7%). Families with probands aged <10 had cascade testing more often in comparison to those with older probands (4.6%; p = 0.0069).

Conclusion: One in six children referred for cardiogenetic testing received a positive result, highlighting opportunities for management. Cascade testing was pursued more often than in families with probands <10 years in comparison to those with adult probands. These findings provide rationale for family-based care to identify patients who can benefit from genetics-guided interventions.

References:.

Grants:.

Conflict of Interest: Robert Nussbaum: None declared, Flavia Facio Invitae, Ana Morales Invitae, Asia Mitchell Invitae, John Garcia Invitae, Dianalee McKnight Invitae, Tom Callis Invitae, Chad Moretz Invitae, Matteo Vatta Invitae, Swaroop Aradhya Invitae.

P06.024.B Identification of novel loss-of-function genes associated with carotid intima-media thickness

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Background/Objectives: Ultrasound measurement of the carotid intima-media thickness (cIMT) of the carotid artery is frequently employed as a biomarker for subclinical atherosclerosis and the extent of vascular remodelling. Large-scale genetic studies identified common genetic risk factors for cIMT. Here, we aimed to identify loss-of-function (LoF) genes that are inactive in one or two copies (i.e. full knockouts) associated with cIMT.

Methods: We used LoFTK (https://github.com/CirculatoryHealth/LoFTK) to annotate LoF genes from UK Biobank exomes and UCC-SMART imputed genotypes. We performed LoF-wide association studies (LoFWAS) in both cohorts and conducted a meta-analysis to identify genes associated with cIMT. We explored tissue- and cell-specific expression in carotid atherosclerotic plaques.

Results: We identified four genes associated with cIMT in our meta-analysis. Three of these genes (*PPP1R21*, *HSD3B2* and *ULK2*) were inactive in one copy, while one gene (*KIR3DL1*) was a full knockout. Single-cell RNA sequencing revealed cellular subtype-specific expression patterns of *PPP1R21*, *ULK2*, and *KIR3DL1* in carotid plaques.

Conclusion: We identified 4 novel genes associated with cIMT. Our study provides insights into LoF genes and cIMT that underpin the genetic and biological mechanisms of atherosclerosis.

References:.

Grants:.

Conflict of Interest: None declared.

P06.025.C A novel rare pathogenic variant in TLN1 in a family with systemic capillary leak syndrome

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Background/Objectives: Systemic capillary leak syndrome (SCLS) is a rare life threatening disorder that presents with episodes of severe hypotension, hypoalbuminemia, and hemoconcentration due to profound vascular leak. The condition was first described by Clarkson in 1960, yet its etiology remains unknown. Current hypotheses include abnormal endothelial cell response to normal stimulation, or normal endothelial cell response to dysregulated or excessive systemic signaling or inflammation. We describe three individuals with SCLS from an extended pedigree suggestive of AD inheritance with incomplete penetrance.

Methods: We conducted exome sequencing analysis on peripheral blood of two family members, followed by Sanger sequencing for segregation in additional family members. cDNA analysis from blood and fibroblast samples was used to determine the consequence of a variant of interest.

Results: A rare novel heterozygous variant in the *TLN1* gene (c.7188+2T>C) was identified in all three family members with SCLS. This variant causes in-frame skipping of exon54 and is predicted to affect the c-terminal actin binding domain in the Talin-1 protein (domain ABS3).

Conclusion: Talin-1 (*TLN1*) has a key role in cell adhesions via the linkage of integrins to the actin cytoskeleton and by activation of integrins. Studies have shown that Talin-1 regulates VE-Cadherin localization, which plays an important role in endothelial cell barrier function. Based on our findings we suggest that pathogenic variants in *TLN1* underlie SCLS. Future studies are warranted to further investigate the mechanism of the disease and to explore possible therapeutic options.

References:.

Grants:.

Conflict of Interest: None declared.

P06.026.D Phenotypic and genetic factors modifying the risk of developing cardiomyopathy symptoms for PLN-p.Arg14del carriers

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Background/Objectives: The phospholamban (PLN) p.Arg14del Dutch founder variant has been associated with dilated and arrhythmogenic cardiomyopathy.[1] Some carriers show life-threatening symptoms, while others remain asymptomatic or show only mild symptoms at old age. To understand the mechanisms behind this incomplete penetrance, we aimed to identify protective factors in asymptomatic PLN-Arg14del carriers in a large population cohort.

Methods: We identified 74 (0.2%) carriers in 36,339 genotyped individuals of the Dutch Lifelines cohort[2], of which 48 were asymptomatic according to their ECG and questionnaires. We interrogated 38 quantitative measurements and 97 polygenic scores (PGS) of cardio-metabolic traits, performed a GWAS, and a rare variant burden analysis in 82 risk loci. We used the non-standard approach of comparing asymptomatic carriers to asymptomatic non-carriers, and confirmed the results by comparing each group to symptomatic carriers.

Results: Compared to the asymptomatic non-carriers, the asymptomatic carriers showed lower QRS ($p = 0.002$), an effect that replicated in another Lifelines subset ($N = 20,221$) and in the ACM patient registry ($N = 592$). Furthermore, symptomatic carriers showed a higher correlation between PGS_{PR} and PR ($p = 0.022$) and between PGS_{QRS} and QRS ($p = 1.9x10^{-5}$). These results indicate symptomatic PLN-Arg14del carriers have an increased sensitivity for common genetic variation affecting cardiac rhythm.

Conclusion: We have used a population cohort to identify protective factors for PLN-Arg14del carriers. These results can improve risk prediction models for cardiac outcomes and guide future studies on genetic diseases with incomplete penetrance.

References: [1]van der Zwaag et al, Eur J Heart Fail 2012.
[2]Scholtens et al, Int J Epidemiol 2015.

Grants: UMCG-HAP-2017-2 324.

Conflict of Interest: None declared.

P06.027.A NGS analysis in a patient with conduction disorders and dilated cardiomyopathy: a case report of R222Q SCN5A variant, benefiting from mutation specific target therapy

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Background/Objectives: Mutations in *SCN5A*, which encodes the alpha-subunit of cardiac sodium channel Nav1.5., are reported non only in patients with Brugada Syndrome but also in those with dilated cardiomyopathy (DCM). In these patients, cardiac dilation is preceded by conduction system abnormalities. Genetic diagnosis in these cases can determine therapeutic choices with consequences on patient's quality of life and survival.

Methods: We performed Next Generation Sequencing (NGS) analysis as part of the routine work-out of a 53-year-old woman with a history of ventricular extrasystoles (burden > 50%) since the age of 20, syncopal sinus pauses with required PM implantation, atrial fibrillation and hypokinetic cardiomyopathy with dilation of left ventricle and atrium and mild left ventricular ejection fraction reduction. Due to worsening of NYHA class, several ablation procedures targeting the frequent ventricular arrhythmia were performed, with later recurrence. Genetic analysis focused on genes associated with DCM and arrhythmogenic cardiomyopathy.

Results: We identified a heterozygous pathogenic variant (p.Arg222Gln) in *SCN5A*, previously reported to be responsive to sodium channel blockers. Hydroquinidine treatment led to normalization of ventricular cell potentials. Patient showed almost complete regression of arrhythmic manifestations and NYHA class improvement.

Conclusion: Identification of R222Q in *SCN5A* was fundamental for the patient's prognosis. This paradigmatic case underlines the importance of NGS analysis for optimal therapeutic management of patients with conduction disorders and DCM. For these patients, genetic studies should be considered integral components of the diagnostic phase.

References: Mann SA et al, JACC 2012.

Grants: Ministero dell'Istruzione, Progetto Strategico di Eccellenza Dipartimentale #D15D18000410001.

Conflict of Interest: None declared.

P06.028.B Yield of 3 years of diagnostic testing of TNNI3K in Arrhythmia and Cardiomyopathy Patients

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Background/Objectives: Genetic variants in TNNI3K have been associated with supraventricular arrhythmias (SVTs), conduction disease (CCD), and dilated cardiomyopathy (DCM) in a limited number of papers thus far. The yield in diagnostic screening of this gene in cardiac diseases has not yet been evaluated, we here aim to fill this gap. As pathogenic variants in TNNI3K have been shown to affect the level of autophosphorylation, we evaluated this for identified variants.

Methods: We collected and analysed clinical data of individuals with variants in TNNI3K. These variants were identified by (i) systematic clinical genetic screening of gene panels in patients with cardiomyopathies and/or arrhythmias between 08-2018 and 12-2021 at the Amsterdam UMC, and (ii) collaboration with multiple centres in the Netherlands. Functional in vitro studies were performed to assess the effects of the identified variants on TNNI3K auto-phosphorylation.

Results: We identified 19 rare coding variants in TNNI3K in 24 probands. Among these two novel likely pathogenic variants (p.H592T and p.I512T) were found in eight families. Individuals harbouring these variants demonstrated SVTs, CCD, DCM, and/or out-of-hospital cardiac arrest. Functional studies revealed significantly increased auto-phosphorylation levels in both TNNI3K variants.

Conclusion: Screening of rare variants in TNNI3K adds to the diagnostic yield for cardiac diseases. However, considering the current lack of validation of the functional tests, co-segregation analysis is paramount for the classification of identified variants.

References: The Diverse Roles of TNNI3K in Cardiac Disease and Potential for Treatment. Doi: Caroline Pham, Noelia Muñoz-Martín, Elisabeth Lodder.

Grants: Netherlands Organisation for Scientific Research (VIDI-91718361).

Conflict of Interest: None declared.

P06.029.C Common rearrangements of the LDLR gene in the Czech population likely arise from one mutational event

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Background/Objectives: Mutations in the low-density lipoprotein receptor gene (LDLR) are the most common cause of familial hypercholesterolemia in the Czech Republic. Out of all Czech probands with an LDLR mutation, nearly 10% are carriers of a deletion or duplication spanning whole exons. We characterized the breakpoints of 8 large rearrangements of the LDLR gene. One of the goals was to analyze the breakpoint of all probands carrying a specific rearrangement to verify the hypothesis that all probands of each rearrangement have identical breakpoints inherited from a common ancestor.

Methods: The breakpoint sequence was determined by PCR amplification and Sanger sequencing.

Results: We sequenced the breakpoint of 8 rearrangements of the LDLR gene, including the four most common rearrangements in Czech population (number of probands ranging from 8 to 28), and four less common rearrangements (1-4 probands). For all analyzed rearrangements, all Czech probands with a specific rearrangement shared identical breakpoint position and sequence, suggesting shared origin from a common ancestor. All breakpoints except for one were located inside an Alu element. In 5 out of 8 breakpoints, there was high homology (>75%) between the two Alu repeats in which the break occurred.

Conclusion: Most common rearrangements of the LDLR gene in the Czech population likely arise from one mutational event. Alu elements likely played a role in generation of the majority of rearrangements within the LDLR gene, but not all.

References:.

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Conflict of Interest: None declared.

P06.030.D Characterising the phenotype and outcomes of cascade-tested individuals carrying a pathogenic sarcomere-variant

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Background/Objectives: Cascade testing of relatives of individuals with pathogenic genetic variants causing hypertrophic cardiomyopathy is recommended. Little is known of the clinical outcomes in cascade-identified individuals. We quantified clinical endpoints in such individuals.

Methods: All individuals reviewed by Genetics in NHS Tayside for a personal or family history of hypertrophic cardiomyopathy between January 2010 and December 2018 were included. Case-note review included genetic testing, echocardiography and clinical outcomes.

Results: 282 individuals from 64 families were included with a total follow-up of 2493 patient years (mean: 8.8±6.3). Of 200 patients under review; 121 were probands (mean age: 65.6 ± 13.9) and 79 cascade-identified (mean age: 48.3 ± 17.0). Causative genes were: MYBPC3 (n = 46);MYH7 (n = 12);TNNT2 (n = 2);TNNI3(n = 1); PKP2 (n = 1);CSRP3 (n = 1);GLA (n = 1).

Baseline asymmetrical myocardial hypertrophy was recorded in 45.6% of cascade-identified individuals. The mean age-adjusted interventricular septal size for cascade-identified individuals (13.8mm;95% CI:12.6-15.0) was lower than that of probands (19.8mm,95%CI:18.8-20.7; $p < 0.001$) but higher than the control group (10.5mm,95% CI:9.1-11.9; $p < 0.001$). Age-adjusted multivariant event analysis demonstrated decreased risk of adverse cardiac events in cascade-identified patients compared to probands (hazard ratio 0.27;95% CI:0.15-0.49; $p < 0.001$), and increased compared to controls (hazard ratio:0.26;95% CI:0.09-0.71; $p = 0.009$). The lifetime incidence of atrial fibrillation, ventricular tachycardia, heart failure and acute coronary syndromes are higher in proband patients versus cascade-tested carrier patients ($p < 0.05$).

Conclusion: Although cascade-tested individuals carrying pathogenic sarcomere gene variants exhibit a milder phenotype and decreased risk of major adverse cardiac events compared to probands, these individuals still have a high rate of complication justifying their identification and follow-up.

References:.

Grants:.

Conflict of Interest: None declared.

P06.031.A Prenatal Long QT syndrome associated with homozygous KCNH2 pathogenic variants

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Background/Objectives: Long QT syndrome type 2 is an inherited autosomal dominant disorder usually caused by heterozygous pathogenic variants in the *KCNH2* gene. However, homozygous *KCNH2* variants have been proposed to cause a more severe cardiac phenotype associated with QT prolongation and functional atrioventricular conduction disturbances before and immediately after birth. Only three families with homozygous *KCNH2* variants have been reported so far.

Methods:.

Results: We report an asymptomatic Portuguese consanguineous couple, whose first child, presented in the prenatal period chaotic foetal rhythm (periods of tachycardia and complete atrioventricular block) and hydrops. She was born at 33 weeks of gestation requiring a pacemaker implantation at day one of life. Postnatal echocardiogram showed left ventricular non-compaction cardiomyopathy. She died at 18 months of age from sudden death. Dilated cardiomyopathy NGS panel study did not identify any pathogenic variant. The second child was a healthy female, now 3 years old. During their third gestation, foetal hydrops was detected associated with global left ventricular dysfunction and cardiomegaly. The pregnancy was terminated at 24 weeks of gestation. Necropsy confirmed foetal hydrops and dilated cardiomyopathy. Considering a potential disease recurrence, a comprehensive cardiomyopathy NGS panel was performed in the index case and a homozygous frameshift pathogenic variant [c.785del, p.(Gly262Alafs*98)] was identified in the *KCNH2*. The same variant was found in the foetus (homozygous) and in the mother (heterozygous). Genetic screening of the father and healthy sister is ongoing.

Conclusion: This report illustrates the severe cardiac phenotype, of prenatal onset, associated with a homozygous *KCNH2* pathogenic variant.

References:.
Grants:.
Conflict of Interest: None declared.

P06.033.C Mutations in the GTPBP3 are associated with hypertrophic cardiomyopathy with rapid progression to burn out phase complicated by severe systolic dysfunction and ventricular tachycardia

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Background/Objectives: About 100 genes have been associated with cardiomyopathies with genotype-phenotype correlations often hard to establish. Genetic testing may help to confirm the genetic diagnosis and assess the risk of inheritance in the family.

Methods: A 26-year old Caucasian male with hypertrophic cardiomyopathy (HCM) and suspected Wolff-Parkinson-White syndrome was referred for genetic testing by his cardiologist. Whole-exome sequencing (WES) was performed, followed by Sanger sequencing segregation analysis in the family.

Results: The targeted PRKAG2 gene screening turned out to be negative. WES revealed the following variants: c.247G>C (p.Ala83Pro) and c.1265C>T (p.Thr422Met) in the GTPBP3 gene in heterozygous state, as well as c.752C>T (p.Thr251Ile) and c.1760C>T (p.Pro587Leu) in the POLG gene. Family segregation analysis showed that the patient's mother is a carrier of the c.247G> C variant and the patient's paternal grandmother is a carrier of the variant c.1265C> T in the GTPBP3 gene. The findings of the family segregation analysis are in accordance with an autosomal recessive model of inheritance of the disease. Both variants in the POLG gene are found paternally inherited in the patient's healthy half-brother, thus are not considered disease-causing.

Conclusion: WES led to the detection of two heterozygous variants in the GTPBP3 gene. Variants in this gene have been reported in patients with HCM, associated with combined oxidative phosphorylation deficiency 23. These heterozygous variants represent the probable cause of the observed clinical symptoms in the patient.

References:.

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Conflict of Interest: None declared.

P06.034.D Novel ALPK3 variants cause infantile and late onset hypertrophic cardiomyopathy in a single family

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Background/Objectives: Cardiomyopathies are clinically heterogeneous disorders that impair heart function and are the leading cause of cardiovascular morbidity and mortality in both children and adults. Of these, hypertrophic cardiomyopathy (HCM) characterized by increased left ventricular wall thickness and dilated cardiomyopathy (DCM) displaying ventricular dilatation, are most common. Alpha-protein kinase 3 (ALPK3) plays an essential role in sarcomere organization. Its mutations have been recently reported to be causative of the recessively inherited severe pediatric-onset DCM and HCM, accompanied by skeletal involvement and facial dysmorphism, as well as of the dominantly inherited adult-onset cardiomyopathy. We have ascertained kindred with multiple family members affected by infantile or late onset cardiomyopathy and aimed to elucidate the genetic basis of cardiomyopathy in the affected individuals.

Methods: The family members underwent clinical assessment and genotyping using whole-exome sequencing and Sanger sequencing.

Results: Compound heterozygosity for two novel variants in ALPK3 has been identified in two identical twin sisters, with severe infantile onset HCM and facial dysmorphism. Both their father and grandfather died by sudden death at young age, without specific diagnosis. Their uncle was diagnosed with adult-onset cardiomyopathy at the age of 56, and has been found to be heterozygous for the truncating ALPK3 variant, identified in both of his nephews.

Conclusion: We report two novel variants in ALPK3 in a kindred with individuals affected by both infantile onset and adult onset cardiomyopathy, and provide additional evidence for the reported phenotypic spectrum of ALPK3-related cardiac disease.

References:.

Grants: The Morris Kahn Family Foundation.

Conflict of Interest: None declared.

P06.035.A Multidisciplinary and standardised post-mortem genetic analysis in a representative Czech cohort of sudden cardiac death (SCD) victims, together with genetic screening of their living relatives, yields high diagnostic yield in cases with positive family history and renders primary prevention of SD in affected families

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Background/Objectives: Post mortem genetic analysis in SCD, together with the cardiologic examination of victim´s relatives, represents a multidisciplinary approach for its prevention. We assessed the molecular aetiology of SCD in a representative cohort and evaluated how its results facilitate prevention of SCD.

Methods: A total of 115 SCD cases (34 females/81 males; av. age 34.1 years; period 2016-2021) was ascertained. Following genetic consultation and cardiological examination of victims´ living relatives 100 candidate genes in 106/115 cases on the MiSeq platform (Illumina) followed by SOPHiA GENETICS DDM bioinformatics (Switzerland) were examined. Class IV-V variants were validated by Sanger DNA sequencing and segregation analyses.

Results: In total 20 sudden arrhythmic death (SADS), 22 -sudden unexplained (infant) death (SUD/SUID), 12- hereditary cardiomyopathy (HCM) and 14 - dilated cardiomyopathy/left

ventricular noncompaction (DCM/LVNC), 22 - arrhythmogenic right ventricular cardiomyopathy (ARVC), 7 – sudden infant death (SIDS) and 11 - acute aortic dissection were diagnosed. Positive family history (pFH) of cardiac disease or SCD was identified in 27/115 (23.4 %; median age 41.0 years) of victims, whereas in cases without pFH their median age was significantly lower – 30.0 years. Molecular aetiology (i.e. presence of Class 4 -5 variants) was detected in a total 21/106 cases (19.8%) in *RYR2*, *KCNH2, KCNQ1, SCN5A, FLNC, GLA, TTN, TNNT2, RBM 20, MYPN, MYBPC3, FHL1, TGFBR1* and *COL3A1*. Diagnostic yield in cases with pFA reached 13/27 cases (48.1 %).

Conclusion: We demonstrate the utility of post mortem cardiogenetic examination in individuals older than 40 years of age.

References:.

Grants: NV18-02-00237 and the Czech SCD Research Consortium.

Conflict of Interest: None declared.

P06.036.B Molecular analysis of cardiomyopathies using next generation sequencing technologies in Slovak patients

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Background/Objectives: The aim of the study was to analyze a panel of genes associated with dilated or hypertrophic cardiomyopathy based on previously published results in order to identify the subjects at risk.

Methods: The method of next-generation sequencing by IlluminaHiSeq 2500 platform was used to detect sequence variants in 28 individuals diagnosed with dilated or hypertrophic cardiomyopathy. Detected variants were filtered and the functional impact of amino acid changes was predicted by computational programs.

Results: DNA samples of the 28 patients were analyzed by whole exome sequencing. We identified six nonsynonymous variants that were shown to be pathogenic in all used prediction softwares: rs3744998 (EPG5), rs11551768 (MGME1), rs148374985 (MURC), rs78461695 (PLEC), rs17158558 (RET) and rs2295190 (SYNE1). Two of the analyzed sequence variants had minor allele frequency (MAF)<0.01: rs148374985 (MURC), rs34580776 (MYBPC3).

Conclusion: Our data support the potential role of the detected variants in pathogenesis of dilated or hypertrophic cardiomyopathy; however, the possibility that these variants might not be true disease-causing variants but are susceptibility alleles that require additional mutations or injury to cause the clinical phenotype of disease must be considered.

References: Rocarati,M.,Latronico,M.V.G.,Musumeci,M. et al.: Unexpectedly low mutation rates in beta-myosin heavy chain and cardiac miosin finding protein genes in Italian patients with hypertrophic cardiomyopathy. J Cell Physiol, 226,2894-2900, 2011.

Yamada T, Nomura S. Recent Findings Related to Cardiomyopathy and Genetics. Int J Mol Sci. 2021 Nov 20;22(22):12522. https://doi.org/10.3390/ijms222212522. PMID: 34830403; PMCID: PMC8623065.

Grants: KEGA 032PU-4/2021.

Conflict of Interest: None declared.

P06.037.C Aortic rupture force in mice modelling hereditary aortic diseases

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Background/Objectives: Individuals suffering from hereditary aortic diseases (ADs) are at increased risk for aortic dissections and ruptures. We established an objective approach to measure the rupture force of the murine thoracic aorta, thereby explaining the outcomes of clinical studies and assessing an added value of old drugs in vascular Ehlers-Danlos syndrome (vEDS). Here, we applied our approach to six additional mouse AD models.

Methods: We used two mouse models for Marfan syndrome (MFS) as well as one smooth-muscle-cell-specific *Efemp2* knockout (SMKO) and three CRISPR/Cas9-engineered knock-in models (*Ltbp1*, *Mfap4*, and *Timp1*). Moreover, one mouse MFS model was subjected to 4-week-long losartan treatment previously shown to reduce aneurysm growth. As previously described, 1.5-mm-long sections of the murine thoracic aorta were mounted on a tissue puller and uniaxially stretched until rupture.

Results: The aortic rupture force was significantly lower in both MFS and SMKO models, but mice with knock-in mutations in the genes *Ltbp1*, *Mfap4*, and *Timp1* did not present with an impaired aortic integrity. As expected, the losartan treatment of MFS-modelling mice led to the reduction of aneurysm formation, which, surprisingly, had no impact on the aortic rupture force.

Conclusion: We show for the first time that our read-out system is able to characterize the aortic biomechanical integrity of mice modelling not only vEDS but also related ADs. Furthermore, aneurysm progression alone may not be a sufficient read-out for aortic rupture, as blood-pressure-lowering therapies preventing aortic aneurysms might still not strengthen the weakened aortic wall. These results may contribute to better medical therapies of hereditary ADs.

References:.

Grants:.

Conflict of Interest: None declared.

P06.038.D Functional characterization of iPSC-derived cardiomyocytes from Brugada syndrome patients of a genetically unresolved family reveals distinct underlying mechanisms

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Background/Objectives: Brugada syndrome (BrS) is an inherited cardiac arrhythmia characterized by a specific ECG pattern of ST-segment elevation, predisposition to ventricular fibrillation and sudden cardiac death. Over 20 genes have been associated with the disorder, however roughly 70% of patients remains without a genetic diagnosis.

Methods: In a large family with six mutation-negative BrS patients, we performed a SNP-based linkage analysis and whole-genome sequencing (WGS) on three patients. From two patients, one unaffected relative and an unrelated healthy control individual iPSC-derived cardiomyocytes (iPSC-CMs) were created. Molecular and electrophysiological characterization of the iPSC-CMs was performed using qPCR, immunocytochemistry, patch-clamping and calcium imaging.

Results: We detected significant linkage with a chromosome 2 locus (LOD-score 3.16). In the WGS-data, no interesting shared candidate variants were identified, also not in the linked locus. Studying the iPSC-CM models, one patient showed significantly reduced sodium current density, a positive shift in sodium channel voltage dependence of activation and reduced action potential amplitude and upstroke velocity. The second patient showed changes in calcium transient duration and rise time, while in both patients arrhythmia-like events occurred during the calcium recordings.

Conclusion: Characterization of iPSC-CMs of two patients from a BrS family showing significant linkage on chromosome 2, showed one patient with a sodium current loss-of-function phenotype consistent with previously reported BrS phenotypes, while the other one only showed calcium handling abnormalities. These results suggest different underlying disease mechanisms within one family, underscoring the complex nature of BrS.

References:.

Grants: Research Foundation Flanders, University of Antwerp BOF, ERC.

Conflict of Interest: None declared.

P06.039.A WES and its application for diagnostic purposes in hypertrophic cardiomyopathy

Albena Todorova[1], *Petya Angelova*[1], *Slavena Atemin*[1;2], *Nikolay Stoyanov*[3], *Tihomir Todorov*[2], *Mila Sleptsova*[2], *Krasimira Hristova*[4], *Mariana Gospodinova*[5], *Vanyo Mitev*[1], *Vasil Velchev*[3]

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Background/Objectives: Hypertrophic cardiomyopathy (HCM) is a common genetically heterogeneous disorder with an autosomal dominant inheritance and incomplete penetrance. More than 50 genes are associated with HCM and it is the most common cause of cardiac death due to cardiac failure. Genetic testing is strongly recommended for patients with clinically suspected HCM due to echocardiography/ECG data, family history for the disease and/or anamnestic data. The phenotype-genotype correlations are difficult to interpret: patients with the same genetic variant could express different clinical presentation, even in the same family.

Methods: A group of 20 HCM patients was screened for pathogenic variants by use of WES (Whole Exome Sequencing). The analysis included a panel of 242 genes associated with cardiomyopathy. The WES data is interpreted by GenesearchNGS software.

Results: The genetic diagnosis was clarified in 9 cases. Pathogenic variants were detected in the following genes: MYBPC3 - 4 variants; TNNI3; LAMP2; RBM20; MYLK2 and JPH2. The suspected clinical diagnosis of Danon disease was genetically confirmed by mutation in the LAMP2 gene. Severe hypertrophic and restrictive cardiomyopathy is provoked by TNNI3 mutation. The gene RBM20 seems to be a promising candidate gene for HCM.

Conclusion: The application of WES for diagnostic purposes in HCM cases turned out to be very useful in order to study the molecular bases of cardiomyopathies. In total 45% of our cases are genetically diagnosed. Moreover, the affected families are offered adequate genetic counselling and prenatal diagnostics.

References:.

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Conflict of Interest: None declared.

P06.040.B APOE molecular spectrum in a French cohort with primary dyslipidemia

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Background/Objectives: Primary hypercholesterolemia is characterized by elevated LDL-cholesterol (LDL-C) levels isolated in case of autosomal dominant hypercholesterolemia (ADH) or associated with elevated triglycerides levels in case of familial combined hyperlipidemia (FCHL), both leading to cardiovascular diseases (CVD). Along with *LDLR-APOB-PCSK9*, rare *APOE* gene variants were reported in ADH and FCHL. We explored the *APOE* molecular spectrum in a French ADH/FCHL cohort of 5,743 unrelated probands.

Methods: NGS was performed on coding DNA sequence and flanking introns (exon padding+/- 30 bp) of the *LDLR*, *PCSK9*, *APOB* and *APOE* genes and on the 12 SNPs of the polygenic score..

Results: The *LDLR*, *PCSK9*, *APOB* and *APOE* sequencing revealed 76 carriers of a rare *APOE* variant, without a mutation in the first three genes. Among the 31 variants 5 were described in ADH/FCHL: p.Leu167del, p.Leu46Pro, p.Arg163Cys, p.Arg269Gly and p.Gly145Asp. Twelve novel missense, five synonymous, two intronic and seven variants in regulatory regions were also identified. Sixteen variants were predicted in silico pathogenic or likely pathogenic, and their carriers had a significantly lower polygenic risk score than carriers of predicted benign variants. We did not observe any correlation between the LDL-C levels and the polygenic risk score which is in favour of a major effect of *APOE* potentially pathogenic variants. The p.Leu167del carriers were associated with a more severe phenotype and our data suggest that *APOE* variant carriers are better responders to statins than carriers of a *LDLR* mutation.

Conclusion: Altogether, we show that the *APOE* variants account for a significant part of ADH and FCHL.

References:.

Grants:.

Conflict of Interest: None declared.

P06.041.C Discovery of rare variants associated with resting heart rate

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Background/Objectives: Resting heart rate (RHR) is associated with cardiovascular disease. Genome-wide association studies (GWAS) have identified common variants at 437 loci, but their biological processes are not fully characterised. Identification of rare variants may help to pinpoint candidate genes.

Methods: We first performed a rare variant GWAS (RV-GWAS) for RHR in 388,223 individuals filtering by allele count > 10. Next, whole exome sequencing (WES) analysis in 161,539 individuals was done to explore consistency of findings and discover coding variants. All participants were of European ancestry from UK Biobank.

Results: The RV-GWAS identified 29 rare variants (9 at known RHR loci). At novel loci, we observe three missense variants in genes AKTIP, TBX5 and DBH. AKTIP encodes an AKT interacting protein, and a knockout mouse model has shown heart abnormalities. The rare variants at TBX5 and DBH are identical to those reported for blood pressure traits. The WES analysis identified 6 rare variants. Three variants overlapped the RV-GWAS and three variants were novel. We observe a missense variant in HIGD1B, a gene with no prior associations with cardiovascular phenotypes. At a second novel locus, a knockout mouse model of TBC1D32 demonstrates several cardiovascular abnormalities.

Conclusion: We identified 35 rare variants associated with RHR across the RV-GWAS and WES, highlighting potential novel candidate genes. Ongoing studies are focused on gene-based testing and validation.

References: (1) Mensah-Kane et al. Front Genet 18; 12:569323 (2021); (2) Lee et al. AJHG 95, 1, 5-23 (2014); (3) Silvestri V et al. Cancer 123, 210-8 (2017).

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Conflict of Interest: None declared.

P06.042.D Cardiac Arrhythmia Syndrome with ST-Segment Depression

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Background/Objectives: We report a large three-generation Italian family presenting with a peculiar ECG pattern and sudden cardiac death (SCD) in several individuals.

Methods: The proband was referred at 59 y.o. after a resuscitated cardiac arrest. She presented with recurrent atrial fibrillation and an abnormal ECG pattern, showing a concave-upward ST-segment depression in leads I, II, aVL, aVF and V2-6. Coronary angiographic studies were normal. She soon after died of SCD as well as her younger sister and several other family members, including her son, father, paternal grandmother, two uncles and three cousins. The proband's older sister and her son were clinically screened and presented similar ECGs patterns.

Results: Firstly, genomic DNA was extracted from peripheral blood of the proband and Next Generation Sequencing (NGS) analysis was performed through a targeted panel of 61 genes associated with channellopathies and cardiomyopathies. NGS analysis failed to evidence the underlying genetic cause in the proband. Secondly, genomic DNA was extracted from peripheral blood of the proband's older sister, her affected son and the healthy father. Trio-based Exome Sequencing (ES) was performed but failed to evidence the underlying genetic cause.

Conclusion: The intriguing ECG pattern of the present family fulfilled the proposed clinical criteria of ST-segment depression syndrome recently observed in five other families and segregating as an autosomal dominant trait[1]. Extensive examination of the NGS data revealed no coding variants segregating with the disease in any of the families reported to date. Genome sequencing is ongoing to reveal the likely underlying genetic cause.

References: [1]PMID:30380381.

Grants: None.

Conflict of Interest: None declared.

P06.043.A The genetic landscape and clinical implication of pediatric Moyamoya angiopathy in a multiethnic cohort

Paolo Zanoni[1], *Katharina Steindl*[1], *Heinrich Sticht*[2], *Beatrice Oneda*[1], *Pascal Joset*[1], *Ivan Ivanovski*[1], *Anselm Horn*[2], *Elena Cabello*[1], *Alessandra Baumer*[1], *Julia Laube*[1], *Markus Zweier*[1], *Anita Rauch*[1;3;4], *Nadia Khan*[5]

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Background/Objectives: To perform a comprehensive genotype-phenotype analysis in paediatric Moyamoya Angiopathy (MMA)[1,2] patients with focus on clinical implications.

Methods: We performed molecular karyotyping, exome sequencing and automated structural assessment of missense variants on a series of 88 paediatric MMA patients and correlated genetic, angiographic and clinical findings.

Results: The two largest subgroups consisted of *RNF213* and neurofibromatosis patients. While deleterious *RNF213* variants were associated with a severe MMA clinical course with early symptom onset, frequent posterior cerebral artery involvement and higher stroke rates in multiple territories, NF1 patients had a similar infarct burden compared to non-NF1 individuals and were often diagnosed incidentally during routine MRIs. Additionally, we found that MMA-associated *RNF213* variants have lower predicted functional impact compared to those associated with aortic disease. We also raise the question of MMA as a feature of recurrent as well as rare chromosomal imbalances and further support the association of MMA with *STAT3* deficiency.

Conclusion: We provide a comprehensive characterization at the genetic and clinical level of a large exclusively pediatric MMA population. Due to the clinical differences we found across genetic subgroups we propose genetic testing for risk stratification as part of the routine assessment of pediatric MMA patients.

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2. Kim SK, Cho BK, Phi JH, et al. Pediatric moyamoya disease: An analysis of 410 consecutive cases. Ann Neurol. 2010;68(1):92-101.

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Conflict of Interest: None declared.

P06.044.B Prevalence and clinical consequences of multiple pathogenic variants in dilated cardiomyopathy

Sophie Stroeks[1], Ida Lunde[2;3], Debby Hellebrekers[4], Godelieve Claes[4], Ingrid Krapels[4], Hiroko Wakimoto[2], Els Vanhoutte[4], Arthur van den Wijngaard[4], Michiel Henkens[1], Anne Raafs[1], Maurits Sikking[1], Jos Broers[5], Miranda Nabben[1;4;5], Elizabeth Jones[1;6], Stephane Heymans[1;6], Han Brunner[4;7], ***Job Verdonschot****[1;4]*

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Background/Objectives: Dilated cardiomyopathy (DCM) was considered a monogenetic disease which can be caused by over 50 genes. Evidence suggests that the combination of multiple pathogenic variants leads to greater disease severity and earlier onset. So far, not much is known about the prevalence and disease course of multiple pathogenic variants in patients with DCM. To gain insight in these knowledge gaps, we 1) systematically collected clinical information of a well-characterized DCM cohort and 2) created a mouse model.

Methods: Complete cardiac pheno- and genotyping was performed in 854 consecutive DCM patients. Patients were followed for an average of 54 months. Endomyocardial biopsies were taken from patients for RNA-sequencing. Compound heterozygous digenic (LMNA/TTNΔA), monogenic (LMNA/WT) and WT/WT mice were created and phenotypically followed over time. Heart tissue was used for RNA-sequencing.

Results: 131 (likely) pathogenic (LP/P) variants were found in 854 consecutive tested DCM patients (15.4%). Three of the 131 patients had a second LP/P variant (2.3%). These three patients had a comparable disease onset, disease severity, and clinical course compared to DCM patients with one LP/P. The LMNA/TTNΔA mice also had no functional differences compared to the LMNA/WT mice after 40 weeks of follow-up, although RNA-sequencing suggests increased cardiac stress and sarcomere insufficiency in the LMNA/TTNΔA mice.

Conclusion: 2.3% of DCM patients with one LP/P also have a second LP/P in a different gene. Although the second LP/P does not seem to influence the disease course of DCM in patients and mice, the finding of a second LP/P can be important for their relatives.

References:.

Grants:.

Conflict of Interest: None declared.

P06.045.C Specific de novo variants in RNF213 cause a monogenic early-onset multisystemic disease ranging from childhood stroke to Leigh-like syndrome

Theresa Brunet*[1;2], Victoria Lieftüchter[3], Dominic Lenz[4], Philipp Peters[3], Robert Kopajtich[2], Benedikt Zott[5;6;7], Hanna Zimmermann[8], Irina Hüning[9], Diana Ballhausen[10], Christian Staufner[4], Alyssa Bianzano[4], Joanne Hughes[11], Robert Taylor[12;13], Robert McFarland[12;13], Tamara Žigman[14], Danijela Petković Ramadža[14], Rohlfs Meino[3], Ute Hehr[15], Neal Sondheimer[16;17;18], Stacy Hewson[16], NIKOLAOS MARINAKIS[19], Konstantina Kosma[19], Jan Traeger-Synodinos[19], Holger Prokisch[1;2], Thomas Meitinger[1], Ingo Borggräfe[20;21], Juliane Spiegler[22], Ivo Baric[14], Marco Paolini[23], Lucia Gerstl[20], Matias Wagner[1;2;20]*

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Background/Objectives: *RNF213*, encoding a giant E3 ubiquitin ligase, has been recognized for its role as key susceptibility gene for Moyamoya disease (MMD). To date, only single case descriptions have also implicated an association of specific variants in *RNF213* with an early-onset form of MMD with full penetrance. We aimed to systematically elucidate the clinical spectrum associated with de novo variants in *RNF213* and to evaluate genotype-phenotype correlations.

Methods: Patients were identified through reanalysis of exome sequencing data of an unselected cohort from a single tertiary care center. Additional patients were ascertained via Genematcher and ClinVar. Phenotypic characterization including cMRI analyses and segregation analysis were performed.

Results: Nine individuals from eight unrelated families with heterozygous (8 de novo, 1 inherited) missense variants in *RNF213* clustering within or around the RING domain were identified. 6/9 individuals had ischemic stroke between the age of birth and 23 years, five of which showed MRI alterations suggestive of Moyamoya disease. In contrast, 2 individuals had bilateral basal ganglia

T2 hyperintensities and high CSF lactate. Additionally, five individuals had recurrent episodes of elevated liver enzymes and three cases had cardiomyopathy. Secondary structural epileptic encephalopathy was frequent (6/9) requiring antiepileptic medication.

Conclusion: De novo missense variants in *RNF213* clustering in the E3 RING or a region distal to it lead to a monogenic syndrome with two clinical presentations. Most patients presented with neonatal or childhood onset stroke and Moyamoya alterations whereas a second group had a Leigh-like phenotype. Hereby, we establish *RNF213* as a Mendelian Disease Gene with symptoms beyond MMD.

References:.

Grants:.

Conflict of Interest: None declared.

P06.048.B The challenging choice of gene panel size: our experience with hypertrophic and dilated cardiomyopathy

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Background/Objectives: Implementation of next generation sequencing (NGS) has led to a rapid expansion in the number of genes included in diagnostic genetic testing for cardiomyopathies. This tendency is changing with the evidence-based assessment of genes conducted by ClinGen. Our aim was to evaluate the mutation detection rate and the involved genes in patients with isolated hypertrophic or dilated cardiomyopathy (HCM and DCM) according to the gene panel size.

Methods: Our NGS approach consist of targeted exome sequencing. Before September 2019, we have used in-house gene panels including 65/78 cardiomyopathy genes. Since October 2019, we switched to Genomics England PanelAPP, including 144/140 high-evidence based genes causative for the different subtypes of cardiomyopathy.

Results: Between 2015 and 2021, a total of 135 patients with non-syndromic HCM or DCM underwent genetic testing. Our mutation detection rate was 49 % (36/74) with the in-house panels, 38% (23/61) with the PanelAPP panel and 44 % (59/135) overall. Comparing the two panel sizes we didn't see any difference in the genes found with causative variants. All the genes with pathogenic or likely pathogenic variants identified with the large PanelAPP panel were already included in our smaller in-house panels.

Conclusion: Our results confirm the lack of major clinical benefit of large panels in isolated HCM or DCM and support the recommendations of the 2021 European Society of Cardiology heart failure guidelines, which suggest to use small evidence-based panels and to consider additional large panel only if there is a clear family history or a specific phenotype.

References:.

Grants:.

Conflict of Interest: None declared.

P07

METABOLIC AND MITOCHONDRIAL DISORDERS

P07.001.C Loss of centrosomal gene ALMS1 alters cell metabolism through the TGF-β pathway

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Background/Objectives: Alström syndrome (AS) is a rare autosomal recessive disease that is associated with mutations in the ALMS1 centrosomal gene. The main manifestations of this syndrome are retinitis pigmentosa, obesity and type 2 diabetes mellitus. The depletion of the ALMS1 gene has been associated with the alteration of different processes regulated through the primary cilium, such as the Notch signalling pathway or TGF-β. Despite this, little is known about which cellular processes associated with the TGF-β pathway can be altered in the absence of ALMS1.

Methods: In this study we analyse the gene expression profile by RNA-seq of a cell line hTERT-BJ-5ta deficient in ALMS1, after stimulating the TGF-β pathway. We also performed a LFQ proteomic analysis by LC-MS/MS under the same conditions, to integrate the level of gene and protein expression. Finally, we validate the data obtained by western blot and fluorescence microscopy.

Results: RNA-seq data showed an enrichment mainly associated with TGF-β coordinated pathways such as PI3K/AKT, MAPKs or p53. Proteomic analysis showed an association between ALMS1 deficiency and alterations in cell metabolism and the lumen of intracellular organelles such as the endoplasmic reticulum. Observing the overlap of both datasets reinforced the relationship of ALMS1 with the endoplasmic reticulum and lipid-dependent signalling pathways. Finally, an over-activation of the AKT pathway was observed in the absence of ALMS1.

Conclusion: ALMS1 deficiency disrupted cross-signalling between the TGF-β pathway and other dependent pathways in immortalised fibroblasts. Furthermore, altered cross-signalling has implications for cellular metabolism and leads to over-activation of the AKT pathway.

References:.

Grants:.

Conflict of Interest: None declared.

P07.002.D ASAH1-related disorders: Expanding phenotypic spectrum requires updated thinking on diagnostic testing

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Background/Objectives: Pathogenic variants in the ASAH1 gene cause acid ceramidase deficiency, which causes a spectrum of phenotypes. Farber disease, with typical symptoms including joint disease, subcutaneous nodules, and dysphonia, along with other symptoms like osteolysis; and SMA-PME (spinal muscular atrophy with progressive myoclonic epilepsy).

Methods: Data including range of symptoms, age at symptom onset, time to diagnosis and outcome were assessed. Sources included recent literature and the Observational and Cross-Sectional Cohort Study of the Natural History and Phenotypic Spectrum of Farber Disease (NCT03233841). This systematic clinical study of patients with Farber disease included prospective evaluations, with 45 patients enrolled.

Results: 266 cases of ASAH1-related disorders were reviewed for phenotypic classification. 129 cases were classified as severe Farber disease with onset of symptoms and death before the age of 4 years; 66 cases as moderate to attenuated Farber disease with symptom onset in childhood and survival ranging from late childhood to the 6th decade; and 17 cases as unclassified Farber disease. 46 cases were classified as SMA-PME. Additional patients included 3 cases of late-onset SMA without epilepsy, one case of PME without SMA, 2 cases of SMA who later developed Farber disease symptoms, and 2 cases with both SMA-PME and Farber disease symptoms in childhood.

Conclusion: ASAH1-related disorders including Farber disease, SMA-PME, or variations of these phenotypes, are likely underdiagnosed. The broad phenotypic spectrum highlights the need for increased clinical suspicion and expanded inclusion of the ASAH1 gene in genetic testing for individuals presenting without textbook symptoms of acid ceramidase deficiency.

References:.

Grants:.

Conflict of Interest: Kathleen Crosby Aceragen, Aceragen, Alexander Solyom Aceragen, Aceragen.

P07.003.A Use of whole genome sequencing to determine the genetic basis of suspected mitochondrial disorders

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Background/Objectives: Diagnosis of mitochondrial disorders is challenging because they are clinically and genetically heterogeneous. WES has been effective for diagnosing mitochondrial disorders and discovering new disease genes. However, it is not possible to make a diagnosis in ~40%. WGS can diagnose pathogenic variants affecting the mtDNA and the nuclear genome, so it has the potential to make diagnoses in more families and shorten the 'diagnostic odyssey'.

Methods: 319 families with suspected mitochondrial disorders were referred to the 100,000 Genomes Project after excluding common genetic causes. Human Phenotype Ontology terms were recorded, median 7 per participant. Short read WGS was analysed. Variants were prioritised using phenotype-based gene panels, Exomiser and comparison to ClinVar. mtDNA variants were called using an in-house pipeline. Copy number variants and short tandem repeats for thirteen neurological disorders were analysed.

Results: A definite or probable genetic diagnosis was identified in 98 families (31%), with an additional 6 possible diagnoses (2%). In total, 95 different genes were implicated. 37.5% of families had a mitochondrial diagnosis and 62.5% had a non-mitochondrial diagnosis. Diagnostic yield was higher in trios/quads (62/148) compared to singletons (23/102) ($p = 0.005$) and in children (64/143) compared to adults (46/202) ($p < 0.001$).

Conclusion: WGS is a useful diagnostic test in patients with suspected mitochondrial disorders yielding a diagnosis in a further 31% after excluding common causes. The majority of diagnoses were non-mitochondrial disorders, including developmental disorders with intellectual disability, epileptic encephalopathies, metabolic disorders, cardiomyopathies and leukodystrophies. These would have been missed using a targeted approach, with some having specific treatments.

References: BMJ 2021;375;e066288.

Grants:.

Conflict of Interest: Katherine Schon Addenbrooke's Charitable Trust. Medical Research Council (MRC) International Centre for Genomic Medicine in Neuromuscular Disease (MR/S005021/1)., Rita Horvath Wellcome Trust Investigator (109915/Z/15/Z).

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Modis Therapeutics, Inc., a wholly.

owned subsidiary of Zogenix, Inc. - Consultancy fee.

Pretzel Therapeutics, Inc. - Consultancy fee.

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Senior Editor, Annals of Human Genetics.

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Research Centre based at Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge.

P07.004.B Genome-wide expression analysis in Fabry disease human podocyte cell line

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Background/Objectives: Fabry disease (FD) is an X-linked lysosomal disease due to alpha-galactosidase A deficiency. This enzyme is involved in glycosphingolipid metabolism and its alteration leads to cellular dysfunction and microvascular pathology. Different organs are involved with kidney as a main organ target. The rise of omics sciences has prompted a paradigm shift, in both research and medicine. These approaches may open new insights into the pathophysiology of multifactorial complex diseases. We performed genome-wide expression analysis in an FD human podocyte model.

Methods: RNAseq-based genome-wide expression analysis was done on human immortalized alpha-galactosidase A deficient podocytes generated using CRISPR/Cas9 technology, and control podocytes. Differential expression analysis was performed using DESeq2 package.

Results: Two hundred and forty-seven genes were differentially expressed, with 111 genes overexpressed and 136 under-expressed in FD compared to controls. Genes known to be involved in angiogenesis (*ITGB3*), autophagy (*TIA1*, *SMG1*) and oxidative stress (*CBR3*, *BLVRA*) were among these genes. The STRIPAK complex, related to autophagy, and the NADP/NADPH pathway (oxidative stress) were among the most altered pathways. The downregulated genes included *MOB1A* linked to the Hippo pathway which participates in kidney podocytes homeostasis maintenance.

Conclusion: These preliminary results unveil Fabry disease-related transcriptomic expression patterns. Further characterization of these disrupted cellular pathways could enable deeper understanding of FD pathophysiology.

References:.

Grants: None.

Conflict of Interest: Sarah Snanoudj University Hospital of Rouen, University of Rouen Normandy, celine derambure University of Rouen Normandie, Céline Lesueur University Hospital of Rouen, Lénaïg Donval: None declared, Stéphane Marret University Hospital of Rouen, University of Rouen Normandie, Soumeya Bekri University Hospital of Rouen, University of Rouen Normandy, Abdellah Tebani University Hospital of Rouen, University of Rouen Normandy.

P07.005.C Rapid exome sequencing for children with severe acute encephalopathy, a case series

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Background/Objectives: Increasingly, next generation sequencing (NGS) is becoming an invaluable tool in the diagnosis of acute neurological disorders where a monogenic etiology is not suspected such as acute encephalopathy\encephalitis.

Here, we describe a brief series of pediatric patients hospitalized in the pediatric intensive unit. All presented with severe acute encephalopathy initially suspected to be of infectious or inflammatory origin, but subsequently diagnosed with a monogenic disorder.

Methods: Rapid exome sequencing was performed during the initial hospitalization of three unrelated patients. Data analysis and initial report was performed in-house in a few hours. All patients were of Muslim Arab descent with a history of consanguinity, previously healthy ranging from 1.5-3 years. One patient presenting with acute necrotizing encephalopathy (ANEC) had a sister who presented with ANEC one year prior.

Results: Exome sequencing was diagnostic in all three cases. One patient had a homozygous pathogenic variant in MOCS2, c.3G>A p.(Met1Ile) associated with late onset Molybdenum cofactor deficiency B. A second patient harbored a homozygous likely pathogenic variant in NDUFS8 c.441G>C p.(Met147Ile). Surprisingly, the initial work-up was not suggestive of this disorder. Finally, a likely pathogenic homozygous missense variant c.359T>C p.(Ile120Thr) in the DBR1 gene was identified in the patient presenting with ANEC which segregated as expected in the family.

Conclusion: This case series demonstrates use of rapid exome sequencing is shifting the paradigm of diagnostics even in critical care situations and should be considered early on in children with acute encephalopathy. Timely diagnosis can direct initial treatment as well as informing decisions regarding long term care.

References:.

Grants:.

Conflict of Interest: None declared.

P07.006.D Analysis of the mitochondrial 13513G>A mutation: A case report of five Hungarian patients

Vera Várhegyi[1], *Fruzsina Szabo*[1], *Zoltan Grosz*[1], *Viktor Molnár*[1], *Noemi Agnes Varga*[1], *Petra Zsidegh*[2], *Agnes Herczegfalvi*[3], *Viktória Szabó*[4], *Anita Maasz*[5], *Judit Bene*[5], *Kinga Hadzsiev*[5], *Aniko Gal*[1], *Maria Judit Molnar*[1]

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Background/Objectives: Pathogenic mutations in the mitochondrial DNA (mtDNA) underlying primary mitochondrial disease result in a wide variety of clinical phenotypes. The m.13513 G>A (p.D355N) mutation in the MT-ND5 gene has been reported in the literature in the background of Leigh syndrome, MELAS, and MELAS / LHON overlap syndrome. In the present study, we sought to answer the question about the proportion of this mtDNA alteration that occurs among the Hungarian population.

Methods: In this study, 537 patients were examined for the m.13513 G>A mutation by bidirectional sequencing. DNA isolation was performed in 390 cases from blood and in 147 cases from postmitotic tissue (muscle, urinary squamous cell).

Results: Among the studied patients, the mtDNA G13513A mutation was detected in five cases. The heteroplasmy ratio was in 50 - >95%. Clinical symptoms ranged from a broad spectrum of phenotypes such as Leigh syndrome, ataxia, strabismus,

neuropathy, visual impairment, and renal failure. In four cases, the disease was associated with early onset Leigh syndrome, while in one patient, clinical symptoms manifested in adulthood with multisystemic involvement, with severe visual and renal impairment and hypoacusis.

Conclusion: The m.13513 G>A mtDNA alteration was present in about 1% of the total investigated cohort, while in the cases examined from postmitotic tissues this proportion was found to be 2.88%. Based on the above, we recommend a more comprehensive study of the m.13513 G>A mutation from postmitotic tissue.

References:.

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P07.007.A Severity of ATAD3A-related pontocerebellar hypoplasia correlates with severity of mutations

Martina Skopkova[1], *Vibhuti Rambani*[1], *Hana Stufkova*[2], *Viktor Stranecky*[3], *Katarina Brennerova*[4], *Miriam Kolníková*[5], *Michaela Pietrzykova*[6], *Hana Hansikova*[2], *Daniela Gasperikova*[1]

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Background/Objectives: Diagnostics of ATAD3A-related disorders is challenging from two reasons. Firstly, the ATAD3 locus contains three paralogous genes, making it difficult target for both sequencing and CNV analysis. Secondly, the clinical picture and severity are heterogeneous, ranging from recessive neonatal-lethal pontocerebellar hypoplasia through milder dominant Harel-Yoon syndrome to, again, neonatal-lethal but dominant cardiomyopathy. Fourteen different recessive variants in 17 families have been described with so far.

Methods: We report two families, each with two affected children. Patients and their parents were analysed using WES followed by CNV analysis (ExomeDepth). Variants were verified using Sanger sequencing and long-range PCR. Enzymological and biochemical studies were performed in muscle and cultivated fibroblasts of one proband.

Results: Decreased activity of complex IV and decreased levels of nuclear-encoded subunits of respiratory chain confirmed mitochondriopathy in the Family 1 proband. Compound heterozygous p.Leu77Val and exon 3-4 deletion in the ATAD3A gene were found in all four affected members of both families. The p.Leu77Val variant had previously been described as mild by functional studies (Yap et al., 2021), exon 3-4 deletion is considered loss-of-function with severe impact. This novel combination of high- and mild-impact variants resulted in strikingly homogenous phenotype in our patients - less severe and with longer life-span than in case of bi-allelic loss-of function variants.

Conclusion: Clinical picture and severity of ATAD3A-related disorders are dependent on the type of mutation and correlate with predicted severity of variants and their combinations.

References: Yap et al., Genome Med 2021.

Grants: APVV-17-0296, AZV MZCR NV19-07-00136.

Conflict of Interest: None declared.

P07.008.B Unraveling a case of Niemann-Pick disease using long read sequencing and adaptive sampling

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Background/Objectives: These last years, Oxford Nanopore Technology (ONT) emerged as a promising actor for structural variants identification and repetitive regions analysis by long read sequencing. Moreover, ONT recently added the adaptive sampling option on its sequencing devices, i.e. a software-controlled enrichment method which allows to target genomic regions of interest without any specific wet-lab enrichment procedure. This method has been shown to identify structural variants not detected by conventional genetic testing (1). Therefore, we used this approach to analyze the unsolved case of a patient affected by the autosomal recessive Niemann-Pick disease, but in whom only one pathogenic mutation was previously identified in NPC1 gene despite extensive genetic testing.

Methods: Adaptive sampling of ONT was performed using 1.5 µg of DNA, on the basis of a custom bed file targeting NPC1 and surrounding genes.

Results: We reached a mean fold-enrichment of 7 on targeted region, along with mean depth coverage of 25.5X. Our result suggested an inversion of 70kb, involving NPC1 and its adjacent gene ANKRD29. This was then confirmed by PCR and Sanger sequencing. Moreover, segregation study showed that this inversion was in trans with the known point mutation, confirming Niemann-Pick diagnostic at molecular level.

Conclusion: The adaptive sampling option proposed by ONT appears extremely flexible and allows easy case-by-case adjustment and analysis. While still perfectible, we believe that this approach could be a valuable help in daily practice of genetics experts, in conjunction with classic genetic testing.

References: 1. Miller et al., PMID: 34216551.

Grants: Institut de Pathologie et Génétique Research Funds.

Conflict of Interest: None declared.

P07.009.C Follow-up of family members of children with mtDNA-associated Leigh syndrome

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Background/Objectives: Leigh syndrome (LS) is a genetic neurometabolic disorder characterized by central nervous system degeneration. Some patients have missense pathogenic variants in mtDNA inherited from the matrilineal lineage. Disease´s expressivity is dependent on the heteroplasmy level at a given tissue. Given this variable expressivity, we reviewed the features of family members of a cohort of patients with mtDNA-associated LS (MALS) in their matrilineal lineage. With this revision, we aim to understand the necessity of a structured follow-up for extended family members.

Methods: We reviewed five families with children with MALS syndrome followed at Centro Hospitalar e Universitário do Porto.

Results: All mothers were asymptomatic at the time of their children´s diagnosis, but two mothers initiated potentiality mitochondrial-related complains after their child diagnosis. The mother of a child with MALS associated to m.10191T>C variant, presented headaches and abnormal subcortical white-matter T2 MRI signal, suggestive of adult onset-LS, with exclusion of autoimmunity and infections. The familial variant was not detected in this woman peripheral blood. A mother of a child with MALS associated to m.8993T>G variant, showed a heteroplasmy level of 75%, and presented complains of headaches and fatigue. Her MRI showed left cerebellar cortico-subcortical hypodense area, suggestive of previous ischemic event. In this second family, two family members by the matrilineal lineage present complains suggestive of neuropathy, ataxia, and retinitis pigmentosa.

Conclusion: Follow-up guidelines should be established for MALS family members at risk and genetic counseling should be offered to all family members in the matrilineal lineage of an index case.

References:.

Grants:.

Conflict of Interest: None declared.

P07.010.D ATAD3 knockout model in zebrafish

Shlomit Ezer[1;2], *Nathan Ronin*[3;4], *Shahar Rotem-Bamberger*[3;4], *Shira Yanovsky-Dagan*[1], *Adi Inbal*[3;4], *Tamar Harel*[1]

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Background/Objectives: ATAD3A encodes a mitochondrial expressed protein which spans the inner and outer mitochondrial membranes. It is involved in mitochondrial dynamics, mtDNA maintenance, cholesterol metabolism, ER-mitochondria interaction and more. ATAD3A pathogenic variants have been shown to cause distinct neurological diseases in humans. We aimed to generate an atad3-null line in zebrafish, in order to further characterize the gene and its role in health and disease and to test possible remedies.

Methods: Using CRISPR-Cas9 genome editing, we created two lines of heterozygous fish with frameshift variants which can be bred to produce atad3-null embryos. We examined the phenotype and mitochondrial content of the mutant embryos, and compared the transcriptome of wild-type and mutant embryos at 3 days post-fertilization (dpf) via RNAseq. Results were validated by quantitative real-time PCR at 3dpf and 5dpf.

Results: Atad3-null embryos demonstrated microcephaly, small eyes, pericardial edema and thinning of the musculature, closely correlating with the human disease. Mitochondrial content was reduced. Transcriptome analysis revealed an expected decline in most mitochondrial pathways in the mutant embryos. In addition, we witnessed an unexpected global upregulation of cytosolic tRNA synthetases, presumably secondary to ER stress.

Conclusion: Zebrafish atad3-null embryos can be used as a reliable model of human ATAD3A-associated disorders. ER stress signals seem to have a role in the pathogenesis, and blocking ER stress by small compounds may serve as a potential therapeutic pathway.

References: Harel, T., et al. (2016). Am J Hum Genet 99, 831-845.

Grants: ISF 1663/17 to TH.

Conflict of Interest: None declared.

P07.011.A Impact of GCSH-deficiency: a protein at the crossroad of one-carbon metabolism and cellular respiration

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Background/Objectives: Post-translational lipoylation of the 2-ketoacid dehydrogenases (2KDH) is essential in the bioenergetics metabolism of eukaryotes. The GCSH-protein, lipoylated subunit of the glycine cleavage enzyme, has been proposed as the only acceptor of lipoate for transferring to other 2KDH in mammals but, this dual function remains unproven. We present an extensive analysis of the nucleotide variations identified in a cohort of six patients with genetic confirmation of bi-allelic changes in the *GCSH* gene.

Methods: The patient's phenotyping included clinical record evaluation, biochemical analysis and brain MRIs. Genetic analysis was based on exome sequencing. Pathogenicity of nucleotide changes was assessed by in silico modelling and functional analysis in patient's cells, knock-down models and purified recombinant proteins.

Results: The clinical presentations ranged from very severe early presentation with a fatal outcome to late milder courses. Patients had increased glycine levels in plasma and cerebrospinal fluid and mostly normal lactate. Genetic analysis identified four missense changes, one nonsense and two genomic rearrangements. Functional studies showed decreases in GCSH-protein amount, lipoylation status and mitochondrial respiration either in patient's cells or after overexpressing missense GCSH-proteins in two *GCSH* knock-down models generated in COS7 and *S. cerevisiae*. Purified recombinant GCSH protein studies resembled those results.

Conclusion: We unravel the final step in post-translational lipoylation in mammals and describe a cohort of patients with pathogenic *GCSH* variants responsible for a new variant-form of NKH combining deficient GCS complex and bioenergetics defects.

References:.

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Conflict of Interest: None declared.

P07.013.C Biallelic variants in PYROXD2 cause a severe infantile metabolic disorder affecting mitochondrial function

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Background/Objectives: Pyridine Nucleotide-Disulfide Oxidoreductase Domain 2 (PYROXD2) is a mitochondrial inner membrane/matrix-residing protein reported to regulate mitochondrial function. Little is known about the clinical importance or precise biological function of PYROXD2. We report biallelic variants in *PYROXD2* in a patient with suspected mitochondrial disease.

Methods: A male infant presented with acute unresponsive episodes and extreme metabolic acidosis. He developed progressive neurological deterioration on a background of postnatal-onset poor growth and microcephaly. He died at 6.5 months age. Magnetic resonance brain imaging showed changes resembling Leigh syndrome. The proband and his unaffected parents underwent genome sequencing and RNA-seq analysis. Functional assays were conducted to assess mitochondrial function using patient fibroblasts. Proteomic differences between the proband and control fibroblasts were investigated with high-resolution tandem mass spectrometry and quantitative protein analysis.

Results: No causative variants were found through standard trio analysis of known disease-causing genes, including analysis of the mitochondrial genome. A genome-wide analysis identified compound heterozygous variants in *PYROXD2*.

Functional assays revealed increased mitochondrial superoxide levels and a heightened sensitivity to culturing in galactose media, a known mitochondrial stressor, indicating impaired mitochondrial activity in fibroblasts.

Proteomic results demonstrated decreased levels of subunits of the mitochondrial respiratory chain complex I, and both the small and large subunits of the mitochondrial ribosome, suggesting a mitoribosomal defect.

Conclusion: Our findings support the critical role of PYROXD2 in human cells, suggest that the biallelic *PYROXD2* variants are associated with mitochondrial dysfunction and are the likely cause of the proband's clinical presentation.

References:.

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Conflict of Interest: None declared.

P07.014.D MITODIAG: A French network of diagnostic laboratories for mitochondrial diseases

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Background/Objectives: Mitochondrial diseases (MD) are characterized by a huge heterogeneity which poses significant diagnostic challenges for clinicians and around 50% of patients are still undiagnosed. Since 2000, the French Network of Mitochondrial Diseases Diagnostic Laboratories, called MITODIAG, has been created and works in close collaboration with the two National Reference Centers, CARAMMEL and CALISSON, and the Neuromuscular rare disease network FILNEMUS, in order to improve the diagnosis and health care for patients with MD.

Methods: We describe here the organization of the MITODIAG network, the evolution of genetic diagnosis in MD in France these last years and the interactions with national platforms of genome core sequencing facilities named AURAGEN and SEQOIA.

We also report the first clinical and genetic description of a cohort of more than 400 patients tested by NGS, in whom a diagnosis could be confirmed by the identification of pathogenic variants in nuclearly-encoded genes.

Results: 3/4 of the patients are children under 18 with often early and severe phenotypes, of mostly autosomal recessive inheritance. In these patients, 30% of pathogenic variants are located in complex I genes or genes involved in mitochondrial translation. In adults, diseases are more heterogeneous, from moderate to very severe, mainly with neuromuscular symptoms due to pathogenic variants in mtDNA maintenance machinery.

Conclusion: These data will be implemented in the MITOMATCHER database in order to improve phenotype-genotype correlations and facilitate development of therapeutic strategies. Collaborations between MITODIAG and FILNEMUS will also facilitate patient follow-up in diagnostic wandering and will facilitate the identification of diagnosis for these patients.

References:.

Grants:.

Conflict of Interest: None declared.

P07.015.A Interpreting the pathogenicity of genetic variants in rare diseases: lessons from Fabry disease

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Background/Objectives: Fabry disease (FD) is an X-linked genetic disease due to pathogenic variants in *GLA* (Gene ID: 2717). Over 1,000 *GLA* variants were identified, a significant number through screening protocols in newborns and at-risk populations more susceptible to disclose variants of unknown significance (VUS). This, together with the non-specificity of symptoms, challenges physicians at the time of diagnosis. Here, a panel of FD specialists convened to study how expertise compares with the traditional approach in interpreting variants.

Methods: Several highly controversial GLA VUS (p.Ser126Gly, p.Ala143Thr, p.Asp313Tyr), were re-analyzed through the review of patients' records.

Results: Experts' input was found to significantly contribute to an accurate interpretation of variants. The proper use of surrogate biomarkers can optimize the interpretation of variants. When a (likely) benign *GLA* variant is disclosed, other genes (e.g., sarcomeric genes in case of HCM) should be investigated. Comparing allele frequencies (AF) of *GLA* VUS is useful in excluding VUS which AF is higher than the disease prevalence or the frequency of the most common pathogenic allele. While databases and in silico prediction softwares are useful tools, they may yield conflicting results.

Conclusion: In genetics, the traditional approach to interpreting the pathogenicity of variants was developed by the American College of Medical Genetics. Our data suggest that through their in-depth knowledge of disease phenotypes, biomarkers, alleles frequencies, and literature data, highly specialized experts bring an important additional value. These lessons from FD give insights for better interpreting the pathogenicity of allelic variants in other genetic diseases.

References:.

Grants:.

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P07.016.B Detection of single nucleotide and copy number variants in the Fabry Disease-associated GLA gene using nanopore sequencing

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Background/Objectives: More than 900 variants have been described in the GLA gene. Some intronic variants and copy number variants in GLA can cause Fabry disease but will not be detected by classical Sanger sequence. We aimed to design and validate a method for sequencing the GLA gene using long-read Oxford Nanopore sequencing technology.

Methods: 57 male and 42 female Fabry patients were blindly analyzed, both by conventional Sanger sequence and by long-read sequencing of a 13kb PCR amplicon. We used minimap2 to align the long-read data and Nanopolish and Sniffles to call variants.

Results: All the variants (100%) detected by Sanger (including a deep intronic variant) were also detected by long-read sequencing.

One patient had a deletion that was not detected by Sanger sequencing but was detected by the new technology.

Conclusion: Our long-read sequencing-based method was able to detect multiple missense variants and an exonic deletion, with the added advantage of intronic analysis. It can be used as an efficient and cost-effective tool for screening and diagnosing Fabry disease. Furthermore, our approach can be easily implemented for other monogenic diseases and improve diagnostic precision and efficiency.

References:.

Grants:.

Conflict of Interest: None declared.

P07.018.D An interactive website visualizing newborn screening programs worldwide

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Background/Objectives: Newborn screening (NBS) strategies have been proven to be successful diagnostics for several life-threatening inherited metabolic disorders and other genetic conditions. Governments are expanding nationwide NBS programs to improve public health. However, due to various challenges, worldwide NBS programs are not yet optimal. Monitoring worldwide NBS programs could accelerate the ongoing efforts for optimization.

Methods: We reviewed the last 10 years of literature to reveal country-level NBS programs. A user-friendly website (www.nbsww.org), consisting of an interactive world map, a filter table, and individual pages for each country, has been developed. Data sources are available with a link to corresponding publications. A data collection form is placed to receive user contributions to keep the content of the website up-to-date.

Results: We identified 115 countries' nationwide NBS programs. The current status of NBS programs is heterogeneous, covering conditions ranging from a minimum of 0 to a maximum of 41 conditions. Expanded NBS is implemented in 35 (30%) of these countries while 57 (50%) screens less than 5 conditions. Most screened conditions are primary congenital hypothyroidism, phenylketonuria, congenital adrenal hyperplasia, screened by 82 (71%), 75 (65%), 50 (43%) programs, respectively.

Conclusion: The nationwide NBS programs were reported by presenting a website that allows monitoring and facilitates evaluation of the current situation.

References: Martínez-Morillo, E., Prieto García, B., & Álvarez Menéndez, F. V. (2016). Challenges for Worldwide Harmonization of Newborn Screening Programs. Clinical chemistry, 62(5), 689–698.

Grants: The Scientific And Technological Research Council Of Turkey grant 2209-A.

Conflict of Interest: None declared.

P07.019.A Psychosine in dried blood spots of newborns at risk of Krabbe disease due to GALC p.Y319C homozygosity

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Background/Objectives: GALC p.Y319C (c.956A>G) is considered a variant of uncertain significance. However, several patients homozygous for p.Y319C presented with symptoms consistent with Krabbe disease (KD) at 3 years of age and older (1). We report on psychosine (PSY) in dried blood spots (DBS) from GALC p.Y319C homozygous neonates.

Methods: PSY concentrations were measured in DBS (2) collected in the neonatal period from individuals homozygous for p.Y319C and identified through newborn screening for KD ($n = 11$).

Results: Neonatal DBS PSY was >2.0 nM (2.1-2.8 nM) in four of eleven p.Y319C homozygotes. Ten of eleven subjects are asymptomatic to date (age range: 4 months – 6.3 years, average age: 2.5 years). One patient identified through newborn screening (PSY: 2.8 nM) displayed clinical signs by 4 years of age and received a hematopoietic stem cell transplant.

Conclusion: A third of p.Y319C homozygotes had PSY concentrations above the reference threshold of 2 nM. The infant with the highest neonatal PSY DBS concentration was the only case developing signs of KD. Ongoing clinical monitoring of these individuals is required to determine whether they are at risk for KD. When KD is considered for newborn screening, programs should define whether all or specific KD forms (infantile vs. later onset KD) are the primary target of screening because this decision will determine if molecular genetic testing of GALC is required as part of the screening process.

References: 1. Bascou et al. al. Front Neurol. 2020;11:563724.
2. Herbst et al. Int J Neonatal Screen. 2020;6(2):29.

Grants:.

Conflict of Interest: Amy White: None declared, Joseph Orsini: None declared, Maria Escolar Forge Biologics, NIH R01NS061965-01(PI). The Legacy of Angels Foundation (PI), Forge Biologics, Dawn Peck: None declared, Gisele Bentz Pino: None declared, April Studinski: None declared, Dimitar Gavrilov: None declared, Devin Oglesbee: None declared, Matthew Schultz: None declared, Silvia Tortorelli: None declared, Dietrich Matern Neurogene advisory board (4 hrs; compensation passed on to Mayo Clinic).

P07.020.B Mitochondrial encephalomyopathy stroke-like episodes and lactate acidosis (MELAS), disease spectrum, lessons from large cohort

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Background/Objectives: Evaluate MELAS mutation cohort clinical spectrum, clinical/genetic factors associated with severe or later presentation.

Methods: Retrospective review 81 Mayo Clinic patients with/without meeting MELAS criteria. Clinical, MRI characteristics, mutations/heteroplasmy reviewed. MELAS, standard onset SLE <40 or late >40.

Results: 42 MELAS, 30 symptomatic non-MELAS, and 9 asymptomatic. MELAS, significantly lower BMI (mean 18.6 vs 25.1, 22.0), trend higher heteroplasmy (means 39.3%, 29.3%, 21.8%). MELAS and non-MELAS had similar age of first symptom (mean 20.3 vs 21.5); non-MELAS higher SNHL presentation (51.6% v24.4%, $p = 0.014$). Neurologic (seizures/SLE/ataxia/dementia) common MELAS first symptom (39%), versus non-MELAS (19.4%). MELAS, significant seizure prevalence (88.1% vs 16.7%), and higher mortality (43% vs 13%). **MELAS cohort, 13 late and 29 standard-**

onset. Mean age of first symptom 14.2y in standard (range 0-36), 34.1 in late (range 16-53). Trend ($p = 0.18$) towards higher heteroplasmy in standard (mean 44.8% vs 25.3%). Late-MELAS, significantly longer time from first symptom to SLE (mean 16.6 vs 9.3y), no different duration to death. Longer life expectancy in late-MELAS (mean death 62 vs 30y). Standard-MELAS higher incidence neurologic symptoms at onset (51.7% vs 15.4%); late-onset higher prevalence diabetes (69.2% vs 13.8%), nephropathy (53.8% vs 10.3%). Late-MELAS tended more systems involved (mean 4.1 vs 2.7).

Conclusion: Standard-MELAS more likely to present with neurologic symptoms compared to later onset, who are more likely to suffer diabetes, nephropathy, greater organ-involvement. Many MELAS present late >40y. **N**on-MELAS suffer substantial neurologic symptoms. Lower BMI in MELAS and higher rates SNHL in non-MELAS may be useful differentiator at onset.

References: N/A.

Grants: N/A.

Conflict of Interest: Ralitza Gavrilova Mayo Clinic, Benjamin Cox Mayo Clinic, Neurology fellow.

P07.021.C Development and validation of a new neonatal screening test

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Background/Objectives: Many genetic conditions affect normal development in newborns, infants or young children. These disorders can cause death, serious life-long disability or chronic disease if not treated early. Also, they have a vast array of symptoms, making diagnosis complicated. Therefore, prompt identification and timely treatment is essential to prevent or minimize the impact of the conditions. Our aim was to develop an NGS-based product, which tests for 106 neonatal conditions including: metabolic, endocrine, haemoglobin, hearing loss, pulmonary and musculoskeletal disorders.

Methods: Custom target capture sequences (TACS) were designed to capture all gene exons, and intron-exon boundaries. The TACS were immobilized on streptavidin-coated magnetic beads. Sequencing libraries were constructed and subjected to in-solution hybridization with the immobilized TACs. The captured sequences were amplified and sequenced using NGS. A blind validation study was performed to assess the sensitivity and specificity of single nucleotide variant (SNV), indel and copy number variant (CNV) detection.

Results: SNVs and indels were detected at sensitivity of 100% (CI: 87-100%) and specificity of 100% (CI: 99.8-100%). The algorithm was designed to detect CNVs at high-resolution with estimated high sensitivity and specificity when applied to single or few exon CNV. Each positive call was confirmed with an orthogonal method.

Conclusion: We have developed and validated a neonatal screening test for 106 disorders that when detected early, can prevent or reduce serious health consequences such as developmental delay, cognitive impairment, neurological and physical problems and premature death.

References:.

Grants:.

Conflict of Interest: Michaella Georgiadou NIPD Genetics, Charalambos Loizides NIPD Genetics, Skevi Kyriakou NIPD Genetics, Achilleas Achilleos NIPD Genetics, Christos Lemesios NIPD Genetics, Michalis Nicolaou NIPD Genetics, Chrisovalando Soteriou NIPD Genetics, Haris Kkoufou NIPD Genetics, Louiza Constantinou NIPD Genetics, Krystallo Christou NIPD Genetics, Antonia Matsentidou NIPD Genetics, Michalis Spyrou NIPD Genetics, Stelia Pissaridou NIPD Genetics, Demetra Panayiotou NIPD Genetics, Kyriakos Tsangaras NIPD Genetics, Elena Kypri NIPD Genetics, Marios Ioannides NIPD Genetics, George Koumbaris NIPD Genetics, Philippos Patsalis NIPD Genetics.

P07.022.D Beyond the exome: identification and characterization of non-coding variants in inborn errors of metabolism

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Background/Objectives: Inborn errors of metabolism (IEM) are a large group of rare diseases that include more than 1450 defects. Historically, the search for mutations contributing to IEM has been limited to exons, whereas regulatory elements have remained poorly investigated. Our aim here consisted in applying a combination of multiple omics to identify non-coding variants responsible for disease-state which scape exome sequencing, thus reducing the diagnostic gap.

Methods: Genomic analyses by whole exome or whole genome sequencing and functional studies (minigenes and luciferase reporters) were carried out in DNA and RNA extracted from patients' fibroblasts. The enzymatic activity was also evaluated in these cells when possible.

Results: We have selected a cohort of unsolved patients with clinical or biochemical diagnosis of different IEM (hyperphenylalaninemia, galactosemia, mucopolysaccharidosis or citrullinemia among others). Human Phenotype Ontologies (HPO) and biochemical signatures were used to filter the obtained variants. We prioritized either no candidate variants or only one. Therefore, all remaining undiagnosed. We have attained a complete genetic diagnosis for several patients by combination of transcriptomic and genomic analyses and functional genomics. We have identified pseudoexons insertions caused by deep intronic variants such as c.[83+658C>G;83+758T>A] in *PTS* or c.598-757G>A in *ASS1* and novel variants located in promoter regions like c.-82_-71delins-103_-86 in *PTS* or c.-87T>C in *IDUA*. The pathogenic role of all of these changes was assessed by minigenes studies or luciferase reporter studies confirming their clinical significance.

Conclusion: The non-coding portion of the genome should be interrogated when searching for disease-causing mutations.

References:.

Grants: PI19/01155, ERT18TRL746, B2017/BMD3721.

Conflict of Interest: None declared.

P07.023.A Three novel SLC2A1 mutations in GLUT1DS pediatric patients

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Background/Objectives: GLUT1 deficiency syndrome (GLUT1DS; #606777) is a rare genetic metabolic disease, characterized by infantile seizures, neurodevelopmental delay, and movement disorders. GLUT1DS is caused by heterozygous (or rarely, homozygous) mutations in the *SLC2A1* gene, which encodes GLUT1, a glucose transporter across the blood-brain barrier. Most commonly these variants arise de novo resulting in sporadic cases, although several familial cases with AD pattern have been described.

Methods: We performed *SLC2A1* sequencing analysis on 26 pediatric patients with clinical suspicion of GLUT1DS, and relatives.

Results: Herein, we reported three novel *SLC2A1* mutations causing GLUT1DS: two sporadic cases (a, b), and one familial case (c).

In the proband of the first sporadic case (a), characterized by 3 months-onset epileptic encephalopathy, we found a novel de novo c.114+1G>A splicing variant in *SLC2A1* gene. In silico analysis showed that the identified variant affects the donor splice-site of intron 2-3, leading probably to exon 2 skipping and exon 1-3 junction.

In the other sporadic case (b), which the proband presents epileptic discharges and movement disorders, the analysis revealed in *SLC2A1* a novel de novo frameshift variant c.370delC (p.(Leu124TrpfsTer12)), predicted to result in NMD.

The proband of the familial case (c) shows febrile seizures, while the father and grandfather present paroxysmal exercise-induced dyskinesia without epilepsy. Despite the clinical familial heterogeneity, we identified a novel *SLC2A1* heterozygous missense variant c.1363A>G (p.Thr455Ala), reported as disease causing in all three patients.

Conclusion: Our report described three novel variants in both sporadic and familial cases, thus expanding the genotypic spectrum of *SLC2A1* mutations causing GLUT1DS.

References:.

Grants:.

Conflict of Interest: None declared.

P07.024.B Establishing the mutational spectrum of Hungarian patients with familial hypercholesterolemia

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Background/Objectives: Familial hypercholesterolemia (FH) is one of the most common autosomal dominantly inherited diseases affecting the cholesterol metabolism which, in the absence of treatment, leads to the development of cardiovascular complications. The disease is still underdiagnosed, even though an early diagnosis would be of great importance for the patient to receive proper treatment and to prevent further complications. No studies are available describing the genetic background of Hungarian FH patients.

Methods: In this work, we present the clinical and molecular data of 44 unrelated individuals with suspected FH. Sequencing of five FH-causing genes (LDLR, APOB, PCSK9, LDLRAP1 and STAP1) has been performed by next-generation sequencing (NGS). In cases where a copy number variation (CNV) has been detected by NGS, confirmation by multiplex ligation-dependent probe amplification (MLPA) has also been performed.

Results: We identified 47 causal or potentially causal (including variants of uncertain significance) LDLR and APOB variants in 44 index patients. The most common variant in the APOB gene was the c.10580G>A p.(Arg3527Gln) missense mutation, this being in accordance with literature data. We detected 40 mutations in the LDLR gene in a total of 37 patients of which 3 patients carried mutations in compound heterozygous form.

Conclusion: Our study revealed one novel mutation in the LDLR gene, while most of the mutations that are present in the Hungarian population, have already been described. Indicating the importance of establishing a genetic diagnosis, the timing of initiation of lipid-lowering therapies may greatly influence the onset and severity of expected complications.

References:.

Grants:.

Conflict of Interest: None declared.

P07.025.C Biochemical and clinical effects of vitamin E supplementation in Hungarian Smith-Lemli-Opitz syndrome patients

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Background/Objectives: Smith-Lemli-Opitz syndrome (SLOS) is a severe monogenic inborn error of cholesterol biosynthesis resulting in low cholesterol and high 7-dehydrocholesterol (7-DHC) levels. 7-DHC-derived oxysterols likely contribute to disease pathophysiology, thus antioxidant treatment might be beneficial because of high oxidative stress.

Methods: In a 3-year prospective study we investigated the effects of vitamin E supplementation in six SLOS patients already receiving dietary cholesterol treatment. Plasma vitamin A and E concentrations were determined by a HPLC method. At baseline plasma 7-DHC, 8-DHC and cholesterol levels were determined by a LC-MS/MS method. The clinical effect of the supplementation was assessed by performing structured parental interviews.

Results: At baseline, patients were characterized by low or low-normal plasma vitamin E concentrations (7.19-15.68 µmol/L), while vitamin A concentrations were found to be normal or high (1.26-2.68 µmol/L). Vitamin E supplementation resulted in correction or significant elevation of plasma vitamin E concentration in all patients. We observed reduced aggression, self-injury, irritability, hyperactivity, attention deficit, repetitive behavior, sleep disturbance, skin photosensitivity and/or eczema in 3/6 patients, with notable individual variability. Clinical response to therapy was associated with a low baseline 7-DHC+8-DHC/cholesterol ratio (0.2-0.4).

Conclusion: We suggest that determination of vitamin E status is important in SLOS patients. According to our results, the lower

level of vitamin E is most likely attributable to increased consumption due to oxidative stress, rather than malabsorption. Supplementation of vitamin E should be considered and might be beneficial.

References:.

Grants:.

Conflict of Interest: None declared.

P07.026.D Missense variant in PDK1 associated with severe developmental delay and epilepsy

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Background/Objectives: The pyruvate dehydrogenase complex (PDC), located in the mitochondrial matrix, is responsible for the conversion of pyruvate into acetyl-CoA, used for energy production in the cells. PDC activity is tightly regulated by phosphorylation, via kinases and phosphatases (PDK/PDP), that respond to, for example, the amount of substrate in the cell (e.g. pyruvate or NADH) and hypoxia. Mutations in all subunits of the PDC and in PDK3 have been reported and the clinical presentation often includes lactic acidosis, neurodevelopmental delay, and seizures. Here we report a missense mutation in PDK1, a gene not previously associated with disease.

Methods: Genetic analysis was done using trio genome sequencing. Functional studies in zebrafish were done by overexpressing human wild type and mutant *PDK1* in early-stage embryos.

Results: A de novo missense variant in *PDK1* (c.1139G>A, p.Gly380Asp) was identified in a patient with developmental delay and seizures. In zebrafish, mutant PDK1 fails to phosphorylate PDHE1 (pyruvate dehydrogenase) resulting in abnormal PDC activity which, consequently, affects mitochondria activity. This, in turn, affects muscle activity, seen by reduced bouts of movement when compared to control embryos, and neuronal development delay.

Conclusion: Here, we report genetic and functional evidence suggesting that loss of function of PDK1 causes a similar clinical presentation as other PDC genes.

References:.

Grants:.

Conflict of Interest: None declared.

P07.027.A History of a diagnostic errancy: how new technologies may allow for a diagnosis after 40 years

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Background/Objectives: This clinical case demonstrates that subtle metabolic impairments may not be detected by basic screening performed in routine diagnostics. In addition to metabolic diagnostics, it's imperative to use more precise molecular biology techniques.

Methods: 46 year old woman, born at full term after a pregnancy and normal delivery, of non-consanguineous parents presented with mild developmental delay since infancy. A waddling walk was noted at the age of 5.Legge-Calvé-Perthes disease diagnosed at the age of 6, necessitating a bilateral hip prosthesis. She underwent surgery for severe scoliosis in childhood.

In her twenties, she was diagnosed with a demyelinating sensory-motor polyneuropathy leading to amyotrophy of the 4 limbs with predominance in the lower limbs.

At age 45, she presented cardiac arrhythmias diagnosed as probable arythmogenic right ventricular dysplasia.

Clinical and genetics investigations over a period of over 40 years had not revealed an explanation for the developmental delay, neuropathy and cardiac condition (Normal EEG and brain MRI).

Results: Normal caryotype and CHG-array, normal metabolic analysis including mucopolysaccharides and oligosaccharides, GJP1 gene analysis.

Genetic analysis of a cardiac arrhythmia panel was normal.

Analysis of a neurodevelopmental panel (859 genes) revealed a compound heterozygous GNPTAB mutation, confirming a diagnosis of mucolipidosis type III. Re-evaluation of skeletal X-rays confirmed associated skeletal changes.

Conclusion: Due to her mild phenotype, a clinical diagnosis of classic mucolipidosis was never evoked.Routine metabolic diagnostic testing may not be sensitive enough to diagnose mild forms of metabolic conditions, gene panels must absolutely cover the genes for storage diseases.

References:.

Grants:.

Conflict of Interest: None declared.

P07.028.B Discovery of Type 2 Diabetes genes using an accessible tissue

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Background/Objectives: Transcriptome Wide Association Study (TWAS) methods use reference expression datasets to link genes to disease. Ideally, these should use samples from a disease relevant tissue, which may be difficult to collect, limiting sample size. We evaluate the power to discover Type 2 Diabetes (T2D) causal genes using a well powered whole blood expression reference against smaller reference datasets from disease relevant tissues.

Methods: Predictive models were built using whole blood expression and proteomic data (DIRECT consortium, $n = 3029$), pancreatic islets eQTL data (INSPIRE consortium, $n = 420$) and eQTL data from 49 tissues (GTEx consortium). These models were combined with GWAS summary statistics (DIAGRAM consortium, $n = 898{,}130$) to calculate gene-T2D association scores using S-PrediXcan.

Results: We found 97 significant associations between T2D and predicted gene expression levels using whole blood from DIRECT. We recapitulate known T2D genes such as *CAMK1D, PAM*, and *CKDN1C* and find more associations using the larger reference

dataset (43 using islets and from 2 to 84 in GTEx). There is a correlation between the sample size and the discovery power ($R^2 = 0.9$, $p = <2e^{-16}$). We also find DIRECT implicated genes to be enriched around GWAS loci compared to GTEx tissue derived genes, meaning a higher proportion of DIRECT genes have supporting genetic evidence. However, the DIRECT reference also misses important T2D genes, such as *TCF7L2* or *IGF2BP2*, which were only captured when using islets models.

Conclusion: Our findings indicate that large studies in non-relevant tissues identify disease causal genes but miss relevant tissue specific signals.

References:.

Grants:.

Conflict of Interest: None declared.

P07.029.C Mitochondrial ATP-synthase deficiency causes a clinical, biochemical and genetical disease spectrum

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Background/Objectives: Mitochondrial Complex V is composed by 18 protein subunits of which 16 are nuclear DNA encoded and 2 mitochondrial DNA (mtDNA) encoded (MT-ATP8 and MT-ATP6). Damaging genetics variants in protein subunits causes enzymatic activity deficiency and, consequently, severe mitochondrial encephalomyopathy.

Methods: Biochemical, histochemical and molecular genetics analyses including next generation sequencing of the whole mitochondrial genome were performed in biological samples (skeletal muscle, urine epithelium and blood) of probands and relatives from two unrelated families.

Results: Proband of family 1, is a 9 years-old male presenting with cerebellar ataxia, psychomotor delay and hypertrophic cardiomyopathy; brain MRI imaging showed cerebellar vermis hypoplasia and corpus callosum dysmorphism. The latter were also presented in an asymptomatic sister. Proband of family 2, is a 48 years-old male presenting with progressive retinitis pigmentosa, cataract, sensorineural deafness and ataxia. Our analyses revealed in both patients a biochemical deficiency of mt-ATP-synthase confirmed by the inheritance of novel pathogenetic variants in MT-ATP8 gene in family 1 (m.8535A>G (p.Lys57Ter) and MT-ATP6 gene (m.8858G>A (p.Gly111Asp) in family 2 with high level of hereroplasmy (95-98%) in patients' tissues while absent or very low level of heteroplasmy in unaffected family members.

Conclusion: Our study identifies novel variants in a very rare mitochondrial disorder due mitochondrial ATP-synthase deficiency and describes a clinical spectrum with a common and predominant feature represented by cerebellar ataxia and a variable multisystemic involvement.

References:.

Grants: Italian Minister of University and Research - Rita Levi Montalcini Program - Rientro cervelli RLM2017; CARISBO ricerca medica traslazionale e clinica 2021.

Conflict of Interest: None declared.

P07.031.A Biallelic SLC25A36 mutation causes hyperinsulinism/hyperammonemia syndrome

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Background/Objectives: Hyperinsulinism/Hyperammonemia (HIHA) syndrome, the second most common form of congenital hyperinsulinism, is characterized by recurrent symptomatic hypoglycemia combined with persistently elevated serum ammonia levels. HIHA syndrome has been shown to be caused by dominant activating mutations in GLUD1, encoding the intra-mitochondrial enzyme glutamate dehydrogenase (GDH). In this study, we present four children from consanguineous Bedouin kindred that were diagnosed with an autosomal recessive consistent HIHA syndrome phenotype with no mutations in GLUD1.

Methods: Genome-wide linkage analysis of ten family members was performed using 750K SNP arrays, HomozigosityMapper and SuperLink softwares. Whole exome sequencing was performed for one affected individual and analyzed using the Qiagen Clinical Insight software and our in-house database of ~700 samples.

Results: A single ~16 Mbp homozygous disease-associated locus was identified on chromosome 3, between rs6439033 and rs55940906 (maximal LOD score of 2.65). Using our filtering analysis pipeline only one homozygous variant was found within the locus: a novel highly conserved SLC25A36 c.284+3A>T splice site mutation. The variant was validated by Sanger sequencing and segregated as expected within the family. The mutation changes the 5′ splice site consensus sequence and leads to exon 3 skipping.

Conclusion: In parallel to two recent independent studies, we report a homozygous mutation in SLC25A36 as causing HIHA syndrome phenotype. The SLC25A36/PNC2 encodes a mitochondrial transporter that imports/exports pyrimidine and guanine nucleotides. Our study reinforces the involvement of SLC25A36 in the development of the HIHA syndrome through a recessive mode of inheritance.

References:.

Grants: The Morris Kahn Family Foundation.

Conflict of Interest: None declared.

P07.032.B Reverse phenotyping enables diagnosis of Smith-Lemli-Opitz syndrome in an oligosymptomatic female

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Background/Objectives: Smith-Lemli-Opitz syndrome (SLOS) is a multiple congenital malformation and intellectual disability syndrome. It is caused by biallelic sequence variants in DHCR7, which encodes the enzyme 7-dehydrocholesterol (7-DHC) reductase.

Biochemically, cholesterol concentration is low, whereas concentration of 7- and 8-DHC is elevated. The spectrum of clinical severity of SLOS is wide and some patients with mild manifestations have been reported.

Methods: Next generation sequencing and analysis of the DHCR7 gene (NM_001360.2) by Sanger sequencing were performed on two siblings with clinically diagnosed SLOS and their parents. Analysis of 7- and 8-DHC in the affected individuals was performed using gas chromatography mass spectrometry.

Results: We describe a 33-year-old female with normal psychomotor development and apart from mild microcephaly no major or minor anomalies. Her two daughters were clinically diagnosed with SLOS. Analysis of *DHCR7* revealed the same paternally inherited pathological variant c.452G>A;p.(Trp151*) in both affected daughters. Each daughter carried a different, maternally inherited pathogenic variant: c.89G>C;p.(Gly30Ala) and c.278C>T;p.(Thr93Met), for which the mother was compound heterozygous. Plasma concentrations of 7- and 8-DHC in this female were elevated, confirming the diagnosis of SLOS.

Conclusion: Biallelic pathogenic *DHCR7* variants c.89G>C;p.(Gly30Ala) and c.278C>T;p.(Thr93Met), accompanied by the typical biochemical features are associated with mild microcephaly as sole manifestation for SLOS. Both variants have been previously described in combination with other pathogenic variants, respectively. The genotype of this female leading to only mild microcephaly has not been reported in the literature.

References: Opitz et al., 1987; Fitzky et al., 1998; Blahakova et al., 2007.

Grants:.

Conflict of Interest: None declared.

P07.033.C Trio-exome sequencing reveals Infantile Liver Failure Syndrome Type 1 (ILFS1) in an infant with recurrent hepatorenal failure and status epilepticus

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Background/Objectives: Biallelic variants in *LARS1*, coding for the cytosolic leucyl-tRNA synthetase, cause infantile liver failure syndrome 1 (ILFS1), a rare disorder of aminoacylation. Especially in infancy-onset of inborn errors of metabolism an early diagnosis is crucial to guide downstream clinical management and treatment decisions.

Methods: We report on a female child born in the 32nd week of gestation with intrauterine growth restriction, metabolic acidosis, hypoglycemia showing in the further course failure to thrive, developmental delay, hepatomegaly, low albumin and microcytic anemia. Many metabolic and endocrine causes were ruled out. At the age of 12 and 16 months episodes of acute liver and renal failure occurred most probably after viral infections with extreme hyperglycemia under moderate diazoxide dosing (6 mg/kg/d) during the first episode and status epilepticus and prolonged encephalopathy during the second episode. A third episode with infection-triggered status epilepticus but without liver failure occurred at the age of 35 month. Trio exome sequencing was performed during the first episode and revealed compound-heterozygous variants of unknown significance in the *LARS1* gene.

Results: With the exclusion of other probable causes and *LARS1* activity in fibroblasts of 8 % of control fibroblasts we concluded the pathogenicity of the *LARS1* variants. A high-protein diet as recommended was probably protective.

Conclusion: In children with acute liver failure rare genetic causes should be considered early. Next generation sequencing shows advantages over a step-wise approach because even a distinct phenotype can be recognized only in retrospect in extremely rare disorders.

References: PMID: **30349989**.

Grants: No affiliations.

Conflict of Interest: None declared.

P07.034.D Next-generation diagnosis in patients with suspected mitochondrial neuromuscular diseases through a deep phenotyping and a in silico panel approach

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Background/Objectives: Mitochondrial neuromuscular diseases are highly heterogeneous disorders occurring at any age and with several clinical features including neurologic, muscular, cardiac, visual and auditory symptoms. These characteristics make the traditional diagnostic approach long, expensive and often inconclusive. Whole Exome Sequencing (WES) has revolutionized the study of rare genetic diseases and has found a wide application in clinical setting allowing to end the diagnostic odyssey typical of many heterogeneous genetic disorders, such as mitochondrial diseases.

Methods: WES strategy analysis based on standardized phenotyping by the Human Phenotype Ontology (HPO) terms and the use of in silico gene panels, in a heterogeneous cohort of 153 patients with suspected mitochondrial neuromuscular diseases. The cohort can be subdivided in patients with isolated optic atrophy (AO, 54), syndromic optic atrophy (AOplus, 16), progressive external ophthalmoplegia isolated or syndromic (CPEO, 34), adult-onset (EM, 16) and neonatal-onset encephalomyopathy (EMped, 22) and myopathy (MIO, 11).

Results: This strategy allowed to reach a diagnostic yield of 46.4%. In patients who had already undergone pre-sequencing panel (79%), very rare genes were identified. In addition, candidate genes could be identified in 4.5% of patients. The diagnosis of mitochondrial disease was confirmed in 50% of patients. The phenotypic traits most associated with mitochondrial genes are optic atrophy and ptosis, while the presence of phenocopies was high in the EMped and in the MIO patients.

Conclusion: The application of WES and a diagnostic algorithm that relies on phenotyping and filtering by in silico panels represents an effective diagnostic strategy in patients with suspected mitochondrial diseases.

References:.

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Claudio Fiorini: None declared, Mariantonietta Capristo: None declared, Maria Lucia Valentina: None declared, Giulia Severi: None declared, Leonardo Caporali: None declared, Gaetano Cantalupo: None declared, Caterina Garone: None declared, Marco seri: None declared, Valerio Carelli Stealth BioTherapeutics, Chiesi, GenSight Biologics, Stealth.

BioTherapeutics, Santhera Pharmaceuticals, and Chiesi.

P07.035.A Investigation of the role of the DNM2 gene in mitochondrial dynamics by siRNA gene silencing

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Background/Objectives: The Dynamin2 protein (DNM2) has diverse roles in cell functions, including in clathrin mediated endocytosis at the plasma membrane and together with DRP1, in mitochondrial division. DNM2 depletion blocks mitochondrial division and results in an elongated, hyper-fused mitochondrial network.

Methods: The silencing was performed for 72 hours. The efficiency of siRNA gene silencing was analysed by real-time PCR and Western blotting. RNA sequencing was performed on the properly transfected samples on Illumina NextSeq platform. The bioinformatics analysis focusing on mRNA expression changes.

Results: Gene silencing of siRNA was performed in 3 parallel measurements with 3 different siRNAs. Scrambled siRNA and non-transfected HeLa cells were used as controls for the experiments. After a bioinformatical analysis very strong significance for 12 genes were found. These genes are involved in regulation of cytoskeletal function and trafficking, muscle function, and steroid biogenesis.

Conclusion: In DNM2 depletion samples, the downregulation of the FBLIM1, KRT13, KRT19, TMEM139, TMEM45A genes are involved in cytoskeletal function and trafficking, so they might be indirectly modify mitochondrial dynamics. TNNC1 plays a major role in the regulation of muscle function, so the decrease in expression found may be related to the formation of centronuclear nuclei. CYP4F3 gene expression was also significantly decreased. It is a monooxidase involved in cholesterol and steroid biosynthesis. Its relationship with DNM2 is currently in question. A validaton of RNASeq results currently in process.

References: -.

Grants: The study was supported by the Semmelweis University StartUp, NKFIH_ 132812 and UNKP-21-5 grants.

Conflict of Interest: None declared.

P07.036.B Correlation between GLA rare variants and phenotype in Hungarian patients with Fabry disease

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Background/Objectives: Fabry disease (FD) is the second most common metabolic disorder with high morbidity and mortality. Hundreds of mutations and non-coding haplotypes in the *GLA* gene have been described; however, many are variants of unknown significance, prompting doubts about the diagnosis and treatment.

Methods: We identified *GLA* mutations in patients with suspicion of FD in Hungary for the last couple of decades. Identification of patients' genotype was done with Sanger sequencing. Patients tested were participating in FD screening projects or showed typical signs of FD or had low enzyme activity. The detected variants were classified using the current ACMG guideline, Fabry databases and literature.

Results: We found 24 different rare variants in 51 patients overall, of which 16 were classified as pathogenic, 4 likely pathogenic and 4 likely benign. Of the identified variants, 13 cause classic and 7 later onset phenotype. We also identified 2 variants that have conflicting interpretations of pathogenicity. Four of the damaging rare variants were only found in Hungarian patients so far.

Conclusion: We present a descriptive clinical study including 51 patients with 24 different *GLA* variants. We identified 4 novel rare damaging variants of the *GLA* gene. In order to better characterise VUS, not only probands but also all asymptomatic variant carriers from Fabry families should be followed prospectively. Data sharing has great importance. These data, in the future, will help to distinguish symptoms attributable to FD from nonspecific comorbidities in benign *GLA* variants carriers.

References:.

Grants: Nothing to disclose.

Conflict of Interest: None declared.

P07.037.C Molecular diagnosis of Fabry disease in patients with chronic renal failure of unknown etiology

Marina Parezanovic[1], *Maja Stojiljkovic*[1], *Marina Andjelkovic*[1], *Nina Stevanovic*[1], *Vesna Spasovski*[1], *Milena Ugrin*[1], *Jovana Komazec*[1], *Natasa Tosic*[1], *Sonja Pavlovic*[1], *Dejan Celic*[2], *Jelica Vucenovic*[3], *Anita Skakic*[1]

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Background/Objectives: Fabry disease (FD) is a rare X-linked disorder caused by variants in the *GLA* gene leading to the deficiency of lysosomal α-galactosidase-A and progressive accumulation of globotriaosylceramide affecting the heart, nervous system, and kidneys. FD has overlapping phenotypes and often remains undiagnosed. Therefore, the precise molecular-genetic diagnosis and the earliest possible treatment are essential to avoid significant disease progression.

Methods: We analyzed 95 (34 female and 61 male) hemodialysis patients with clinical suspicion of FD using Sanger sequencing of all coding exons (7) and flanking intron regions of the *GLA* gene, and measured the relative expression of the *GLA* gene in available samples.

Results: The genetic analysis revealed 3 patients with a missense variant (p.Asp313Tyr), and 10 patients with combinations of non-coding variants, described as complex intronic haplotypes (ClHs). ClH1 (c.-10C>T, c.370-81_370-77delCAGCC, c.640-16A>G,

c.1000-22C>T), the most frequent haplotype, was detected in 7 (7.4%) patients. Lyso-Gb3 biomarker levels were within the normal range in each tested patient. However, RT-qPCR analysis revealed decreased relative expression of *GLA* gene in PBMC of 2 female patients with CIH1 and one female patient carrying only c.-10C>T variant by 9,1%, 7,4%, 46,3%, respectively, pointing out that further analyses are needed to confirm/exclude FD in these patients.

Conclusion: Because the effects of CIHs are not yet fully understood, our work highlights the importance of analyzing intronic regions of the *GLA* gene as genetic modifiers and the need to include expression analysis in the diagnostic algorithm.

References:.

Grants: Genetic and biomarker analyses are sponsored by Takeda GmbH, Serbia.

Conflict of Interest: None declared.

P07.038.D An unusual case of combined hypolipidaemia and premature peripheral vascular disease

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Background/Objectives: Monogenic hypobetalipoproteinemias include a heterogeneous group of disorders characterized by very low plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and apolipoprotein B (apoB) that are relatively uncommon in general population. It is thought that low LDL can protect from CVD, but this is not what we found in a case we present. We report on a 57 years old male patient (with BMI 21 kg/m^2) with combined hypolipidaemia who presented with premature peripheral vascular disease. We also presented his two sons aged 32 and 27 years, who also manifested tendency to low lipid levels.

Methods: We used Illumina exome analysis in all three individuals. Variant filtering by QCI was performed using two different approaches: one based on candidate genes and the other one based on clinical symptoms.

Results: No *pathogenic* or *likely pathogenic* variants within main hypocholesterolaemia/dyslipidaemia candidate genes (*ANGPTL3, SAR1B, APOB*, *PCSK9* or *MTTP*) were found in any of them. However, all three individuals share a novel *ABCA1* variant, possibly responsible for decreased HDL levels. The proband and one of his sons share also the splicing variant rs138326449 within the *APOC3* gene, shown to be associated with decreased TG levels.

Conclusion: We can hypothesize that in our patient despite his low TGs and LDL levels obtained thanks to the presence of the protective *APOC3* variant, atherosclerosis developed due to the impaired cholesterol efflux caused by *ABCA1* variant.

References:.

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Conflict of Interest: None declared.

P07.039.A Untreated PKU patients without intellectual disability: SHANK gene family as a candidate modifier

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Background/Objectives: Phenylketonuria (PKU) is an inborn error of metabolism caused by variants in the phenylalanine hydroxylase (PAH) gene. Although PKU is a monogenic disease, decades of research and clinical practice have shown that the correlation between the genotype and corresponding phenotype is not simple at all. Attempts have been made to discover modifier genes for PKU cognitive phenotype but without any success so far.

Methods: We conducted whole genome sequencing of 4 subjects from unrelated non-consanguineous families who presented with pathogenic mutations in the PAH gene, high blood phenylalanine concentrations and near-normal cognitive development despite no treatment.

Results: We used cross sample analysis to select genes common for more than one patient. Thus, the SHANK gene family emerged as the only relevant gene family with variants detected in 3 of 4 analyzed patients. We detected two novel variants, p.Pro1591Ala in SHANK1 and p.Asp18Asn in SHANK2, as well as SHANK2:p.Gly46Ser, SHANK2:p.Pro1388_Phe1389insLeuPro and SHANK3:p.Pro1716Thr variants that were previously described. Computational analysis indicated that the identified variants do not abolish the function of SHANK proteins. However, changes in posttranslational modifications of SHANK proteins could influence functioning of the glutamatergic synapses, cytoskeleton regulation and contribute to maintaining optimal synaptic density and number of dendritic spines.

Conclusion: Our findings are linking SHANK gene family and brain plasticity in PKU for the first time. We hypothesize that variant SHANK proteins maintain optimal synaptic density and number of dendritic spines under high concentrations of phenylalanine and could have protective modifying effect on cognitive development of PKU patients.

References:.

Grants: MESTD-RS 451-03-68/2022-14/200042.

Conflict of Interest: None declared.

P07.040.B Clinical, genetic and therapeutic aspects in Menkes disease: study of a French cohort and systematic literature review

Paul Rollier[1;2], *Marie-Pierre Moizard[3;4], Annick Toutain[5], Sophie Blesson[5], Eric Bieth[6], Chrystèle Bonnemains[7], Aline Cano[8], Brigitte Chabrol[8], Annabelle Chaussenot[9], Léna Damaj[10], François Feillet[7], Sylvie Joriot[11], Manoelle Kossorotoff[12], Christian Richelme[13], Dominique Bonneau[14], Sylvie Odent[1], Magalie Barth[14]*

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Background/Objectives: Menkes disease has multi-organ involvement with neurological, cutaneous, urological, vascular and bone complications and historically death before 3 years. Copper histidinate (CuHis) is the only specific treatment but with variable results. The objective was to describe clinical and genetic characteristics of a French cohort and discuss therapeutic management.

Methods: A cohort was constructed from genetic diagnoses (*ATP7A*) of Menkes in France and compared with a literature systematic review.

Results: Diagnostic yield was 81% (71/88 cases) with 50% undescribed variants. 24.6% are intragenic deletions/duplications, 50.8% are loss-of-function and 24.6% are missense. Missenses are distributed exclusively from exon 7 onwards and can result on splicing defect. Of the 24 individuals (clinical subcohort), average age at diagnosis was 4.68±2.16 months. Symptoms at diagnosis are hypotonia (96%), epilepsy (88%), pili torti (38%), skin pallor (44%). Neonatal hypothermia (53%) and cephalhaematomas (27%), although non-specific, are overrepresented. CuHis was started on 55% of individuals (mean age 5.1±2.8 months), without significant improvement on survival or development, or of genotype-phenotype correlation distinguishing best responders. Comparing with the literature, CuHis is only effective (survival and neurodevelopment) when initiated in infants who are not yet neurologically impaired. Epilepsy occurred in all individuals in our cohort even when CuHis was started before first seizures. CuHis may also provide better control of urological complications but not on vascular and bone phenotypes.

Conclusion: Menkes disease remains a non-curable disease with limited therapeutic range and poor prognosis. CuHis should only be offered in first intention in presymptomatic individuals.

References:.

Grants: No funding source.

Conflict of Interest: None declared.

P07.041.C Novel MECR mutation in a Czech patient with childhood-onset dystonia, optic atrophy, and basal ganglia abnormality

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Background/Objectives: Gene *MECR* encodes a highly conserved mitochondrial trans-2-enoyl-coenzyme-A-reductase which has a crucial role in mitochondrial fatty acid synthesis (mFAS). Here we report a 12-year-old patient with recessive mutations in *MECR* who presented with childhood-onset dystonia, hypoplasia and atrophy of the optical nerves, and abnormal MRI signaling of basal ganglia and dorsal mesencephalon.

Methods: Family-based whole-exome sequencing of DNA extracted from peripheral blood lymphocytes was followed by bioinformatics analysis of the TRIO using the software VarAFT. Functional measurements of mitochondrial oxidative phosphorylation (OXPHOS) were performed on skin fibrocytes via high-resolution respirometry (OroborosOxygraph). Western blot was used to detect individual mitochondrial proteins and to study the assembly of OXPHOS complexes.

Results: Within gene *MECR*, we found a novel variant c.610G>C p.(Ala204Pro) and previously described variant c.772C>T p.(Arg258Trp) in a compound heterozygous state. Functional measurements of OXPHOS on fibrocyte cell line found a statistically significant decrease of the mitochondrial electron transport chain (ETC) capacity, complex I and IV capacity, and overall mild decrease of respiration. Western blot showed a decreased quantity of complexes I and IV.

Conclusion: We described a novel variant c.610G>C in *MECR* in an individual with a phenotype consistent with previously described cases of the *MECR*-related neurologic disorder (1). The pathogenicity of the variant was confirmed by functional analysis.

References: 1. Heimer G, Kerätär JM, Riley LG, et al. MECR Mutations Cause Childhood-Onset Dystonia and Optic Atrophy, a Mitochondrial Fatty Acid Synthesis Disorder. *Am J Hum Genet*. 2016;99(6):1229-1244. https://doi.org/10.1016/j.ajhg.2016.09.021.

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Conflict of Interest: None declared.

P07.042.D False positive newborn screening for maple syrup urine disease in patients with hydroxyprolinemia

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Background/Objectives: Newborn screening (NBS) for maple syrup urine disease (MSUD) by tandem mass spectrometry utilizes leucine as primary marker. The isobaric amino acids leucine, isoleucine, alloisoleucine and hydroxyproline (OH-Pro) are not distinguished by this method. Hydroxyprolinemia is an autosomal recessive benign condition (1) and therefore should not be identified by NBS.

Methods: Of six healthy newborns with NBS results presumptive positive for MSUD two were hospitalized until MSUD was excluded. The other four patients had plasma and urine collected for amino acid (PAA) and organic acid (UOA) analyses at an outpatient visit. Original NBS specimens of two cases were available for retrospective measurement of leucine, isoleucine, valine, alloisoleucine and OH-Pro (2).

Results: PAA and UOA analyses excluded MSUD and disclosed elevations of OH-Pro (237-424 mmol/L; controls <61) in all six cases. OH-Pro but not leucine, isoleucine, valine or allo-isoleucine, was elevated in the NBS specimens of two cases. Molecular genetic analysis of *PRODH2* is underway.

Conclusion: MSUD is a critical condition that requires rapid initiation of treatment. However, hydroxyprolinemia is more common and therefore a cause of false positive NBS results (1). Total parenteral nutrition can also cause false positive results (2). Employing a second-tier NBS test in the original dried blood spot specimen to differentiate between branch-chain amino acids and OH-Pro can avoid the cost and anxiety associated with false positive results of NBS for MSUD.

References: 1. Staufner et al. J Inherit Metab Dis. 2016;39:625-32. 2. Oglesbee et al. Clin Chem 2008;54:542-9.

Grants:.

Conflict of Interest: Dimitar Gavrilov: None declared, Amy White: None declared, Faisal Asumda: None declared, Noemi Vidal

Folch: None declared, Laura Davis-Keppen: None declared, Isum Ward: None declared, Kari Casas: None declared, Matthew Schultz: None declared, Devin Oglesbee: None declared, Silvia Tortorelli: None declared, Dietrich Matern Advisory board, Novogene, 4 hrs (honorarium passed on to my employer, Mayo Clinic).

P07.043.A Clinical and metabolomic peculiarities in monogenic disorders affecting urea cycle or mitochondrial ATP synthase

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Background/Objectives: The clinical-biochemical findings in born errors of metabolism (IEMs) are sometimes nonspecific. A metabolomic approach involves a quantitative analysis of a set of metabolites present in urine or/and plasma linking them to a selected metabolic pathway and/or particular metabolic condition.

Methods: Results from plasma and/or urine amino acids analyzed by a two-dimensional thin-layer-chromatography (2D-TLC) as an urgent selective screening in patients from pediatric clinical departments were compared with urinary NMR spectroscopic results in several IEMs. ^{1}H-NMR urine spectroscopy constitutes a complementary technique in the diagnosis of several IEMs, providing an overall view of metabolism, giving a "fingerprint" of almost all hydrogen nuclei in a metabolite.

Results: We summarize clinical data and biochemical comparative markers obtained using these two methods in several urea cycle disorders (ornithine transcarbamylase - OTC and argininosuccinic aciduria - AAS), and a mitochondrial disorder - the TMEM70 defect evolving with mild or severe hyperlactataemia. In TMEM70 defect, several plasma amino acids were outside the normal ranges as nonspecific changes, but the diagnostic was established by molecular DNA investigation. Contrary, the orotic acid and argininosuccinic acid were rapidly detected using urinary NMR spectroscopy in ornithine transcarbamylase defect. In AAS, the urinary 2D-TLC method was useful for rapid identification of high concentration of argininosuccinic acid.

Conclusion: The 2D-TLC and ^{1}H-NMR spectroscopy methods available for a rapid evaluation may be a versatile association for several specific molecules. Identification of such bio-markers demonstrated important potential for including more rare diseases into current national screening programme and research.

References:.

Grants:.

Conflict of Interest: None declared.

P07.045.C Shifting the border of pathogenicity with a DEL9 mutation in the CEL gene

Maria Grossmann[1], *Janek Wilk*[2], *Andrea Bier*[1], *Silke Reif*[1], *Manuela Timmer*[1], *Christian Jänecke*[1], *Vivien Klaschka*[1], *Beate Küchler*[2], *Albrecht Kobelt*[3], *Stefan Krüger*[1], *Jens Plaschke*[1;2]

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Background/Objectives: Maturity-onset diabetes of the young (MODY) is a group of autosomal dominantly inherited disorders of non-autoimmune diabetes mellitus with usual onset in adolescence or young adulthood. Most MODY mutations are located in genes *GCK, HNF1A*, *HNF4A* and *HNF1B*. Rarely, mutations in other contributing genes (*APPL1, ABCC8, BLK, CEL, INS, KCNJ11, KLF11, NEUROD1, PAX4, PDX1*) are causative for MODY.

In the last exon of the *CEL* gene, single-base deletions within the 33bp VNTR (Variable Number of Tandem Repeat) region causing different C-terminal aberrant proteins have been described. The pathogenic potential is higher for mutations located in more 5′ VNTR parts. For DEL1 (named after the affected repeat number) and DEL4 a negative gain-of-function effect has been established by functional studies. In contrast, DEL13 was considered as benign variant due to a behavior like wildtype, whereas DEL9 showed intermediate effects and was concluded to be likely benign because of observation in healthy controls.

Here we report two sisters (34 and 37 years) and their mother (61 years) affected by MODY.

Methods: NGS analysis for all above-mentioned 14 genes was performed.

Results: For all three patients we identified the *CEL* mutation NM_001807.6:c.1941delC, p.(Val648Cysfs*45) in a heterozygous state affecting VNTR repeat 9 (DEL9 regarding individual VNTR haplotype).

Conclusion: Due to co-segregation in this family we consider the *CEL* DEL9 as pathogenic. This shifts the previously assumed border of pathogenicity to a more 3′end of the gene.

References: Gravdal et al. J Biol Chem. Jan-Jun 2021;296:100661.

Grants:.

Conflict of Interest: None declared.

P07.046.D MELAS: clinical and laboratory-based diagnostic algorithm

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Background/Objectives: Mitochondrial Encephalopathy with Lactic Acidosis Syndrome (MELAS) is a mitochondrial disease caused by point mutations or deletions in the mitochondrial or even nuclear genome. The pathogenesis of MELAS is not fully understood, so several hypotheses have been proposed. Some of the most obvious processes involved are related to the accumulation of RNA19 in active metabolic tissues, mitochondrial

proliferation due to energy and nitric oxide deficiency, impaired glucose metabolism with lactic acidosis.

Methods: Following a review of the current literature on the diagnosis methods and criteria of MELAS, we have developed a quick algorithm for helping clinicians in establishing the diagnosis of MELAS.

Results: The Hirano et al. (1992) and Yatsuga et al. (2012) criteria remain the benchmark for selecting patients suspected of MELAS, based on their clinical picture such as: seizures, headache with vomiting, and myopathy. However, to secure the diagnosis, performing PCR-RFLP in a common set of affected-genes, including the prevalent m.3243A>G substitution, should be done first on a MELAS-suspected, only then followed by full mitochondrial and even nuclear genomic testing or, if sequencing is not possible, by allele-specific oligonucleotide (ASO) dot blot hybridization, which is more sensitive than PCR.

Conclusion: Beside the proposed clinical algorithm, muscle biopsy/blood lactate cannot provide certain evidence for MELAS diagnosis, which is why molecular testing should be prioritized over other clinical tests when establishing a diagnosis; yet a genetic test positive for a mutation in the mitochondrial DNA should still be correlated with any of the first two tests.

References:.

Grants:.

Conflict of Interest: None declared.

P08

IMMUNOLOGY AND HEMATOPOIETIC SYSTEM

P08.001.A The value of genetic data from 665,460 individuals in predicting anemia and ability to donate blood

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Background/Objectives: We aim to find out whether genetic information has significant effect in predicting iron deficiency anemia or blood donation ability.

Methods: Genetic data from FinnGen release 6 (230,000 participants), Blood Service Biobank (30,000 participants) and UK Biobank (400,000 participants) was analyzed. In addition, age, sex, weight, height, anemia status and blood donation histories were used when available.

We performed GWAS for anemia and blood donation ability and computed polygenic risk score weights for anemia, ferritin[1] and hemoglobin[2]. A Bayesian logistic regression for anemia was fitted on all FinnGen participants and for donation ability on blood donors, stratified into three demographic groups.

Results: A single significant SNP rs199598395 in gene RNF43 was revealed by our anemia and deferral GWAS. The meta-analysis from FinnGen and UKBB for anemia provided three more significant lead SNPs. The largest effect of the genetic data in anemia model was by the RNF43 SNP for pre-menopausal females (OR 4.3, CI 2.6 – 6.7) and in the deferral model again the RNF43 SNP for pre-menopausal females (OR 3.2, CI 1.9 – 5.4). PRSs didn't have significant effects in either model.

Conclusion: A single SNP can have a strong effect on prediction of both anemia and blood donation ability. PRSs are not yet informative enough to be usable predictors.

References: [1] Bell et al. A genome-wide meta-analysis yields 46 new loci associating with biomarkers of iron homeostasis. *Commun Biol* **4**, 156 (2021).

[2] Vuckovic et al. The Polygenic and Monogenic Basis of Blood Traits and Diseases. Cell. 2020 Sep 3;182(5):1214-1231.e11.

Grants:.

Conflict of Interest: Jarkko Toivonen Full-time employee at Finnish Red Cross Blood Service. Significant contribution., Johanna Castrén Full-time employee at Finnish Red Cross Blood Service. Significant contribution., FinnGen Consortium: None declared, Mikko Arvas Full-time employee at Finnish Red Cross Blood Service. Significant contribution.

P08.002.B Added value of reanalysing whole exome- and whole genome sequencing data with an extended gene panel and structural variant calling in patients suspected of having primary immune deficiency

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Background/Objectives: Knowledge of genetic variation underlying Primary Immune Deficiency (PID) is steadily increasing(1). Reanalysis of genome-wide sequencing data from patients suspected of PID may improve the diagnostic rate.

Methods: We included patients monitored at the Department of Infectious Diseases, Rigshospitalet, Denmark, for a suspected PID, who had been analysed during 2015-2020 using a targeted PID gene panel (457 PID-related genes) on whole exome- (WES) or whole genome sequencing (WGS) data. A literature review was performed to extend the PID gene panel used for reanalysis of single nucleotide variation (SNV) and small indels. Structural variant (SV) calling was added on WGS data.

Results: In total, genetic data from 94 patients (86 adults) was reanalysed a median of 23 months after the initial analysis (38 WES and 56 WGS). The extended gene panel included 208 additional PID-related genes. The total proportion of patients with ACMG class 3-5 variants increased from 43 (46 %) to 50 (53 %). The proportion of patients with a causal genetic diagnosis was constant, but after reanalysis 13 patients (14 %) had a new potentially disease causing/contributing variant of unknown significance (VUS) identified. Among these, 8 of 94 (9 %) had a SNV and 5 of 56 (9 %) patients had a SV.

Conclusion: These data indicate a possible diagnostic gain of reassessing WES/WGS data from patients with suspected PID. Reasons for the possible gain included improved knowledge of genotype-phenotype correlation, expanding the gene panel, and adding SV analyses.

References: (1) PMID: 31953710.

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P08.003.C Copy number variants in pediatric patients with suspected inborn errors of immunity

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Background/Objectives: Exome sequencing (ES) results do not rule out genetic contribution to disease, including contribution from structural variation. We evaluated 332 pediatric patients with suspected inborn errors of immunity (IEI) to determine the relative contribution of ES and chromosomal microarray (CMA).

Methods: ES and CMA customized for exonic coverage of immune-related genes were performed to study 332 unrelated pediatric probands referred to the National Institute of Allergy and Infectious Diseases Centralized Sequencing Program for suspected IEI.

Results: Nearly one-third (107/332) of patients' phenotypes involved 10 or more top-level Human Phenotype Ontology categories, most commonly the immune system, integument, respiratory system, digestive system, and blood/blood-forming tissues, in decreasing frequency.

Of the 332 probands, 131 (39.5%) received at least one molecular diagnosis. In total, 113/131 (86.2%) probands were diagnosed by ES alone; 15/131 (11.5%) were diagnosed by CMA alone, including two de novo changes. ES and CMA both contributed to diagnoses for 3/131 (2.3%) probands, including two compound heterozygotes.

Nine of the 18 patients with CMA contribution to diagnosis had copy number variants in at least one gene not listed by the International Union of Immunological Sciences as causative of IEI. Six were primarily neurological, one was cardiovascular, one was metabolic, and one was respiratory.

Conclusion: CMA findings contributed to over one-in-ten (18/131) molecular diagnoses. Although most diagnoses were made by ES, CMA can increase the likelihood of molecular diagnosis when ES is inconclusive. Half of CMA diagnoses at least partially involved non-immune phenotypes, and so would not typically appear on commercial panels for immune disorders.

References:.

Grants:.

Conflict of Interest: None declared.

P08.004.D Trio-based sequencing in patients with sporadic inborn errors of immunity: a retrospective cohort study

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Background/Objectives: De novo variants (DNVs) are currently not routinely evaluated as part of diagnostic whole exome sequencing (WES) in patients with inborn errors of immunity (IEI). This study explored the additional value of systematic DNV assessment in a retrospective cohort of 123 patients with sporadic PIDs.

Methods: Patient-parent trios sequenced at the Radboud University Medical Center were eligible for inclusion when 1) the IEI in-silico gene panel was analysed and 2) the phenotype of the index patient suggested sporadic disease. Exome-wide analysis was performed to retain rare, coding, non-synonymous de novo SNVs. Variants were further prioritized based on gene and variant level metrics. In immune cells from one selected patient, functional validation experiments were performed at the level of RNA splicing, NF-κB signalling and cytokine production.

Results: Candidate DNVs were identified in 15 (12.2%) trios, in addition to 12 (9.8%) with inherited (likely) pathogenic mutations. These potentially disease-causing DNVs were identified in the known IEI genes *NLRP3* and *RELA*, and novel candidate genes including *PSMB10, DDX1, KMT2C* and *FBXW11*. Furthermore, the *FBXW11* canonical splice site DNV, carried by a patient with autoinflammatory disease, was shown to cause defective RNA splicing, increased NF-κB signalling and elevated IL-1β production.

Conclusion: This retrospective cohort study advocates the implementation of trio-based sequencing in routine diagnostics of patients with sporadic IEI to improve the solve rate. Furthermore, we have provided functional evidence in support of a causal role for *FBXW11* loss-of-function mutations in autoinflammatory disease.

References:.

Grants: This research was part of a Radboud Institute for Molecular Life Sciences PhD grant.

Conflict of Interest: None declared.

P08.005.A Genetic characterization of non-familial hemophagocytic lymphohistiocytosis patients: monogenic defects in HAVCR2, TNFRSF9 and MADD genes

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Background/Objectives: Hemophagocytic lymphohistiocytosis (HLH) is a severe and life-threatening syndrome characterized by a strong hyperactivation of the immune system. Familial HLH (FHL) is caused by biallelic variants in the genes *PRF1*, *UNC13D*, *STX11* and *STXBP2*. Non-familial HLH (nFHL) may be caused by other genetic entities or may be sporadic or secondary to other conditions.

Methods: To elucidate other genetic mechanisms driving nFHL we studied a cohort of 31 nFHL patients (25 European, 5 North-African and 2 Asian). All cases had an immunological diagnosis of HLH but remained unsolved at a genetic level after excluding pathogenic variants in FHL genes.

Results: Using WES data, we first evaluated the possibility of the disease being casued by a monogenic defect. We found the genetic diagnosis for three of the patients i) one patient who carried a compound heterozygous variant at *HAVCR2*, ii) one with a homozygous nonsense variant at *TNFRSF9*, and iii) one with a homozygous deletion at *MADD*. Next, we looked for pathogenic variants over-represented in our cohort in comparison to healthy populations, identified monoallelic variants that could be acting as risk factors, and explored the digenic model in genes of the cytotoxic pathway.

Conclusion: Altogether, we showed that WES is a valuable diagnostic tool in nFHL patients, provided new insights into the physiopathology of the disease, and insisted on the importance of a genetic diagnosis on the clinical management and genetic counseling of the patients and their families.

References:.

Grants:.
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Conflict of Interest: None declared.

P08.006.B First description of a bone marrow failure syndrome in Spain caused by compound heterozygous truncating mutations in ERCC6L2

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Background/Objectives: Biallelic variants in ERCC6L2 have been recently described to cause inherited bone marrow failure syndrome type 2 (BMFS-2) which is characterized by hypocellular bone marrow and, frequently, microcephaly. We describe the case of two siblings diagnosed at 8 and 14 years with medullary aplasia and thrombocytopenia (60-80e9platelets/L) without extra-hematopoietic manifestations. The aim was to identify the molecular defect associated with the patients' phenotype using a whole exome sequencing (WES) strategy.

Methods: WES was performed using the DNA Prep with Enrichment protocol (Illumina) and sequenced in NextSeq500 system (Illumina). Variants were identified with BWA Enrichment and annotated and filtered with Variant Interpreter (Illumina). Family based trio analysis including the affected siblings and their parents (unaffected) was carried out using recessive inheritance pattern.

Results: Two truncating ERCC6L2 pathogenic variants, according to ACMG guidelines, were identified in both patients: 1) NM_020207:c.1930C>T (p.Arg644Ter) in exon 13, the second most recurrent variant, only found in homozygous state; 2) NM_020207:c.2156del (p.Gly719AspfsTer50), located in exon 15, previously described in a single patient.

Conclusion: This is the first description in Spain of medullar aplasia caused by ERCC6L2 mutations. Our findings contribute to the small worldwide cohort of 20 families with BMFS-2, with only four caused by compound heterozygous mutations in ERCC6L2. Early detection of the genetic defect will help in clinical management, which is relevant due to the susceptibility of BMFS-2 patients to myelodysplastic syndrome and acute myeloid leukaemia.

References: PMID: 29633571; 29987015; 29146883; 30936069.

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Conflict of Interest: None declared.

P08.007.C A homozygous STING1 gene variant causes STING-associated vasculopathy with onset in infancy (SAVI) in two patients

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Background/Objectives: Stimulator of interferon response cGAMP interactor 1 (*STING1*) gain of function (GoF) variants cause STING-associated vasculopathy with onset in infancy (SAVI; AD inheritance). While most causative heterozygous variants arise de novo, Lin et al. (2020) showed that *STING1* variant p.(R281W) causes SAVI only in homozygous state. Here we report two further patients carrying *STING1* homozygous variant p.(R281W).

Methods: Whole exome sequencing (WES) and whole genome sequencing (WGS) were applied respectively to identify causative variant in patient 1 and 2.

Results: *STING1* (NM_198282.3): c.841C>T p.(R281W) was identified in two patients and classified as "pathogenic". Patient 1 presented with recurrent severe hypoxaemia, hypoventilation, diffuse alveolar hemorrhage, interstitial lung disease, without skin vasculitis. Segregation analysis within the consanguineous family showed that healthy parents and one brother carried variant heterozygously. The interferon signature was highly elevated in patient 1 but not in healthy heterozygous family members. This correlates to published cases displaying that the heterozygous p.(R281W) variant did not affect the interferon signature. No significant improvement was observed after therapy with Janus kinase inhibitor Baricitinib. Patient 2 showed hypoventilation with scarring fibrotic changes, bronchiectasis, tachypnea, failure to thrive with marked dystrophy, hypocalcemia, and also no skin vasculitis.

Conclusion: Our report supports the possibility that p.(R281W) causes an atypical SAVI phenotype without demonstrating vasculopathy. It is important to consider an autosomal recessive inheritance pattern when evidence of STING-associated vasculopathy is given.

References: No references.

Grants: German Research Foundation (DFG) under Germany's Excellence Strategy - EXC 2155 - RESIST project number 390874280.

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Conflict of Interest: None declared.

P08.009.A Influence of HBG2, BCL11A and HMIP polymorphisms on the clinical phenotype in Iraqis with beta-thalassemia

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Background/Objectives: The variability in β-thalassemia phenotype has been attributed to several genetic modifiers. The significance of the latter varies in different populations. The objective of the current study was to determine the significance of six genetic modifiers as they relate to phenotype in Iraqi β-thalassemia patients and to create a genetic scoring system that could predict phenotype in patients from this part of the world.

Methods: A total of 224 Iraqi patients homozygous or compound heterozygous for β-thalassemia were assessed for five polymorphisms at HbF QTLs, namely: rs7482144 C>T at *HBG2*, rs1427407 G>T and rs10189857 A>G at *BCL11A*, and rs28384513 A>C and rs9399137 T>C at *HMIP*.

Results: The enrolled patients had a median age of 14 years, with 96 males and 128 females. They included 144 thalassemia major (TM) and 80 thalassemia intermedia (TI) patients. Multivariate logistic regression revealed that three genetic modifiers, namely β^+ alleles, *HBG2* rs7482144 and *BCL11A* rs1427407, could significantly predict phenotype (TM versus TI) with an overall prediction rate of 82.1%. A cumulative favorable allele score based on these significant predictors had an area under curve of 0.862 (95% CI 0.816-0.909), which was highly significant (P = 3.2656E-19).

Conclusion: The current study identified three genetic predictors of phenotype in Iraqi patients with β-thalassemia, with an overall prediction rate of 82.1%. Furthermore, the population-specific cumulative favorable allele scoring system created had a good ability to discriminate between TM and TI. The application of the latter may help in providing more informed management and therapeutic options in this region.

References:.

Grants:.

Conflict of Interest: None declared.

P08.012.D Genetics in inborn errors of immunity: pediatric autoinflammatory phenotypes and the underlying genetic causes in 125 families

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Background/Objectives: Diagnosis of monogenic autoinflammatory diseases (AID) requires an accurate description of the patients´ phenotype and the identification of highly penetrant genetic variants in single genes is pivotal.

Methods: In a routine genetic diagnostic setting, we performed whole exome sequencing (WES) of 125 pediatric patients with suspected monogenic AID. Datasets were analyzed in a stepwise approach to identify the most feasible diagnostic strategy: First, we analyzed a virtual gene panel including 13 genes associated with known AID and, if no genetic diagnosis could be established, followed by the analysis of a virtual panel including 420 genes published by the International Union of Immunological Societies associated with all known inborn errors of immunity (IEI). Subsequently WES data were analyzed without pre-filtering for known AID/IEI genes.

Results: Analyzing 13 genes yielded a definite diagnosis in 16.0% (n = 20). The diagnostic yield was increased by analyzing 420 genes to 21.8% (n = 26). Importantly, expanding the analysis to WES data did not increase the diagnostic yield in our cohort, neither in single WES analysis nor in trio-WES analysis.

However variants of unknown significance were detected in 43.2%, (n = 54) which could become clinically relevant in the future.

Conclusion: The study highlights that analyzing virtual gene panels based on WES that include the majority of known genes causing AID or differential diagnosis can rapidly confirm the diagnosis for a large number of pediatric patients. WES data or trio-WES data analysis as a first-tier diagnostic analysis in patients with suspected monogenic AID is of minor use.

References: /.

Grants: /.

Conflict of Interest: None declared.

P08.013.A Transcriptomes of MPO-deficient patients with generalized pustular psoriasis reveals expansion of CD4+ cytotoxic T cells and an involvement of the complement system

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Background/Objectives: Generalized pustular psoriasis (GPP) is a severe psoriatic subtype characterized by epidermal neutrophil infiltration. Although variants in *IL36RN* and *MPO* have been shown to affect immune cells contributing to GPP's pathogenesis, a systematic analysis of neutrophils and of a wide variety of PBMC subsets and their differential gene expression dependent on *MPO* genotypes was not performed yet.

Methods: We assessed transcriptomes of MPO-deficient patients using single cell RNA-sequencing (scRNAseq) of peripheral blood mononuclear cells (PBMCs) and RNA-sequencing of neutrophils in relatively stable disease state.

Results: Cell type annotation by multimodal reference mapping of scRNAseq data was verified by flow cytometry of surface and intracellular markers; proportions of $CD4^+$ cytotoxic T-lymphocytes (CTLs) and other $CD4^+$ effector cells were increased in GPP, while frequencies of naïve $CD4^+$ T cells were

significantly lower. The expression of the marker gene for $CD4^+$ CTLs and $CD8^+$ effector memory T-cells (TEMs) *FGFBP2* was elevated in GPP patients with disease-contributing variants compared to healthy and diseased non-carriers ($p = 0.0015$) based on quantitative RT-PCR. Differentially expressed genes (DEGs) involved in the complement system were enriched in patients' neutrophils.

Conclusion: Future studies assessing affected cell types and pathways will show their contribution to GPP's pathogenesis, and indicate whether findings can be transferred to the situation in affected skin and whether depletion or inactivation of $CD4^+$ CTLs may be a reasonable therapeutic approach.

References:.

Grants: CRC1181, project A05.

Conflict of Interest: None declared.

P08.014.B Filaggrin loss-of-function mutations are associated with the persistence of egg and milk allergy

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Background/Objectives: A genetic defect in the epidermal barrier protein filaggrin plays a major role in the etiology of eczema and associated allergic airways diseases. However, it is still controversial to what extend loss-of-function (LOF) mutations in the filaggrin gene (*FLG*) contribute to the development of food allergies. We tested association of *FLG* LOF mutations with allergic reactions to diverse foods and investigated their effect on the persistence of early food allergies.

Methods: We recruited 890 children with challenge-proven food allergy for the German Genetics of Food Allergy Study (GOFA). All children were genotyped for the four most common LOF mutations in *FLG*; R501X, 2282del4, R2447X, and S3247X. Associations between *FLG* mutations and food allergies were analyzed by logistic regression using the German Multicenter Allergy Study cohort as control population.

Results: *FLG* mutations were associated with allergies to diverse foods including hen's egg (HE), cow's milk (CM), peanut, hazelnut, fish, soy, cashew, walnut, and sesame with similar risk estimates. Effects remained significant after adjusting for the eczema status. Interestingly, *FLG* mutations increased the risk of a persistent course of HE and CM allergy.

Conclusion: Using the gold standard for food allergy diagnosis, we demonstrate that *FLG* LOF mutations confer risk of any food allergy independent of eczema. They predispose to the persistence of HE and CM allergy and should be considered in the assessment of tolerance development.

References:.

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Conflict of Interest: None declared.

P08.015.C Ethnicity-specific GWAS to explore the genomic architecture of platelet count in the Northeast Indian subcontinent

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Background/Objectives: Blood cell traits are highly heritable, yet most known genomic associations have been identified in broad population sub-groups. We compared the genomic determinants of platelet count (PLT) in Pakistanis and Bangladeshis, a genetically distinct population from the Northeast Indian subcontinent where thrombocytopenia (PLT<150 $x10^9$/L) is reported in 35%.[1].

Methods: Data were analysed from British Bangladeshi (BAN, $n = 20{,}292$) and British Pakistani (PAK, $n = 9{,}237$) individuals (https://www.genesandhealth.org/). Associations between TOPMed-imputed genotypes and mean PLT were calculated using BOLT-LMM and LD score regression software. Variants were annotated using the Ensembl Variant Effect Predictor.

Results: PLT was slightly lower in BAN (mean 266.4 $x10^9$/L) than PAK (mean 271.5 $x10^9$/L, $p = 1.16e^{-10}$) but respective rates of thrombocytopenia (1.5% vs 1.8%) and trait heritability (0.214 vs 0.216) were comparable. PLT was lower (mean 255.2 $x10^9$/L) and had higher heritability (0.230) in a previously reported European (EUR) population ($n = 166{,}066$).[2] Genetic correlation for PLT was higher between BAN and PAK (r_g 1.11, SE 0.2) than for BAN and EUR (r_g 0.82, SE 0.07). GWAS-significant variants in BAN were in regions associated with PLT in EUR, except for a *MAST2* intronic loci previously associated with PLT in East Asian populations.

Conclusion: Loci associated with PLT were concordant between BAN and PAK, and largely reproduced previous findings in EUR. Thrombocytopenia observed in the Northeast Indian subcontinent was not reproduced in the British BAN population in which there was also no discernible genomic explanation for lower PLT.

References: 1. Nania et al., J Thromb Haemost. 2005, 2, 2581-1582.

2. Astle et al., Cell 2016, 167, 1415-1429.

Grants:.

Conflict of Interest: None declared.

P08.016.D Italian ancestral HLA haplotype predisposing to severe COVID-19 by low spike and high IFNα binding affinity

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Background/Objectives: Validated association between COVID-19 and the most obvious candidate genes, e.g. *HLA*, is still missing. A weak association with class I HLA- C*04:01 was found for infection in Sardinians and for severity in another mixed population. Auto-antibodies to interferon type I have been implicated in the severity of COVID-19 in two studies.

Methods: The binding affinity between *HLA* molecules and SARS-CoV-2 spike protein and IFNα subunits was evaluated in silico. The presence of antibodies against one or more of the 12 IFNα subunits was evaluated in 160 hospitalized COVID-19 patients. The 10 most frequent haplotypes in the Italian population were tested in 1.997 SARS-CoV-2 infected patients (hospitalized versus not hospitalized).

Results: The presence of auto-antibodies against at least one IFNα subunit was detected in 26% of patients. The haplotype A*24:02-B*35:02-C*04:01-DRB1*11:04-DQB1*03:01 was found to predispose to severity ($p = 0.0018$; $p = 0.07$ after Bonferroni correction) in patients <50 years. The haplotype includes alleles able to bind spike with low affinity (i.e. C*04:01 and DRB1*11:04) and IFNα with high affinity (i.e. DRB1*11:04).

Conclusion: One of the 10 most frequent ancestral haplotype of the Italian population predisposes to severity likely reducing both innate immunity through IFNα auto-antibodies induction and adaptive immunity through weaker spike protein presentation.

References: Daga S et al. Employing a systematic approach to biobanking and analyzing clinical and genetic data for advancing COVID-19 research. Eur J Hum Genet. 2021 Jan 17:1-15.

Grants: FISR 2020 / Tuscany Region COVID-19 / INTERVENE - GA No. 101016775 / Soka Gakkai PAT-COVID.

Conflict of Interest: None declared.

P08.017.A High prevalence of Netherton syndrome in Latvian population caused by founder SPINK5 variant

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Background/Objectives: Netherton syndrome (NTS) is a rare autosomal recessive inborn error of immunity caused by biallelic *SPINK5* loss-of-function variants. NTS is characterized by skin and immune system abnormalities, but with variable expressivity. Therefore, we aimed to clinically and molecularly characterize NTS in individuals with homogenous genotype and ethnicity.

Methods: We selected all Latvian NTS patients diagnosed in Children's Clinical University Hospital by using exome sequencing (ES). Unrelatedness, as well as haplotype lengh for variant's age estimation in individuals with homozygous pathogenic variant was analysed using high-quality variants from ES.

Results: We identified pathogenic biallelic variants in 9 individuals (age 1 month to 17 years) from 7 families, confirming NTS diagnosis. Clinically, all patients have ichthyosis, hair abnormalities, developmental delay, high total IgE levels (371-6977 kU/L), and recurrent skin and respiratory infections with variable severity. Surprisingly, 5/7 families had the same homozygous *SPINK5* variant (NM_006846.4:c.1048C>T,p.(Arg350*)) and two siblings carried the same variant in compound heterozygous state (c.1048C>T,p.(Arg350*); c.1430+4A>G,r.spl.). Therefore, we suspected that the variant is founder in Latvian population. NTS prevalence in Latvian population is higher: 1:256,000 (vs. ~1:1,000,000 in France by Dreifus I. et. Al. 2014) and carrier frequency is estimated 1:300. Assuming a 'correlated' genealogy, the mutation arose 47.4(95%CI 14.9-167.2) generations and 1175 years (assuming one generation 25y) ago.

Conclusion: We report high prevalence of NTS in Latvia due to a common founder variant that arose ~50 generations ago. Interestingly, despite similar ethnical and identical genotype, NTS expressivity was variable among the individuals.

References:.

Grants: Latvian Council of Science project:lzp-2020/1-0269.

Conflict of Interest: None declared.

P08.018.B Microenvironmental determinants of clonal hematopoiesis expansion rate

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Background/Objectives: Clonal hematopoiesis of indeterminate potential (CHIP) is a clonal expansion of blood cells caused by somatic mutations. CHIP confers risk of multiple aging diseases including blood cancer and cardiovascular disease. It is presently unknown what causes CHIP clones with identical driver mutations to expand at different rates in humans. Here, we leverage genetically predicted traits to identify factors that modify CHIP clonal growth rate.

Methods: We used the passenger-approximated clonal expansion rate (PACER) method to quantify clonal growth rate for 4,370 individuals with CHIP mutations in the NHLBI TOPMed cohort. [1] We calculated polygenic risk scores (PRS) for DNA methylation clocks, inflammation related lab values, disease traits, and circulating protein levels. We tested for associations between PRS and PACER score with linear regression, controlling for covariates and correcting for multiple-hypothesis testing.

Results: CHIP clonal growth rate was significantly associated with both genetically predicted and measured methylation clocks ($p < 0.01$). No associations were identified with any of the inflammation-related lab values, proteins or diseases. An unbiased proteome wide search identified circulating levels of myeloid zinc finger 1, anti-müllerian hormone, TIMP metallopeptidase inhibitor 1 and glycine N-methyltransferase as altering CHIP clonal expansion rate ($p < 4.1 \times 10^{-6}$).

Conclusion: An aging microenvironment was associated with increased CHIP expansion rate as well as four specific circulating micro-environment proteins. Contrary to prior murine models, we do not identify inflammation as a root cause of CHIP clonal expansion rate in humans.

References: [1] Weinstock (2021) *bioRxiv* https://doi.org/10.1101/2021.12.10.471810.

Grants: HBPRP T32 HL144446.

Conflict of Interest: Taralynn Mack: None declared, Michael Raddatz: None declared, Joshua Weinstock: None declared, Jaiswal

Siddhartha TenSixteen Bio, TenSixteen Bio, Alexander Bick TenSixteen Bio, TenSixteen Bio.

P08.019.C Assessing new RNA-based biomarkers in multiple sclerosis

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Background/Objectives: Multiple sclerosis (MS) is an autoimmune neurodegenerative disease characterized by chronic inflammation, and demyelination. Accumulating evidence suggests a pathogenic link between MS and abnormalities in alternative splicing (AS) [1], a process that increases the information content of the transcriptome through the expression of different mRNAs from single genes [2]. Our objective is to define an AS-based signature able to discriminate patients from controls, starting from a blood sample.

Methods: RNA was extracted from peripheral blood mononuclear cells of 29 relapsing-remitting MS patients and 20 controls. Fluorescent RT-PCR assays, focusing on three specific AS events were performed, and PCR products were separated using capillary electrophoresis. The relative exon inclusion level (percent-spliced in, psi) was calculated for each sample.

Results: For each AS event, we divided the psi range in quantiles and we assigned each sample to its relevant class. We calculated a risk score for each sample, resulting from the sum of the quantiles they fit in in each AS assay. We used this score to perform a ROC analysis. Using this 3-marker-AS signature, we obtained a good predictive model (AUC = 0.72, specificity = 0.9, sensitivity = 0.48). We are testing the predictive value of our model on an independent cohort (32 MS cases and 50 matched controls).

Conclusion: Considering that the current diagnostic methods for MS are based on complicated procedures, the implementation of a diagnostic test, centered on RNA biomarkers use, would be useful in a clinical perspective.

References: 1. https://doi.org/10.1016/j.autrev.2019.05.010.
2. https://doi.org/10.1146/annurev.biochem.72.121801.161720.

Grants: This work is supported by Fondazione Regionale per la Ricerca Biomedica (FRRB), Early Career Award.

Conflict of Interest: None declared.

P08.020.D Immunodeficiency 68 - a life-threatning primary immunodeficiency

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Background/Objectives: MYD88 deficiency is an inherited disease of the immune system that leads to abnormally frequent severe infections by pyogenic bacteria such as *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* that can develop into sepsis and meningitis. Infections can be fatal in infancy and childhood and become less frequent around the age of 10.

Diagnostic and genetic counselling.

Methods: Case Report: First male child of a healthy non-consanguineous couple, with congenital oculocutaneous hypopigmentation, nystagmus and ophthalmoscopy of the left eye compatible with partial oculocutaneous albinism. WES revealed a heterozygous mutation in *TYR* gene. At ten months, he had an inguinal abscess due to *Escherichia Coli*, with blood culture positive to *Campylobacter*. Normal Immunodeficiency investigation. At fourteen months, the child presented a periorbital cellulitis and, at 21 months, a right thumb recurrent cellulitis to Methicillin-susceptible *Staphylococcus aureus*. He died at 29 months from fulminant meningitis due to *Pseudomonas aeruginosa*.

Results: An WES trio analysis was performed and revealed ac.625C>T(Arg209Cys) in the *MYD88* gene in homozygosity.

Conclusion: The *MYD88* gene encodes a cytosolic adapter protein that plays a central role in the innate and adaptive immune response that interferes in the activation of numerous proinflammatory genes. The phenotypic characteristics observed in the present case are consistent with those described in the literature, with a severe meningitis responsible for the child's death. This condition has an autosomal recessive pattern and both parents are heterozygous, with no signs or symptoms of this disease. To our knowledge, there are less than 30 affected individuals described in the literature.

References:.

Grants:.

Conflict of Interest: None declared.

P08.021.A Differential diagnostic algorithm for Inherited Macrothrombocytopenia enables the genetic characterization of 52 families in a single tertiary referral centre

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Background/Objectives: Inherited Macrothrombocytopenias (IMTP) are platelet (PLT) disorders characterized by reduced PLT counts, increased PLT volume and often disproportionate bleeding, with up to 30 genes currently implicated. We aimed to develop an efficient diagnostic algorithm for IMTP, contributing towards the understanding of these disorders and their underlying defects in our population.

Methods: The proposed algorithm comprises three assessment steps: (i) clinical data (personal/family history, physical examination, bleeding tendency); (ii) preliminary laboratory tests including PLT counts and indices [mean PLT volume (MPV) and immature PLT fraction (IPF)], PLT morphology and function assays (occlusion time by PFA100/200 and lumi-aggregometry); (iii) flow cytometry to assess surface glycoproteins and PLT size (forward scatter indices). PLT phenotypic patterns orientate towards candidate gene(s); in the absence of a suspected underlying cause, targeted next-generation sequencing (NGS) is used.

Results: The algorithm distinguished patient subgroups, guiding appropriate molecular approaches. The genetic cause was

identified in 37 families by direct Sanger sequencing and in 15 families by NGS. Differential diagnosis achieved was: 4 biallelic Bernard-Soulier syndrome (BSS), 5 monoallelic BSS, 1 Platelet-type vWD, 13 ITGB3/ITGA2B-related thrombocytopenia (-RT), 9 ACTN1-RT, 9 TUBB1-RT, 3 GFI1B-RT, 7 MYH9-related disease (-RD) and 1 DIAPH1-RD.

Conclusion: Knowledge emerging from new genotype-phenotype correlations, particularly by reassessment of PLT function and phenotype in NGS-diagnosed cases, will be incorporated into the algorithm for further refining. Genetic diagnosis is essential for prognosis and preventive treatments, to flag syndromic entities with probable multi-organ involvement, as well as for determining carrier status.

References:.

Grants: FCT grants to CM (UI/BD/150741/2020), UMIB and ITR (UIDB/00215/2020; UIDP/00215/2020; LA/P/0064/2020).

Conflict of Interest: None declared.

P08.022.B Making the invisible visible: Understanding what's helpful in learning to live with a suspected Inborn Error of Immunity

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Background/Objectives: Prior research has established the potential for negative quality-of-life consequences among individuals with suspected Inborn Errors of Immunity (IEI). Relatively underrepresented in this literature, however, are qualitative methods that explore patient perceptions of their challenges and opportunities. This study aims to interrogate psychosocial impacts of having an IEI to better understand how patients adapt to their condition.

Methods: A survey including open-ended questions was sent to patients enrolled on the Centralized Sequencing Protocol and were suspected of having an IEI. Here, we present responses to the question: What has been most helpful in learning to live with your illness? And why?.

Results: Out of 1038 invited participants, 252 (24% response rate) responses were coded and analyzed thematically (59% female, mean age = 48 years). Many patients reported that they benefit from competent providers with a nuanced understanding of their disease and who provide effective treatments. Patients also reported seeking support in coping from family, friends, and the disease community. Many also reported the benefit of lifestyle changes and reconceptualizing their illness to gain a sense of control. An important minority of individuals indicated they had not found anything helpful in living with their illness.

Conclusion: Beyond encouraging further biomedical research into new disease associations and therapies, patient responses suggest healthcare providers can promote adaptation by encouraging patients to play an active role in their treatment and connecting them to support systems and psychological interventions. Patients who claim no adaptation can benefit from practices to better identify their challenges and corresponding interventions.

References: None.

Grants: None.

Conflict of Interest: None declared.

P08.023.C Mutation spectrum of Fanconi anemia associated genes in five patients from Azerbaijan

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Background/Objectives: Fanconi anemia (FA) is a rare genetic disorder caused mutations in genes which protein products are involved in replication, cell cycle control and DNA repair. FA proteins are required for the proper repair of DNA interstrand crosslinks (ICL), a deleterious type of DNA damage that covalently binds DNA strands. FA is characterized by congenital malformations, bone marrow failure, and predisposition to malignancies. Presently, 22 autosomal and one X linked genes are held responsible from >85% of the disease and the *FANCA* mutations accounts for almost 60%-70%.

Methods: 5 patients, with FA clinic are included into this investigation. We performed exome sequencing and copy number variant analyses and detected variants considered to be pathogenic are verified by Sanger. Mutation un-identified patients and patients carrying heterozygous pathogenic variants are further tested by MLPA.

Results: Three known variants in four alleles (c.[2679G>A];[2679G>A], c.[1343A>G];[2495_2497delTCT], and two *novel* variants in four alleles (c.[495delC];[495delC], c.[2941T>C];[2941T>C] in *FANCA*, and a *novel* variant in two alleles (c.[283_284delCT];[283_284delCT]) in *FANCF* are identified.

Conclusion: This is the first study looking at clinical and genetic features of FA in Azerbaijan. We aimed to identify the mutation frequencies of FA genes, outcome, overall condition, and genetic features of patients in Azerbaijan to optimize management, identify the most common genes, describe new mutations, and offer prenatal diagnosis and counseling to the affected families.

References: Toksoy, Güven, et al. "Clinical and Molecular Characterization of Fanconi Anemia Patients in Turkey." *Molecular syndromology* 11.4 (2020):183-196.

Grants:.

Conflict of Interest: None declared.

P08.024.D Variant of CXCL8 gene associated with IBD pathogenesis?

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Background/Objectives: Inflammatory bowel diseases (IBD) is a group of chronic pathologies of the digestive system with not well known etiology. Interleukin 8 encoded by the *CXCL8* gene is a cytokine that is a chemotactic factor responsible for the targeted migration of leukocytes to the site of inflammation. Its pro-inflammatory properties suggest that it plays a key role in the development of the inflammatory process. The aim of this analysis was to examine the two variants rs4073 (c.-251A>T) and rs188378669 (c.91G>T, p.Glu31Ter) in *CXCL8* gene distribution in a group of IBD patients and populations.

Methods: Using pyrosequencing, Competitive Allele-Specific PCR (CASP) and Sanger sequencing, the two variants, rs4073 (c.-251A>T) and rs188378669 (c.91G>T, p.Glu31Ter) of the *CXCL8* gene were analyzed in a group of 353 IBD patients and 200 subjects from Polish population in order to check if there is any correlations of them with a disease.

Results: Polymorphism c.91G>T, located in exon 2 of *CXCL8* gene, causing premature termination of the polypeptide chain and consequently forming a non-functional protein was observed significantly more often in a group of IBD patients (MAF of 2.12%)

compared to population group (MAF of 0.25%) $p = 0{,}012$, OR = 8,661, C.I.=[1,140-65,816].

Conclusion: This is the first report concerning the correlation of IBD presence and p.Glu31Ter variant in *CXCL8* gene, what suggest the role of CXCL8 gene in IBD pathogenesis.

References: Dakal TC et al. Predicting the functional consequences of non-synonymous single nucleotide polymorphisms in IL8 gene. *Sci Rep* 2017.

McGovern DP et al. Genetics of Inflammatory Bowel Diseases. *Gastroenterology* 2015.

Grants:-.

Conflict of Interest: None declared.

P09

INTELLECTUAL DISABILITY

P09.001.A Parental mosaicism is underestimated in rare intellectual disability syndromes

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Background/Objectives: De novo variants are a common cause to rare intellectual disability syndromes, associated with low recurrence risk. However, when such variants occur pre-zygotically in parental germ cells, the recurrence risk might be higher. Still, the recurrence risk estimates are mainly based on empirical data and the prevalence of germline mosaicism is often unknown.

Methods: To establish the prevalence of mosaicism in parents of children with intellectual disability syndromes caused by de novo variants, we performed droplet digital PCR on DNA extracted from blood (43 trios), and sperm (31 fathers).

Results: We detected low-level mosaicism in sperm-derived DNA but not in blood in the father of a child with Kleefstra syndrome caused by an *EHMT1* variant. Additionally, we found a higher level of paternal mosaicism in sperm compared to blood in the father of a child with Gillespie syndrome caused by an *ITPR1* variant.

Conclusion: By employing droplet digital PCR, we detected paternal germline mosaicism in two intellectual disability syndromes. In both cases, the mosaicism level was higher in sperm than blood, indicating that analysis of blood alone may underestimate germline mosaicism. Therefore, sperm analysis can be clinically useful to establish the recurrence risk for parents and improve genetic counselling.

References:.

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Conflict of Interest: None declared.

P09.003.C A novel splice site mutation in the CNOT3 gene is associated with syndromic intellectual disability with clinical variability

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Background/Objectives: Variants in CNOT3 have been associated with intellectual developmental disorder with speech delay, autism, and dysmorphic features (IDDSADF). To date, 22 mutated patients are known, and the phenotype is poorly described.

Methods: Via Exome sequencing, we detected the first splice site mutation) in intron 2 in CNOT3 (c.26-2A>G). In silico tools revealed a damaging effect on the splicing due to an alteration on the canonical splice site at 3'end, no longer recognized. Analysis of the transcript confirmed its damaging effect, consisting of intron 2 retention and of an in-frame duplication of exons 1 and 2.

Results: This is the third family with an inherited mutation in CNOT3, associated with a condition displaying intrafamilial variability. In fact, the proband showed intellectual disability (ID), language delay, structural cerebral anomalies, cardiac defects, physical dysmorphisms, whereas his mother manifested a mild ID and shared her son's dysmorphisms.The mutation was related to anorectal dysplasia, firstly detected in CNOT3-patients.

Conclusion: The rectum is generated from the differentiation of the endoderm in the primordial gastrointestinal tract during the 4th week of the fetal life. CNOT3 takes part in the mesendoderm differentiation, which originates mesoderm and endoderm, suggesting a connection between its alterations and rectal atresia.This study contributes to define the CNOT3 phenotypic spectrum and highlights its clinical variability.

References: R. Meyer, et al., Inherited cases of CNOT3-associated intellectual developmental disorder with speech delay, autism, and dysmorphic facies, Clinical Genetics 2020.

Martin R, et al., De novo variants in CNOT3 cause a variable neurodevelopmental disorder. Eur J Hum Genet. 2019.

Grants: None.

Conflict of Interest: None declared.

P09.004.D Missense variants in ANKRD11 cause KBG syndrome by impairment of stability or transcriptional activity of the encoded protein

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Background/Objectives: Although haploinsufficiency of ANKRD11 is among the most common genetic causes of neurodevelopmental disorders [1], the role of rare ANKRD11 missense variation remains unclear. We characterized the clinical, molecular and functional spectra of ANKRD11 missense variants.

Methods: We collected clinical information of individuals with ANKRD11 missense variants and evaluated phenotypic fit to KBG syndrome. We assessed pathogenicity of variants by in silico analyses and cell-based experiments.

Results: We identified 29 individuals with (mostly de novo) ANKRD11 missense variants, who presented with syndromic neurodevelopmental disorders and were phenotypically similar to individuals with KBG syndrome caused by ANKRD11 protein truncating variants or 16q24.3 microdeletions. Missense variants significantly clustered in Repression Domain 2. Cellularly, most variants caused reduced ANKRD11 stability. One variant resulted in decreased proteasome degradation and loss of ANKRD11 transcriptional activity.

Conclusion: Our study indicates that pathogenic heterozygous missense variants in ANKRD11 cause the clinically recognizable KBG syndrome. Disrupted transrepression capacity and reduced protein stability each independently lead to ANKRD11 loss-of-function, consistent with haploinsufficiency. This highlights the diagnostic relevance of ANKRD11 missense variants, but also poses diagnostic challenges, as the KBG-associated phenotype may be mild, and inherited pathogenic ANKRD11 (missense) variants are increasingly observed, warranting stringent variant classification and careful phenotyping.

References: 1. Ockeloen, C.W., et al., Further delineation of the KBG syndrome caused by ANKRD11 aberrations. Eur J Hum Genet, 2015. 23(9): p. 1270.

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P09.005.A A novel pathogenic FMR1 splice site variant in a man with normal intelligence and Klinefelter syndrome - A case report

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Background/Objectives: We describe a 30-year-old man referred to genetic counselling regarding a possible Marfan syndrome. He presented with marfanoid features such as tall stature, an aortic root enlargement, and a systemic score of 7 points.

Methods: We performed whole exome sequencing based on the marfanoid phenotype including HPO-terms for cardiac defects.

Results: No variants associated with Marfan syndrome were detected. The exome based CNV (copy number variation) analysis revealed a duplication of all X-chromosomal genes, strongly suggesting a Klinefelter syndrome (KS) (karyotype 47,XXY), that does not fully explain the patient's phenotype. Exome analysis detected a novel splice-site variant c.1738-1G>A, p.? in the FMR1 gene. The variant has not been described previously. Splice-site in silico prediction tools (SSF, MaxEnt, NNSplice and GeneSplicer) indicated a disrupted splice acceptor site of intron 16.

KS is a sex-chromosomal disorder with a frequency of ca. 1:500 men. Phenotypic features can include tall stature, gynecomastia and often azoospermia.

In more than 99% of patients diagnosed with a Fragile-X-syndrome the mutational mechanism is a CGG-trinucleotid repeat-

expansion in FMR1, whereas other types of FMR1 mutations, affecting the coding region or splicing of FMR1, account for less than 1% of cases. Males previously described to carry FMR1 splice mutations had moderate to severe mental retardation.

Conclusion: We describe a patient with normal intelligence despite a pathogenic splice variant in FMR1, probably due to a skewed X-inactivation because of his Klinefelter syndrome.

References: 1. Intragenic FMR1 disease-causing variants: a significant mutational mechanism leading to Fragile-X syndrome, Quartier et al. (2017).

Grants:.

Conflict of Interest: None declared.

P09.006.B Deep phenotyping of patients with developmental disorders and their relatives to support interpretation of rare inherited copy number variants

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Background/Objectives: In patients with developmental disorders (DD), copy number variants (CNV) inherited from seemingly unaffected parents are typically disregarded in variant interpretation. However, understanding the phenotypic contribution of inherited rare variants is crucial for genetic counselling. Therefore, an extended family-oriented approach is proposed, including deep familial phenotyping and multi-omics in carriers and non-carriers.

Methods: Deep phenotyping (including medical, developmental and behavioral, using standardized instruments) of carriers and non-carriers within the nuclear family, enables familial segregation analysis of a CNV with DD (sub)phenotypes. Trio whole genome sequencing is used to identify additional pathogenic variants causing or contributing to the phenotype. RNA and capture Hi-C sequencing on EBV cell lines is used to examine the regulatory effect of the CNV. This combined approach was applied to interpret a rare paternally inherited deletion ([GRCh37] 4:141693186-142147039x1) in a 15-year-old boy with moderate intellectual disability, ASD, DCD, hypotonia and facial dysmorphic features.

Results: CNV carriers (FSIQ; index 40, father 71) scored significantly lower on cognitive abilities compared to non-carriers (FSIQ; mother 100, sibling 92). Behavioral profiles of carriers were more overlapping than profiles of non-carriers. De novo, recessive, X-linked and paternally inherited variant analyses of SNV, indels and additional CNVs were negative. RNA-seq shows four differentially expressed genes in the CNV or flanking regions (INPP4B, SETD7, MAML3, ZNF330). Their contribution to DD is subject to further study.

Conclusion: This multi-omics approach and correlation through deep familial phenotyping is suggestive of a contributory effect of this CNV to DD in this family.

References:.

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Conflict of Interest: None declared.

P09.007.C Establishing the neurodevelopmental phenotype and genotype-phenotype correlations in individuals with a TRIP12 mutation

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Background/Objectives: Haploinsufficiency of *TRIP12* causes a neurodevelopmental disorder characterized by intellectual disability associated with epilepsy, autism and dysmorphism, also named Clark-Baraitser syndrome. Less than 25 individuals harboring pathogenic *TRIP12* variants have been reported. We aim to further delineate the *TRIP12*-associated phenotype and review genotype-phenotype correlations. In addition, characteristic facial traits are objectified through image analysis based on deep-learning algorithms.

Methods: 37 individuals were recruited through a collaborative call via ERN-ITHACA. Clinical data was collected and the pictures of 21 individuals were uploaded into the GestaltMatcher database for analysis of facial morphology.

Results: One inherited and 35 *de novo TRIP12* variants were identified, including frameshift ($n = 16$), nonsense ($n = 6$), missense ($n = 5$) and splice ($n = 3$) variants as well as intragenic deletions ($n = 5$) and a multigene deletion disrupting *TRIP12*.

Though variable in severity, global developmental delay was noted in all individuals, with language deficit most pronounced. Half of the individuals showed autistic features, but there was no clear correlation with the mutation type. Susceptibility to obesity seemed to be a recurrent feature in older individuals. Seizures were reported in a minority and proved to be refractory in individuals with a missense variant.

Facial analysis shows a clear gestalt including deep-set eyes with narrow palpebral fissures, downturned corners of the mouth and large, low-set ears with prominent earlobes.

Conclusion: We report the largest cohort to date of individuals with pathogenic *TRIP12* variants, further delineating the associated phenotype including introduction of a facial gestalt and expanding genotype-phenotype correlations. These findings will improve future counseling and patient guidance.

References: /.

Grants: /.

Conflict of Interest: None declared.

P09.008.D The clinical benefit of trio-based whole-exome sequencing for the detection of rare pathogenic sequence variants in paediatric patients with undiagnosed neurodevelopmental disorders

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Background/Objectives: Thanks to more than 50% diagnostic yield the whole-exome sequencing (WES) has become an effective and powerful approach to identify molecular genetic causes of neurodevelopmental disorders (NDDs) and multiple congenital abnormalities (MCA).

Methods: We present our experience with WES as an effective tool for the detection of rare and novel pathogenic sequence variant using the commercial kit Human Core Exome (Twist Biosciences) and Illumina NovaSeq 6000. Our pilot study included 45 families (trios or quatros) of children with severe NDDs and MCA.

Results: Using our in-house bioinformatic pipeline and unique algorithm for variant prioritization we identified recurrent de novo pathogenic sequence variants in clinically relevant SHANK3, GRIN1, NSD1, CDK13 genes, novel de novo pathogenic variants in KDM1A, KMT2E, GNAI1, MEIS2, SMARCA2, RAI1 and CHD8 genes, pathogenic X-linked variants in EDA and OPHN1 genes of maternal origin, pathogenic sequence variants in ZGRF1 of paternal origin and biallelic pathogenic sequence variants in the BLM gene. Moreover, two pathogenic sequence variants in the CTNNB1 and DYNC1H genes were present as low-level mosaicism in healthy fathers. All clinically important variants including secondary findings (in "ACMG" genes) were manually verified using Sanger sequencing and interpreted based on relevant information in integrated databases of genomic variants, relevant scientific literature, and individual anamnesis.

Conclusion: With an achieved diagnostic yield of 37.5% (18/48 children with NDDs and MCA), trio-based WES represents as an effective first-tier diagnostic test in the genetic evaluation of children with NDDs.

References:.

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Conflict of Interest: None declared.

P09.009.A RAB11B-associated neurodevelopmental disorder - confirmed and extended to milder manifestations through 13 individuals with 5 novel variants

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Background/Objectives: The family of Rab GTPase proteins are molecular switches acting through GDP/GTP-exchange and involved in vesicle trafficking. RAB11B has been associated with a Mendelian neurodevelopmental disorder (NDD) in 2017 as two recurrent de novo missense-variants (amino acids 22,68) were identified in five children with severe intellectual disability (ID), absent speech, ataxic gait, and decreased cortical white matter. No additional cases have since been reported. We aim at confirming the relevance of RAB11B and delineating the genotypical and phenotypical spectrum.

Methods: Through international matchmaking, we gathered eight individuals with RAB11B variants identified through exome sequencing or array CGH. Clinical details of novel and previously reported five individuals were standardized to HPO-terms. We performed a cross-sectional analysis regarding the clinical manifestations. Three-dimensional analysis using a ProteinDataBank structure was done by manual inspection of variant localization in PyMol as well as cluster calculation according to mutation3d-algorithm.

Results: The variant spectrum comprises six missense-variants (four novel; amino acids 21,33,72,75) as well as one 319kb duplication, all except one confirmed de novo. In silico parameters indicate damaging effects. All variants affect or lay close by the GTP-binding sites and five of them showed significant clustering. Data from 13 individuals confirm main clinical findings to be NDD/ID (75%), muscular hypotonia (75%) and small cerebral cortex (70%). However, cognitive function ranges from severe ID (67%) to few cases with normal cognitive development only with epilepsy.

Conclusion: Confirmation of RAB11B as an NDD gene and extension to milder manifestations, generally de novo-variants within the GTP-binding regions are highly suspicious as disease-causing.

References:.

Grants:.

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P09.010.B Comparison of methylation epi-signatures in KMT2B and KMT2D-related human disorders

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Background/Objectives: Many neurodevelopmental disorders caused by mutations in genes regulating chromatin function and/or structure display abnormal DNA methylation patterns (episignatures) in peripheral blood. DYT-KMT2B is unique among "chromatin neurodevelopmental disorders" in that the most prominent clinical feature and most frequent presentation is childhood onset dystonia rather than developmental delay or congenital anomalies (as seen in many chromatin neurodevelopmental disorders such as Kabuki syndrome).

Methods: To investigate peripheral blood methylation episignatures in KMT2B-related dystonia (DYT-KMT2B), we undertook genome-wide methylation profiling of ~2M CpGs using a next-generation sequencing based assay and compared the findings to those in controls and patients with Kabuki syndrome Type 1 (KS1-KMT2D).

Results: Methylation profiling revealed 1,812 significantly differentially methylated CpG positions (DMPs) (FDR < 0.05) in 10 DYT-KMT2B samples compared to controls, covering 40 CpG. Multi-dimensional scaling analysis showed that the DYT-KMT2B samples clustered together and separately from 29 control individuals and 10 individuals with pathogenic variants in KMT2D. Most DMPs were specific to one disorder and that all (DYT-KMT2B) and most (KS1) methylation alterations in CpG islands were gain of methylation events. Analysis of genes associated with CpG islands suggested potential; candidate genes for the molecular pathogenesis of DYT-KMT2B.

Conclusion: Using higher resolution methodology for methylation profiling, we confirmed a peripheral blood methylation signature for DYT-KMT2B. Methylation episignatures could be used to aid pathogenicity interpretation of KMT2B variants and can complement mechanistic investigations of the pathogenesis of DYT-KMT2B.

References:.

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Conflict of Interest: None declared.

P09.011.C Exome sequencing detects two novel variants in SNRPN in two patients with Prader-Willi/ Prader-Willi-like syndrome

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Background/Objectives: Imprinting disorders (IDs) are a group of congenital disorders caused by (epi)genetic alterations in imprinted chromosomic regions. Prader-Willi syndrome (PWS) is one of the best known IDs, and is consequence of the loss of paternally expressed genes (*MAGEL2*, *NDN*, *SNURF-SNRPN* and *SNORD116*) within the 15q11.2-q13, caused either by a CNV (65–75% of cases), a upd(15)mat (20–30%), or an epimutation (1–3%) (*1*). Recently, point variants in *SNRPN* have been reported as causative of PWS in two independent families (*2*, *3*) and in this report we described two new cases.

Methods: Two independent probands were referred for genetic testing due to seizures, mild intellectual disability and obesity (P1); and clinical suspicion of PWS (P2). After negative MS-MLPA testing for 15q11 region, exome sequencing (ES) was carried out. Variant confirmation and cosegregation studies were analysed by Sanger sequencing. Parental origin of the allele was tested by allele-specific RT-PCR amplification, and sequencing.

Results: ES studies identified a novel missense variant in *SNRPN* in each proband. Complementary studies determined that each variant was carried in the proband's paternal allele.

Conclusion: According to these and previous results (*2*, *3*), genetic testing for *SNRPN* point variants should be performed in PWS patients with negative results for classical causes.

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P09.012.D LoF variants can cause TUBB-associated disorder

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Background/Objectives: TUBB encodes one of nine beta-tubulin proteins and is widely expressed in all tissues, especially in the developing brain. Pathogenic variants in TUBB are associated with two main phenotypes: cortical dysplasia, complex, with other brain malformations 6 (OMIM: 615771) and symmetric circumferential skin creases, congenital, 1 (OMIM: 156610). Both diseases are autosomal dominant and result in impaired intellectual development. There are only 10 pathogenic variants currently described and none of them demonstrate loss of function (LoF) effect. We report for the first time that LoF variants in TUBB may be responsible for impaired intellectual development.

Methods: Whole exome sequencing (WES) was performed using the IlluminaTruSeq® ExomeKit, IDT xGen® Exome Research Panel, and Illumina NextSeq 500.

Results: A 38-year-old man was born from a full-term pregnancy with a weight of 2600 g and length of 48 cm. Motor development was delayed: he could seat at 10 months, walk at 1 year 8 months. He cannot speak. On examination: normal height and weight, thoracolumbar kyphosis, strabismus, ocular hypertelorism, short filter, protruding lower jaw, no focal neurological symptoms. Karyotype is 47, XYY, normal methylation status of the FMR1 promotor. WES revealed variant of unknown significance

NM_178014.4(NP_821133.1):p.(Tyr208*) in TUBB gene, which has low tolerance to LoF (pLI = 0.98). Segregation analysis by Sanger sequencing revealed de novo status of the variant. The proband's clinical features overlap with both TUBB-associated phenotypes.

Conclusion: We propose that LoF variants in TUBB can cause TUBB-associated disorder.

References:.

Grants:.

Conflict of Interest: None declared.

P09.013.A Delineating the MAPK8IP3-related neurodevelopmental disorder reveals consistent variant specific phenotypes

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Background/Objectives: We and others have recently described de novo variants in MAPK8IP3 gene as a cause of neurodevelopmental disorder (NDD) with intellectual disability (ID), seizures, microcephaly, and brain anomalies.

Methods: We present an overview of 18 published and 20 novel individuals with causative MAPK8IP3 variants and a HPO based phenotypic analysis.

Results: Missense variants were identified in 31 individuals, truncating variants in six, and whole gene deletion in one. About 58% (22/38) harbour one of the previously reported recurrent missense variants p.Arg578Cys (10), p.Leu444Pro (3), or p.Arg1146Cys/His (9). ID ranges from mild to profound. Additional symptoms encompass brain anomalies (22/26), obesity (9/16), spasticity or dystonia (16/21), seizures (10/27), ataxia (6/8), microcephaly (5/15), and precocious puberty (4/6). The recurrent variant p.Arg578Cys is consistently associated with severe ID, spasticity, hypoplastic corpus callosum, white matter abnormalities, seizures, small hands and feet and early-onset obesity with insatiable appetite. In contrast, recurrent variants affecting Arg1146 are less frequently linked with obesity or seizures but instead with autism and ataxia. Three individuals do not fall into the MAPK8IP3—related NDD spectrum: two brothers with the de novo variant p.Ser984Leu have recurrent pain during urination with one of them also mildly delayed. Another six year old boy with the de novo variant p.Pro822Ser initially showed infantile spasm, which subsequently resolved with normal development.

Conclusion: MAPK8IP3-related NDD encompasses ID and consistent variant-specific phenotypes including novel observations of obesity, precocious puberty and small hands and feet. Causality of novel de novo variants remain to be elucidated.

References:.

Grants:.

Conflict of Interest: None declared.

P09.014.B BAFfling: Microduplications of ARID1A and ARID1B cause a novel clinical and epigenetic distinct BAFopathy

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Background/Objectives: The clinical relevance of *ARID1B* whole gene duplications has been unclear until now. *ARID1B* and *ARID1A* have the same role within the BAF complex and are mutually exclusive. *ARID1A* duplications appear to lead to a distinct clinical syndrome, while *ARID1B* duplications have not been linked to a clinical phenotype.

Methods: To investigate whether an *ARID1B* duplication phenotype exists, and how this relates to the *ARID1A* phenotype, we included patients with an *ARID1A* or *ARID1B* duplication, compared their phenotypes and determined DNA methylation.

Results: We included data of 9 *ARID1A* and 11 *ARID1B* duplication patients. Duplication size ranges between 0.1-1.2 Mb with 1-44 genes for *ARID1A* and 0.4-10.3 Mb with 2-101 genes for *ARID1B*. Main features shared by *ARID1A* and *ARID1B* patients were intellectual disability, microcephaly, growth delay and cryptorchidism. Even though there is overlap between the two groups, the phenotype of *ARID1A* patients appeared to be more severe compared to that of *ARID1B* patients. DNA methylation Episign analysis showed that *ARID1A* and *ARID1B* duplication patients have a similar DNA methylation pattern in blood, which is different from controls and opposite to the pattern of more common *ARID1A* or *ARID1B* loss-of-function variants.

Conclusion: We report for the first time that duplications of *ARID1B* lead to a clinical phenotype with significant overlap with *ARID1A* duplications, and that these patients have overlapping episignatures providing further evidence for an overlapping phenotype distinct from other BAFopathies. A new type of BAFopathies caused by a duplication, rather than haploinsufficiency, is emerging here.

References:.

Grants:.

Conflict of Interest: None declared.

P09.015.C Expanding Rett syndrome landscape: identification of candidate genes from a WES study

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Background/Objectives: Rett syndrome (RTT) is a neurodevelopmental disorder (incidence of 1:10,000 live female births) most frequently affecting girls during infancy, after an early normal development. The genetic cause is known for about 90% of patients (MECP2 mutations in the classic form, CDKL5 and FOXG1 mutations for the two variants), while the 10% remain without molecular diagnosis.

Methods: We applied whole exome sequencing (WES) analysis to Rett-like probands and their healthy parents. We enrolled in the study patients negative for mutations in RTT genes to identify the genetic cause and expand the knowledge of the pathogenetic mechanisms underlying RTT.

Results: We found one girl (#1) being compound heterozygote for two unreported variants in NBEA gene, encoding for a brain-specific protein involved in vesicle trafficking. In addition, patient #2 was found being carrier of a de novo missense mutation in DYNC1H1, known to be associated to a recently classified neurodevelopmental disorder. Finally, in patient #3 was identified a novel heterozygous missense variant in SLC35F1, mainly expressed in the brain and coding for a putative solute carrier whose role is currently under investigation.

Conclusion: The identification of new RTT genes could expand the genotype-phenotype correlation for RTT syndrome, and the characterization of new candidate RTT genes could give insights into the pathogenesis of this neurodevelopmental disorder.

References: Neul et al., 2010; Mulhern et al., 2018; Poirier et al., 2013; Szafranski et al., 2015; Di Fede et al., 2021.

Grants: Grant Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics and Intramural funding of Università degli Studi di Milano.

Conflict of Interest: None declared.

P09.016.D A novel missense mutation in SLC2A1 gene responsible for Glut1 Deficiency Syndrome

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Background/Objectives: Glut1 Deficiency Syndrome (Glut1DS) is a brain energy failure disease caused by impaired glucose transport across brain tissue barriers. The definite diagnosis of Glut1DS is confirmed by the presence of characteristic clinical features, hypoglycorrhachia, and a pathogenic variant in *SLC2A1 gene*. The type of genetic mutation correlates with severity of disease; missense variants (mild severity); splice site, nonsense variants, insertions, deletions (moderate and severe severity); complete gene microdeletions (severe severity). Heterozygous de novo pathogenic variants in *SLC2A1* responsible for the phenotype of 81-89% of Glut1DS patients. Ketogenic dietary treatment (KDT) can achieve seizure freedom within a couple of days with normalization of EEG changes, frequently allowing for the withdrawal of any antiepileptic medications.

Methods: We present a patient with positive family history (mother with intellectual disability and seizures) and characteristic features for Glut1DS, including mild microcephaly, developmental and speech delay, paroxysmal eye movement abnormality and starvation-induced atypical absence seizures. *NGS*-based targeted custom epilepsy gene panel analysis was performed using QIAGEN QIAseq library kit and Illumina sequencing technology.

Results: NGS analysis identified a c.1288G>T (p.Gly430Cys) heterozygous, likely pathogenic variant in *SLC2A1* gene. This alteration is a missense variant, has never been reported. Application of KDT resulted in marked clinical improvement of the motor and seizure symptoms in our patient.

Conclusion: Accurate molecular diagnosis is critical for the clinical management and prognosis of subjects suspected with Glut1 deficiency, especially in case of mild severity, where early diagnosis and intervention would substantially influence the cognitive and motor functions.

References:.

Grants:.

Conflict of Interest: None declared.

P09.017.A AutoCaSc: Prioritizing candidate genes for neurodevelopmental disorders

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Background/Objectives: Exome sequencing(ES) in individuals with developmental disorders (DD) remains inconclusive in ~50%. Evaluation unsolved cases to identify candidate genes is subjective, slow, and uncomparable between labs. We developed AutoCaSc to prioritize candidate genes.

Methods: webAutoCaSc was developed in Python and vcfAutoCaSc was designed for VCFs scoring from the command line. The tools automate our fine-tuned candidate scoring scheme (CaSc), which is composed of the four categories "Variant attributes", "Inheritance", "Gene constraint" and "Gene plausibility". The first three categories were implemented as decision trees, while "Gene plausibility" is a precomputed score of expression, model organism, protein-protein interaction, literature, and de novo occurrence in DD cohorts.

Results: As a proof of principle, we injected into two public ES trios 79 variants in recently published DD genes, thus simulating the identification of candidate genes and variants. AutoCaSc consistently (94.5%) scored all variants and genes in the top three

ranks (mean rank of 1.5 and 2.3 in the two ES trios). Furthermore, in 93 in-house trios, AutoCaSc identified all previously identified candidate variants by a human evaluator. AutoCaSc placed these in the top ranks while evaluating additional highly scoring variants that were missed in the initial manual evaluation. weAutoCaSc is thus in standard use at our institute and is publically available: https://autocasc.uni-leipzig.de/.

Conclusion: AutoCaSc enables anybody to quickly screen a variant of interest for its plausibility for DDs even if the gene is still not described. We provide usage recommendations, based on our experience in projects describing novel DD genes to accelerate deciphering the genetics of DD.

References:.

Grants:.

Conflict of Interest: None declared.

P09.018.B Novel in-frame deletion in RBMX leads to X-linked intellectual disability by disturbed RNA processing, splicing regulation and potentially reduced SH3 binding

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Background/Objectives: RNA binding motif protein X-linked (*RBMX*) encodes the heterogeneous nuclear ribonucleoprotein G (hnRNP G) important for regulation of splicing, sister chromatid cohesion and genome stability. Deletion of the RGG/RG motif in hnRNP G have been associated with Shashi syndrome, however other hnRNP G domains' association to X-linked intellectual disability (XLID) remains unknown.

Methods: Whole exome sequencing, whole genome sequencing, and X-chromosome inactivation studies were used to genetically characterize a Swedish five-generation family with XLID. The variant effect on biological processes and alternative splicing events were investigated by transcriptomics analyses of an SH-SY5Y cell line overexpressing the mutant or wildtype *RBMX*. The variant effect on hnRNP G was investigated by using prediction tools and fluorescence polarization assays with SH3 domains and peptides spanning the variant.

Results: A novel in-frame *RBMX* deletion segregated with disease in this family, where affected individuals were hemizygous, and asymptomatic individuals were non-carriers or heterozygous females with skewed X-chromosome inactivation. The affected individuals presented poor phenotypic overlap with Shashi syndrome, suggesting a different disease mechanism. Transcriptomics analyses revealed differentially expressed splicing events and enrichment for genes involved in RNA processing and neurodevelopment. Protein analyses imply a novel SH3-binding motif in hnRNP G and potentially lower affinity binding to SH3 domains caused by the variant.

Conclusion: We present a novel in-frame deletion in *RBMX* as a cause to XLID, by disrupted RNA processing, splicing regulation and potentially reduced SH3-binding. The results implies that disruption of different motifs impact the severity of disease.

References:.

Grants: Swedish Society for Medical Research and Sävstaholm Foundation.

Conflict of Interest: None declared.

P09.019.C Genome Sequencing is a sensitive first-line test to diagnose individuals with intellectual disability

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Background/Objectives: Individuals with intellectual disability (ID) and/or neurodevelopmental disorders (NDD) are currently investigated with several different approaches in clinical genetic diagnostics. A molecular diagnosis can facilitate better individualized health care, improved genetic counselling and quality of life.

Methods: We compare the results from three diagnostic pipelines in patients with ID/NDD; genome-first (n = 100), genome as a secondary test (n = 129) or chromosomal microarray (CMA) with or without FMR1 screening (n = 421).

Results: The diagnostic yield was 37% (genome-first), 26% (genome as a secondary test) and 12% (CMA/FMR1). Notably, the age of diagnosis was delayed by 1 year when genome was done as a secondary test and the cost per diagnosed individual was 36% lower with genome-first compared to CMA/FMR1. Furthermore, 91% of those with a negative result after CMA/FMR1 screening (338 individuals) have not yet been referred for additional genetic testing and remain undiagnosed.

Conclusion: Our findings strongly suggest that genome analysis outperforms other testing strategies and should replace traditional CMA and FMR1 screening as a first-line genetic test in individuals with ID/NDD. Genome is a sensitive, time- and cost-effective method that results in a confirmed molecular diagnosis in 37% of all referred patients.

References: None.

Grants: The Swedish Research Council, the Swedish Brain Foundation, the Swedish Rare Diseases Research Foundation, the Hållsten Research Foundation.

Conflict of Interest: None declared.

P09.020.D Characterization of de novo variants in PPP2R5C expands the spectrum of PP2A-related neurodevelopmental disorders

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Background/Objectives: Protein Phosphatases of type 2A (PP2A) regulate brain function and development by catalyzing phospho-Ser/Thr dephosphorylations in various substrates. PP2A holoenzymes comprise a catalytic C, scaffolding A and regulatory B-type subunit, which determines substrate specificity and enzyme regulation. De novo mutations in genes encoding Aα-, B56δ- and Cα-subunits were recently identified as new genetic causes of intellectual disability and (neuro)developmental delay (ID/NDD). A single case report describes an overgrowth phenotype associated with a de novo mutation in *PPP2R5C*, encoding the regulatory B56γ-subunit.

Methods: Matchmaker Exchange and international collaborations enabled us to identify 12 additional individuals with *de novo PPP2R5C* mutations, and 2 individuals with a *PPP2R5C* variant of unclear inheritance. Variants were biochemically characterized for interaction with other PP2A subunits and a potential PP2A substrate, and for phosphatase activity.

Results: Besides ID/NDD, clinical features of *PPP2R5C*-affected cases included hypotonia and commonly, epilepsy, behavioral and brain size abnormalities (macrocephaly). Most *PPP2R5C* variants affected the same, highly conserved B56 acidic loop or other orthologous amino acids that are also recurrently mutated in *PPP2R5D*-affected cases. Six variants were new. All de novo variants showed varying defects in A, C and/or substrate binding, while phosphatase activity, measured on phospho-peptide substrates, did not seem majorly affected. Both variants of unsure inheritance behaved normally in these assays, and were thus classified as likely non-pathogenic (VUS).

Conclusion: We report the first cohort of patients with pathogenic *de novo PPP2R5C* variants that show impaired functionality and are a novel cause of ID/NDD, with high clinical and biochemical similarities to *PPP2R5D*-affected cases.

References:.

Grants: JGA/FWO.

Conflict of Interest: None declared.

P09.021.A Copy number detection from exome sequencing data for patients with neurodevelopmental disorder: an effective approach

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Background/Objectives: Copy number variant (CNV) sequencing and exome-based gene panel analysis became state-of-the art in routine diagnostics. However, small CNVs may not be routinely detected or reported and in recessive disease, the combination of both a CNV and single nucleotide variant (SNV) affecting the causal gene may underlie the phenotype.

Methods: In our center, we implemented ExomeDepth, a read depth-based analysis tool (Plagnol, 2012) for CNV detection in exome sequencing data.

Results: ExomeDepth analysis established the molecular diagnosis in seven patients suffering from a neurodevelopmental disorder (NDD) for whom SNV and CNVseq analysis did not provide conclusive results. A patient with a clinical diagnosis of Johanson-Blizzard syndrome harboured a ~30 kb triplication in UBR1, with both parents carrying the mono-allelic intragenic UBR1 duplication. In a second patient a de novo 350bp deletion of exon 9 of the MYT1L gene was detected and confirmed by qPCR. The deletion was not detected by former molecular karyotyping (CNVseq analysis). In five other patients read-depth analysis revealed a CNV in a gene related to an autosomal recessive NDD, following the identification of a pathogenic SNV on the other allele (FARSA2, KIAA0586, MMACHC, VPS13B, SLC7A7).

Conclusion: We conclude that ExomeDepth is an efficient tool to detect CNVs in exome sequencing data, even for CNVs with sizes below the detection limit of CNVseq. Given the added value of exome-based CNV detection via ExomeDepth in the diagnostic yield for NDDs patients, we implemented this tool in our routine exome analysis strategy.

References:.

Grants:.

Conflict of Interest: None declared.

P09.022.B Retrospective analysis of molecularly diagnosed KBG syndrome in Estonia during 2015-2021

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Background/Objectives: KBG syndrome (MIM # 148050) is an autosomal dominant inherited syndrome characterized by cognitive impairment, behavioral issues, short stature, characteristic facial features and macrodontia of the upper central incisors. It is caused by a heterozygous variant in *ANKRD11* gene or deletion of chromosomal region 16q24.3 that includes *ANKRD11* gene.

Methods: Retrospective analysis of performed NGS analyses during 2015-2021 in order to obtain detection estimation and a clinical summary of all molecularly diagnosed KBG syndrome patients.

Results: We diagnosed KBG syndrome in 12 patients (7 males, 5 females). In all patients a frameshift pathogenic variant in *ANKRD11* gene was identified; in 7/12 of them, the detected variant was a novel one. The age of diagnosis was 8 months - 41 years (average age 9 years). The following common clinical features were evaluated: cognitive impairment (6/7), speech delay (10/11), behavioral issues (4/6), short stature (4/11), epilepsy (3/12), mild hearing loss (2/11), hypertelorism (6/11), protruding ears (6/11), thin upper lip or thin lips (6/11), macrodontia (2/3), clinodactyly of F5 (5/11) and brachydactyly (4/11). Average age of autonomous walking was 16 months.

Conclusion: Clinically relevant variants in *ANKRD11* gene were found in 0.2% (12/6076) of the total amount of NGS panels done in Estonia. KBG syndrome is one of the most common monogenic autosomal-dominant neurodevelopmental syndromes, which we have detected by NGS panel. We present a patient with a previously described pathogenic variant in *ANKRD11* gene with no cognitive delay or behavioral problems.

References: N/A.

Grants: Estonian Research Council grant PRG471.

Conflict of Interest: None declared.

P09.023.C White matter abnormality as a rare feature in Helsmoortel-van der Aa syndrome

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Background/Objectives: Helsmoortel-Van der Aa syndrome (HVDAS) is a neurodevelopmental disorder caused by de novo mutations in the *ADNP* gene, characterized by autism spectrum disorder (ASD), impaired intellectual development, hypotonia and dysmorphic facial features. The global developmental delay is often associated with attention deficit-hyperactivity disorder and delayed speech. Among the heterogeneous clinical picture, numerous brain abnormalities have been described, although white matter abnormalities were less frequent. Hereby we demonstrate two patients with HVDAS who showed significant signal intensity change in their white matter.

Methods: Whole-exome sequencing (WES) and brain magnetic resonance imaging (MRI) was applied in both patients. Libraries were sequenced on Illumina NovaSeq 6000 instrument with 100bp paired-end chemistry. Library preparation was carried out with Twist Human Core Exom Kit Library Prep Kit. Targeted sequencing was applied for confirmation and verification.

Results: WES data analysis revealed two frameshift mutations, a known (c.64dupA) mutation in Patient 1, and a novel (c.1223dupA) mutation in Patient 2. Patient 1 presented ASD, delayed speech, psychomotor development. Patient 2 manifested ASD, delayed speech, psychomotor development, intellectual disability, facial dysmorphism, hypotonia, epilepsy, visual disturbance, hirsutism. MRI showed significant aspecific white matter signal intensity change in both patients, furthermore corpus callosum dysplasia in Patient 2.

Conclusion: *ADNP* is a human transcription factor essential in brain development, however, according to our current knowledge only a few cases have been associated with abnormality of the white matter so far. Both of our patients presented such abnormality, and it suggests that it could be a potential rare feature in HVDAS.

References:.

Grants:.

Conflict of Interest: None declared.

P09.024.D Deep phenotypic characterisation in patients with proximal 22q11.2 duplication

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Background/Objectives: 22q11.2 duplication (22q11.2dup) is associated with variable clinical and neurodevelopmental features and a high rate of familial transmission. This study contributes to the phenotypic characterisation of 22q11.2dup.

Methods: In this retrospective study, we analysed digital medical records of 38 patients with proximal 22q11.2dup, focusing on physical and neurodevelopmental features, including longitudinal data in a subgroup (n = 17). Phenotypes of patients with de novo (n = 12) and inherited (n = 19) duplications were compared.

Results: Common clinical features include nutritional problems (59%), transient hearing impairment (50%), congenital heart defects (29%) and neurological abnormalities (44%). Developmental delays are reported in infancy, while learning (64%), attention (64%), speech-language (56%) and motor problems (56%) are present in primary school. ADHD is diagnosed in 33%. Average IQ is in the borderline range (IQ77), with 23% having mild intellectual disability (IQ55-70). Longitudinal IQ-data indicate that 53% show a growing into deficit trajectory. No significant differences between the group with inherited and de novo 22q11.2dup were observed, apart from a trend towards more failure to thrive in the latter.

Conclusion: This study confirms a heterogeneous phenotype in patients with 22q11.2dup and provides for the first time longitudinal IQ-data. Only index patients were included, potentially resulting in ascertainment bias. Therefore, future studies should also include family members diagnosed through segregation analysis. When children are diagnosed with 22q11.2dup, healthcare professionals should be aware of an increased risk of nutritional, neurological and hearing problems, and initiate neurodevelopmental support early in life, given the high risk of developmental delay, speech-language, motor and attention problems.

References:.

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Conflict of Interest: None declared.

P09.025.A Low-level parental somatic mosaicism detected by exome sequencing in cohort of patients with neurodevelopmental disorders

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Background/Objectives: Low-level somatic mosaicism leading to neurodevelopmental disorders (NDDs) and autism is more prevalent than previously thought. Due to limitations in its detection by routine molecular techniques, e.g. chromosomal microarrays or Sanger sequencing, detected single-nucleotide variants (SNVs) or small indel variants are often misinterpreted as de novo in the affected offspring. However, low-level somatic mosaicism in parents may significantly increase the risk of passing pathogenic variants to the offspring. We present our experience with exome sequencing as an effective approach for the detection of low-level parental somatic mosaicism for clinically relevant SNVs in patients with NDDs.

Methods: Our study included 45 families (trios or quatros) of infants with NDDs examined using the commercial kit Human Core Exome (Twist Biosciences) and Illumina NovaSeq 6000.

Results: Using a customized bioinformatic pipeline, we detected SNVs or indel variants classified as pathogenic or likely pathogenic in 18/48 children (37.5%). Two SNVs classified as pathogenic in probands were detected in parental blood samples as low-level mosaicism (10-15%), both in fathers. The father of patient with c.7059G>C variant in *DYNC1H* gene exhibited learning disabilities, a phenotype possibly related to the mosaic change. The father harbouring mosaic variant c.911dup in *CTNNB1* gene was clinically unaffected.

Conclusion: Our data demonstrate the importance of considering the possibility of parental mosaicism whenever exome sequencing is performed for precise genetic counselling.

References:.

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Conflict of Interest: None declared.

P09.026.B GenIDA, an international participatory database to better understand the natural history and comorbidities of genetic forms of neurodevelopmental disorders

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Background/Objectives: GenIDA is an international online research project initiated to better characterise the clinical manifestations and natural history of genetic forms of intellectual disability with or without autism or epilepsy.

Methods: Clinical information reported and updated by the patient's family using a structured questionnaire is analysed to identify new medically relevant information for families and professionals concerned with a given condition. The current questionnaire consists of 41 multiple-choice questions exploring physical parameters, cognitive and behavioural aspects, the presence or absence of neurological disorders or problems affecting major physiological functions (cardiac, renal, etc.). Five open-ended questions explore families' perception of the events that most affect their relative's health and quality of life, the secondary effects of treatments, etc.

Results: Currently, the questionnaire is available in 7 languages and has been completed for 1415 patients, the main cohorts being Koolen-de Vries/KdVS (n = 244), Kleefstra (174) and KBG (43) syndromes. For the KdVS, epilepsy, which affects almost 50% of patients, is of much greater concern than sleep problems. Other cohorts have grown significantly over the past year (DDX3X: 45; MED13L: 43; DYRK1A: 23; KMT2A: 32; POGZ: 18). Comparing several aspects of these 5 conditions reveals major differences: behavioural problems and sleep disorders appear to be more frequent in KMT2A and POGZ patients, while movement disorders are more frequent in DDX3X patients.

Conclusion: This validates the interest of our participatory approach: through their direct involvement, families can reveal aspects of the pathology that were previously underestimated.

References:.

Grants:.

Conflict of Interest: None declared.

P09.027.C Optical Genome Mapping Analysis of FMR1 Expansions in Fragile X Syndrome and Multi-site Validation

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Background/Objectives: Fragile X syndrome (FXS) is associated with intellectual disability, and is usually due to CGG expansion in FMR1 [1]. Phenotype severity being correlated with expansion size, accurate sizing is crucial. The repetitive nature of these regions presents difficulties: 1. PCR is unable to traverse through long repeats; 2. Sequencing is limited short-read lengths; 3. Southern-blot is inaccurate, time-consuming, and expensive. Optical genome mapping (OGM) has the potential to address some of these shortcomings [2].

Methods: OGM images ultra-long DNA molecules, labeled at specific motifs linearized in nanochannel arrays, and can be used for SV and CNV calling. We developed a targeted analysis workflow for

FMR1 analysis. To evaluate the capability of measuring repeat arrays in ranges consistent with normal, premutation, and full mutation, we analyzed 75 FXS samples and 20 control subjects.

Results: In annotated samples, we observed alleles consistent with annotation across the entire range of repeat counts. Sensitivity was measured at 97% with 100% PPV for expansions >200 repeats [3]. The largest expansion detected was ~1000 repeats. In controls, we measured CN below the full mutation cutoff in all cases. Repeatability studies were carried out to show analytical consistency. EnFocus™ analysis report provides pass/fail for QC metrics as well as analytic measurement quality using internal control regions on each autosome chromosome.

Conclusion: OGM performance for FMR1 repeat lengths show a much higher dynamic range compared to PCR, NGS, and higher precision compared to Southern-blot.

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Grants:.

Conflict of Interest: Alessio Venier Full-time employee Bionano Genomics, Martin Muggli Bionano Genomics Employee, Bahar Ramandi Bionano Genomics Employee, Neil Miller Bionano Genomics Employee, Joyce Lee Bionano Genomics Employee, Andy Wing Chun Pang Bionano Genomics Employee, Henry B. Sadowski Bionano Genomics Employee, Yannick Delpu Bionano Genomics Employee, Alex Hastie Bionano Genomics Employee, Mark Oldakowski Bionano Genomics Employee.

P09.028.D An atypical Rubinstein-Taybi syndrome 2 associated with EP300 exon 20 skipping

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Background/Objectives: *EP300* gene encodes p300, a ubiquitously expressed histone acetyltransferase that regulates transcription via chromatin remodeling. *EP300* haploinsufficiency is associated with Rubinstein-Taybi syndrome 2 (RSTS2, MIM#613684). The phenotypic and mutational spectrum of this condition is broad and genotype–phenotype correlations have been proposed, suggesting clinical features depend upon the protein domain involved.

Methods: Trio exome analysis and genematcher exchange were conducted. Splicing analysis on cDNA from patient#1-derived PBMCs and Sanger sequencing on single bands extracted from agarose gel were performed.

Results: We identified two unrelated patients with de novo heterozygous variants in *EP300* (NM_001429.4) affecting exon 20 splicing: patient#1 with c.3671+5G>C, and patient#2 with c.3671+5_3671+8delGTAA. Both patients presented a severe RSTS2-like clinics, including ASD, speech delay, hearing loss, microcephaly, developmental delay and intellectual disability, accompanied with ocular, respiratory and cardiovascular abnormalities. Patient#2 developed colorectal cancer and deceased at 35 years old. Both splicing variants were predicted to affect exon 20 donor site (splice site score: -39% and -41%, respectively; visual Alamut). Splicing analysis on cDNA from patient#1 showed an in-frame exon-20 skipping, also expected for patient#2. In silico 3D protein modeling showed that loss of exon 20 (27 a.a.) leads to a possible alteration of p300 structure between RING_CBP-p300 and HAT-KAT11 domains. To further corroborate our data, we will study DNA methylation profiles and p300 protein expression.

Conclusion: We suggest a novel *EP300*-related RSTS2 associated with *in-frame* exon 20 skipping. Our results further highlight the need of assessing the pathogenic role of splicing variant surrounding the invariant donor/acceptors splicing sites.

References:.
Grants:.

Conflict of Interest: None declared.

P09.029.A Heterozygous and homozygous variants in STX1A cause a neurodevelopmental disorder with or without epilepsy

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Background/Objectives: The neuronal SNARE complex is responsible for synaptic vesicle exocytosis. Therefore, one of its core proteins syntaxin 1A (*STX1A*) has long been suspected to play a role in neurodevelopmental disorders.

Methods: We assembled eight individuals harbouring single amino acid deletions, missense and splicing variants in *STX1A* who present with a spectrum of neurodevelopmental delay and epilepsy. For all missense variants and single amino acid deletions, we applied in silico modelling of functional effects on interaction with other SNARE components and STXBP1.

Results: In our detailed phenotypic description, we observed an epileptiform characterized phenotype in the individuals with missense variants in contrast to intellectual disability with autistic behaviour in carriers of both single amino acid deletions and the homozygous splicing variant. In silico modelling showed impaired interaction with STXBP1 due to missense variants and

impaired SNARE complex function caused by single amino acid deletions.

Conclusion: Different lines of evidence presented here support that rare heterozygous and homozygous variants in *STX1A* cause a neurodevelopmental disorder with two different phenotypic presentations: (1) an *STX1A*-related developmental epileptic encephalopathy and (2) an *STX1A*-related intellectual disability and autism spectrum disorder. Our description thus expands the group of disorders called SNAREopathies.

References:.
Grants:.
Conflict of Interest: None declared.

P09.030.B The performance of GS as a first-tier test for neurodevelopmental disorders

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Background/Objectives: Genome sequencing (GS) can identify novel diagnoses for patients who have exhausted routine diagnostic procedures. We tested whether GS is a better first-tier genetic diagnostic test than current exome-based standard of care (SOC) by assessing the technical and clinical validity of GS for patients with neurodevelopmental disorders (NDD).

Methods: Using a prospective parallel design, we performed both GS and exome sequencing in 150 consecutive NDD patient-parent trios. Diagnostic yield was calculated from disease-causing variants affecting exonic sequence of known NDD genes as primary outcome measure.

Results: GS (30%, n = 45) and SOC (28.7%, n = 43) had similar diagnostic yield. All 43 conclusive diagnoses obtained in SOC were also identified by GS. These 43 conclusive diagnoses included a mixture of single nucleotide variants (SNVs, n = 26), insertion-deletion variants (InDels, n = 13), copy number variants (CNVs, n = 3), and a repeat expansion (n = 1). All 31 possible diagnoses obtained by SOC were also identified by GS. SOC, however, required integration of multiple test results (average 1.5; range 1-6) to obtain these conclusive and possible diagnoses. GS yielded two more conclusive diagnoses, and four more possible diagnoses than ES-based SOC (35 vs 31). Interestingly, all six likely pathogenic variants detected only by GS were CNVs.

Conclusion: Our data provide the technical and clinical validity of GS to serve as routine first-tier genetic test for patients with NDD. The additional diagnostic yield from GS is limited. Still, GS comprehensively identified all variants in a single experiment, suggesting that GS could constitute a more efficient genetic diagnostic workflow for patients with NDD.

References:.
Grants:.
Conflict of Interest: None declared.

P09.031.C Systematic evaluation of EHMT1 protein altering variants uncovers unexpected insights on EHMT1 functions and Kleefstra syndrome pathogenesis

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Background/Objectives: EHMT1 is a histone-3 lysine-9 (H3K9) methyltransferase acting in heterodimer with EHMT2. EHMT1 haploinsufficiency causes Kleefstra syndrome (KS), but the role of *EHMT1* protein altering variants' (PAV) is unknown. Therefore, we have systematically collected and evaluated EHMT1 PAVs.

Methods: We collected 30 different EHMT1 PAVs, identified in 40 individuals with neurodevelopmental disorders (NDDs), and evaluated them on: 1)patients' phenotype similarity to KS, 2)predicted effects on the protein's 3D structure, and 3)KS-episignature on DNA methylation array. Additionally, we studied EHMT1-EHMT2 heterodimerization capacity and methyltransferase-activity in-vitro for selected variants.

Results: Out of the 30 analysed PAVs, we concordantly classified nine as benign and 17 as pathogenic. All pathogenic PAVs located in the ANK-repeat domain (n = 11), a H3K9me1-2 "reader", or in the pre-SET/SET-domain (n = 6), which is responsible for the EHMT1-EHMT2 heterodimerization and methyltransferase-activity. Surprisingly, in four individuals with non-KS NDD and without KS-episignature, we identified three clustering SET-domain PAVs (two *de-novo* and one sib-pair) predicted to disrupt methyltransferase-activity, but not EHMT1-EHMT2 heterodimerization. This suggests that loss of only EHMT1 methyltransferase-activity is not responsible for KS. Indeed, unlike other PAVs, only KS-causing SET-domain PAVs, disrupted EHMT1-EHMT2 heterodimerization in-vitro. Finally, we identified one individual without KS-phenotype and KS-episignature but with a *de-novo* PAV that completely disrupted the RING-like domain (functions unknown).

Conclusion: Disruption of EHMT1 "reader" or EHMT1-EHMT2 heterodimerization functions is sufficient to cause KS. However, the effects of loss of EHMT1 methyltransferase-activity or of RING-like domain are unknown but may result in novel syndromic NDDs e.g. via loss of the recently-described EHMT1-mediated non-histone protein methylation.

References:.

Grants:.

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P09.032.D A novel, recurrent TCF4 missense variant causes non-specific intellectual disability without Pitt-Hopkins syndrome

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Background/Objectives: Haploinsufficiency of TCF4 by deletions, truncating variants or loss-of-function missense variants clustering within the DNA-binding and protein interacting bHLH domain cause Pitt-Hopkins syndrome (PTHS). This specific neurodevelopmental disorder (NDD) is characterized by severe intellectual disability (ID), epilepsy, postnatal microcephaly, hyperbreathing and typical facial dysmorphism. Only few deletions and variants, mostly located in the very N-terminal part of TCF4 have been associated with milder or atypical phenotypes.

Methods: By personal communication, we now assembled four cases with the novel, recurrent, de novo missense variant c.1165C>T, p.(Arg389Cys) in TCF4. This variant was independently identified by diagnostic exome or ID panel sequencing in different centers and is located upstream of the bHLH domain.

Results: In three of the individuals, the variant was initially classified as of unknown significance due to its location outside the bHLH domain and a rather unspecific neurodevelopmental phenotype not suggestive of PTHS. Recurrence of the identical variant in four individuals allowed reclassification to pathogenic. All four individuals presented with moderate to severe ID with prominent language delay and impaired speech. Microcephaly occurred only in one of the four individuals, and no breathing anomalies or epilepsy were reported. Facial gestalt was unsuspicious. Interestingly, the variant is located in the AD2 activation domain next to a highly conserved Φ-x-x-Φ-Φ-motif and might alter interaction with coactivator proteins independently from the bHLH domain.

Conclusion: Our findings of a recurrent missense variant outside the bHLH domain in four individuals with a rather non-specific ID phenotype delineates a novel genotype-phenotype correlation for TCF4-related NDDs.

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Conflict of Interest: None declared.

P09.033.A Update on the phenotype of symptomatic females with ARX pathogenic variants, including 9 new patients

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Background/Objectives: The X-linked Aristaless-Related Homeobox (ARX) gene encodes a transcription factor that is essential for brain development. ARX-related disorders are well-described in male patients and encompasses syndromic and nonsyndromic intellectual disability with or without brain malformations and epilepsy. By contrast, the phenotype in females is poorly delineated.

Methods: We report the clinical and molecular data of nine novel female patients with de novo ARX pathogenic variants and review the data of the 63 females with truncating or missense variants from the literature.

Results: Half of the female carriers have a normal neurodevelopment, whereas the other half have a neurodevelopmental disorder (NDD) comprising: isolated learning disabilities (8.3%), mild to moderate ID (20.8%), developmental and epileptic encephalopathy (19.4%). NDD was significantly more prevalent in the group of women carrying de novo variants (84%). Agenesis of the corpus callosum (ACC) was observed in 65% of cases and affected more significantly the group of patients with NDD. Among the 10 previously reported de novo variants in female patients, all but two were truncating variants. We report here five de novo missense variants, which are all located in the homeodomain of the protein, affecting the ARX transcriptional activity. None of the de novo cases from our cohort had skewed XCI in blood.

Conclusion: ARX pathogenic variants in female patients are characterized by a broad clinical spectrum ranging from no symptoms to DEE phenotype, frequently associated with ACC. These new data will help prenatal counseling when an ARX variant is found in a female fetus with corpus callosum anomaly.

References:.

Grants:.

Conflict of Interest: None declared.

P09.034.B BRAT1 biallelic variations: phenotypic spectrum and phenotype-genotype correlation from 91 cases

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Background/Objectives: Biallelic variations in *BRAT1* were described in 40 patients from 29 families, presenting with epileptic encephalopathy or cerebellar ataxia.

Methods: Thanks to an international collaboration, we collected data of 51 new patients, allowing us to describe a large series of 91 patients.

Results: Data analysis showed two distinct phenotypes and an intermediate one. In the first group, 43 patients exhibited a neonatal epileptic encephalopathy. Prenatal signs were present in 16 of them (57%). None of them had any psychomotor acquisition and all had epilepsy with neonatal onset. Neurological examination showed microcephaly (91%) and limb rigidity (90%). Brain MRI showed cerebral atrophy (58%). They all died prematurely.

In the second group, 36 patients presented cerebellar ataxia, with acquisition of walking (85%) and language (69%). Axial hypotonia was found in 23 patients (85%). Epilepsy was rare (14%). Brain MRI always showed cerebellar atrophy. No patient in this group died.

Study of phenotype-genotype correlation showed that patients with epileptic encephalopathy harbour two predicted amorphic variations in most cases (25/33; 76%) and a recurrent inframe deletion/duplication in a tenth of cases (4/33; 10%). In contrast, patients with cerebellar ataxia present at least one missense variation (23/27; 85%).

Conclusion: *BRAT1* bi-allelic variations are associated with a broad phenotypic spectrum whose most severe end, observed in patients with two amorphic variations, is associated with epileptic encephalopathy and early death. On the opposite, patients with at least one missense variation seem to have a moderate phenotype including variable intellectual disability, ataxia and cerebellar atrophy.

References:.

Grants:.

Conflict of Interest: None declared.

P09.036.D Clinical interpretation of aCGH results in patients with neurodevelopmental disorders by geneticists: impact on diagnostic yield

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Background/Objectives: Neurodevelopmental disorders (NDD) are complex diseases with significant health and social implications. Genetic alterations play an important role in their pathogenesis and array comparative genomic hybridization (aCGH) is recommended as first-tier genetic test. We aimed to evaluate the diagnostic yield (DY) of aCGH in our cohort of NDD patients, comparing different clinical groups.

Methods: We enrolled 420 patients with developmental delay (DD), intellectual disability (ID) or autism spectrum disorder (ASD) who underwent aCGH analysis and genetic counseling between 2017 and 2021. Copy number variants (CNVs) were classified according to American College of Medical Genetics (ACMG) guidelines. Parental investigation, when necessary, was carried out by quantitative PCR.

Results: We identified at least one class 3, 4 or 5 CNV in 207 patients. After clinical evaluation, considering patients' phenotype and CNV inheritance, DY of aCGH in our cohort was 18,1% (18,6% in patients with DD/ID, 14,1% in patients with ASD associated with DD/ID and 9,1% in patients with isolated ASD). DY was 17,3% in non-syndromic patients and 18,4% in patients with NDD associated with congenital malformations, abnormal brain MRI, dysmorphisms or epilepsy (19,6% in patients with 2 or more of the conditions listed above).

Conclusion: We observed a global DY comparable to that reported in published large cohorts and a reduction of DY in isolated ASD. As expected, DY was slightly higher in patients with more complex clinical phenotype. This highlights the importance of accurate evaluation by a clinical geneticist to identify patients with NDD who should be tested by aCGH.

References:.

Grants:.

Conflict of Interest: None declared.

P09.037.A Further characterization of Borjeson-Forssman-Lehmann syndrome in females due to de novo variants in PHF6

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Background/Objectives: While inherited hemizygous variants in PHF6 cause X-linked recessive Borjeson-Forssman-Lehmann syndrome (BFLS) in males, de novo heterozygous variants in females are associated with an overlapping but distinct phenotype. This includes moderate to severe intellectual disability, characteristic facial dysmorphism, dental, finger and toe anomalies and linear skin hyperpigmentation.

Methods: By personal communication with colleagues, we assembled clinical and mutational data on ten additional female individuals with BFLS due to variants in PHF6. Testing was either performed targeted by Sanger sequencing or MLPA or by exome or panel sequencing. X-inactivation in blood was determined in six individuals. Structural modeling was performed for missense variants.

Results: We confirm the distinct female phenotype of BFLS to include variable intellectual disability, a recognizable facial gestalt and various other anomalies. Skewed X-inactivation in blood and streaky skin pigmentation point to a functional mosaicism. Variants occurred de novo in nine individuals, of whom one was only mildly affected and inherited it to her more severely affected daughter. The mutational spectrum comprises a 2-exon deletion, five truncating and three missense variants, the latter all located in the PHD2 domain and predicted to severely destabilize the domain structure. This observation supports the hypothesis of more severe variants in females contributing to gender-specific

phenotypes in addition to or in combination with effects of X-inactivation and functional mosaicism.

Conclusion: Our findings therefore further delineate the clinical and mutational spectrum of female BFLS and provide further insights into possible genotype-phenotype correlations between females and males.

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Conflict of Interest: None declared.

P09.038.B The largest cohort of KBG patients: defining the evolving phenotype

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Background/Objectives: KBG syndrome is characterized by distinctive facial gestalt, skeletal features, short stature, and variable clinical findings. With aging, some clinical features become more recognizable, allowing a differential diagnosis. We aimed to better characterize KBG-associated clinical features progression.

Methods: In the context of an ERN ITHACA collaborative study, we collected the largest cohort of KBG patients (47) followed over time. A combined array-CGH and NGS approach on genomic DNA investigated both genomic CNV and SNV.

Results: Intellectual disability (81%) ranged from mild to moderate with severe ID identified in one patient. Epilepsy was present in 25%. Short stature was consistent over time, while OFC, about -2SD at birth, normalized over years. Macrodontia, oligodontia and dental agenesis was present in 30,2%, representing the second most relevant clinical feature along with skeletal anomalies (44%, 28% with 5th finger clinodactyly). Two patients presented microdontia. Cerebral anomalies, among which enlarged cisterna magna and broad anterior fontanella, were identified in 35%. Heart defects were reported in 16,3%. In 28% of cases prenatal ultrasound anomalies were reported (increased NT, polyhydramnios, IUGR). Except for three splicing variants, leading to a premature termination, mutations were almost all frameshift, scattered along ANKRD11.

Conclusion: Our results, broadening the spectrum of KBG phenotype progression, suggest to consider a wider range of dental anomalies including microdontia before excluding diagnosis. Cerebral anomalies, previously sporadic reports, represent a moderately frequent feature. NGS approaches, following evidence of increased NT, should include *ANKRD11*.

References:.

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Conflict of Interest: None declared.

P09.039.C Inherited pathogenic variants in neurodevelopmental disorders: a potential pitfall in trio-based analysis of clinical exomes

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Background/Objectives: The use of clinical exome sequencing (ES) has led to a steep increase in the diagnostic yield in patients with neurodevelopmental disorders (NDDs).

Methods: ES data of > 2000 patients and both parents (when available) were analyzed for a panel of 1441 NDD related genes. Variant classification was based on ACMG/AMP and ACGS guidelines.

Results: We identified a class 4 or 5 variant explaining the phenotype in 21% of cases. In 60% of these probands, the variant occurred de novo, while in 40% the causal variant(s) was/were inherited. Of the probands with inherited variants, 12.5% had an X-linked disorder, 46% an autosomal recessive disorder, and 41.5% an autosomal dominant disorder. Furthermore, we reported a variant of unknown significance (class 3) in an additional 25% of the probands.

Conclusion: With a diagnostic yield between 21% and 46%, we confirm that exome sequencing is a game-changer in the diagnostic workup of patients with NDDs. Although the majority of causal variants occurred de novo, several (likely) pathogenic variants were observed in seemingly healthy parents. Analysis pipelines focusing solely on de novo or bi-allelic defects, may therefore miss molecular diagnoses. Our data add evidence that reduced penetrance is currently underreported in several NDDs and we should be cautious to rely on the "de novo paradigm for neurodevelopmental disorders" both in laboratory analysis and genetic counseling.

References:.

Grants:.

Conflict of Interest: None declared.

P09.040.D Altered gene expression profiles impair the nervous system development in patients with 15q13.3 microdeletion

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Background/Objectives: The 15q13.3 microdeletion has a wide range of pleiotropic effects ranging from apparently healthy to severely affected individuals with intellectual disability, epilepsy, and neuropsychiatric disorders. Previous studies have investigated several genes within the deletion as potential drivers, but the pathomechanism and the underlying basis of the variable phenotype remain elusive.

Methods: We analyzed the effects of the 15q13.3 microdeletion using native RNA-seq data from blood of 3 probands and 4 control subjects. We assessed differentially expressed genes (DEGs), gene ontology (GO) enrichment, protein-protein interaction (PPI) functional modules, as well as gene expression in different brain developmental stages.

Results: The haploinsufficiency of genes within the deleted region was not transcriptionally compensated, suggesting a dosage effect may contribute to the pathomechanism. We observed network-wide dysregulatory effects implying the phenotype is not caused by a singular critical gene. A significant proportion of DEGs, which are silenced in the adult brain, have maximum expression during the prenatal stage of brain development. Based on DEGs and their PPI partners we identified altered functional modules related to immune and inflammatory processes, as well as nervous system development.

Conclusion: 15q13.3 microdeletion has an ubiquitous impact on transcriptome pattern, especially dysregulation of genes involved in brain development. The impact on immune and inflammatory responses cannot be fully assessed based on the test material, i.e. blood. Possibly, the high phenotypic variability could stem from an increased vulnerability during brain development, without an underlying driving pathomechanism.

References:.

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P09.041.A Clinical description, molecular delineation, and genotype-phenotype correlation in KBG syndrome: 67 new patients and review of the literature

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Background/Objectives: KBG syndrome is a highly variable neurodevelopmental disorder caused by loss-of-function sequence variants and large deletions involving *ANKRD11*. Despite several attempts, to date no correlation has been reported between KBG features and *ANKRD11* aberration. We present the clinical and molecular characteristics of 67 new patients with KBG syndrome and perform a genotype-phenotype correlation leveraging data on 273 patients previously published.

Methods: 67 patients with KBG syndrome were recruited through a Spanish collaborative effort. 43 clinical features were assessed using a phenotypic questionnaire developed for this project. The frequency of the features was calculated and those with a frequency >50% of the patients were used to perform a genotype-phenotype correlation in the 67 patients and 273 KBG patients from the literature comparing the main features of the syndrome in patients with four different types of *ANKRD11* variants.

Results: The most frequent features were those related to neurodevelopment (95%), comorbidites (82.8%), macrodontia (80.9%), triangular face (71%), characteristic ears (76%), nose (75.9%) and eyebrows (67.3%). The genotype-phenotype correlation yielded significant associations with the triangular face (71.1% in SNVs vs 45.2% in CNVs, $p = 0.015$), short stature (62.5% inside exon 9 vs. 27.8% outside; $p = 0.009$) and macrodontia (with larger deletions, $p = 0.028$). ID/ADHD/ASD (70.4% in c.1903_1907del vs. 89.4%; $p = 0.012$) and a higher phenotypic score in patients with SNVs ($p = 0.005$).

Conclusion: We present a detailed phenotypic description of KBG syndrome in the largest series of patients reported to date and provide evidence of a genotype-phenotype correlation between some KBG features and specific *ANKRD11* aberrations.

References:.

Grants:.

Conflict of Interest: Elena Martinez-Cayuelas: None declared, Isabel Lorda: None declared, Fiona Blanco-Kelly: None declared, Rosario Lopez-Rodriguez: None declared, Fermina Lopez-Grondona: None declared, Saoud Tahsin-Swafiri: None declared, Rebeca Losada: None declared, Beatriz Moreno: None declared, Maria Rodrigo: None declared, Carmen Ayuso: None declared, Aitor López-González: None declared, Antonio Martinez-Monseny: None

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P09.042.B De novo missense variants in SLC32A1 cause a neurodevelopmental disorder with epilepsy due to impaired GABAergic neurotransmission

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Background/Objectives: Rare inherited missense variants in SLC32A1, the gene that encodes the vesicular GABA transporter (VGAT), have recently been shown to cause genetic epilepsy with febrile seizures plus. We aimed to clarify if de novo missense variants in SLC32A1 can also cause a neurode-velopmental disorder with epilepsy.

Methods: Using exome sequencing, we identified four individuals with a neurodevelopmental disorder with epilepsy and de novo missense variants in *SLC32A1*. To assess causality, we performed functional evaluation of the identified variants in a murine neuronal cell culture model.

Results: The main phenotype comprises moderate to severe intellectual disability, early onset epilepsy within the first 18 months of life and a choreatic, dystonic or dyskinetic movement disorder. In silico modeling and functional analyses reveal that three of these variants, which are located in helices that line the putative GABA transport pathway, result in reduced quantal size, consistent with impaired filling of synaptic vesicles with GABA. The fourth variant, located in the VGAT N-terminus, does not affect quantal size, but increases presynaptic release probability, leading to more severe synaptic depression during high frequency stimulation. Thus, variants in VGAT can impair GABAergic neurotransmission via at least two mechanisms, by affecting synaptic vesicle filling and by altering synaptic short-term plasticity.

Conclusion: This work establishes de novo missense variants in *SLC32A1* as a novel cause for a neurodevelopmental disorder with epilepsy.

References:.

Grants:.

Conflict of Interest: Konrad Platzer: None declared, Heinrich Sticht: None declared, Caleb Bupp: None declared, Mathily Ganapathi: None declared, Elaine Pereira: None declared, Gwenaël Le Guyader: None declared, Frederic Bilan: None declared, Lindsay Henderson GeneDx, Holger Taschenberger: None declared, Nils Brose: None declared, Maximilian Radtke: None declared, Rami Abou Jamra: None declared, Sonja Wojcik: None declared.

P09.044.D Whole exome sequencing for the diagnosis of genetically unexplained severe or syndromic neurodevelopmental disorders

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Background/Objectives: Neurodevelopmental disorders (NDDs), including intellectual disability (ID), global developmental delay (GDD), and autism spectrum disorder (ASD), represent the most prevalent chronic medical conditions encountered in pediatric primary care. Identification of an underlying genetic etiology may improve prognostic predictions, ensure personalized management, clarify recurrence risk, and direct patients and families to condition-specific resources and supports. Whole exome sequencing (WES) was reported to reach a definite diagnosis in about 40% NDD patients (1). Here we assess the diagnostic yield reached by WES in our diagnostic lab for unexplained severe non-syndromic and syndromic ID, GDD, or ASD patients, negative at array-GCH and fragile-X testing.

Methods: WES was performed on 30 patients with severe non-syndromic GDD/ID and/or level 3 ASD (12 complicated with epilepsy), and on 67 syndromic NDD patients recruited over a 3-year period regardless of their age (mean 13 years), gender and familial history.

Results: A definite genetic diagnosis was reached in 44/97 (45%) probands, including 9/30 (30%) with severe non-syndromic NDD, and 35/67 (52%) with syndromic NDD. Of note, for syndromic patients, the diagnostic rate increased linearly with the number of concurrent clinical features, raising from 48% (2 concurrent features) up to 75% (4 features).

Conclusion: This study confirms that WES is a powerful diagnostic tool for NDDs, especially in case of severe or syndromic presentations, and should therefore be included in the diagnostic workout for these common disorders.

References: 1. Srivastava S et al. 2014. https://doi.org/10.1002/ana.24251.

Grants:.

Conflict of Interest: None declared.

P09.045.A Array-CGH in a large cohort of unexplained syndromic and non-syndromic neurodevelopmental disorders

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Background/Objectives: Neurodevelopmental disorders (NDDs), including intellectual disability (ID), global developmental delay (GDD) and autism spectrum disorder (ASD), are non-progressive disorders typically diagnosed from infancy to adolescence, and characterized by deficits in cognition, language, behavior, and/or motor skills. Here we aimed at comparing the diagnostic yield of array-comparative genomic hybridization (aCGH) for the diagnosis of syndromic versus non-syndromic NDDs in our diagnostic laboratory.

Methods: aCGH was performed using a 180K platform (Agilent Technologies) on 156 patients with syndromic NDDs and 117 with non-syndromic NDDs recruited over a 3-year period regardless of their age (mean 11 years), gender (M:F = 174:96) and family history.

Results: 30 pathogenic unbalanced rearrangements (21 deletions, 9 duplication) were identified in 25 patients. The most frequent rearrangements affected chromosomes 15 and 16; four probands carried more than one pathogenic copy number variants. The overall detection rate was 9% (25/270), with a similar rate in syndromic (13/156, 8%) and non-syndromic cases (12/114, 10%). In three patients, a double diagnosis was achieved through an integrated approach which combined aCGH and whole-exome sequencing (WES).

Conclusion: Our data show that aCGH remains a cost-effective, rapid and powerful first-level diagnostic strategy, able to diagnose about 10% NDDs patients regardless of their clinical presentation.

References:.

Grants:.

Conflict of Interest: None declared.

P09.046.B Genetics of anomalies of the corpus callosum: lessons from a cohort of 403 individuals

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Background/Objectives: The corpus callosum is the main cerebral commissure. Its complex development occurs between the 11th and 15th week of gestation and involves multiple cellular and molecular events. Anomalies of the corpus callosum (AnCC) are among the most common brain malformations in humans. Their etiologies are numerous and involve many different genetic causes.

AnCC are currently identified by ultrasonography during the pregnancy which leads to difficulties in terms of prenatal and genetic counseling. Indeed, the neurodevelopment prognosis associated is very large, from normal development to severe intellectual disability (ID). The fetal prognosis depends to the underlying etiology of the AnCC.

Methods: We describe the clinical and genetic aspects of a cohort of 403 individuals with AnCC. 38 (9.4%) of them had a chromosomal anomaly and 105/292 (36%) had a least one sequence variant (found by exome/genome sequencing or by targeted sequencing) explaining the AnCC.

Results: In our series, genetic causes of AnCC were identified (50 %) when it was associated with developmental delay and/or ID, mostly in genes responsible for monogenic syndromes with ID. In all "solved" cases, AnCC was a trait with incomplete penetrance. By contrast, when AnCC was associated with a normal neurodevelopment, the diagnosis rate falls with only few recurrent genes identified (including DCC).

Conclusion: This confirmed that AnCC may result from the disruption of multiple developmental steps from early midline telencephalic patterning to neuronal specification and guidance of commissural axons. Our poor knowledge of genetic causes of isolated AnCC leads us to consider other pathogenic mechanisms such as oligo/polygenic inheritance.

References:.

Grants:.

Conflict of Interest: None declared.

P09.047.C Assessment of the transcriptomic consequences of NIPBL pathogenic variants in edited induced pluripotent stem cells

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Background/Objectives: Cohesins play a critical role in gene expression regulation. Cornelia de Lange syndrome (CdLS) is a transcriptomopathy related to cohesin complex alterations. *NIPBL*, the main CdLS gene, encodes the cohesin loading factor. Gene expression consequences of *NIPBL* pathogenic variants might be used as potential CdLS biomarkers.

Methods: By CRISPR/Cas9, we introduced each of the following *NIPBL* pathogenic variants in an induced pluripotent stem cell (iPSC) line, in a heterozygous state: p.Arg45*, p.Arg834*, p.Ser1466Lysfs*13 and p.Arg2298Cys, as well as frameshift indels in the corresponding exons. We assessed *NIPBL* total mRNA levels

by RT-ddPCR, performed RNAseq and measured protein levels of NIPBL and its partner MAU2 by western-blotting.

Results: Both RT-ddPCR and RNAseq showed a decrease in *NIPBL* RNA levels for all cells carrying premature stop codon variants, except for the p.Arg45* variant, where *NIPBL* mRNA level was slightly increased. The differential expression analysis highlighted a pattern of up or downregulated genes. For all conditions, including the missense variant, a 50% decrease of MAU2 protein levels was observed, without *MAU2* mRNA modification.

Conclusion: We propose new edited iPSC models for CdLS. Our results support previously established data suggesting that variations in the 5′ end of *NIPBL* coding sequence escape nonsense mediated decay, possibly due to translation reinitiation[1]. We will confront our results to RNAseq and MAU2 western blot on patients' blood, to assess their diagnostic potential.

References: (1) Parenti et al., PMID 32433956.

Grants:.

Conflict of Interest: None declared.

P09.048.D Identification of a causal mutation in DLG3 in the MRX20 family

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Background/Objectives: Here, we describe one of the 105 historical families that received MRX numbers. MRX20 is one of the larger pedigrees which presents with intellectual disability. In 1995 Lazzarini et al. (AJMG 57: 552-7) could map the causal region to the centromeric region on the X-chromosome. In this study we identified the causal gene and investigated its possible effects with contemporary techniques.

Methods: DNA and RNA was extracted from Epstein-Barr virus (EBV) cell lines of 19 family members. Whole exome sequencing (WES) was performed on two affected and one unaffected sample. Results were confirmed using Sanger Sequencing in all available members of the pedigree.

RNA sequencing (RNAseq) was performed in 7 affected and 4 unaffected male samples. Pathway analysis was done using the ingenuity pathway analysis (IPA) tool. Differential expression of 11 genes were validated using quantitative PCR (qPCR). Data analysis was performed using qBASE+ and Graphpad.

Results: A causal c.194delC mutation in the *DLG3* gene was identified, aside from two other variants of unknown significance that also segregated in the family; *SSX1* (c.358G>T) and *USP27x* (c.56A>G). Fourteen differentially expressed genes were identified. Currently, 7 out of 11 were confirmed using qPCR: *BCL11A, PPP1R16B, WWTR1, LDHA, NMT2, PEX26* and *CDCA4*. Pathway analysis resulted in 4 related networks.

Conclusion: A causal mutation in *DLG3* was identified in the MRX20 family. Fourteen genes were found to be significantly differentially expressed, of which 7 out of 11 could be confirmed. Further research is ongoing.

References:.

Grants:.

Conflict of Interest: None declared.

P09.049.A Multiomics approach identifies altered molecular pathways in MECP2-duplication syndrome and Rett syndrome patients

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Background/Objectives: Methyl CpG binding protein 2 (*MECP2*), located in the Xq28, is a multifunctional gene. Loss-of-function mutations in *MECP2* trigger Rett syndrome (RTT), whereas its duplication causes *MECP2* duplication syndrome (MDS). Both syndromes are characterized by neurodevelopmental delay and intellectual disability. Although the disease-causing genetic alterations are known, the molecular pathomechanism remains unknown. We aim to understand which are the molecular pathways altered in RTT and MDS.

Methods: RNA and protein were extracted from skin fibroblasts of 18 MDS patients, 21 RTT patients and 15 healthy controls. We performed transcriptomics and proteomics analysis using DESeq and LIMMA in a case-control approach. Enrichment of the differentially expressed genes (DEG) was evaluated.

Results: Transcriptomics and proteomics analysis showed a number of pathways that are significantly altered in both syndromes. Since *MECP2*'s functionality is dysregulated in an opposed way, we checked whether significantly upregulated genes in MDS were downregulated in RTT. The analysis revealed pathways involved in DNA replication and RNA processing.

Conclusion: We present the multiomics analysis of one of the biggest cohorts of RTT and MDS patients described so far. We could identify altered pathways in RTT and MDS patients regulated in opposite direction and, hopefully, contribute to the understanding of these two syndromes.

References:.

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Conflict of Interest: None declared.

P09.050.B Uncovering BOD1 mutation effect and its correlation to syndromic intellectual disabilities

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Background/Objectives: We and others showed that inherited autosomal recessive nonsense mutations in *BOD1* gene resulted a syndromic intellectual disability mostly combined with endocrine dysfunction. *BOD1* has shown to: direct chromosome orientation

in mitosis, form a complex with a methyltransferase *SET1B* that regulates expression of some fatty acid metabolic genes and its downregulation showed also synaptic defects and learning deficiency is drosophila.

Our project aims to determine the consequences of p.R151* nonsense mutation and to start investigating at molecular, cellular and organism levels the mechanisms linking BOD1 deficiency to syndromic ID.

Methods: Two HEK293 cell models were obtained by either introducing p.R151* *BOD1* mutation or deleting *BOD1* coding region by CRISPR/Cas9. We are also developing *BOD1* knockout mouse model to investigate its developmental and physiological function.

Results: HEK293 cells with p.R151* mutation express a truncated protein that was observed by Western blot analysis when proteasome is inhibited, indicating that mutated allele mutation is probably amorph, with complete loss of function. HEK293T clones with *BOD1* KO display an altered morphology suggesting modified cell adhesion properties. The electrophoretic profile of *BOD1* KO clones indicate an alteration of protein expression that remains to investigate. Contrary to what was observed, after RNA interference-mediated knockdown of *BOD1*, we didn't observe alterations of ADIPOR1 expression in the kO cells.

Conclusion: Our observations indicate that p.R151* mutation corresponds to an amorpha allele. We showed that *BOD1* is not essential for survival or proliferation of HEK293T cells but its deprivation leads to an altered colony morphology on uncoated plastic surface.

References:.

Grants: University of Namur.

Conflict of Interest: Nadine Hamdan: None declared, Olivier De Backer Professor Full time Job, University of Namur, Eliane Choueiry: None declared, Cybel Mehawej: None declared.

P09.051.C MED13L missense variations cause multifaceted functional consequences underlying severe phenotypes

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Background/Objectives: Pathogenic *MED13L* variants cause a neurodevelopmental disorder characterized by a moderate-to-severe intellectual disability. The majority of the patients carry a protein-truncating variation leading to haploinsufficiency and a typical phenotype. Few patients with a missense variant were reported with a broader and more severe phenotype. The exact biological consequences underlying atypical phenotypes remain unknown.

Methods: We selected five *MED13L* reported pathogenic variants located in exon 15, 17, and 30, causing either a severe phenotype (p.Pro866Leu, p.Pro869Ser and p.Cys1131Tyr) or a typical phenotype (p.Gly1899Arg and p.Thr2162Met). To unravel pathogenic mechanisms, we analyzed MED13L wild type and mutant subcellular localization, integration into CDK8-module (CKM) and core Mediator complex, proteasome-mediated degradation, and phosphorylation.

Results: The p.Pro866Leu and p.Pro869Ser variants were likely to induce a hyper-phosphorylated status of the MED13L$^{823\text{-}930}$ domain while residues Cys1131 and Thr2162 are predicted to be critical for MED13L proper folding. Cytoplasmic relocalization and reduced integration into CKM and core Mediator complex were observed only with the p.Gly1899Arg.

Conclusion: *MED13L* missense variants are likely to be responsible for different pathogenic mechanisms. *MED13L* variants associated with typical phenotypes (p.Gly1899Arg and p.Thr2162Met) probably induce a loss of function, while variants associated with severe phenotypes probably cause a dominant-negative effect. This study highlights potential novel MED13L functions that should now be properly deciphered.

References:.

Grants:.

Conflict of Interest: None declared.

P09.052.D Biallelic mutations in CACTIN encoding component of spliceosome machinery cause severe intellectual disability, speech impairment and epilepsy

Uzma Abdullah[1], *Maria Asif*[2], *Maryam Anayat*[1], *Ghulam Nasseer Udin*[1], *Muhammad Sajid Hussain*[2]

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Background/Objectives: Intellectual disability (ID) is a condition of significant limitation of cognitive functioning and adaptive behaviour, with 50% of aetiology attributed to genetic predisposition.

Methods: We recruited two consanguineous Pakistani families (A an B) manifesting severe ID and developmental delay. The probands were subjected to whole exome sequencing (WES) and variants were further prioritized based on population frequency, predicted pathogenicity and functional relevance.

Results: The WES data analysis of family A identified a homozygous missense variant *CACTIN* (NM_001080543.1:c.1040A>T;-p.(Asp347Val)), co-segregating with the disease. The gene is highly intolerant to loss of function variants (pLOF = 0.14) and has never been implicated in any inherited disorder. Its encodes Cactin which is a critical component of post-catalytic spliceosome that mediates exon ligation by stabilizing the position of the branch helix. *CACTIN* depleted cells show global splicing defects, while its knock down in zebrafish leads to embryonic lethality or extensive dysmorphogenesis. Family B segregated a novel missense substitution (NM_024298.4:c.757G>A;p.(Glu253Lys)) of *MBOAT7*, a known gene of ID. Both of the identified variants were absent in gnomAD and 1000 Genomes and predicted to be 'deleterious' by several in silico tools.

Conclusion: Our findings indicate that *CACTIN* is novel gene that when disrupted leads to intellectual disability. These also provide additional evidence to the role of *MBOAT7* in aetiology of ID.

References: Nil.

Grants: Nil.

Conflict of Interest: None declared.

P09.054.B Variability in Wolf-Hirschhorn syndrome: two cohorts side by side

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Background/Objectives: Wolf-Hirschhorn syndrome (WHS) results from the loss of genetic material at 4pter. It is a neurodevelopmental disorder of genetic cause that have great phenotypic heterogeneity, including global developmental delay, microcephaly, delayed/absent speech, dysmorphic features, and epilepsy. We compare clinical, genetics, and follow-up characteristics between the Spanish and Latin-American subpopulations.

Methods: We describe a cohort of 134 patients with WHS (Spain, 72; Latino-America, 62). Patients were characterized by deep phenotyping, SNP-arrays, and other genetic approaches. We estimated an individual severity score (The global functional assessment of the patients (GFAP)) in our two cohorts using different features taken from two questionnaires and weighed them by HPO-term frequencies, on a numerical scale. Principal Component analysis was performed to validate our GFAP scale.

Results: The whole cohort (134) has a mean age of 7.81 years and shows a predominance of female sex over males (2:1). Although no differences between the two cohorts were observed at neonatal variables nor dysmorphic traits or physical aspects, we do find significant differences at several comorbidities, different items affecting developmental delay and clinical management of the epilepsy, although both subpopulations showed similar GFAP functional numbers.

Conclusion: No great functional differences can be made comparing both subpopulations, but do see significant changes at some of the variables collected (mainly related to the clinical and genetic managements of the cases).We used our GFAP scale as a base to develop a potential Clinical Outcome Assessment for each individual.

References: None.

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Conflict of Interest: None declared.

P09.056.A Supporting evidence on the role of a novel STXBP1 variant underlying discordant phenotypes in a family with a common apparently balanced translocation

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Background/Objectives: Familial apparently balanced translocations (ABTs) segregating with discordant phenotypes are challenging for interpretation and counselling. We report an individual with intellectual disability, epilepsy and a t(1;7)(p36.1;q22) inherited from his non-affected mother. All possible mechanisms that could explain the differential phenotypes were thoroughly investigated; whole-genome mate-pair sequencing further demonstrated that the common translocation was identical and truly balanced. Subsequent family-based Whole-Exome Sequencing (WES) revealed a novel, de novo splice donor variant (NM_003165.6:c.1110+2T>G) in the *STXBP1* gene, which is essential for neurotransmitter release through syntaxin regulation.

Methods: In order to assess the impact of this candidate variant on mRNA splicing, RNA was extracted from lymphoblastoid cell lines of the patient and a control sample. Reverse transcription PCR (RT-PCR) was performed, using *STXBP1*-specific primers flanking the identified variant and β-actin primers, followed by bidirectional sequencing.

Results: The expected wild-type *STXBP1* allele was observed in both samples but lower quantities were seen in the patient. However, when a primer specifically amplifying the mutated allele was used, a PCR product was obtained only in the patient, indicating the incorporation of intronic sequences into the *STXBP1* transcript. Sequencing of the RT-PCR products revealed that fifteen new amino acids are eventually inserted at position 371 of the STXBP1 protein (NP_003156.1) followed by a premature stop codon.

Conclusion: This study supports our previous findings describing familial ABTs as coincidental in individuals with discordant phenotypes. The novel, *de novo STXBP1* variant identified by WES affects normal splicing and segregates with the patient's phenotypes, thus expanding the mutational spectrum of the *STXBP1* gene.

References:.

Grants:.

Conflict of Interest: None declared.

P10 NEUROGENETIC AND PSYCHIATRIC DISORDERS

P10.001.D High diagnostic rate of trio exome sequencing in consanguineous families with neurogenetic diseases

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Background/Objectives: Consanguineous marriages are common in Turkey (~25%). They increase the risk for autosomal recessive genetic diseases most of which mainly affect the nervous system and muscles. Access to molecular diagnostic methods and other advanced diagnostic tools are limited in the majority of children with neurogenetic diseases in Turkey. Although the next-generation sequencing reduces the diagnostic odyssey, the diagnosis success rate is still low for neurogenetic diseases which impose a substantial clinical and economic burden.

Methods: We recruited 246 children with clinically undiagnosed neurogenetic diseases from 190 consanguineous families. All patients underwent deep phenotyping and trio exome sequencing. Advanced bioinformatics platforms were used to integrate the data (RD-Connect GPAP).

Results: We identified causative variants in 119 known disease genes (72%) and likely pathogenic variants in 27 novel genes (14%) with an 86% overall diagnostic yield. The majority of the causative variants were homozygous (82%) and the remaining were de novo (9.3%), X-linked recessive (5.2%), and compound heterozygous (3.5%) variants. Protein synthesis/degradation defects and metabolic disease-related pathways were mostly revealed by pathway analysis. We also provided prevention of transmission and targeted treatments in 24 patients (10%).

Conclusion: We generated an important genomic data resource to better understand the genetic causes of childhood neurogenetic disorders, provided improved diagnosis and targeted treatments. We also demonstrated the superiority of trio exome sequencing over singleton exome sequencing and targeted gene panels.

References: Kurul, S. H. et al. High diagnostic rate of trio exome sequencing in consanguineous families with neurogenetic diseases. Brain (2021). https://doi.org/10.1093/brain/awab395.

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P10.002.A Genetic overlap between dystonia and other neurologic disorders: a study of 1,100 exomes

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Background/Objectives: Although shared genetic factors have been previously reported between dystonia and other neurologic conditions, no sequencing study exploring such links is available. In a large dystonic cohort, we aimed at analyzing the proportions of causative variants in genes associated with disease categories other than dystonia.

Methods: Gene findings related to whole-exome sequencing-derived diagnoses in 1,100 dystonia index cases were compared with expert-curated molecular testing panels for ataxia, spastic paraplegia, neuropathy, epilepsy, and intellectual disability.

Results: Among 220 diagnosed patients, 21% had variants in ataxia-linked genes; 15% in spastic-paraplegia-linked genes; 12% in neuropathy-linked genes; 32% in epilepsy-linked genes; and 65% in intellectual-disability-linked genes. Most diagnoses (76%) were related to genes listed in ≥1 studied panel; 69% of the involved loci were found in the non-dystonia panels but not in an expert-curated gene list for dystonia.

Conclusion: Our study indicates a convergence in the genetics of dystonia and other neurologic phenotypes, informing diagnostic evaluation strategies and pathophysiological considerations.

References:.

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Conflict of Interest: None declared.

P10.003.B Contribution of schizophrenia polygenic burden to longitudinal phenotypic variance in 22q11.2 deletion syndrome

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Background/Objectives: While the recurrent 22q11.2 deletion is one of the strongest genetic risk factors for schizophrenia (SCZ), variability of its associated neuropsychiatric endophenotypes reflects its incomplete penetrance for psychosis development.

Methods: To assess whether this phenotypic variability is linked to common variants associated with SCZ, we studied the association between SCZ polygenic risk score (PRS) and longitudinally acquired phenotypic information of the Swiss 22q11.2DS cohort (n = 97, 50% females, mean age 17.7yr, mean visit interval 3.8yr).

Results: The SCZ PRS with the best predictive performance was ascertained in the Estonian Biobank (n = 201,146) with LDpred. The infinitesimal SCZ PRS model showed the strongest capacity in discriminating SCZ cases from controls with one SD difference in SCZ PRS corresponding to an odds ratio (OR) of 1.74 (95% CI 1.59–1.90, P = 2.99 × 10-33). Social anhedonia (OR = 2.09, P = 0.0002), avolition (OR = 1.61, P = 0.001) and ideational richness (OR = 1.78, P = 0.003) within negative symptoms course, and dysphoric mood (OR = 2.00, P = 0.002) and stress intolerance (OR = 1.76, P = 0.0002) within general symptoms course showed the strongest associations with SCZ PRS in 22q11.2 patients. Genetic liability for SCZ was additionally associated with full scale cognitive decline (β = –0.25, P = 0.02) and with longitudinal volumetric reduction of the right and left hippocampi (β=–0.28, P = 0.005; β=–0.23, P = 0.02, respectively).

Conclusion: Our results indicate that the polygenic contribution to SCZ acts upon the threshold-lowering first hit (i.e., the deletion). It modifies the endophenotypes of 22q11.2DS and augments the derailment of developmental trajectories for negative and general symptoms, cognition, and hippocampal volume.

References:.

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P10.004.C Get your molar tooth right: Joubert syndrome misdiagnosis unmasked by whole exome sequencing

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Background/Objectives: Joubert Syndrome (JS) is a recessively inherited ciliopathy, characterized by a specific cerebellar and brainstem malformation recognizable on brain imaging as the "Molar Tooth Sign" (MTS). Clinical signs include infantile hypotonia, developmental delay and intellectual disability of variable degree, ataxia, ocular-motor apraxia and variable organ involvement. In recent years, the genetic diagnosis of JS has been mostly based on the analysis of next-generation-sequencing targeted gene panels. Recognition of the MTS is crucial to address the patient to the appropriate genetic testing. However, the MTS is not always properly diagnosed, resulting either in false negative diagnoses (patients with the MTS not addressed to JS genetic testing) or in false positive diagnoses (patients with a different brain malformation wrongly addressed to JS genetic testing).

Methods: We report on six cases referred for JS genetic testing based on inappropriate recognition of MTS.

Results: While the analysis of JS-related genes was negative, whole exome sequencing (WES) disclosed pathogenic variants in other genes causative of distinct brain malformations: LAMA1 (Poretti-Boltshauser syndrome), SHANK3 (Phelan-McDermid syndrome), GTPBP2 (Jaberi-Elahi syndrome), NLGN4X (X-linked mental retardation and autism susceptibility) and 16p11.2 deletion. Reassessment of brain MRIs confirmed that the initial suspicion of MTS was incorrect.

Conclusion: This study highlights that the diagnostic yield of NGS-based targeted panels is strictly related to the accuracy of the diagnostic referral based on clinical and imaging assessment, and that WES has an advantage over targeted panel analysis when the diagnostic suspicion is not straightforward.

References: PMID: 34846692.

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Conflict of Interest: None declared.

P10.005.D A complex chromosome 21 rearrangement causes APP triplication and autosomal dominant early onset Alzheimer disease

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Background/Objectives: Amyloid-β precursor protein (*APP*) gene copy number variation can cause early onset autosomal dominant Alzheimer disease (EO-ADAD). We present a parent and a child with symptom onset at age 64 and 34 years respectively. Neuropathology confirmed the AD diagnosis with Braak stages V-VI and extensive cerebral amyloid angiopathy (CAA).

Methods: Array comparative genomic hybridization (array-CGH) of genomic DNA from blood identified increased *APP* copies, with seemingly more copies in the child. Follow-up analysis with genome sequencing (GS) allowed for a detailed analysis of the *APP* copy number and the breakpoint junctions in both individuals.

Results: The GS analysis, using FindSV (TIDDIT, CNVnator) and visualization in IGV, resolved the genomic architecture of the complex structural variant with novel breakpoints; a triplication with three *APP* copies that was mosaic in the parent (20% of cells). All involved segments, including an inverted middle segment, are in *cis*. Further characterization of mosaicism will be explored using digital droplet PCR on tissue from different body organs and brain regions.

Conclusion: Structural chromosomal rearrangements in neurodegenerative disorders (NDS) likely contribute to the missing heritability. A GS-first approach allows for efficient screening of structural variants and provides more patients with a genetic diagnosis. In our study, the presence of the triplication in 100% of the cells in the child, while the parent was mosaic, is the explanation for the anticipation with 20 years earlier onset of AD in the child.

References:.

Grants: Emma Ehn was supported by the Region Stockholm (combined residency and PhD training program).

Conflict of Interest: None declared.

P10.006.A Sensorimotor polyneuropathy is an underestimated feature of ENTPD1 associated spastic paraplegia

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Background/Objectives: Spastic paraplegia type-64 (SPG64; OMIM615683) is an ultra-rare form of complicated hereditary spastic paraplegia. SPG64, caused by biallelic *ENTPD1* mutations, is associated with impaired purinergic neurotransmission. Here, we report a novel *ENTPD1* variant which adds novel dysmorphic findings, thalamus atrophy and sensorimotor polyneuropathy to phenotypic spectrum of SPG64.

Methods: Clinical exome sequencing was conducted utilizing Illumina-TruSight-One panel and analyzed by SophiaDDM.

Results: The previously unreported nonsense *ENTPD1* variant NM_001776: c.1174C>T (p.Gln392Ter) is located in the extracellular loop domain and is linked to a severe SPG64 phenotype in the proband, associated with early age-of-onset (2 years) and total loss of ambulation at 6 years. Segregation was in accordance with autosomal recessive inheritance in the family.

Conclusion: Only 9 individuals with SPG64 from 5 families have been described with clinical details and the full phenotypic spectrum remains inadequately characterized. Based on our comprehensive follow-up for 4 years and comparison with reported individuals, the clinical spectrum of SPG64 can include cognitive decline (9/10), speech abnormality (7/10), dystonia (3/10) and brain abnormalities (3/7). Additionally, presence of sensorimotor polyneuropathy in the proband, accompanied by thalamus atrophy possibly secondary to sensory neuropathy, suggests that the clinical spectrum of SPG64 is broader than previously reported. Early-stage neuropathy suspected in a previous individual with SPG64 supports that neuropathy may be an overlooked phenotypic component of SPG64. Moreover, neuropathy may also be associated with impaired purine metabolism since purines are acting as neurotransmitters in peripheral nervous system. Our study highlights the importance of evaluating all future patients for clues of neuropathy.

References: PMID:33771085.

Grants:.

Conflict of Interest: None declared.

P10.007.B Intermediate and expanded HTT alleles and risk for α-synucleinopathies

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Background/Objectives: Previous studies suggest a link between CAG repeat number in the HTT gene and non-Huntington neurodegenerative diseases. The main objective is to analyze whether expanded HTT CAG alleles and/or their size are associated with the risk for developing α-synucleinopathies or their behavior as modulators of the phenotype.

Methods: We genotyped the HTT gene CAG repeat number and APOE-Ɛ isoforms in a case-control series including patients with either clinical or neuropathological diagnosis of α-synucleinopathy.

Results: We identified three PD patients who carried low-penetrance HTT repeat expansions representing 0.29% of the PD cohort, whereas none of the DLB or MSA patients nor any healthy control carried pathogenic HTT expansions. In addition, a clear increase of the number of HTT CAG repeats was found among DLB and PD groups influenced by the male gender, and with the presence of APOE4 allele among DLB patients. HTT Intermediate alleles' (IAs) distribution frequency was increased in the MSA group compared with controls (8.8% vs 2.9%, respectively). These differences were indeed statistically significant in the MSA group with neuropathological confirmation. Two MSA HTT CAG IAs carriers with ≥ 32 HTT CAG repeats showed isolated polyQ inclusions in pons and basal nuclei, which are two critical structures in the neurodegeneration of MSA.

Conclusion: Our results pointed to a link between, HTT CAG number, HTT IAs and expanded HTT CAG repeats with other non HD brain pathology and also support the hypothesis that they can share common neurodegenerative pathways.

References:.

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Conflict of Interest: None declared.

P10.008.C Founder effect in Joubert syndrome: identification of four shared haplotypes enriched in distinct European geographical regions associated to recurrent variants

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Background/Objectives: Joubert syndrome (JS) is a neurodevelopmental ciliopathy characterized by peculiar mid-hindbrain malformation, the "molar tooth sign". The >40 known causative genes account for 65-75% cases. While most pathogenic variants are novel or extremely rare, we report on 10 recurring variants in six genes, including three already known "founder variants".

Methods: We compared the frequencies of these variants in two unrelated JS cohorts (one Italian-European and one from the United States), in three Italian control cohorts and in the gnomAD database.

Results: Six variants were markedly enriched in both JS cohorts compared to controls, while three others were enriched only in European JS. Genotyping of microsatellite markers across the gene loci identified four novel founder haplotypes: (1) a 2.29Mb Mediterranean haplotype across MKS1 c.1476T>G; (2) a 2.02Mb Sardinian haplotype around KIAA0586 c.1006C>T; (3) a ~1.4Mb European haplotype encompassing KIAA0586 exon 8-10 deletion; (4) a 747Kb Italian haplotype across RPGRIP1L c.1843A>C.

Of note, we and others showed that at least two (MKS1 c.1476T>G and KIAA0586 c.428delG) are hypomorphic variants which do not cause JS when homozygous, but only when in trans with a more deleterious variant. Functional studies for MKS1 c.1476T>G, showed a similarly reduced percentage of ciliated cells between healthy homozygous parent and compound-heterozygous affected son, but a much more severe reduction of ciliary length in the patient, confirming the hypomorphic effect.

Conclusion: This study contributes to understand the complex genetic landscape of rare genetically heterogeneous diseases such as JS, and to explain their variable prevalence in distinct areas.

References:.

Grants:.

Conflict of Interest: None declared.

P10.010.A Perinatal stroke: the importance of genetic testing

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Background/Objectives: Perinatal stroke is a group of cerebrovascular disorders with an incidence ranging from 1/1600 to 1/4000 live births. It is related to several neonatal and maternal risk factors, such as coagulation disorders, infection, asphyxia, etc. However, in some patients no traditional risk factors can be identified, hence a genetic cause might underlie the pathophysiology of stroke in these patients.

Methods: In our cohort of 76 cases with (presumed) perinatal stroke, 36 cases with ischemic stroke and 40 cases with intracranial haemorrhage were identified. All cases underwent genetic investigations using single nucleotide polymorphism array and a whole exome sequencing based gene panel.

Results: A genetic diagnosis was confirmed in 7/76 cases (9.21%). The diagnostic yield was highest in cases with haemorrhagic stroke (6/40 patients, 15%), with an important contribution of COL4A1 pathogenic variants (4/6 diagnosed cases with haemorrhagic stroke, 66.67%). Furthermore, trisomy 12p and cardiofaciocutaneous syndrome due to a de novo pathogenic BRAF variant were diagnosed in two haemorrhagic stroke cases. In the ischemic stroke group, only one diagnosis was made, comprising a de novo mosaic JAG1 variant in an atypical case of Alagille syndrome.

Conclusion: Our findings highlight the added value of genetic testing in cases with (presumed) perinatal stroke. A higher yield

was present in patients with haemorrhagic stroke, where we could establish a significant contribution of COL4A1 pathogenic variants. The added value in ischemic stroke seems less substantial, however needs further investigation.

References:.

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P10.011.B Evaluation of microRNA from serum extracellular vesicles in adolescents at risk for psychiatric disorders

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Background/Objectives: Exosomes are small extracellular vesicles (EVs) detected in body fluids, their content commonly enriched for microRNAs. They can cross the blood-brain barrier and therefore may reflect alterations in the brain. Here, we longitudinally evaluate microRNA content in serum EVs from adolescents, to identify those associated with incidence and remission of Psychiatric Disorders (PDs).

Methods: We selected 119 individuals who were evaluated twice over a three-year follow-up: controls (N = 30); incident cases (N = 34); remitted cases (N = 29); and persistent cases (N = 26). EVs were isolated from serum using differential centrifugation and precipitation kit. We characterized EV size, concentration and membrane proteins. microRNA was isolated from EV samples and sequenced.

Results: EV characterization indicated the presence of general and brain-associated EV protein markers. miR-4433b-5p, miR-584-5p, miR-625-3p, miR-432-5p and miR-409-3p were differentially expressed comparing Major Depression cases (n = 38) vs. controls (n = 56) in the second timepoint (FDR-adjusted p-value <0.1). miR-625-3p and miR-432-5p were previously associated with Schizophrenia and miR-409-3p with Major Depression and cognition. miR-382-5p, miR-625-3p, miR-134-5p and miR-151a-3p were differentially expressed longitudinally in male individuals regardless of diagnosis (n = 66). miR-134-5p regulates synaptic plasticity processes, considered important in pubertal brain development.

Conclusion: We were able to observe differences in EV microRNA expression associated with diagnosis of Major Depression, as well as with age in males, and characterize EV samples. We expect to identify more microRNA associated with incidence and remission of PDs.

References: Salum, et al., IntJ MethodsPsychiatrRes. 2015; Geaghan M, Cairns MJ., BiolPsychiatry. 2015; Rao, et al., NeurosciBiobehav Rev. 2016.

Grants: NARSAD Young Investigator Grant 573974/2008-0; FAPESP 2020/02247-7; CAPES Scholarship.

Conflict of Interest: None declared.

P10.012.C Familial 1p36.31p36.23 deletion encompassing CAMTA1 associated with variable intellectual disability and ataxia phenotype

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Background/Objectives: Intragenic *CAMTA1* rearrangements are rare and have been associated with varied neurobehavioral phenotypes, including developmental delay/intellectual disability (DD/ID) and nonprogressive cerebellar ataxia (OMIM#614756). From the few families reported, the phenotypic spectrum appears to be very variable, with cognition ranging from normal to moderate ID, and cerebellar signs from unsteady gait to overt ataxia.

Methods: The proband is a 9-year-old girl, referred due to autism spectrum disorder (ASD) and learning difficulties, with a history of impairment in gross and fine motor skills and unsteadiness while running. Brain MRI was normal. Her maternal half-sister had mild ID, ASD, attention deficit hyperactivity disorder (ADHD) and a diagnosis of intrauterine CMV infection causing postlingual unilateral deafness. She also had marked impairment of gross and fine motor skills; brain MRI showed possibly CMV-related abnormalities, but no cerebellar involvement. Their mother had no ID or ataxia, but reported mild global DD, learning difficulties, and ADHD in childhood, plus a history of frequent falls.

Results: ArrayCGH identified an interstitial deletion in 1p36.31p36.23 (6967159_8402554; hg19) encompassing exons 4 to 23 of *CAMTA1*. FISH studies confirmed the deletion to be maternally inherited and present in the proband's half-sister.

Conclusion: This family illustrates the variable phenotype associated with intragenic rearrangements in *CAMTA1*, namely regarding cognition and motor impairment. Although, to our knowledge, the deletion found in this family is larger than previously reported (encompassing several genes downstream of *CAMTA1*), the phenotype is similar to other families, further contributing to establish *CAMTA1* as a gene associated with a neurodevelopmental phenotype with variable cerebellar involvement.

References:.

Grants:.

Conflict of Interest: None declared.

P10.013.D Fine-mapping and transcriptome wide association study of anorexia nervosa prioritises WDR6 and immune pathways as potential causal factors

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Background/Objectives: Anorexia nervosa is the leading cause of mortality among psychiatric disorders worldwide. Currently no medications are approved for anorexia treatment, and thus, identification of clinically actionable risk factors for this disease is pivotal to reducing disease burden.

Methods: We performed a transcriptome wide association study (TWAS) and conditional analysis across 17 tissues, leveraging mRNA, protein, and mRNA alternative splicing weights to identify genes, proteins, and transcripts, respectively, associated with anorexia risk. We utilised the largest anorexia GWAS available to date (N = 72,517). Probabilistic finemapping was then applied to further prioritise genes that may represent true causal signals.

Results: TWAS identified 134 genes, 4 proteins, and 16 transcript isoforms significantly associated with anorexia after

Benjamini-Hochberg correction. A conditional analysis of these significantly associated genes considering other proximal genes resulted in 97 genes that remained independently associated. Moreover, finemapping prioritised five genes with strong evidence of a causal effect on anorexia (PIP > 0.8 in 90% credible set), with eight other genes exhibiting moderate evidence (PIP > 0.4). Upregulation of the gene encoding WD repeat domain 6 (WDR6) was identified as an anorexia risk gene through both finemapping and conditional analysis. Genes prioritised by finemapping were also overrepresented amongst the immune system process gene ontology pathway.

Conclusion: We identify novel candidate risk genes for anorexia through integration of anorexia genetic associations with models of genetically regulated mRNA, protein, and splicing. There was strong evidence to support a role for WDR6 in anorexia pathogenesis, whilst other prioritised genes were found to be plausibly linked to immunological function.

References:.

Grants:.

Conflict of Interest: Danielle Adams: None declared, William Reay Employee of the University of Newcastle, Murray Cairns Employee of the University of Newcastle.

P10.014.A Fatal familial Insomnia in the Basque Country: descriptive analyses of a case series

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Background/Objectives: Fatal familial insomnia (FFI) is a rare prionopathy characterized by sleep disturbances, dysautonomia, motor and cognitive dysfunction, thalamus neuronal loss and gliosis. Although, the most prevalent genetic form of prion disease worldwide occurs at codon 200 of the PRNP resulting in the typical genetic Creutzfeldt-Jakob disease (CJD)(1), in some regions as Germany and Basque Country(2), the most common mutation is located at the codon 178 of the PRNP (D178N) causing FFI.

Methods: The Basque Country Brain Bank database was screened for patients with FFI diagnosed from 2010 to 2021 according to standard diagnostic criteria, including genetics and neuropathological data. Epidemiological and clinic-genetic data were analyzed.

Results: A total of 16 (12 male and 4 female) were detected. All carried the D178N variant. The mean age of onset was 54 years with a disease course of 11 months. The brain MRI (88%) and the electroencephalography (81%) were usually normal, whereas polysomnography was pathological in 92% of the cases. Most (75%) of the patients were homozygous for Met129 polymorphism and in the remaining 25%, the mutation was in cis with Met variant. There was no family history in 25% of the cases.

Most classical diagnostic examinations were normal with homogeneous consistency in this Basque FFI population.

Conclusion: Whereas classical clinical analyses are non-conclusive, genetic study together with the polysomnography are essential in the diagnosis of cases with suspected fatal familial insomnia.

References: 1. Kovács et al., Hum. Genet. 2005 1182. 118, 166–174 (2005).

2. De Pedro-Cuesta et al., Prion. 15, 94 (2021).

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Conflict of Interest: None declared.

P10.015.B Phenotypic spectrum in four cases presenting 16p11.2 reciprocal CNVs associated with additional rare gene variants – pleiotropic clinical outcomes

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Background/Objectives: Chromosomal rearrangements in the 16p11.2 region are considered among the most frequent etiologies of neurodevelopmental and autism spectrum disorders with variable penetrance and highly pleiotropic clinical outcomes. Our study characterizes four cases diagnosed with reciprocal 16p11.2 CNVs and additional rare and novel variants in genes located outside the affected 16p11.2 region.

Methods: Four pediatric patients, two girls (A, B) and two boys (C, D), were referred to the clinic for various symptoms including global developmental delay, nonspecific dysmorphic features, early onset seizures, movement disorders and autistic traits. Brain MRI revealed abnormalities in three of them. Patients were recommended genetic investigations for diagnosis: A performed a WES test, B and C, an epilepsy gene panel test, and D, a chromosomal microarray analysis.

Results: 16p11.2 CNVs were revealed in all cases: microduplication for patient A and microdeletions for the rest. Additionally, VUS variants were detected: one heterozygous PRRT2 missense variant in trans to the CNV for each patient A and B, and a TUBB4A inframe deletion variant for patient C. The additional variants detected for B and C have not been described to date. Testing is about to be continued for D.

Conclusion: Our study highlights the challenges encountered when establishing genotype-phenotype correlations in pediatric patients diagnosed with 16p11.2 CNVs associated with other rare gene variants, emphasizing that such diverse phenotypes are the result of CNVs interaction with the rest of the genome. To our knowledge, this is the first reported case presenting an association between a 16p11.2 CNV and a TUBB4A gene variant.

References:.

Grants:.

Conflict of Interest: None declared.

P10.016.C Evaluation of a nurse led care service for patients with neurofibromatosis

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Background/Objectives: Neurofibromatoses (NF) are a group of complex genetic disorders requiring multidisciplinary care. Inconsistent and fragmented care has been reported to lead to disease complications that could have been prevented. We implemented a nurse led care (NLC) service to provide extra support to NF patients.

The aim of the study was to audit the consultations of the clinical nurse specialist (CNS) and evaluate patient experience, enablement and satisfaction with the NLC service.

Methods: The audit included 163 patient encounters with 114 patients with NF that received NLC (May 2020-May 2021). The 58-item anonymous online survey included validated scales and was distributed to all audit patients.

Results: The CNS' main role was identified as 'coordination of care' (40%). 57 surveys were analysed (50% response-rate) with over 80% of patients reporting a positive or very positive experience. Mean patient satisfaction scores (50/75) were also favourable. Statistically significant association was found between patient satisfaction scores and patients' views and concerns being listened to X 2 (2, (n = 50) = 6.34(p = 0.04); flexibility of getting a time with the nurse X 2 (3, (n = 57) = 12.13(p = 0.007); patients' involvement in making decisions about their treatment plan X 2 (4, (n = 57) = 12.83(p = 0.01) and being given enough information to manage their care at home X 2 (2, (n = 44) = 12.85(p = 0.002).

Conclusion: This study shows nurse led care is associated with positive patient satisfaction and experience and supports its introduction as a model of care for management of patients with rare diseases.

References:.

Grants:.

Conflict of Interest: None declared.

P10.017.D Genotypic spectrum and its clinical implication in disorders with epilepsy in Indian population: A preliminary experience

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Background/Objectives: Rapid advancement in identification of underlying genetic mechanisms in disorders with epilepsy has attributed to improved management including precision medicine as well as informed genetic counseling in families with these disorders.

Methods: We recruited 71 families (75 individuals) with neurodevelopmental disorders with epilepsy from October 2019 till date. Targeted testing and/or genomic testing after detailed clinical evaluation was carried out. The implications on genetic counseling and therapy were evaluated in individuals with definitive molecular diagnosis.

Results: Molecular diagnosis was achieved in a total of 54 out of 71 families (76%): two (4%) by targeted testing, ten (18%) by Mendeliome, and 42 (78%) by exome sequencing. Of these, monogenic disorders were identified in fifty-three families: autosomal recessive in 30 families (56%), autosomal dominant in 19 families (36%), X-linked in four families (9%), and imprinting/ microdeletion syndrome (Angelman syndrome) in one family (Table 1). Thirty (55%) of 57 disease-causing variants were novel. One novel disease-gene association with pathogenic variants in GCSH was identified. We also report genotypic and phenotypic expansion of extremely rare and recently described disorders with pathogenic variants in SHMT2, SLC25A10, SNRPN, TRAPPC12, MINPP1 and FGF13. Reproductive counseling was offered to 72% (39/54) of families. Therapeutic implications were noted in 30% of individuals (16/54) with definitive diagnosis. Strong evidence of recommended therapies was available in 50% (8/16) families, emerging evidence in 6% (1/16), and sparse evidence in 44% (7/16) families.

Conclusion: The above-mentioned cohort is a part of an ongoing study of disorders with epilepsy.

References:.

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Conflict of Interest: None declared.

P10.018.A Identity-by-descent analysis of a large Tourette's syndrome pedigree from Costa Rica implicates genes involved in neuronal development and signal transduction

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Background/Objectives: Tourette Syndrome (TS) is a poorly understood, substantially heritable neuropsychiatric disorder that typically begins in early childhood. Identifying rare variants that make a significant contribution to risk in affected families may provide important insights into the molecular aetiology of this disabling condition. We report data from a large pedigree (>500 individuals), densely affected by TS and co-morbid psychiatric disorders from a genetically isolated Costa Rican population. The pedigree spans 11 generations and shares ancestry from six founder-couples.

Methods: Whole genome sequencing (WGS) data was generated for 19 individuals from this pedigree. Identity-by-descent (IBD) analysis was performed, filtering haplotypes on the following criteria: >1Mb in length; shared by at least three affected individuals sharing ancestry from the same founder couple(s); and absent in Costa Rican control samples.

Results: The IBD pipeline identified eleven haplotypes. Fine-mapping of these haplotypes using the WGS data identified rare (MAF< 0.01) and ultra-rare (MAF< 0.001) coding and non-coding variants in candidate genes. In particular we identified a rare deleterious missense variation in RAPGEF1 and two ultra-rare putatively deleterious intronic variants in ERBB4 and IKZF2.

Conclusion: RAPGEF1 has recently been implicated in a family study of neuropsychiatric symptoms, supported by a zebrafish model of this gene. ERBB4 participates in many critical functions, such as neurodevelopment and synaptic plasticity, while IKZF2 is a transcription factor shown to play a role in neuronal development. Together, these variants represent biologically relevant targets for investigation in other pedigree and population-based TS data.

References:.

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P10.019.B A different light on COL4A2 variant interpretation

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Background/Objectives: Pathogenic variants in *COL4A1* and *COL4A2* are known to cause a phenotype with a broad spectrum of neurological findings. *COL4A2* variants are less frequent compared to *COL4A1* variants. This has caused a biased representation, as *COL4A2* is rarely considered separate from *COL4A1* variants. In this study we re-assessed the current knowledge on *COL4A2* variants.

Methods: After PubMed search, we collected all *COL4A2* variants published until February 2022 and reviewed the LOVD and Clinvar database. All variants were reclassified using current ACMG guidelines.

Results: Twenty-three articles described 97 cases with 49 different *COL4A2* variants. Remarkable was the discordance between published class and the classification using ACMG guidelines in more than 30% of the variant descriptions. In some cases, a strong discordance was noted between pathogenicity based on computational results, functional testing or population frequencies. A striking example is the *COL4A2* variant, p.Glu1123Gly. Functional testing in 2012 showed a cellular phenotype, but current population frequency suggests this variant to be incompatible with monogenic disease.

Interestingly, the prevalence of confirmed de novo cases (12%) was distinctly lower compared to the previously reported 40% in *COL4A1* variants. Screening of family members carrying the familial variant detected brain MRI abnormalities in 59% of the cases, even in clinically unaffected cases.

Conclusion: These results show that *COL4A2* variant interpretation is complicated due to phenotypic variability, and conflicting predictions of pathogenicity. Additional research about the pathogenic mechanism of *COL4A2* variants is imperative for correct interpretation of *COL4A2* variants.

References:.

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Conflict of Interest: None declared.

P10.020.C A novel autosomal dominant locus for essential tremor: when linkage analysis can still drive gene discovery

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Background/Objectives: Essential tremor (ET) is a common movement disorder with a prevalence of approximately 1% worldwide. Although a considerable effort has been made in recent years trying to identify genomic markers for ET, the responsible genes have been elusive.

Methods: We studied a three-generation Italian family segregating early-onset autosomal dominant ET (7 affected, 4 unaffected cases, 1 spouse). Postural and action tremor started slowly and symmetrically in their twenties and progressed gradually with age; the neurological examination was otherwise unremarkable. We performed exome sequencing in two affected cousins, but no pathogenic variant was found. Using the CytoScan HD Arrays (Affymetrix), we performed SNP genotyping. Linkage analysis calculation was done using Merlin software.

Results: We identified an approx. 43 Mb region on chromosome 10p11.21-q22.3, between markers rs7477871 and rs12253494, containing several stretches of SNPs with a LOD score above 2 (up to 2.475). In the region, 313 unique encoding transcripts were identified, 161 of which are OMIM genes. Among them, 104 transcripts are expressed in brain, even if none was suggestive for ET pathology.

Conclusion: Our linkage analysis, even if below the significant threshold, highlighted a single region of 43 Mb on chromosome 10, as potentially in linkage with an autosomal dominant form of ET. An in-depth sequencing analysis of this region in two individuals of the family by whole genome sequencing is undergoing that hopefully will help us with this "gene hunt".

References:.

Grants:.

Conflict of Interest: None declared.

P10.021.D Variant-specific changes in RAC3 function disrupt corticogenesis in neurodevelopmental phenotypes

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Background/Objectives: Variants in *RAC3*, encoding the small GTPase RAC3 which is critical for the regulation of actin cytoskeleton and intracellular signal transduction, are associated with a rare neurodevelopmental disorder with structural brain anomalies and facial dysmorphism.

Methods: We investigated a cohort of 10 unrelated participants with neurodevelopmental phenotypes and an overlapping pattern of complex brain malformations. We performed exome sequencing and examined the pathophysiological significance of novel and previously reported *RAC3* variants (p.P29L, p.P34R, p.A59G, p.Q61L, and p.E62K). We then focused on the four variants in the Switch II region (p.Q61L, p.E62del, p.D63N, and p.Y64C), variation hot spot and essential for the small GTPases biochemical activity.

Results: In vitro analyses revealed that all tested *RAC3* variants were biochemically and biologically active to variable extent, showing different affinities to downstream effectors. Acute expression of the four Switch II variants in embryonic mouse brain using in utero electroporation caused defects in cortical neuron morphology and migration, leading to cluster formation during corticogenesis.

Conclusion: Our results indicate that *RAC3* variants cause morphological and functional defects in cortical neurons during brain development through variant-specific mechanisms, leading to heterogeneous neurodevelopmental phenotypes.

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Guerin: None declared, Wendy Wu: None declared, Elisabeth Gabau Vila: None declared, Bryan C. Mak: None declared, Julian A. Martinez-Agosto: None declared, Michael B. Gorin: None declared, Bugrahan Duz: None declared, Yavuz Bayram: None declared, Claudia M. B. Carvalho Fonseca United States National Institutes of Health (NHGRI/NHLBI UM1HG006542; NHGRI U01HG011758), Jaime Vengoechea: None declared, David Chitayat: None declared, Tiong Yang Tan: None declared, Bert Callewaert: None declared, Bernd Kruse: None declared, Lynne M. Bird: None declared, Laurence Faivre: None declared, Marcella Zollino: None declared, Saskia Biskup: None declared, Undiagnosed Disease Network: None declared, Telethon Undiagnosed Diseases Program: None declared, Pasquale Striano: None declared, Vincenzo Nigro: None declared, MariaSavina Severino: None declared, Valeria Capra: None declared, Gregory Costain: None declared, Koh-ichi Nagata Japan Society for the Promotion of Science (JSPS) KAKENHI Grant-in-Aid for Scientific Research (B) (Grant Number JP19H03629), Grant-in-Aid for Scientific Research (C) (Grant Number JP19K07059), Grant-in-Aid for Research Activity Start-up (Grant Number JP20K22888), Grant-in-Aid for Early-Career Scientists (Grant Number JP21K15895), a grant-in-aid of the Practical Research Project for Rare/Intractable Diseases from Japan Agency for Medical Research and Development (AMED) (15ek0109040h0002).

P10.022.A Elucidating the role of RLS-associated MEIS transcription factors during neural development

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Background/Objectives: The strongest risk variant associated with restless legs syndrome (RLS) is located within the gene encoding the TALE homeobox transcription factor MEIS1. Both *MEIS1* and its close homolog *MEIS2*, also located in a RLS risk locus, are expressed in various neural cell types throughout the developing and adult central nervous system. The functional role of *MEIS* in RLS pathogenesis, as well as the cell types involved, still remain elusive.

Methods: Using lentiviral vectors, we manipulated *MEIS1* and *MEIS2* expression in human neural stem cells in vitro and measured global changes in gene expression and DNA methylation. Furthermore, we performed single-cell RNA-sequencing in *Meis*-deficient developmental mouse models and quantified the effects on gene expression in different neuronal subtypes. Direct target genes of MEIS transcription factors were identified by chromatin immunoprecipitation.

Results: We identified MEIS target genes active in early development in mouse models and human NSCs. In the mouse brain, preliminary results suggest MEIS transcription factors control the differentiation of striatal projection neurons. MEIS targets in hNSC were enriched for regulators of Wnt signalling and axon development.

Conclusion: We linked the genetic risk factors *MEIS1* and *MEIS2* to specific neural cell types and brain regions during development. This brings us one step closer to understanding the complex pathophysiology underlying RLS.

References:.

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Conflict of Interest: None declared.

P10.023.B De novo variants in ATP2B1 lead to neurodevelopment delay

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Background/Objectives: We describe a cohort of 12 unrelated individuals with probable causative variants in ATPase plasma membrane Ca2+ transporting 1 (ATP2B1) ascertained through international matchmaking efforts. All probands share the phenotype of mild to moderate global developmental delay and other common, but non-significant clinical features like autism (5/11), seizures (6/12), and distal limb abnormalities (4/12), such as clinodactyly and arachnodactyly. Nine probands had missense variants of which seven were in specific functional domains; the other

three individuals carry nonsense variants. Nine variants in ATP2B1 were proven de novo, while the parents of the remaining three individuals were unavailable for segregation. ATP2B1 encodes a plasma membrane calcium-transporting protein, which plays a central role in calcium homeostasis and is mainly expressed in the central nervous system.

Methods: All missense variants were structurally analysed via 3D protein modelling. We introduced all nine missense variants in transfected HEK293 cells and investigated intracellular localization. Afterwards we performed Ca2+ imaging to investigate Ca2+ export capacity of the transfected cells.

Results: 3D structural protein modeling suggested that the variants have a destabilizing effect on the protein. All variants lead to a significant decrease in Ca2+ export capacity compared with the wild-type construct. These nine variants also exhibited incorrect intracellular localization of ATP2B1, which suggests a loss of function mechanism.

Conclusion: The genetic and phenotypic similarities among probands as well as the functional analyses imply that de novo variants in ATP2B1 cause a novel monogenic neurodevelopmental disorder.

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P10.024.C GABBR1 monoallelic de novo variants linked to neurodevelopmental delay and epilepsy

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Background/Objectives: GABAB receptors are obligatory heterodimers providing prolonged inhibition in the central nervous system. The two receptor subunits are encoded by the *GABBR1* and *GABBR2* genes. Variants in the *GABBR2* gene have been associated with a Rett/like phenotype (OMIM 617903), epileptic encephalopathy (OMIM 617904) and milder forms of developmental delay with absence epilepsy. To date, however, the phenotypes associated with pathogenic variants of the *GABBR1* gene remain to be established.

Methods: Through GeneMatcher, we have ascertained 4 patients each with a monoallelic *GABBR1 de novo* nonsynonymous variant; these cases presented with neurodevelopmental delay (motor and/or language delay) ranging from mild to profound severity, and in one case, epilepsy. Further phenotypic features include varying degrees of intellectual disability, learning difficulties, psychiatric disorders including autism, ADHD, ODD, sleep disorders and muscular hypotonia.

Results: We functionally characterized the four de novo *GABBR1* variants, E368D, A397V, A535T and G673D in transfected HEK293 cells. All four variants analyzed lead to a deficit in GBR-mediated inhibition that likely leads to an increase in the excitation/inhibition balance in the central nervous system. Variant G673D in TM3 renders the receptor completely inactive, consistent with failure of the receptor to reach the cell surface. E368D is located near the orthosteric binding site and reduces potency and efficacy of GABA at the receptor. GABA exhibits normal potency and decreased efficacy at A397V and A535T.

Conclusion: Characterization of *GABBR1*-related variants provides a rationale for understanding disease phenotypes and points to possible therapeutic strategies.

References:.

Grants:.

Conflict of Interest: None declared.

P10.025.D Development of methods and tools in NPCs and zebrafish towards modeling of DNA sequence variants in patients with pachygyria by using genome editing technologies

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Background/Objectives: The main bottleneck in identification of DNA variants that cause neurogenetic diseases is functional analysis of VUS. The aim of this study was to develop a methodology for modelling candidate causative variants observed in patients with pachygyria by using CRISPR/Cas9 genome editing in NPCs and zebrafish.

Methods: DNA from 20 patients with pachygyria/lissencephaly were analyzed by aCGH and WES, and variants were prioritized. Mutant lines were generated in NPCs and Zebrafish by using CRISPR/Cas9 genome editing, and compared to models where one of three key genes (TUBG1, LIS1, DAB1) that are known to play role in pachygyria/lissencephaly. Characterization of NPCs were performed with a 3D matrigel chamber system (IC-Chip) and phenotypic changes were observed in developing zebrafish at 3 dpf and 5dpf. The comparison of target mutant lines and selected variant lines was made with qPCR.

Results: A delay in migration was observed in mutant NPC lines of 3 selected genes compared to control group. WES identified two candidate variants, CGREF1 and NOL9. Expression changes of lissencephaly and microcephaly-related and neuronal differentiation

genes in CGREF1-KO-zebrafish and CGREF1-KO-NPCs were observed. A severe phenotype including small-head and eyes, and abnormal liver/gut development was observed in Tubg1 mutant zebrafish.

Conclusion: Our results provide evidence that variants that cause defects associated with NPC migration can be tested using NPC and zebrafish models in a time- and cost-efficient manner. Multi-omic analysis could further expand the use of this approach to other groups of neurogenetic defects.

References:.

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P10.026.A Rare compound heterozygous missense variants in CSMD3 are associated with developmental delay, intellectual disability, and structural brain anomalies

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Background/Objectives: CUB and Sushi multiple domains 3 (*CSMD3*) encodes a large transmembrane protein. It is highly expressed in adult and fetal brain tissue. *CSMD* family genes have been associated with neurodegenerative and psychiatric disorders and their connection to neurodevelopment has been suggested. Studies have indicated that *CSMD3* regulates dendritic branching and might regulate dendritic morphology. Recently another gene from this family (*CSMD1*) was associated with cerebellar agenesis and bilateral polymicrogyria.

Methods: We have collected clinical information about 4 patients with rare biallelic variants in *CSMD3* gene.

Results: Three male and one female patient were all Caucasian and they were aged between 6 and 23 years during last evaluation. Phenotypes were varying with the common feature of motor development delay (ranging from mild to severe). Most patients (3/4) had hypotonia. Two patients had mild intellectual disability (ID), one had moderate to severe ID and in one case this information was not available. Cerebral MRI was done in 2 cases – one had type 2 lissencephaly and polymicrogyria, and the other had cerebellar agenesis and hypoplasia of corpus callosum. Microcephaly was present in both cases, but was either not documented or not present in the other two. Whole exome sequencing revealed different compound heterozygous rare missense variants in *CSMD3* in all cases.

Conclusion: In conclusion, *CSMD3* gene is likely associated with neurodevelopmental phenotype and might be connected to brain structural anomalies as well as ID. Further research is needed to confirm this finding.

References:.

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Conflict of Interest: None declared.

P10.027.B A WDR47 variant suggests a phenotypic relation with microcephaly in humans

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Background/Objectives: Microcephaly is defined by a head circumference of more than three standard deviations (SD) below the mean for age and gender. Most of the causative genes for microcephaly-related phenotypes encode proteins that play crucial roles in cellular processes, such as cytokinesis, centromere and kinetochore function, transmembrane or intracellular transport, and autophagy. WDR47 belongs to a group of genes that participate in key microtubule-mediated processes, including neural stem cell proliferation, radial migration, and growth cone dynamics.

Methods: Whole exome sequencing (WES) was performed in a blood sample of a 12-month-old male who presented with microcephaly, persistent head lag, abnormality of ocular smooth pursuit, myoclonic seizure and hyperreflexia. His older sibling died at six years of age due to a similar neurodevelopmental disease.

Results: WES revealed a homozygous missense variant in WDR47 (OMIM: 615734) [NM_001142550.1: c.1973C>T p.(Pro658-Leu)] in the affected boy. Both parents are heterozygous carriers. A crucial role in neuronal development is supported by mouse models.

Wdr47-deficient mice exhibited lethality, primary progressive microcephaly, fiber tract hypoplasia, a reduced corpus callosum area, thinner cortices, hyperactivity and sensory-motor gating abnormalities.

Conclusion: This is the first report of a pathogenic variant in WDR47 in humans. WDR47 may be associated with a neurodevelopmental disorder in which microcephaly is a prominent feature.

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Grants:.

Conflict of Interest: Zafer Yüksel Bioscientia Healthcare GmbH, Saeed Al Tala: None declared, Ira Schwaab Bioscientia Healthcare GmbH, Berit Kerner Bioscientia Healthcare GmbH.

P10.029.D Heterozygous UCHL1 is a novel cause of autosomal dominant neurodegeneration with spasticity, ataxia, neuropathy, and optic atrophy

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Benita Menden[1], Isabell Cordts[22], Joachim K. Krauss[23], Christian Blahak[24], Tim Strom[25], Marc Sturm[1], Bart Van De Warrenburg[13], Holger Lerche[26], Boris Macek[8], Matthis Synofzik[20;21], Stephan Ossowski[1], Dagmar Timmann-Braun[27], Marc Wolf[28;29], Smedley Damien[2], Olaf Riess[1;30], Ludger Schoels[20;21;30], Holger Hengel[20;21], Henry Houlden[2], Tobias Haack[1;30]

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Background/Objectives: Hereditary ataxias and hereditary spastic paraplegias are rare neurodegenerative disorders that often show overlapping clinical features as well as shared genetic basis. Bi-allelic variants in *UCHL1* (Ubiquitin C-terminal hydrolase L1) have been associated with a progressive early-onset neurodegenerative disorder, autosomal recessive spastic paraplegia type 79. Our objective was to investigate candidate variants/genes in extended exome and genome datasets within national and international networks using cohort-based burden analyses.

Methods: Gene burden analyses were performed on exome and genome data in independent cohorts of hereditary ataxia and spastic paraplegia patients from Germany and the UK on a total of 3,169 patients and 33,141 controls. Detailed clinical information including in-depth neurological assessments on affected patients were subsequently collected, and additional independent families were ascertained through national and international collaborators. Mass-spectrometry-based proteomics was conducted on patients' fibroblasts.

Results: Gene burden analysis prioritized UCHL1 in both independent cohorts from Germany and UK as a candidate gene for an autosomal dominant disorder. In total, we identified 33 cases from 17 unrelated families, carrying 12 heterozygous predicted loss-of-function variants (in 14 families) and an inframe insertion (in 3 families). Affected individuals mainly presented with spasticity (23/30), ataxia (27/30), neuropathy (11/20) and optic atrophy (10/17). The mass-spectrometry-based proteomics data showed an approximately 50% reduction of UCHL1 expression in patients' fibroblasts indicating haploinsufficiency as the likely pathological mechanism.

Conclusion: Our bioinformatic analysis, in-depth clinical and genetic work-up and functional studies establish haploinsufficiency of *UCHL1* as a novel disease mechanism in spastic ataxia.

References:.

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research and innovation programme, members of the European Reference Network for Rare Neurological Diseases (ERN-RND), Holger Lerche: None declared, Boris Macek: None declared, Matthis Synofzik This project was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) No 441409627, as part of the PROSPAX consortium under the frame of EJP RD, the European Joint Programme on Rare Diseases, under the EJP RD COFUND-EJP N° 825575 and by grant 779257 "Solve-RD" from the Horizon 2020 research and innovation programme, member of the European Reference Network for Rare Neurological Diseases (ERN-RND), Stephan Ossowski: None declared, Dagmar Timmann-Braun DT received funding from the German Reseach Foundation, EU and Bernd Fink Foundation, unrelated to the present study., Marc Wolf: None declared, Smedley Damien Member of The Genomics England Research Consortium, Olaf Riess This project was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) No 441409627, as part of the PROSPAX consortium under the frame of EJP RD, the European Joint Programme on Rare Diseases, under the EJP RD COFUND-EJP N° 825575 and by grant 779257 "Solve-RD" from the Horizon 2020 research and innovation programme, member of the European Reference Network for Rare Neurological Diseases (ERN-RND), Ludger Schoels members of the European Reference Network for Rare Neurological Diseases (ERN-RND), Holger Hengel HH was supported by the intramural fortüne program (#2554-0-0) and by the DFG under the project number HE 8803/1–1., member of the European Reference Network for Rare Neurological Diseases (ERN-RND), Henry Houlden: None declared, Tobias Haack TBH was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation: 418081722, 433158657).

P10.030.A Link of disorders in the schizo-affective spectrum and comorbidity with neurobiological correlates

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Background/Objectives: Disorders of the schizoaffective spectrum such as major depression, bipolar disorder, schizoaffective disorder and schizophrenia are common mental disorders with overlapping symptomatology and high comorbidity rates. The specific genetic architecture of mental disorder comorbidity and its association with brain phenotypes, however, is poorly understood.

Methods: We focused on a subsample from the FOR2107 study comprising n = 470 patients with a single disorder in the schizoaffective spectrum (SD), n = 310 patients with additionally one or more comorbidities (COM), and n = 649 healthy controls (HC). We investigated group differences regarding a) the global severity index, b) a cross-disorder polygenic risk score (PRS) calculated with PRS-CS, using summary statistics of a large GWAS across mental disorders (Lee et al., 2019), and c) neurostructural alterations in gray matter using a whole-brain approach. An explorative subanalysis investigating only medication-free individuals (n = 246 patients) was also performed.

Results: The global severity index significantly differed between groups (COM > SD > HC). SD and COM patients displayed increased cross-disorder PRS compared to HC. However, they were not different from each other. Patients displayed decreased volume in the insula and the middle temporal gyrus compared to HC. These neurostructural associations did not withstand correction for multiple testing in the medication-free individuals.

Conclusion: Our analyses identified phenotypic and genetic differences between SD and COM compared to HC. They did not provide strong evidence that SD and COM patients differed significantly from each other at the genetic and brain structural levels. Future studies are needed to further assess the neurobiological correlates of comorbidity.

References: Lee et al., 2019, PMID:31835028.

Grants: DFG, grant FOR2107.

Conflict of Interest: Lisa Sindermann: None declared, Udo Dannlowski: None declared, Susanne Meinert: None declared, Elisabeth Johanna Leehr: None declared, Lena Waltemate: None declared, Hannah Lemke: None declared, Alexandrea Winter: None declared, Katharina Thiel: None declared, Katharina Brosch: None declared, Tina Meller: None declared, Frederike Stein: None declared, Per Hoffmann: None declared, Axel Krug: None declared, Tilo Kircher Dr. Kircher received unrestricted educational grants from Servier, Janssen, Recordati, Aristo, Otsuka, neuraxpharm. This funding is not associated with the current work., Till Andlauer: None declared, Eva Beins: None declared, Markus M Nöthen: None declared, Andreas Forstner: None declared.

P10.031.B The usefulness of array CGH for identification copy number variants in children with autism spectrum disorders

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Background/Objectives: Autism spectrum disorder (ASD) is one of the most common neurodevelopmental disorders characterized by impairment in social interaction, communication and stereotypic behavior. Copy number variants (CNVs) are clinically relevant in the pathogenesis of ASD and occur in approximately 7-9% of cases. We aimed to demonstrate the clinical utility of dedicated array Comparative Genome Hybridization (aCGH) in isolated and complex ASD.

Methods: Array CGH was performed using 4x180k CytoSure Autism Research Array (OGT; UK). DNA was extracted from peripheral blood. The tested group comprised 155 pediatric patients divided in two cohorts: 93 patients with ASD and 62 patients with ASD and additional clinical features including dysmorphia, intellectual disability, epilepsy and psychomotor retardation.

Results: The strategy allowed for the identification of 38 rare exonic chromosomal imbalances classified as pathogenic or likely pathogenic in 13 of 155 patients (8.4 %) and variants of uncertain significance (VUS) in 22 of 155 patients (14.2%). The range of CNVs size varies between 284 b and 4.04 Mb. In the group of isolated ASD 11 variants were classified as VUS and one as likely pathogenic. Among patients with complex ASD nine harbored pathogenic variants, three likely pathogenic and 10 VUS imbalances.

Conclusion: Overall, we have evaluated the usefulness of dedicated aCGH in the detection of clinically relevant CNVs in a cohort of 155 patients with ASD. Our results support the relevance of performing chromosomal microarray analysis especially in the case of complex ASD.

References:.
Grants:.
Conflict of Interest: None declared.

P10.032.C A case with 9q33.3 microdeletion pointing to the existence of a new syndrome unrelated to STXBP1 gene deletion

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Background/Objectives: Several microdeletions of the 9q33.3-q34.11 chromosomal region have been reported with a range of clinical phenotypes mostly restricted on patients with seizures and STXBP1 gene point mutations or deletions. New cases showed a variable and incomplete penetrance of clinical features, including seizures in patients with or without STXBP1 gene deletion. Here we report a female patient with microcephaly, hypogenesis of corpus callosum, severe psychomotor development delay, absence of speech, but with no episode of seizures.

Methods: WES analysis was performed on DNA samples from the patient and her parents using the AgilentV6 WES and NEB DNA library preparation kits followed with data analysis using SophiaDDM platform.

Results: A 493 kb microdeletion was identified in the chr9q33.3 region (chr9: 126,914,782-127,407,898) encompassing 3 OMIM genes - RALGPS1, GARNL3 and SLC2A8 related to the region identified in patients with 9q33.3-q34.11 microdeletions. The clinical presentation of the patient was similar to the ones of previously reported cases with several common features including intellectual disability, psychomotor developmental delay with delayed or absent speech. The parents were not carriers of the microdeletion.

Conclusion: In addition to the previously reported cases, the microdeletion identified in our patient is one of the smallest and encompasses the minimal critical region with two genes RALGPS1 and GARNL3. This re-confirms that the minimal causative region responsible for the clinical spectrum with no seizures in patients with 9q33.3 microdeletion does not encompass STXBP1 and instead includes two other genes, and which could be considered as a new 9q33.3 microdeletion syndrome.

References: No.
Grants: No.
Conflict of Interest: None declared.

P10.033.D DNA methylation analysis in precision medicine: Accurate biomarker and predictor of onset. The example of KMT2B-associated dystonia

Nazanin Mirza-Schreiber[1], *Michael Zech*[1;2], *Barbara Schormair*[1;2], *Juliane Winkelmann*[1;2;3], *Konrad Oexle*[1;2]

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Background/Objectives: Alterations of DNA methylation relate to disease-associated changes in cellular organization states. These alterations therefore allow for deriving disease-specific biomarkers. Here we report a blood biomarker for histone lysine methyltransferase (KMT2B)-deficient dystonia. Dystonia is a prevalent, heterogeneous movement disorder, and KMT2B-deficiency is a leading monogenic subtype.

Methods: The biomarker was derived by training a support vector machine classifier on an episignature of 113 DNA CpG sites showing significant epigenome-wide association with KMT2B deficiency.

To refine our classifier and to increase sensitivity we stepwise re- trained the classifier with previously classified VUS samples. With this approach we aim to capture KMT2B mutations with moderate epigenetic effects but still causing dystonia.

Results: All CpG sites of the episignature showed increased methylation levels in cases. The mean of their normalized methylation levels correlated well with the age at onset of dystonia ($p = 0.003$) – being lower in patients with late or incomplete penetrance – thus serving as a predictor of disease onset and severity. The SVM classifier was accurate both when tested on the general population and on samples with various other deficiencies of the epigenetic machinery. It was able to successfully evaluate variants of uncertain significance (VUS) from multiple batches.

Conclusion: Our data show that episignatures do not only function in diagnostics but may also provide predictors of disease course and, potentially, markers of therapeutic success. KMT2B-deficient patients are known to profit exceptionally well from deep brain stimulation. The episignature-based biomarker will serve to identify them with high accuracy.

References:.
Grants:.
Conflict of Interest: None declared.

P10.034.A Uncovering the phenotypic, genetic, and causal relationships of depression with other psychiatric and non-psychiatric disorders

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Background/Objectives: Depression, as one of the most prevalent psychiatric traits and one of the leading causes of health loss worldwide, is known to be highly heritable and is frequently observed comorbid with other mental and physical illnesses. This observation motivated us to look deeper into its genetic and potentially causal connections with other disorders.

Methods: In this study, we utilized data from the UK biobank to systematically evaluate relationships between depression and other heritable traits, from both phenotypic and genetic aspects. We compressed 6,300 ICD codes into 412 heritable PheCode items and constructed a comorbidity network across depression and a wide range of diseases among 300,000+ European ancestry participants. Subsequently, we examined the genetic correlation for each pair of phenotypic connection observed from the network. We also looked into causality relationships through mendelian randomization for all pairs of significantly correlated disorders and further uncovered horizontal pleiotropic genetic variants and genes contributing to disease aetiologies.

Results: We found gastro-oesophageal reflux disease (GORD), body mass index and osteoarthritis as direct causes for depression, with GORD lying in the center of the causal network. Genes broadly expressed in various tissues, such as NEGR1, TCF4 and BTN2A1 were identified to underlie the pathways that lead not only to depression but also other related diseases.

Conclusion: Our work highlights the broad connections between depression and diverse traits, indicating a complex aetiology and possible existence of subtypes for depression. Our findings highlight the value of cross-trait analysis towards better

understanding of the neurobiology of complex psychiatric disease.

References:.

Grants:.

Conflict of Interest: None declared.

P10.035.B The diagnostic yield of NGS-based gene panels in epilepsy

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Background/Objectives: We report the yield of NGS panels applied for routine diagnostics in suspected monogenic epilepsy syndromes during the years 2019-2021.

Methods: We analyzed 181 (23 adult and 158 pediatric) patients with epilepsy of unknown etiology who underwent diagnostic testing using targeted gene panels (range 3-143 genes). The pathogenicity of variants was assessed according to ACMG criteria. Diagnostic yield was determined for all individuals according to age at genetic diagnosis.

Results: Panel analysis identified pathogenic or likely pathogenic variants in 36 of 181 patients, resulting in an overall diagnostic yield of 19,9 %. The yield depended on the age of the patient at diagnosis as well as the epilepsy syndrome. The yield was highest among infants aged 6-12 months (72,7 %, 8/11) and toddlers aged 12-24 months (60 %, 9/15). Accordingly, benign familial infantile epilepsy (BFIE) exhibited the highest diagnostic yield among epilepsy syndromes (69,2 %, 9/13), followed by Dravet syndrome (50 %, 4/8). 17 different genes harbored pathogenic or likely pathogenic variants with PRRT2 (8 patients), PCDH19 (6 patients) and SCN1A (4 patients) being most frequently affected. Missense variants were the most common type contributing to diagnosis (44 %, 16/36). Frameshift variants, nonsense variants and CNVs accounted for 31 % (11/36), 14 % (5/36) and 11 % (4/36) of cases, respectively.

Conclusion: In our diagnostic setting NGS epilepsy gene panels have an overall diagnostic yield of approximately 20 %. We observed the highest probability of genetic diagnosis in infants whereas the detection rate decreased with increasing age at the time of genetic diagnosis.

References:.

Grants:.

Conflict of Interest: None declared.

P10.036.C Deciphering new genetic components of psychiatric disorders through multivariate association studies

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Background/Objectives: The study of imaging-derived phenotypes and their associated genetic and environmental factors has become central to mental disorder research. However, the dimensionality and high correlation of imaging phenotypes causes severe computational and analytical challenges. Here, we applied novel methods we recently developed to investigate shared genetic components between neuroanatomical phenotypes and psychiatric disorders.

Methods: We first performed multitrait genome-wide association study (GWAS) of brain volume phenotypes in 26,000 individuals from the UKBIOBANK and ENIGMA cohorts using JASS, a robust and computationally efficient multitrait analysis pipeline. We next conducted an unsupervised clustering of top univariate and multivariate association signals to identify clusters of variants displaying similar multitrait association patterns. Genetic variants from each cluster were then assessed for association with psychiatric disorders.

Results: Our multitrait analysis detected substantially more associated variants than univariate brain volume GWAS, with over 80% increase in power. Among top variants, we identified multiple clusters with marked enrichment for association with specific psychiatric diseases. In particular, a cluster of highly pleiotropic variants associated with all brain volumes considered was associated with bipolarity ($P = 1.8e\text{-}7$). Another cluster, capturing shared genetics between putamen, pallidum and caudate volume, showed strong association with schizophrenia ($P = 1.5e\text{-}17$), supporting the role of genetics in the previously reported association between basal ganglia and schizophrenia.

Conclusion: Our analyses demonstrate that data-driven multitrait analysis of imaging-derived phenotypes can help decipher the genetic components of psychiatric disorders.

References:.

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Conflict of Interest: None declared.

P10.037.D Phenotypic spectrum overview of patients with neurodevelopmental disorders sharing one recurrent copy number variant (CNV) and carrying different additional CNVs

Martina Servetti[1], *Livia Pisciotta*[1;2], *Elisa Tassano*[3], *Maria Cerminara*[1;4], *Marco Fontana*[4], *Chiara Bagliani*[1], *Lino Nobili*[1;5], *Silvia Boeri*[1;5], *Giulia Rosti*[1;4], *Margherita Lerone*[4], *Maria Teresa Divizia*[4], *Federico Zara*[1;4], *Patrizia Ronchetto*[3], *Aldamaria Puliti*[1;4]

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Background/Objectives: Patients with neurodevelopmental disorders (NDDs) may have different clinical presentation even within the same diagnostic category. We aimed to characterize the phenotype of patients sharing one recurrent CNV and carrying additional different CNVs. To evaluate possible synergistic effects among multiple CNVs and their fall-out on patient's phenotype.

Methods: We re-evaluated array-CGH results from 518 patients with NDDs with non-benign CNVs classified as pathogenic, likely pathogenic and variants of uncertain significance (1). Pathogenic variants included syndromic/recurrent CNVs (according to Decipher and SFARI databases), CNVs > 3 Mb and those < 3 Mb and encompassing known NDD-associated genes. Protein-protein interaction and gene enrichment analyses were used to show potential interplay among CNV-encompassed genes.

Results: We found that among patients with pathogenic variants (131), 8% (11) have one additional, inherited or de novo CNV. These double hits could explain the complex phenotypic spectrum of the patients. Among major results, three patients sharing a syndromic 15q13.3 deletion associated with additional different CNVs, and patients sharing one small CNV, involving a known NDD

gene, in addition to different CNVs involving other NDD-associated genes with potential synergistic effects.

Conclusion: It is worth to further investigate patients with pathogenic CNVs and analyse modifier effects of multiple CNVs on patient's phenotype. A better understanding and classification of these patients can be useful for disease prognosis, management of the patients, keeping with the developing idea of precision medicine.

References: 1. Servetti et al, Front. Genet. 2021.

Grants:.

Conflict of Interest: None declared.

P10.038.A Modelling the combined effects of rare and common genetic variants, with opposite effects on cognition, on autism spectrum disorder risk

Zoe Schmilovich[1;2], *Elise Douard*[3;4], *Guillaume Huguet*[3;4], *Patrick Dion*[2;5], *Sebastien Jacquemont*[3;4], *Guy Rouleau*[2;5]

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Background/Objectives: Common and rare variants contribute to autism spectrum disorder (ASD) [1]. Rare de novo and inherited CNVs that substantially increase ASD-risk also have negative effects on cognition [2,3,4]. Conversely, there is a positive genetic correlation between ASD and cognition, such that common variants which increase ASD-risk also increase intelligence (IQ) in the general population [5].

Methods: Deletion and duplication CNVs, annotated by a constraint score (LOEUF), and PRS for intelligence (PRSIQ) were identified in 6,929 ASD cases and 32,663 unaffected individuals. The sum of LOEUF represented the individual-level burden of deleterious rare variants. A sliding window modelled the risk for ASD across LOEUF and PRSIQ categories. The cumulative risk for ASD across both LOEUF and PRSIQ categories was modelled using a double-sliding window approach.

Results: Intolerant (LOEUF<0.35) and tolerant (0.35≤LOEUF<1) genes disrupted by gene dosage significantly increased ASD-risk, even after adjusting for their effects on cognition. High and low PRSIQ increased and decreased ASD-risk, respectively, and this effect was heightened after adjusting for cognition. A combination of highly intolerant genes (LOEUF < 0.2) and a high PRSIQ (≥1 standard deviations from the control population mean) was associated with the highest risk for ASD for deletions (OR = 13.81;p = 2e-02) and duplications (OR = 1.81;p = 2e-02).

Conclusion: Our results suggest that a combination of genetic variants that both increase and decrease cognition may be key elements to ASD-risk.

References: 1-Weiner (2017); 2-Satterstrom (2020); 3-Huguet (2018); 4-Douard (2020); 5-Grove (2019).

Grants: Transforming Autism Care Consortium (Fonds de Recherche Québec - Santé).

Conflict of Interest: None declared.

P10.039.B GRIN2A null variants confer a high risk for early-onset mental disorders and potentially enable precision medicine approaches

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Background/Objectives: Mental disorders are considered complex and highly polygenic and numerous loci have been associated with a small risk increments for mental disorders, each. One of these loci is 16p13.2 spanning *GRIN2A*, a gene encoding the GluN2A subunit of the NMDA receptor.

Methods: Therefore, we investigated the presence of psychiatric diagnoses within our registry of 236 individuals with *GRIN2A*-related disorders.

Results: Carriers of null variants were significantly more likely to develop mental disorders compared to carriers of missense variants. *GRIN2A*-related mental disorders comprised the whole spectrum of psychiatric diseases, but most prominently mood and anxiety disorders followed by psychotic disorders. Literally all *GRIN2A*-related mental disorders manifested in childhood or adolescence and thus had a significantly earlier onset compared to the general population. In some individuals, the mental disorder appeared to be the foremost clinical diagnosis.

Conclusion: Thus, we identified *GRIN2A* to be the first gene ever associated with a monogenic mental disorder, demonstrating that the genetic background of this disease spectrum is not necessarily as polygenic or complex as previously thought. Likewise, we uncovered a completely novel phenotypic facet of this genetic entity, changing our perception of isolated seemingly non-syndromic mental disorders in general. Moreover, we recently observed that treatment with NMDA receptor co-agonists appears to be beneficial in *GRIN2A*-related disorders due to null variants, even with respect to psychotic features. Thus, the NMDA receptor appears to be a promising target of precision medicine approaches to ameliorate mental disorders due to *GRIN2A* null variants and maybe even beyond.

References:.

Grants:.

Conflict of Interest: None declared.

P10.040.C Generation and characterisation of induced pluripotent stem cell-derived microglia to study the influence of genetic risk for depression on microglial functions

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Background/Objectives: Microglia, the resident immune cells of the brain, have been implicated in the pathology of psychiatric disorders, including depression. These findings are mostly based on animal models, while results from humans are limited. Recent advances in induced pluripotent stem cell (iPSC) research provide new possibilities to study human microglia. Here, we established the differentiation of iPSC into microglia (iMG) to study the influence of genetic risk for depression on microglial function.

Methods: In pilot experiments, sequential differentiation of iPSC into primitive macrophage precursors (PMP) and iMG was established following published protocols[1].The progression of differentiation was monitored at several stages using flow cytometry, immunocytochemistry and transcriptomics (3'mRNA-Seq). Furthermore, iMG were stimulated with lipopolysaccharide (LPS) and/or dexamethasone to study inflammatory and glucocorticoid ("stress") signalling. Prospectively, we plan to analyse iMG of depressed patients and healthy controls, selected based on

genome-wide genotype data and polygenic risk scores for depression.

Results: First results indicate successful differentiation from iPSCs to PMP and iMG, based on surface expression of macrophage/microglia markers (CD11b, CX3CR1, P2RY12) and in vitro microglia-like morphology. Transcriptome analysis confirmed cell type-specific gene expression signatures throughout differentiation as well as differential expression of inflammatory and glucocorticoid-response genes after stimulation.

Conclusion: The generation of iMG from donors with different genetic backgrounds will provide a valuable tool to study human microglial function in the context of depression or other psychiatric disorders and enable analyses of gene-environment interactions (e.g. "stress") in a defined cellular model.

References: [1]Reich et al. 2021 (PMID: 33613545).

Grants: DFG EXC2151–390873048.

Conflict of Interest: None declared.

P10.041.D Eating disorders: Age of onset and its associated genetic risk factors

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Background/Objectives: Eating disorders have a heritability of 16-83%[1] and a median age of onset of 18 years[2]. UK-based studies on age of onset of eating disorders and how this is influenced by genetics are lacking.

Methods: Participants self-reported eating disorder diagnoses, symptoms, and age of onset in either the Eating Disorders Genetics Initiative UK (EDGI UK) or the Genetic Links to Anxiety and Depression (GLAD) Study (n = 8,945). We associated polygenic scores calculated with PRS-CS with age of onset.

Results: Median age of onset was younger in females than males: binge eating (18 vs. 21 years), low weight (18 vs. 20 years), and purging (16 vs. 19 years). More males reported onset older than 25 years for binge eating (41.3% vs. 18.4%), purging (29.1% vs. 10.3%), and low weight (33.2% vs. 17.6%), and onset below 10 years for low weight (2.9% vs. 0.3%). Preliminary polygenic score analyses indicate that a one standard deviation increase in educational attainment polygenic score was associated with earlier age of onset across all phenotypes: purging by 0.41 years ($p = 0.04$), low weight by 0.51 years ($p = 0.04$), and binge eating by 0.52 years ($p = 0.01$).

Conclusion: Eating disorders primarily begin in the 16-25 age group. Genetics driving educational attainment may be associated with younger age of onset. Our results highlight the need for sufficient investment into adult clinical services, increased awareness efforts about eating disorders in adulthood, and early screening.

References:.

Grants: Funded by NIHR BioResource and the NIHR Maudsley Biomedical Research Centre.

Conflict of Interest: Helena Davies: None declared, Christopher Hübel: None declared, jonathan kelly: None declared, agnes ayton: None declared, rachel Bryant-waugh: None declared, molly davies: None declared, Jessica Mundy: None declared, janet treasure: None declared, Gerome Breen Prof Breen has received honoraria, research or conference grants and consulting fees from Illumina, Otsuka, and COMPASS Pathfinder Ltd.

P10.042.A Population-level study of CNVs and their associated risk of psychiatric disorders in a Danish case-cohort

Seyedmorteza Vaez[1;2], *Simone Montalbano*[1;2], *Xabier Calle Sanchez*[1;2], *iPSYCH Collaborators*[2], *Armin Raznahan*[3], *Wesley Kurt Thompson*[2;4], *Thomas Werge*[1;2;5], *Dorte Helenius Mikkelsen*[1;2], *Andres Ingason*[1;2;5]

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Background/Objectives: Recurrent copy number variants (CNVs) have been shown to increase the risk of neuropsychiatric disorders in clinical case-control studies. Yet, little is known about their pathogenic impact at the level of an individual or an entire population. We sought to estimate the true population prevalence of recurrent CNVs and their associated risk of psychiatric disorders.

Methods: We applied the iPSYCH2015 case-cohort (1), including all individuals born in Denmark in 1981-2008 and diagnosed with a major psychiatric disorder by 2015, and a random comparison sample from the same birth cohort. Samples were genotyped and recurrent CNVs at 30 loci called with PennCNV (2) and verified by visual inspection. Population-representative hazard ratios (HR) were derived using a Cox proportional hazard model with inverse probability of sampling (IPS) weights (3).

Results: The population prevalence of several CNVs was higher than in the UK Biobank (4). Overall, the carrier rate in cases was higher than in the comparison sample with HR varying widely across disorders and CNVs.

Conclusion: The population-based iPSYCH study enables estimating of true prevalence and associated risk conferred by CNVs of ascertained psychiatric disorders, thus paving the way for implementing genetic predictions into clinical practice.

References: 1. Byberg-Grauholm et al., 2020 (https://doi.org/10.1101/2020.11.30.20237768). 2. Wang et al., 2007 (https://doi.org/10.1101/gr.6861907). 3. Barlow et al., 1999 (https://doi.org/10.1016/s0895-4356(99)00102-x). 4. Crawford et al., 2019 (https://doi.org/10.1136/jmedgenet-2018-105477).

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Conflict of Interest: None declared.

P10.043.B Investigation of polygenic scores in patients with social anxiety disorder

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Background/Objectives: Social anxiety disorder (SAD) is a common anxiety disorder (AD). It has a heritability of around 10-50% and is highly comorbid with depressive symptoms and major depressive disorder (MDD). Large AD genome-wide association studies (GWAS) have been conducted, but sample sizes in genetic analyses of SAD have been limited. Therefore, the genetic basis of SAD and its subphenotypes is still largely unknown.

Methods: We computed polygenic risk scores (PRS) using PRSice-2 and summary statistics of depression (Howard et al., 2019) and MDD GWAS (Wray et al., 2018). PRS were calculated in a large cohort comprising n = 1304 patients with SAD from our Social Phobia Research project and external collaborators, and n = 4140 controls from the Heinz Nixdorf Recall study. The association between the PRS and case-control status as well as SAD subphenotypes (depressive, SAD-specific, anxiety symptoms) was investigated by using R.

Results: Preliminary analyses revealed a significant association between the PRS of depression and SAD case-control status. The depression PRS was also nominally associated with depressive symptoms in SAD patients, but this association did not withstand stringent Bonferroni correction.

Conclusion: Our preliminary results suggest that the genetic risk for depression might contribute to depressive symptoms in SAD patients. Beyond that, we did not identify strong contributions of genetic risk factors for depression/MDD to SAD subphenotypes. Further systematic analyses of PRS of AD and additional subphenotypes (e.g., comorbid MDD) are currently ongoing and will be presented at the upcoming conference.

References: Howard et al. (2019), PMID: 30718901.
Wray et al. (2018), PMID: 29700475.

Grants: Else Kröner-Fresenius-Stiftung (2019_A127).

Conflict of Interest: None declared.

P10.044.C Investigation of the association between loci with opposite directional effects across multiple neuropsychiatric disorders and human brain structure

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Background/Objectives: In a genome-wide association study (GWAS) meta-analysis, Lee et al. (2019) reported eleven SNPs to show opposite effects on multiple neuropsychiatric disorders. The pathogenic mechanisms of these antagonistic effects, i.e. increased risk for one disorder along with reduced risk for another, and their potential influence on brain structure have not been explored yet.

Methods: To investigate the association between antagonistic SNPs and brain structural phenotypes, we reviewed the summary statistics of large ENIGMA GWAS of subcortical volume, cortical thickness (CT), surface area (SA), and total intracranial volume (ICV). After false discovery rate correction for all SNP-phenotype pairs, we assessed for each significant association if large ENIGMA case-control brain imaging studies have previously reported those structural alterations in patients with neuropsychiatric disorders.

Results: Seven antagonistic SNPs were significantly associated with at least one structural phenotype. The SNPs were associated with the SA in widespread cortical regions (e.g. rs6748341 with the SA in the bilateral pars opercularis), the CT in the cingulum, and the inferior parietal lobule, the volume of the caudate nucleus, and ICV. Analyzing case-control imaging studies provided further evidence that some of the identified SNP-brain phenotype associations might be involved in disease development.

Conclusion: Our study supports the notion that several antagonistic SNPs associated with neuropsychiatric disorders influence brain structure, which might explain how antagonistic SNP alleles modulate the risk of developing a specific disorder. Future case-control genomic imaging analyses and a more refined characterization of underlying brain networks are required to strengthen the preliminary findings.

References: Lee et al. (2019) PMID:31835028.

Grants:.

Conflict of Interest: None declared.

P10.045.D Whole exome sequencing followed by reverse phenotyping in patients with neurodevelopmental disorders identifies rare genetic syndromes and reveals novel clinical and genetic variants

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Background/Objectives: The complex nature of neurodevelopmental disorders offers a real challenge when it comes to clinical and molecular diagnostics. The classical gene panel approach to the whole exome sequencing data analysis is hindered by the "blurred" clinical picture of complex, overlapping and variable clinical phenotypes. Looking outside a particular gene panel and back to clinical data made possible to "recognize" rare syndromes and to identify novel clinical and genetic variants.

Methods: Whole exome sequencing analysis using gene panels and non-targeted analysis followed by reverse phenotyping in 10 patients with neurodevelopmental disorders who had already been excluded for the common types of genetic defects for the suspected particular condition.

Results: We have identified potentially disease-causing variants in six cases. Three of them are due to de novo mutations in genes *GFAP*, *KCNC3* and *KMT2E*. The other three were associated with homozygous or compound heterozygous variants in *TTC37*, *RUBCN* and *ALDOA* genes that relate to disorders with autosomal recessive inheritance. The patient carrying the *KCNC3* variant exhibit non-characteristic for this locus clinical phenotype with a major clinical feature of focal epilepsy underlined by a mild developmental delay. A homozygous mutation in the *ALDOA* gene was found in patient with autism and mental retardation. Up to the moment, the role of this gene in autistic spectrum disorders has been only discussed but not established clear-cut.

Conclusion: The combined whole exome sequencing – reverse phenotyping approach is very effective method for the diagnostics that could provide new insides in of rare genetic disorders.

References: -.

Grants: D83/04.06.2021, D01-285/17.12.2019, D01-395/18.12.2020, D01-302/17.12.2021.

Conflict of Interest: None declared.

P10.046.A Dysfunctional CTDP1 impairs the cell cycle indicating its essential role in neurodevelopment

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Background/Objectives: Malformations of cortical development (MCDs) constitute a diverse range of disorders that are common causes of neurodevelopmental delay and epilepsy. The underlying molecular mechanism of several MCDs have been elucidated, although many still remain unknown.

Methods: Trio exome analysis in a girl with congenital arthrogryposis, unilateral auditory neuropathy, absent optic chiasma and grey matter heterotopia revealed the presence of two novel variants in the C-terminal domain phosphatase 1 (CTDP1) gene. CTDP1 functions as a phosphatase which dephosphorylates the C-terminus of RNA polymerase II (RNAPII) and may potentially be involved in MCD, re-enforced by its essential role for normal embryo development.

CTDP1 expression was analysed both on mRNA level using qPCR and protein (FCP1) level by western blot (WB) analysis. The protein's phosphatase activity on the C- terminal domain was studied using WB. Finally, cell cycle analysis was performed by DNA staining using propidium iodide.

Results: qPCR data showed a significant downregulation of *CTDP1* expression in patient versus controls. WB revealed a decreased protein level and more phosphorylated RNAPII in patient cells. Lastly, a disturbed cell cycle was observed in the patient compared to the controls.

Conclusion: Our preliminary data show that the expression of *CTDP1*, as well as FCP1 and the phosphatase activity are altered in the patient. Alterations caused by abnormal FCP1 function in the cell cycle are associated with disturbed cell cycle proteins (p27, cyclin B). Together, these results provide us a preliminary evidence that FCP1 plays a role in early brain development and cell growth by regulating the cell cycle.

References:.

Grants:.

Conflict of Interest: Hamide Yildirim Marguerite-Marie Delacroix foundation, Boyan Dimitrov: None declared, Elyssa Cannaerts: None declared, Karen Sermon: None declared, Alexander Gheldof: None declared, Katrien Stouffs: None declared, Anna Jansen: None declared.

P10.047.B X-chromosome inactivation is an easy assay to further investigate cases without a diagnostic exome sequencing

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Background/Objectives: Non-random X-chromosome inactivation (skewed-XCI) is suggestive of an X-linked genetic disease if detected in mothers of affected males (skewing towards the normal allele) or affected females (skewing towards the

deleterious allele). We explored skewed-XCI in a cohort of unsolved neurodevelopmental disease (NDD) cases, negative at FRAXA, array-CGH and trio Exome Sequencing (ES).

Methods: XCI test by methylation-sensitive fluorescent PCR screening on blood-extracted DNA. Episignature analysis using Episign.

Results: We examined 66 NDD females and 115 mothers of affected NDD males and found an excess of extreme XCI (>90%) in both groups (7/66: 11%; $p = 0.03$; 10/115: 9%; $p = 0.04$). We re-analyzed ES data and found overlooked causal X-chromosome variants in 6/17. Five occurred in genes encoding chromatin remodeling proteins; in four, we confirmed the corresponding episignature: (i) [c.1204G>A;p.(D402N)] likely pathogenic (LP) variant in KDM5C (female; 90:10 XCI); (ii) deletion of exons 3-4 in *ATRX* (male; mother 100:0 XCI); (iii) [c.1322G>A;p.(Arg441Glu)] LP variant in *ZMYM3* (male; mother 100:0 XCI); (iv) [c.1526C>T;p.(Pro509Leu)] LP variant in *OTUD5*, a recently described novel disease gene (two brothers, mother 100:0 XCI).

We also found the de novo [c.890G>T;p.(Cys297Phe)] VoUS in *PHF6* in a 10-year-old girl with DD/ASD whose episignature apparently did not confirm PHF6-associated disease. Finally, we found the *TAF1* VoUS [c.805G>A;p.(Gly269Arg)] in a female (100:0 XCI) and her brother with ID, still under investigation, since no affected female has been described so far.

Conclusion: Our work recommends XCI as an easy assay to further study cases without a diagnostic ES, guiding molecular re-evaluation and further analysis for X-linked genes.

References:.

Grants:.

Conflict of Interest: None declared.

P10.048.C Association of pathogenic variants in SCN2A and COL4A2 mimics the neurometabolic phenotype - a case of SCN2A-related encephalopathy and COL4A2-related brain small vessel disease

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Background/Objectives: *SCN2A* gene encodes Na(v)alpha1.2 protein – alpha-2 subunit of voltage-gated type 2 sodium channel, expressed in central nervous system excitatory neurons. Pathogenic variants of *SCN2A* gene are associated with variable spectrum of neurodevelopmental and paroxysmal phenotypes as developmental and epileptic encephalopathy, benign familial infantile seizures, episodic ataxia, intellectual disability, and autism spectrum disorders with or without seizures. *COL4A2* gene encodes alpha-2 chain of type IV collagen, ubiquitously present in basement membranes. *COL4A2* pathogenic variants are associated with broad spectrum of systemic disorders affecting various systems; central nervous system affecting phenotypes include brain small vessel disease and intracerebral hemorrhages.

Methods: Here we report a case of male infant with neurometabolic-like presentation: drug-resistant epilepsy, pyramidal tetraplegia, creatine kinase and lactic acid elevation, abnormal NMR neuroimaging, the course was complicated by Rotavirus infection, *Bordatella pertussis* pneumonia, and *Klebsiella pneumoniae* sepsis.

Results: Whole exome sequencing (WES) in the proband revealed two clinically significant de novo variants (absent in both biological parents) in *SCN2A* (NM_021007.3): c.4972C>T (p.Pro1658Ser) and *COL4A2* (NM_001846.4): c.2989G>A (p.Gly997Arg).

Conclusion: Congenital dysfunction of *SCN2A* and *COL4A2* genes mimics neurometabolic-like phenotype in described patient. As both *SCN2A*- and *COL4A2*-related disorders are characterized by variable penetrance and diverse expression, clinical features and molecular findings presented here, broaden the knowledge of both disorders pathogenesis and their role as modifying factors for each other.

References:.

Grants:.

Conflict of Interest: None declared.

P10.049.D The influence of polymorphisms of selected genes on human chronotype

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Background/Objectives: Circadian rhythms are the very important human biorhythms. Their disorders can have unpleasant health, psychological and social consequences. However, the rhythm of life in conflict with the physiological setting of the "biological clock" can also bring problems, including health problems. On the contrary, respecting these rhythms can be used positively in medicine, but also in economics. According to their individual settings, we can distinguish people of different chronotypes (so-called „larks", „owls" and neutral), whereas this chronotype is largely determined genetically.

Methods: We genotyped thirteen polymorphisms in seven candidate genes (clock genes *CLOCK*, *PER1*, *PER2*, *PER3* and *BMAL1* and clock-related metabolic genes *SIRT1* and *PGC-1α*) in 57 probands with extreme chronotype (38 „larks" and 19 „owls") and analyzed the potential association between the polymorphisms and the probands chronotype (defined using MEQ score). We also used the relative risk ratio (RR).

Results: The analyzes show two results statistically significant at $\alpha = 0{,}05$, and some more results at $\alpha = 0{,}1$). We found a strong association between the minor allele A in rs3736265 (*PGC-1α*) and the extreme evening chronotype ($p = 0{,}0331$; $RR = 2{,}98$) and a strong association between the minor allele C in rs1801260 (*CLOCK*) and the extreme morning chronotype ($p = 0{,}0165$; $RR = 1{,}17$). From the other findings the most interesting is an association between the minor allele C in rs2640909 (*PER3*) and the extreme morning chronotype ($p = 0{,}0683$; $RR = 2{,}06$).

Conclusion: Our findings identify new polymorphisms associated with the human chronotype and confirm the need to focus not only on clock genes but also on clock-related metabolic genes.

References: No.

Grants: No.

Conflict of Interest: Roman Solc Charles university, Faculty of science.

P10.050.A Investigating the efficacy of combined ketogenic diet and anti-inflammatory therapy in a mouse model of early-onset Tay-Sachs disease

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Background/Objectives: Tay-Sachs disease is a rare genetic disorder caused by abnormal GM2 ganglioside accumulation predominantly in the central nervous system (CNS) due to β-hexosaminidase A (HEXA) enzyme deficiency. Recently, our research group generated Hexa-/-Neu3-/- mouse model that mimics neuropathology of early-onset Tay-Sachs disease (1). We showed that undegraded GM2 accumulation resulted in neuronal cell death and activated neuroinflammation inducing astrogliosis and microgliosis-based pro-inflammatory cytokines and chemokines secretion (2). The high fat, low-carbohydrate ketogenic diet (KD) has broad potential usage in the treatments of neurological disorders and there is growing evidence that KD is also anti-inflammatory. In this study, we aim to show KD and anti-inflammatory drug therapy in the treatment of neuroinflammation in Hexa-/-Neu3-/-.

Methods: Hexa-/-Neu3-/- mice were fed until 4.5 months old under the following groups: (i) control diet (CD), (ii) ad-libitum KD (10-day), (iii) ad-libitum CD with propagermanium (8mg/kg/daily for 21-day) and (iv) KD with propagermanium (21-day). Neuroinflammation markers were analyzed by qRT-PCR and immunohistochemical (IHC) analysis.

Results: Administration of KD and propagermanium in Hexa-/-Neu3-/- mice significantly reduced the expression levels of Ccl2, Ccl3, Ccl5, Cxcl10 in the cortex and cerebellum compared to Hexa-/- mice. Consistent with qRT-PCR analysis, IHC staining for glial fibrillary acidic protein (GFAP) displayed decreased astrogliosis.

Conclusion: Altogether, our results clearly suggest that ketogenic diet and propagermanium could be potential combined therapeutic strategy to reduce neuroinflammation in an early-onset Tay-Sachs disease mouse model.

References: (1) Seyrantepe V et al 2018 (2) Demir SA et al 2020.

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Conflict of Interest: None declared.

P10.051.B High diagnostic yield in children and adolescents with psychiatric disorders

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Background/Objectives: Psychiatric disorders are more prevalent in children with mild to borderline intellectual functioning. Rare pathogenic variants in neurodevelopmental genes increase the risk for psychiatric disorders and may explain the comorbidity. Despite these patients represent up to 35% of those attended at mental health services, genetic diagnosis is usually not offered. The identification of mentioned variants could lead to improved clinical care.

Methods: Whole exome sequencing was performed on 50 out of 150 enrolled young affected by a psychiatric condition diagnosed following DSM-5 criteria, and either mild intellectual disability(IQ 55-69) or borderline intellectual functioning(IQ 70-85). Severity and interference of IQ and psychiatric comorbidity was evaluated using several psychometric tests. Inheritance pattern was assessed through Sanger sequencing. ACMG/AMP guidelines were used for variant classification.

Results: Inheritance pattern was assessed on 43 candidate variants after applying a stringent customized variant filtering and prioritization process. 15 were classified as pathogenic/likely pathogenic resulting in a 30% diagnostic yield. In details, 80% of the variants were de novo, 3 inherited. Among the reported variants we identified 8 loss of function, 4 missense and 3 copy number variants. Most of the genes harbouring these variants have been involved in neurodevelopmental disorders.

Conclusion: The high diagnostic yield obtained from our exome sequencing approach demonstrates the need to offer genetic testing in children with psychiatric disorders and comorbid mild to borderline intellectual functioning.

References: Homann OR, et al. Molecular Psychiatry.2016;21(12):1690-5.

Wolfe K, et al. Journal of Applied Research in Intellectual Disabilities.2018;31(2):273-84.

Grants: Instituto de Salud Carlos III, 2019,PI19/01902.

Conflict of Interest: None declared.

P10.053.D Czech family confirms the new 1p36.13-1p36.12 microdeletion syndrome and the involvement of the critical region with UBR4 and CAPZB genes

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Background/Objectives: In 2020 Aagaard Nolting and coauthors described a new 1p36.13-1p36.12 microdeletion syndrome characterized by learning disability, behavioral abnormalities and ptosis caused by a deletion of 1 Mb in the smallest region of overlap. They described seven patients from 5 families with these characteristic features and considered the genes *UBR4* and *CAPZB* as the most likely candidate genes for the features of this new syndrome.

Methods: Clinical, molecular cytogenetic (array CGH; Agilent SurePrint G3 8x60K) and genotype-phenotype analysis.

Results: We present a Czech family where the daughter and her mother have congenital ptosis, intelectual subnormality and behavioral abnormalities and recently detected and electrophysiologically verified also demyelinating polyneuropathy. Array CGH revealed a 2,2 Mb microdeletion at 1p36.13 (chr1:17815790-20018021 (GRCh37/hg19)) in both patients affecting also the *UBR4* and *CAPZB* genes. This deletion was not detected in the healthy grandmother of the daughter and the grandfather already died on cancer. Polyneuropathy was not reported in the seven patients in the original publication.

Conclusion: Our Czech family with two similarly affected patients, daughter and mother confirms the new 1p36.13-1p36.12 microdeletion syndrome and the involvement of the critical region with *UBR4* and *CAPZB* genes. The demyelinating polyneuropathy detected in both our patients clinically and electrophysiologically may be part of this new syndrome, but may also have another independent cause in our family. The microdeletion at 1p36.13-1p36.1 seems to be very rare.

References: Aagaard Nolting et al. A new 1p36.13-1p36.12 microdeletion syndrome characterized by learning disability,

behavioral abnormalities, and ptosis. Clin Genet. 2020 Jun;97(6):927-932. https://doi.org/10.1111/cge.13739.

Grants:.

Conflict of Interest: None declared.

P10.054.A Polygenic relationships between human longevity and psychiatric disorders

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Background/Objectives: Psychiatric disorders (PD) have complex, polygenetic architectures involving interrelationships among common and rare genetic factors and environmental exposures. There are broad genetic correlations among PDs and psychological and behavioral traits that represent risk (RF) or protective factors (PF). Psychiatric patients have increased mortality and genes associated with longevity (PLS) may capture PF. Genetic relationships among PDs, RFs, PFs and longevity have not been fully described.

Methods: LD-score regression to estimated genetic correlations between PDs and RF, PF, and longevity using published GWAS. We estimate correlations among polygenic scores (PGS) from these GWAS in >100,000 individuals from the iPSYCH study. Multiple logistic regression and sequential model fitting estimate contributions of multiple PGS for PDs, RFs, PFs, and PLS in models for each PD.

Results: Most genetic correlations among PDs, RFs, PFs, and longevity are in expected directions (e.g., longevity negative with ADHD), but some are opposite (e.g., longevity positive with Autism). Each class of PGS makes significant, independent contributions in joint models predicting PDs, especially for ADHD and MDD, where PLS remain significant after adjustment for all other PGS.

Conclusion: We present genetic relationships among longevity and PDs, RFs, and PFs. We show PGS from multiple domains, including longevity, significantly increases explained variance. This has implications for the role of PF and PGS in psychiatric clinical and research applications.

References:.

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Conflict of Interest: None declared.

P10.056.C Deciphering the mechanism of a de-novo GRIA2 mutation related to a neurodevelopmental disorder

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Background/Objectives: *GRIA2* encodes the GluA2 unit of the AMPA-type glutamate receptors. AMPA receptors are a major mediator of fast excitatory neurotransmission in the brain. They promote the formation of new synapses and trigger activity-dependent processes that underlie learning and memory. *GRIA2* mutations cause intellectual disability (ID) and intractable seizure. We studied a de-novo missense variant in *GRIA2* (L772W) detected by exome sequencing in a 1.5-year-old girl with severe ID, microcephaly without seizures.

Methods: The GluA2(L772W) homomeric mutation was expressed in Xenopus laevis oocytes. Currents were recorded using the two-electrode voltage clamp configuration. Receptor maturation and surface expression were determined by Western-blot analysis, coupled with glycosylation (oocytes) and biotinylation (HEK293 cells) assays.

Results: The mutant protein was expressed normally, it reached the cell membrane and maturated into functional receptor but had altered kinetics. This variant causes faster desensitization, thus limiting the duration of channel opening.

Conclusion: We believe that reduction of channel opening duration due to this *GRIA2* mutation may explain the neurological phenotype, either by directly reducing neuronal excitability or by compensatory mechanisms which may cause a reactive increase in the calcium permeable GluA1 receptors, thus increasing the neuronal excitatory activity leading to cell death. We plan to investigate this variant in its natural surrounding using human induced pluripotent stem cells (iPSCs) derived from our patient and a healthy close relative.

References:.

Grants:.

Conflict of Interest: None declared.

P10.057.D Identification of shared genes and pathways between psychiatric GWAS and monogenic neurodevelopmental disorders

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Background/Objectives: Large genome-wide association studies (GWAS) rapidly identified novel risk genes for psychiatric disorders. Mendelian neurodevelopmental disorders (NDD) caused by single gene mutations show overlapping behavioural symptomatology, however the extent to which genes are shared between psychiatric GWAS and monogenic NDD needs to be fully explored.

Methods: We carry out genetic overlap analyses between five large-scale psychiatric GWAS (ADHD, autism, bipolar disorder, depression, schizophrenia, sample size range: $N = 46{,}077-807{,}553$) and monogenic neurodevelopmental disorders (NDDs). Consistent gene-mapping was done in FUMA for each GWAS. Overlap analyses were carried out with monogenic disease genes in the Developmental Disease Gene - Phenotype (DDG2P) database. Using various bioinformatic resources, we analysed gene properties and gene-expression of genes implicated in psychiatric disorders, NDDs or both.

Results: We found significant enrichment of psychiatric GWAS genes in all monogenic NDDs (enrichment = 1.18, $P = 1.70 \times 10^{-5}$), and NDDs genes related to abnormal brain development (enrichment = 1.39, $P = 1.10 \times 10^{-9}$). Of all psychiatric disorders, ADHD showed the strongest overlap with NDDs ($P = 0.009$), and bipolar the lowest ($P = 0.96$). We show that differences in gene function (synaptic and neurogenesis), properties (pLI score, missense sensitivity) and expression patterns predict involvement of genes in GWAS, NDD or overlapping in both. No enrichment of psychiatric GWAS genes was observed in known copy number variant regions that are associated with a high risk of psychiatric symptoms.

Conclusion: Our findings demonstrate novel insights into partly shared genetic etiologies between polygenic psychiatric

disease and monogenic NDDs, and highlight mechanisms and gene properties that explain overlap seemingly separate monogenic and polygenic disease etiology.

References:.

Grants: ZonMW VENI grant (NWO:09150162010138).

Conflict of Interest: None declared.

P10.058.A PCDH19 is regulated by neurosteroids and their receptors

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Background/Objectives: *Protocadherin 19* (*PCDH19*) Clustering Epilepsy (CE) is a disorder of cellular mosaicism. We and others identified altered steroidogenesis and deregulated Nuclear Hormone Receptor (NHR)-related gene expression in the affected individuals. We reported PCDH19 as a co-regulator of Estrogen Receptor (ER) α activity on gene expression. Little is known if *PCDH19* is regulated by steroids and what is the effect of protocadherins, not just PCDH19, on NHRs Androgen Receptor (AR) and/or Progesterone Receptor (PR). We utilised disease relevant models to investigate (a) the impact of neurosteroids on *PCDH19* gene and (b) functional interaction between delta2 protocadherins and NHRs.

Methods: We treated T47D and embryonic mouse neurons with estradiol (E2), progesterone (P4) or dihydrotestosterone (DHT) separately or in combination and assayed *PCDH19* expression via RT-qPCR, and performed RNA-sequencing on CE and control primary skin fibroblasts. We determined Protocadherin-NHR interactions by co-immunoprecipitation using epitope-tagged protocadherins (PCDH10, PCDH12, PCDH17 and PCDH19) and NHRs (ERα, AR or PR).

Results: *PCDH19* expression was repressed in T47D cells treated with E2, P4 and DHT, and embryonic mouse neurons with E2. Congruent with this, RNA-sequencing of skin fibroblasts of CE girls revealed significant AR upregulation. AR (but not ERα or PR) co-immunoprecipitated with PCDH10, PCDH12, PCDH17 and PCDH19.

Conclusion: We show that NHRs play a major role in the regulation of *PCDH19* expression and provide evidence of an interaction of PCDH19 with AR. This further supports our hypothesis that dysregulation of the steroid hormone receptor pathway contributes to CE pathogenesis.

References:.

Grants: PCDH19 Alliance Grant, USA.

Conflict of Interest: None declared.

P10.059.B Relative prevalence of AD SCA subtypes in the Czech republic – data from the Centre for Hereditary Ataxias in Prague

Emílie Vyhnálková[1], *Martin Vyhnálek*[2], *Alena Zumrová*[2], *Jaroslav Jeřábek*[2], *Jaroslava Paulasová Schwabová*[2], *Michaela Danková*[2], *Lucie Šťovíčková*[2], *Markéta Havlovicová*[1], *Zuzana Musova*[1]

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Background/Objectives: The classification of autosomal dominant spinocerebellar ataxias (AD SCA) is based on findings of specific gene variants. Testing of the most common forms of AD SCA has been introduced since 2000 in the Czech Republic.

Methods: Initial testing for SCA1-3, 6-8, 12, 17, and 28 is used routinely in patients with suspicion of AD SCA. We present a summary of AD SCA subtypes diagnosed in the Centre for Hereditary Ataxias in Motol University Hospital Prague, member of the ERN-RND network.

Results: The centre performed testing in 1700 probands with chronic and slowly progressive ataxia. In total, 162 patients from 111 unrelated families were diagnosed with AD SCA. The most common subtypes are SCA2 (70 patients from 39 families), SCA17 (22 patients from 14 families), and SCA1 (18 patients from 11 families). SCA28 was suspected in 9 patients from 6 families (1 patient with known pathogenic variant, rare variants of unknown significance in others). SCA8, a subtype with reduced penetrance, was detected in 30 patients from 28 families. Other SCA subtypes were rare (SCA6 was found in 9 patients, SCA3 detected in 3 patients, and SCA7 found in one patient). SCA12 has not been detected.

Conclusion: The most common AD SCA subtypes in the Czech Republic are SCA2, SCA17, and SCA1. The estimated prevalence of genetically confirmed AD SCA is 1.5 /100 000. Rare variants revealed in SCA28 gene need further investigation.

References:.

Grants: The authors are members of the European Reference Network for Rare Neurological Diseases, Project ID 739510.

Conflict of Interest: Emílie Vyhnálková Motol University Hospital Prague, Martin Vyhnálek Motol University Hospital Prague, Alena Zumrová Motol University Hospital Prague, Jaroslav Jeřábek Motol University Hospital Prague, Jaroslava Paulasová Schwabová Motol University Hospital Prague, Michaela Danková Motol University Hospital Prague, Lucie Šťovíčková Motol University Hospital Prague, Markéta Havlovicová Motol University Hospital Prague, Zuzana Musova Motol University Hospital Prague.

P10.060.C Brain mosaic mutations as a cause of drug-resistant epilepsy including mesial temporal lobe epilepsy

Katherine Benson[1;2], *Robert Carton*[2], ***Michael Doyle***[2;3;4], *Hugh Kearney*[2;3], *David Henshall*[2], *Gianpiero Cavalleri*[1;2], *Norman Delanty*[1;2;3]

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Background/Objectives: The contribution of somatic mutations in epilepsy is becoming apparent, in particular in malformations of cortical development (MCD). The aim of this study was to evaluate the diagnostic yield of brain somatic mutations in germline epilepsy genes, somatic epilepsy genes, and brain expressed pik3k-mtor genes in drug-resistant epilepsy.

Methods: Surgical epilepsy cases, were clinically phenotyped and sub-divided based on the histopathology of resected brain tissue. Matched blood-derived and brain-derived DNA samples, were sequenced using high coverage (~500X) targeted next-generation sequencing for 229 genes. Variants were identified using MuTect-2 and annotated using ANNNOVAR. Variants were filtered for those which had a variant allele frequency >0.05%, were absent in gnomAD, matched the inferred inheritance pattern and passed manual quality control inspection in Integrative Genome Viewer. Candidate variants were confirmed using high-coverage amplicon sequencing.

Results: 41 patients were successfully sequenced. 9 patients yielded 13 candidate somatic variants. 5 variants in 5 patients were validated using amplicon sequencing (genes: *CBL*, *KCNB1*,

ALG13, MTOR and *FLNA*). The overall diagnostic yield across 41 patients was 12%. Within the hippocampal sclerosis (HS), MCD and non-lesional focal patient sugroups, the yield was 13.6%/20%/0% respectively.

Conclusion: This study has provided new insights into the aetiology of HS, a condition not previously associated with somatic variants. The diagnostic yield in MCD is consistent with previous studies, confirming the importance of *MTOR* and related genes in this condition.

References:.

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Conflict of Interest: None declared.

P10.061.D Joint effects of rare GBA variants and polygenic risk score on Parkinson's disease risk

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Background/Objectives: Rare homozygous mutations within the Glucocerebrosidase (*GBA*) gene were linked to lysosomal storage-disorder, whereas rare heterozygous mutations in *GBA* contribute to an increased risk for Parkinson's disease (PD). We aimed to investigate how a polygenic risk scores (PRS) defined by common GWAS variants and different types of rare *GBA* (GBAr) variants (benign, mild, severe) jointly influence the PD risk.

Methods: We used genotyping and rare variants data from a Luxembourgish PD cohort of 805 controls and 792 patients, and stratified these samples based on PRS percentile and GBAr carrier status. The PRS was calculated genome-wide (gPRS) and for genes involved in lysosomal (lysoPRS) and mitochondrial (mitoPRS) pathways. We compared GBAr carrier frequencies in each PRS category and different groups of PRS risk (low, intermediate, high), and assessed the combined effect of PRS and GBAr carrier status on PD risk.

Results: Overall GBAr carriers were more frequent in the high-risk compared to the low-risk group for gPRS and lysoPRS, but not for mitoPRS. For all types of PRS, severe GBAr were less frequent in the high-risk than in the low-risk PRS group. PD risk was higher among GBAr carriers than non-carriers in low-risk and high-risk for gPRS and lysoPRS. However, for mitoPRS, PD risk was higher among GBAr carriers compared to non-carriers in low but not in the high PRS group.

Conclusion: Our results indicate that the GBAr-conferred risk could be significantly influenced by PRS. Specifically, lysoPRS could give more insight into how lysosomal dysfunction leads to neurodegeneration in PD.

References:.

Grants:.

Conflict of Interest: None declared.

P10.062.A Unique Ataxia oculomotor apraxia 2 (AOA2, OMIM #606002) case in Israel: Expanding the phenotypic spectrum, highlighting novel variants and possible identification of a poison exon

Penina Ponger[1], *Alina Kurlop*[1], *Hofit Gadot*[1], *adi mory*[1], *Yael Winai*[1], *Nir Giladi*[1], *Tatyana Gurevich*[1], *Daphna Marom*[1], *yuval yaron*[1], *Hagit Baris Feldman*[1]

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Background/Objectives: SCAN2 is a rare progressive adolescent-onset cerebellar ataxia characterised by elevated serum alpha-fetoprotein (AFP) and oculomotor apraxia. SCAN2 is caused by bi-allelic variants in SETX, encoding senataxin, involved in DNA repair and RNA maturation.

Methods: WES trio was followed by SETX RNA analysis, extracted from peripheral blood mononuclear cells and converted to cDNA to enable splicing analysis. qRT-PCR was performed to assess overall and mutant SETX mRNA expression.

Results: Proband is of non-consanguineous Persian Jewish family. Phenotype includes early childhood clumsiness, functional deficits in late adolescence, ocular apraxia, dysarthria, appendicular and truncal ataxia and cerebellar atrophy, especially of the vermis and anterior lobes in MRI. Serum AFP levels are within normal range. WES trio of proband and parents revealed two novel *SETX* variants in trans, maternal nonsense variant in exon 6 (c.568C>T; p.Gln190*), and paternal deep intronic variant in intron 12 (c.5549-107A>G). Intronic variant analysis and *SETX* mRNA expression revealed activation of a cryptic exon. This insertion introduces a premature stop codon (p.Met1850Lysfs*18). qRT-PCR analysis revealed that the intronic c.5549-107A>G variant induces aberrant splicing and leads to 20-30 times higher levels of cryptic exon activation compared to WT samples (mother and healthy controls). In combination with a second deleterious allele, this variant leads to low levels of *SETX* mRNA and disease manifestations.

Conclusion: Our case expands the phenotypic spectrum of SCAN2 and emphasizes the need for tailored molecular work-up to ensure timely diagnosis. Deep-intronic variant analysis reveals a previously undescribed poison exon in the SETX gene which may contribute to therapy development.

References:.

Grants:.

Conflict of Interest: Penina Ponger: None declared, Alina Kurlop: None declared, Hofit Gadot: None declared, adi mory: None declared, Yael Winai: None declared, Nir Giladi: None declared, Tatyana Gurevich: None declared, Daphna Marom: None declared, yuval yaron Geenox, Hagit Baris Feldman: None declared.

P10.064.C CANVAS: molecular analysis, expansion size determination, and clinical features in Italian patients

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Background/Objectives: CANVAS is an adult-onset, slowly-progressive neurodegenerative disorder, whose phenotypic spectrum is expanding, ranging from late-onset ataxia to sensory neuropathy, caused by a biallelic intronic AAGGG expansion in the *RFC1* gene. Expansions with alternative sequence conformations have been identified whose pathogenic role remains to be established.

Objectives: 1) To screen Italian patients with full or partial CANVAS phenotype (n = 223) or late-onset (n = 216) ataxia for *RFC1* expansion; 2) to assess the frequency of *RFC1* expansion carrier in a control population; 3)to sequence the expanded alleles to determine their repeat structure and role in disease.

Methods: The *RFC1* locus was analysed by a PCR flanking the repeat, followed by fluorescent repeat-primed PCRs (Cortese, 2019). A long-read sequencing approach has been set up for the sequencing of the expanded allele.

Results: Biallelic *RFC1* expansion was identified in 32.3% (72/223) probands with a CANVAS spectrum phenotype, and in 12.5% (27/216) of patients with ataxia. Heterozygous carrier rate was 10.6% in patients (16/151) and 10% (36/360) in controls. Approx. 5% of alleles showed complex repeat conformations with uncertain pathogenicity.

Conclusion: Biallelic *RFC1* expansion accounts for a large proportion of ataxia phenotypes in Italian patients (12-32%), and should always be considered in the diagnostic algorithm of patients with sporadic ataxia, ranking first in late-onset cerebellar and sensory ataxia. Given the high carrier rate, *RFC1* expansion might be the most common cause of ataxia in Italian patients. Sequencing data confirm the complex architecture of the expanded repeat which may have crucial implications for diagnosis and counseling.

References: Cortese A (2019) Nature Genetics 51:649-658.

Grants: FRRB-CP-20/2018.

Conflict of Interest: None declared.

P10.065.D A newly implemented NGS-based methods to detect GBA variants in patients with Parkinson disease

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Background/Objectives: Heterozygous variants in the GBA gene, encoding for the lysosomal enzyme β-glucocerebrosidase, are the commonest genetic risk factor for Parkinson disease (PD), accounting for 5-15% of all PD cases. Sequencing of the whole GBA coding region (11 exons) is a cumbersome task, both employing conventional techniques such as Sanger sequencing as well as more innovative strategies such as next-generation-sequencing (NGS). In particular, the high degree of homology (96-98%) between GBA and its pseudogene GBAP1 often leads to recombination events that eventually produce complex alleles which are misaligned through the standard NGS pipeline. Here we tested an implemented NGS-based technology on a selected pool of 22 patients, including 15 patients known to carry different GBA variants (identified through Sanger sequencing) and 7 controls.

Methods: The NGS experiment was designed starting from a long-range PCR to amplify a unique 12kb amplicon encompassing GBA. This was used as template to create libraries, which were amplified using Nextera technology and then run on an Illumina MiSeq instrument. In parallel to standard bioinformatic analysis, we employed a custom pipeline, forcing the alignment of reads against the genomic coordinates of GBA gene only.

Results: All GBA variants were correctly called and identified using this approach; furthermore, comparing (*.bam) files obtained with standard vs forced alignment, the latter showed a significant increase in read depth and mapping quality.

Conclusion: The proposed NGS-based approach appears a reliable and valid alternative for GBA sequencing, holding promise to increase the speed and the accuracy of variant detection compared to conventional strategies.

References:.

Grants:.

Conflict of Interest: None declared.

P10.066.A Combining extended genealogies and genotypes for a unique view of the genetic architecture of major depressive disorder

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Background/Objectives: State-of-the-field complex disorder genetics leverages population-scale biobanks with broad demographic, health, survey, and genetic data. This presents an opportunity for Major Depressive Disorder (MDD), where genetic predictors underperform, clinical heterogeneity is etiologically enigmatic, and missing heritability persists. Combining genotypes, family history, and adjacent phenotypes in large data can advance each aim, but requires new approaches.

Methods: We introduce Pearson-Aitken framework for Family Genetic Risk Scores (PA-FGRS) to estimate individual liability scores from uniquely complex pedigrees of individuals in the iPSYCH case-cohort. We compute multiple psychiatric PA-FGRS and polygenic scores (PGS) for N = 37,555 MDD cases and 49,303 controls. We test discriminative utility of PA-FGRS and PGS with regression, compare GWAS on diagnoses and PA-FGRS, and compare multivariate PA-FGRS+PGS profiles of MDD cases with different clinical outcomes using multinomial regression.

Results: We construct genealogies including >2,000,000 relatives of iPSYCH individuals and show PA-FGRS has important advantages in simulations. Combining PA-FGRS with PGS explains as much additional variance in MDD as PGS alone (~3%). Clinical heterogeneity is widely associated with variability in genetic profiles, e.g., recurrent MDD increased PA-FGRS and PGS for MDD and bipolar. In iPSYCH, GWAS on PA-FGRS liabilities identified 3 more loci than binary diagnoses.

Conclusion: Approaches that integrate multiple data types in population biobanks give important new perspectives on genetic architectures of complex disorders like MDD. Clinical and research aims could be more data integrative and better attend to heterogeneity.

References:.

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Conflict of Interest: None declared.

P10.067.B Genetic variability of inflammation and oxidative stress in Alzheimer's disease biomarkers

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Slovenia; [2]University Medical Centre Ljubljana, Department of Neurology, Ljubljana, Slovenia.

Background/Objectives: Neuroinflammation and oxidative stress are important processes involved in Alzheimer's disease (AD). Numerous risk factors, including complex genetic background, can affect the immunological processes of the brain and further promote disease progression from mild cognitive impairment (MCI). Our aim was to evaluate the association of common polymorphisms with cerebrospinal fluid (CSF) biomarkers and cognitive test results in patients with dementia and AD.

Methods: Our study included 54 AD patients, 14 MCI patients with pathological CSF biomarker levels and 20 MCI patients with normal CSF biomarker levels. Isolated DNA from blood was genotyped for polymorphisms in *SOD2*, *CAT*, *GPX1*, *IL1B*, *MIR146A*, *IL6*, *TNF*, *CARD8*, *NLRP3*, *GSTP1*, *NOS1*, *KEAP1* and *NFE2L2* using competitive allele-specific PCR. Association of polymorphisms with CSF biomarker levels and cognitive tests was evaluated using nonparametric tests.

Results: In the whole cohort, carriers of two polymorphic *IL1B* rs16944 alleles had higher CSF $A\beta_{1-42}$ ($p = 0.025$), while carriers of at least one polymorphic *NFE2L2* rs35652124 allele had lower CSF $A\beta_{1-42}$ ($p = 0.040$) levels. In AD group, only *IL1B* rs16944 remained significant ($p = 0.029$). Significant associations with mini-mental state exam (MMSE) were observed for *CAT* rs1001179 ($p = 0.022$), *GSTP1* rs1138272 ($p = 0.005$), *KEAP1* rs1048290 and rs9676881 (both $p = 0.019$), *NFE2L2* rs35652124 ($p = 0.030$). In AD group, significant association of *IL1B* rs1071676 ($p = 0.004$), *KEAP1* rs1048290 and rs9676881 (both $p = 0.035$) with MMSE were observed.

Conclusion: Polymorphisms in inflammation and antioxidant genes may be associated with CSF biomarkers and cognitive test score and could serve as additional biomarkers contributing to early diagnosis of cognitive decline.

References:.

Grants: ARRS P1-0170.

Conflict of Interest: None declared.

P10.068.C Genetic modulators of GBA expressivity in synucleinopathies

Giada Melistaccio[1], *letizia straniero*[1], *valeria rimoldi*[1], *Alessio Di Fonzo*[2;3], *Giulia Solda*[1;4], *anna zecchinelli*[5], *stefano duga*[1;4], *gianni pezzoli*[6], *Rosanna Asselta*[1;4]

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Background/Objectives: Heterozygous mutations in GBA are associated with an increased risk of the synucleinopathies Parkinson's disease (PD) and Lewy body dementia (LBD), which encompasses Parkinson's disease dementia and dementia with Lewy bodies (DLB). As it is not clear why individuals with the same GBA variant could eventually develop PD or LBD we focused on genetic modulators of GBA mutations expressivity. To this end, we: compared the burden of rare deleterious variants (minor allele frequency <1%) in dementia-related genes; assessed the role of a second GBA variant; and evaluated the impact of the APOE-ε4 haplotype, among LBD cases, PD patients, and healthy controls all carriers of at least one GBA mutation.

Methods: We studied a cohort of 203 healthy individuals, 196 PD and 139 LBD patients heterozygous for at least a GBA mutation using an NGS custom panel including 10 dementia-related genes. The frequency of APOE haplotypes was assessed through a TaqMan™ Genotyping Assay.

Results: We showed a significantly increased burden of rare mutations in dementia-related genes in the DLB cohort compared to healthy controls (12.82% vs 3.86%, $P = 0.02$). We also found that a second GBA variant is more frequent in LBD vs PD patients (9.56% vs 3.96%, $P = 0.04$), and that the APOE-ε4 allele is significantly enriched in the DLB cohort compared to healthy controls (17.57% vs 8.13%, $P = 0.018$).

Conclusion: Our data suggest that there is a correlation between the analyzed risk factors and the development of a dementia phenotype in GBA mutation carriers.

References:.

Grants: PRIN Grant no:2017228L3J.

Conflict of Interest: None declared.

P10.069.D Two de novo pathogenic variants in different genes identified by a whole exome sequencing TRIO approach explain and complement the phenotype in a patient with syndromic intellectual disability and autism

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Background/Objectives: Five-years-old boy presented with a complex phenotype, including intellectual disability, autism, and attention deficit spectrum disorder with dysmorphic features as microcephaly and hypertelorism. CGH-array, karyotype and X-fragile tests were normal. Whole exome sequencing (WES) TRIO diagnostic was performed to elucidate the genetic cause underlying his phenotype.

Methods: WES for proband and progenitors were performed from DNA using Nextera DNA Flex Pre-Enrichment Library Prep and Illumina Exome Panel and sequenced on the NovaSeq 6000 System (Illumina). Raw data were processed by the Igenomix in-house bioinformatics pipeline.

Detected variants were prioritised according to their possible pathogenicity following the recommendations of the American College of Medical Genetics using the in-house developed software GPDxViewer.

Results: Two de novo pathogenic variants were found in the proband: c.1139_1140delTT; p.(Phe380fs) in the *CUL3* gene and c.1001+2T>A in the *NARS1* gene. *CUL3* is associated with Neurodevelopmental disorder with or without autism or seizures (MIM#619239) and *NARS1* with Neurodevelopmental disorder with microcephaly, impaired language, epilepsy, and gait abnormalities (MIM#619092), both with autosomal dominant inheritance pattern.

Conclusion: Each variant contributes to different parts of the complex phenotype present in the proband and as a result, the patient has a combination of two different disorders.

These results also highlight the importance of being thorough in the variant analysis to avoid missing those variants that could complement or complete the explanation of the phenotype as a whole.

In disorders with such a heterogeneous genetic basis as autism and intellectual disability, WES-TRIO emerges as the diagnostic approach with the highest efficiency.

References:.

Grants:.
Conflict of Interest: None declared.

P10.070.A Novel mutation in ENG causes autosomal dominant isolated cerebral arteriovenous malformation at young age

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Background/Objectives: Cerebral arteriovenous malformation (CAVM) constitute abnormal vessels shunting blood from the arterial to the venous circulation, resulting in high flow lesions prone to rupture. CAVMs account for ~2% of all haemorrhagic strokes. Most CAVM cases are sporadic, although few are due to autosomal dominant inheritance of a genetic mutation, most commonly in the context of Hereditary Hemorrhagic Telangiectasia (HHT), an autosomal dominant genetic disorder characterized by epistaxis, telangiectasias, and multiorgan vascular dysplasia. We studied a case of father and daughter with isolated CAVM ruptures at young age, aiming to identify mutations that cause CAVM.

Methods: Magnetic resonance angiography, whole exome sequencing and validation through PCR and Sanger sequencing.

Results: A father and daughter presented with isolated CAVM, with ruptures at an early age. Both were examined clinically and did not present telangiectasias. A frameshift mutation in ENG (endoglin) chr9:127816006 AG>A HET. (hg38) was found in both affected father and daughter.

Conclusion: Mutations in ENG have been reported to cause HHT1, a subtype of HHT with higher risk for developing CAVM than other subtypes of the disease. This new mutation is unique in causing CAVM at a very young age and without skin presentation of telangiectasias. Thus, genetic testing is important for diagnosis of HHT in families with history of isolated CAVM ruptures.

References: 1) C. Stapf et. al. (2002). Cerebrovascular Diseases. 2) Abdalla, S. A., & Letarte, M. (2006). Journal of Medical Genetics.

Grants: The Morris Kahn foundation.
Conflict of Interest: None declared.

P10.071.B Genetic diagnoses for complex neurodevelopmental disorders in the international NeuroWES-Macedonia project

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Background/Objectives: Neurodevelopmental disorders (NDD) are genetically and clinically heterogeneous diseases that include Autism Spectrum Disorders (ASD), intellectual disability (ID), and epilepsy.

Methods: To improve Whole Exome Sequencing (WES) diagnostics in NDD, we exploited detailed clinical characterization, X-chromosome inactivation (XCI) profiles, epigenetic signatures and novel in vitro functional models.

Results: We collected and deeply phenotyped 203 NDD cases from Macedonia with ASD (69/203), ASD/ID (31/203), ID (42/203), or syndromic NDD (61/203). We solved 26% (52/203), with the highest diagnostic rate in syndromic NDD (31/61; 51%), and the lowest in ASD cases (5/69; 7%). Notably, we found two cases with a dual molecular diagnosis, and one family with two different de novo variants in two similarly affected brothers (*TRIP12*:L1044Ffs*3, and *FBN*:A1728V).

In three unrelated boys, whose mothers each had a 100% skewed XCI, we found variants in novel candidate genes, previously overlooked by WES analysis (*ZMYM3*:R441Q, *PDZD4*:K730N, and *AMOT*: G343R). Because chromatin remodeling genes were frequently mutated (14/52; 27%), we exploited episignatures, and confirmed the pathogenic role of variants in *EZH2, HIST1H1, PQBP1*. We found 18 strong novel disease-gene candidates (8%), which included *PHLPP1, AARSD1, UBE2I* and *CASKIN1*. The *PHLPP1*-mutated cases showed functional alteration of the AKT-mTOR pathway using dental pulp stem cells (SHED); the variants in *AARSD1*, involved in aminoacyl-tRNA editing, caused growth impairment in a mutagenized yeast model.

Conclusion: The combination of deep phenotyping, XCI analysis and episignature profiling not only improved the WES diagnostic yield but identified several potentially novel NDD genes.

References:.
Grants:.
Conflict of Interest: None declared.

P10.072.C Novel de-novo heterozygous H3-3A mutation causes neuro-developmental disorder

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Background/Objectives: A non-consanguineous Jewish Moroccan family presented with a phenotype of a neuro-developmental disorder (NDD) of global developmental delay, low muscle tone and epilepsy, affecting an individual at the latest generation.

Methods: Whole exome sequencing data of the affected individual and healthy parents (Trio) were analyzed and filtered for known benign variants using our in-house databases along with open access databases (1000 genomes, NHLBI ESP, ExAC etc.).

Results: No mutations or likely-pathogenic variants were found in genes previously associated with NDDs. A novel de-novo heterozygous p.Q126R missense mutation in H3.3 Histone A (H3-3A) was identified. The mutation was verified by Sanger sequencing and was found to fully segregate in the affected kindred as expected for a dominant effect of a de-novo mutation. In-silico analysis of the p.Q126R variant showed putative multiple effects on the mature protein. Proliferation assay (XTT) of patient-derived fibroblasts versus matched controls indicated decreased proliferation of cells harboring the mutation.

Conclusion: Variants in H3-3A were mainly associated with cancer and only recent studies have shown H3-3A as a novel contributor to NDDs1. Our data suggest the de-novo p.Q126R mutation is the likely cause of the aforementioned NDD.

References: 1 - Bryant L et al. Histone H3.3 beyond cancer: Germline mutations in Histone 3 Family 3A and 3B cause a previously unidentified neurodegenerative disorder in 46 patients. Sci Adv. 2020 Dec 2;6(49): eabc9207.

Grants:.

Conflict of Interest: None declared.

P10.073.D Interaction between polygenic risk score and rare variants in amyotrophic lateral sclerosis

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Background/Objectives: The genetic background of amyotrophic lateral sclerosis (ALS) consists of common small-effect alleles and rare penetrant alleles. Here, we tested whether risk for ALS was affected by high polygenic risk score (PRS), carrying rare ALS-associated variants, or both.

Methods: 330 ALS cases and 349 unaffected controls from Québec, Canada were included. SNP-chip genotyping was generated for all samples, and targeted or whole exome sequencing was used to screen 26 ALS genes. We used ALS GWAS summary statistics[1] to estimate PRS for ALS. From sequencing, only protein-altering variants rarer than 0.1% were included. Individuals were subdivided into cases and controls, and rare variant carriers or non-carriers. Logistic regression was used to assess association of ALS risk with PRS and rare variant status.

Results: 4,309 SNPs were included in the PRS, which explained 1.16% of phenotypic variation. The upper PRS quartile showed an odds ratio of 2.05 for ALS risk. Risk for ALS was significantly associated with PRS and rare variants independently ($p = 2.58 \times 10^{-5}$ and 4.90×10^{-3}, respectively), but the interaction was not significant. PRS only differed significantly between non-carrier cases and non-carrier controls ($p = 0.0087$).

Conclusion: ALS PRS was informative only between cases and controls not carrying a rare variant, suggesting that rare variants in ALS genes have a substantial effect on disease risk. Rather than modifying the penetrance of rare risk variants, ALS PRS differentiates cases and controls only in the absence of these variants.

References: 1. van Rheenen. 2016. Nat Genet.

Grants: Canadian Institutes for Health Research – FRN 159279.

Conflict of Interest: None declared.

P10.074.A GIPR and GLP1R genetic polymorphisms and alcohol dependence

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Background/Objectives: Alcohol dependence affects approximately 4% of the general population. It leads to decreased quality of life for patients and their families and present a huge burden to society. Gastric inhibitory polypeptide receptor (GIPR) and glucagon-like peptide one receptor (GLP1R) are expressed in the reward-related brain areas and modulate dopamine levels and glutamatergic neurotransmission, which results in behavioral changes. The present study investigated the association of common genetic polymorphisms in *GLP1R* and *GIPR* with alcohol dependence.

Methods: Three groups of male Slovenian participants have been included: 88 hospitalized alcohol-dependent patients, 96 abstinent alcohol-dependent patients, and 94 healthy blood donors and were genotyped for *GLP1R* rs10305420 and rs6923761 and *GIPR* rs1800437. In the statistical analysis, Kruskal-Wall and Mann-Whitney tests were used for additive and dominant genetic models.

Results: Polymorphic *GIPR* rs1800437 GC+CC genotypes were more frequent in hospitalized alcohol-dependent than controls ($P = 0.020$). The association remained statistically significant after adjustment for age, education, smoking, environment, and partnership ($P = 0.040$). Similar associations were observed when comparing alcohol-dependent patients with controls ($P = 0.047$), but the significance was lost after adjustment ($P = 0.086$). Regarding psychosymptomatology, rs1800437 GC+CC genotypes were associated with Yale-Brown Obsessive Compulsory Scale (YBOCS) obsession ($P = 0.045$), YBOCS compulsion ($P = 0.032$), and Buss-Durkee Hostility Inventory (BDHI) ($P = 0.046$) scores. No significant associations were observed for *GLP1R* rs10305420 and rs6923761.

Conclusion: Our data indicate that *GIPR* rs1800437 may play some role in susceptibility to alcohol dependence, and obsession, compulsion, aggression, and hostility symptoms. However, further studies with larger cohorts are needed to confirm these preliminary findings.

References:.

Grants: ARRS P1-0170.

Conflict of Interest: Evangelia Eirini Tsermpini: None declared, Anja Plemenitaš Ilješ: None declared, Katja Goricar: None declared, Vita Dolzan ARRS P1-0170.

P10.076.C Expanded phenotype of Snijders Blok-Campeau syndrome related to CHD3 pathogenic variant with precocious puberty and pituitary microadenoma

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Background/Objectives: Snijders Blok-Campeau syndrome is an autosomal dominant genetic disorder often associated with missense mutations in the CHD3 gene that affects chromatin remodelling in human cells. This syndrome is also

characterized by a distinct neurodevelopmental phenotype. Here, we present a case of a young female diagnosed with Snijders-Block Campeau syndrome with previously unreported clinical features, including precocious puberty and pituitary microadenoma.

Methods: The patient was investigated by a multidisciplinary team, she had extensive genetic testing, including CGH, metabolic work-up and targeted exome sequencing.

Results: Sequencing data revealed a heterozygous de novo likely pathogenic variant in the CHD3 gene and no other notable structural nor punctual variants. This case demonstrates a unique clinical manifestation of Snijders Blok-Campeau syndrome related to a de novo likely pathogenic variant in the CHD3 gene: NM_001005271.2: c.5609G>A.

Conclusion: We believe that central precocious puberty can be part of the clinical manifestation of Snijders Blok-Campeau syndrome and this finding can expand the phenotypic spectrum of this disorder.

References: Snijders Blok, L., Rousseau, J., Twist, J., Ehresmann, S., Takaku, M., Venselaar, H., Rodan, L. H., Nowak, C. B., Douglas, J., Swoboda, K. J., Steeves, M. A., Sahai, I., Stumpel, C. T., Stegmann, A. P., Wheeler, P., Willing, M., Fiala, E., Kochhar, A., Gibson, W. T., … Campeau, P. M. (2018). CHD3 helicase domain mutations cause a neurodevelopmental syndrome with macrocephaly and impaired speech and language. Nature Communications, 9(1). https://doi.org/10.1038/s41467-018-06014-6.

Grants: New Brunswick health research foundation.

Conflict of Interest: radu parascan: None declared, eric allain: None declared, philippe pierre robichaud: None declared, BEN AMOR MOUNA NBHRF clinical research scholarship.

P10.078.A genetic testing in turkish pituitary adenoma patients: preliminary findings

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Background/Objectives: Pituitary adenomas are the third most common intracranial tumors after meningioma and glioma. Its incidence was determined as 15%. There is no familial predisposition in 95% of the patients, while the remaining 5% is familial. Although germline mutations of genes such as *MEN1, MEN4, AIP, NF1, RET, CDKN1B, SDHs* can be detected, these mutations are not found in most of the patients.

Methods: DNA was isolated from peripheral blood after obtaining informed consent from patients. AIP gene sequencing (Illumina Miseq) was performed to eight patients and customized gene panel (50 genes-Illumina NovaSeq) was performed to 32 patients. Variants with MAF <%0,1 were filtered and retained variants were searched in Clinvar. ACMG classification was used to determine the pathogenicity.

Results: Fourty patients (19 women and 21 men) with age range between 15-71 were included in our study. Five patients had microadenoma and 35 patients had macroadenoma. The most common pathological subtype was GHoma (14/37). Two patients had atypical adenomas with high Ki67 and p53 levels. In four patients pathogenic variants were detected in *MEN1, AIP, MLH1* and *MUTYH* genes. In one patient who also had pheochromocytoma, variant of unknown significance (VUS) was detected in SDHD gene. In another patient, missense VUS was detected in AIP gene.

Conclusion: We detected pathogenic variants in 10% of our patients, contributing to the follow-up and treatment of both patients and their families which implicate the importance of genetic testing in pituitary adenoma patients.

References: Aflorei ED, Korbonits M. Epidemiology and etiopathogenesis of pituitary adenomas. J Neurooncol.2014;117(3):379-394. https://doi.org/10.1007/s11060-013-1354-5.

Grants:.

Conflict of Interest: None declared.

P10.079.B Identification of molecular signatures and pathways involved in Rett syndrome-spectrum disorders using a multi-omics approach

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Background/Objectives: Rett syndrome (RTT) is a severe neurodevelopmental disorder whose classical form is mainly caused by mutations in the *MECP2* gene. There are also atypical forms, generally caused by mutations in *CDKL5* or *FOXG1*, and RTT-like forms, with considerable clinical overlap with typical RTT but caused by mutations in other genes. Since the pathomechanism by which the dysfunction of these genes causes disease is not yet fully understood, there is no treatment available. Therefore, the aim of this study is to characterize the molecular alterations in RTT-spectrum patients to identify common and specific molecular signatures and discover pathways involved in the pathogenesis of these disorders.

Methods: Primary fibroblast cell cultures were obtained from 33 RTT-spectrum patients (21 with typical RTT, 6 with atypical RTT and 6 with RTT-like) and 15 healthy controls. RNA and protein were isolated and genome-wide differential expression was evaluated by RNAseq and quantitative TMT proteomics.

Results: Differential expression analysis revealed common molecular signatures in RTT-spectrum patients and enrichment analysis identified altered pathways associated with these signatures. Spliceosome components and genes involved in oxidative phosphorylation were significantly enriched within altered genes in all RTT-spectrum patients. Moreover, we found specific alterations that distinguish patients with mutations in certain genes from other RTT-spectrum patients with overlapping phenotypes.

Conclusion: Integrative multi-omics contribute to the understanding of the pathomechanisms behind RTT-spectrum disorders and could uncover new biomarkers to monitor disease status as well as potential therapeutic targets for treating these disorders.

References:.

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Conflict of Interest: None declared.

P10.081.D Digenic inheritance of STUB1 variants and TBP polyglutamine expansions solves the enigma of SCA17 and SCA48 incomplete penetrance

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Torino, Dipartimento di Scienze Mediche, Turin, Italy; [3]A.O.U. Città della Salute e della Scienza, Unità di Genetica Medica, Turin, Italy.

Background/Objectives: Spinocerebellar ataxia type 17 (SCA17), characterized by cerebellar-cognitive-behavioural features, is caused by a CAG/CAA repeat expansion in the *TBP* gene. *TBP* alleles with >49 CAG/CAA are fully penetrant. By contrast, intermediate *TBP* alleles with 41-49 CAG/CAA ($TBP_{41\text{-}49}$), are characterized by reduced penetrance, since ~50% of carriers in SCA17 families are healthy and ~1% of subjects in the general population carry $TBP_{41\text{-}47}$ alleles. Objective: To unravel the genetic factors underlying missing heritability in SCA17.

Methods: Using NGS approaches, we investigated 40 $TBP_{41\text{-}54}$ index patients, their affected ($n = 55$) and unaffected ($n = 51$) relatives, and a cohort of ataxia patients ($n = 292$).

Results: All but one (30/31) index cases with intermediate $TBP_{41\text{-}46}$ alleles carried a heterozygous pathogenic variant in the *STUB1* gene encoding the chaperone-associated E3 ubiquitin ligase CHIP. No *STUB1* variant was found in patients carrying $TBP_{47\text{-}54}$ alleles. *TBP* expansions and *STUB1* variants cosegregate in all affected family members, while the presence of either one alone was never associated with disease. *STUB1* pathogenic variants were previously associated with the autosomal recessive spinocerebellar ataxia SCAR16 and the autosomal dominant spinocerebellar ataxia SCA48.

Conclusion: Our data reveal an unexpected interaction between *STUB1* and *TBP* in the pathogenesis of SCA17 and raise questions on the existence of SCA48 as a monogenic disease, with crucial implications for diagnosis and counselling. They provide a convincing explanation for the incomplete penetrance of intermediate *TBP* alleles and demonstrate a dual inheritance pattern for SCA17, which is a monogenic dominant disorder for $TBP_{\geq 47}$ alleles and a digenic *TBP*/*STUB1* disease (SCA17-DI) for intermediate expansions.

References:.

Grants: FRRB_CP2_20_2018.

Conflict of Interest: None declared.

P10.083.B Loss of C-terminal Mediator Complex subunit-11 impairs fetal brain development and cause severely progressive neurodegeneration

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Background/Objectives: The Mediator (MED) is an evolutionarily conserved multi-subunit protein complex that modulates the activity of several transcription factors and different critical components of the overall transcriptional machinery. Genetic defects of different MED subunits have been implicated in severe brain developmental disorders with microcephaly and neurodegeneration.

Methods: Exome or genome sequencing were performed in five unrelated families identified via different research networks and Matchmaker Exchanges. Deep clinical and brain imaging evaluations were performed by pediatric neurologists and neuroradiologists. The functional impact of the candidate variant on both MED11 RNA and protein was assessed by RT-PCR and Western Blotting using patient-derived fibroblast cell lines and by computational approaches.

Results: A recurrent, segregating homozygous variant in MED11 (c.325 C>T; p.Arg109Ter) was identified in all eight affected children. The variant results in a premature stop codon and a putative protein lacking the last nine residues of MED11 C-terminal. The phenotype was characterized by microcephaly, profound neurodevelopmental delay, exaggerated startle reaction, refractory myoclonic epilepsy and diffuse severe brain degeneration with premature death. Semi-quantitative RT-PCR and Western blot revealed levels of protein and transcript similar to healthy carriers and age- and sex-matched controls, suggesting the variant does not cause non-sense mediated decay (NMD) but instead disrupts the C-terminal domain of MED11.

Conclusion: Loss of the C-terminal of MED subunit 11 may affect its binding efficiency to other MED subunits, thus impacting the complex stability and function and leading to a rare and severe prenatal-onset developmental and degenerative neurological condition.

References:.

Grants: Wellcome Trust (WT093205MA and WT104033AIA).

Conflict of Interest: None declared.

P10.084.C The genetics of manic symptoms assessed by the Mood Disorder Questionnaire

Jessica Mundy[1], *Christopher Hübel*[1], *jonathan coleman*[1], *Brett Adey*[1], *Gursharan Kalsi*[1], *Henry Rogers*[1], *Lee Sang Hyuck*[1], *molly*

davies[1], Thalia Eley[1], Robin Murray[2], Evangelos Vassos[1], Gerome Breen[1]

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Background/Objectives: Mania affects ~1% of the population. Together with depression, it is characteristic of bipolar disorder, a severe psychiatric disorder closely related to other disorders, including depression and schizophrenia [1-2]. The Mood Disorder Questionnaire (MDQ) is used to screen for manic symptoms in at-risk groups and the general population [3]. We investigated the heritability of mania and calculated genetic correlations with other traits.

Methods: We created a continuous mania trait using MDQ data from the Genetic Links to Anxiety and Depression Study and healthy volunteers in the NIHR BioResource (n = 26,450) in the UK. We performed a genome-wide association study of mania and calculated genetic correlations using Linkage Disequilibrium Score Regression, applying multiple testing correction.

Results: The continuous mania trait had a SNP-based heritability of 0.06 (SE = 0.02). Mania showed a significant, positive genetic correlation with major depressive disorder ($r_g = 0.45$, SE = 0.10, $p = 4.5x10^{-6}$), attention deficit hyperactivity disorder ($r_g = 0.54$, SE = 0.11, $p = 8.8x10^{-7}$), posttraumatic stress disorder ($r_g = 0.61$, SE = 0.12, $p = 8.5x10^{-7}$), alcohol dependence ($r_g = 0.48$, SE = 0.12, $p = 8.6x10^{-5}$), and number of sexual partners ($r_g = 0.26$, SE = 0.06, $p = 1.6x10^{-5}$). Mania showed a significant, negative genetic correlation with educational attainment ($r_g = -0.44$, SE = 0.06, $p = 3.41x10^{-12}$). However, mania did not show significant genetic correlations with bipolar disorder or its subtypes.

Conclusion: Caution should be paid when assessing bipolar disorder quantitative traits via the MDQ in epidemiological studies. When applied to the general population, the MDQ may capture symptoms of general distress rather than mania.

References: [1] Daly (1997).

[2] Mullins et al. (2021).

[3] Hirschfeld et al. (2000).

Grants: Funded by the NIHR Maudsley Biomedical Research Centre and the Lord Leverhulme Charitable Grant.

Conflict of Interest: Jessica Mundy: None declared, Christopher Hübel: None declared, jonathan coleman: None declared, Brett Adey: None declared, Gursharan Kalsi: None declared, Henry Rogers: None declared, Lee Sang Hyuck: None declared, molly davies: None declared, Thalia Eley: None declared, Robin Murray: None declared, Evangelos Vassos: None declared, Gerome Breen Prof Breen has received honoraria, research or conference grants and consulting fees from Illumina, Otsuka, and COMPASS Pathfinder Ltd., Prof Breen has received honoraria, research or conference grants and consulting fees from Illumina, Otsuka, and COMPASS Pathfinder Ltd., Prof Breen has received honoraria, research or conference grants and consulting fees from Illumina, Otsuka, and COMPASS Pathfinder Ltd.

P10.085.D Neuropsychiatric risk in children with intellectual disability of genetic origin: IMAGINE - The UK National Cohort Study

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Background/Objectives: Children with intellectual disability (ID) frequently have multiple co-morbid neuropsychiatric conditions and poor physical health. Genomic testing is increasingly recommended as a first-line investigation for these children. We aimed to determine the impact of genomics, inheritance and socioeconomic deprivation on neuropsychiatric risk in children with intellectual disability of genetic origin as compared to the general population.

Methods: IMAGINE is a prospective study using online mental health and medical assessments in a cohort of 2770 children with ID and pathogenic genomic variants, identified by the UK's National Health Service.

Results: Assessments completed on 2397 young people with ID (4-19 years, M 9.2, SD 3.9) with a rare pathogenic genomic variant. 1339 (55.9%) were male. 1771 (73.9%) of participants had a pathogenic copy number variant (CNV), 626 (26.1%) a pathogenic single nucleotide variant (SNV). Participants were representative of the socioeconomic spectrum of the UK general population.

The relative risk of co-occurring neuropsychiatric diagnoses, compared with the UK national population, was high: Autism Spectrum Disorder 29.2 (95% CI 23.9 to 36.5), Attention Deficit Hyperactivity Disorder 13.5 (95% CI 11.1 to 16.3). In children with a CNV, those with a familial variant tended to live in more socioeconomically deprived areas. Both inheritance and socioeconomic deprivation contributed to neuropsychiatric risk in those with a CNV.

Conclusion: Children with genomic variants and ID are at a greatly enhanced risk of neuropsychiatric difficulties. CNV variant inheritance and socioeconomic deprivation also contribute to the risk.

References:.

Grants: UK Medical Research Council and Medical Research Foundation.

Conflict of Interest: Jeanne Wolstencroft: None declared, Marianne Van Den Bree Grant from Takeda Pharmaceuticals, Jeremy Hall Grant from Takeda Pharmaceuticals, Michael Owen Grant from Takeda Pharmaceuticals, David Skuse: None declared, Imagine Consortium: None declared, F. Lucy Raymond: None declared.

P10.086.A Single-cell deconvolution of cerebellar cortex uncovers increased proportions of astrocytes and granule cell loss in essential tremor

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Background/Objectives: Essential tremor (ET) is one of the most common movement disorders, affecting 1% of the worldwide population. Not much is known about its pathophysiology. Some histopathological studies of ET cerebellum have noted reduced numbers of Purkinje cells (PCs), but this finding remains controversial [1]. We sought to replicate these findings using deconvolution of bulk-RNA sequencing (bulk RNA-seq) data to compare cell type proportions between ET and healthy control cerebellar cortices.

Methods: MuSiC was used to deconvolute bulk RNA-seq of 16 ET and 16 control cerebellar cortex using cerebellar single-cell data [2]. A permutation test (100k iterations without replacement) was used to test cell type proportions between ET and control samples.

Results: Most cell types including both PC subtypes did not differ between ET and control samples. Astrocytes proportions were significantly increased in ET samples compared to controls (Beta = 0.0382, q-val = 0.0196). Granule cells were significantly decreased in ET cerebellum compared to control cerebellum (Beta = -0.0563, q-val = 0.0221).

Conclusion: Using deconvolution of bulk RNA-seq, we found increased proportions of astrocytes as well as decreased numbers of granule cells in ET cerebellum compared to controls. PC proportions did not differ between ET and control samples. Although astrogliosis has been previously described in ET, decreased numbers of granule cells is a novel finding in ET.

References: [1] Rajput, A.H., Parkinsonism Relat. Disord. (2013). [2] Lake B.B., Nature Biotechnology. (2018).

Grants: Canadian Institutes of Health Research - CGSM.
Fonds de recherche du Québec en santé - Formation de maîtrise.

Conflict of Interest: None declared.

P10.090.A Biallelic variants in the ESCRT-II subunit SNF8 cause either early-onset lethal leukoencephalopathy or childhood onset optic atrophy

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Background/Objectives: We report five individuals from three unrelated families presenting with severe leukoencephalopathy and three adults from two unrelated families presenting with mild developmental delay and optic atrophy in which biallelic variants in the gene encoding the ESCRT-II subunit SNF8 could be identified.

Methods: Exome sequencing was performed in affected individuals which were collected using GeneMatcher. Patients were phenotyped including MRI and neuropathologic examinations. Proteomic profiling of patient derived fibroblasts as well as immunofluorescence and electron microscopy studies were used to assess ligand-induced EGFR degradation and autolysosome formation.

Results: Cases with leukoencephalopathy had severe developmental delay and secondary microcephaly, three of four passed away within the first two years of life. Loss of ESCRT II subunits was confirmed in patient derived fibroblasts by proteomic profiling. Patient derived fibroblasts showed no detectable defects in cytokinesis or degradation of growth factor receptors but displayed an accumulation of autolysosomes which could be reproduced by knockdown of SNF8 in control fibroblasts. Electron microscopy showed accumulation of vesicular structures and immunohistopathological evaluation of brain tissue in one individual revealed increased LC3B staining both in line with autolysosome accumulation. Additionally, adult cases presented with loss of function variants in compound-heterozygosity with a recurrent missense variant. This variant could be observed in homozygosity in a control individual suggestive of a hypomorphic effect.

Conclusion: Loss of ESCRT II due to biallelic SNF8 variants is associated with two allelic diseases characterized by impaired autophagic flux: early-onset leukoencephalopathy and adult-onset optic atrophy the latter associated with a hypomorphic effect.

References:.

Grants:.

Conflict of Interest: None declared.

P10.091.B Genetic abnormalities in neurodevelopmental disorders with multidimensional impairment

Cyril Hanin[1], *Paloma Torres[1], Isabelle Millet[1], Joana Matos[1], Marianna Giannitelli[1], Cora Cravero[1], Anne Sophie Pellen[1;2], DELPHINE HERON[2], Boris Keren[3], Cyril Mignot[2], Charline Grossard[1], Ingrid Zammouri[1], Astrid De Foucaud[1], Angele Consoli[1], Claudine LAURENT-LEVINSON[1], David Cohen[1]*

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Background/Objectives: Many neurodevelopmental disorders (NDD) in children are complex with multiple domains of dysfunction (Xavier and Cohen, 2020). These cases have been termed Multidimensional Impairment (MDI), but they could be considered "diagnostically homeless" because DSM-5 does not include MDI. Here, we evaluate the prevalence of genetic abnormalities in MDI as a step toward establishing the utility of this diagnosis.

Methods: We evaluate complex NDD cases using both categorical and dimensional approaches (e.g. intelligence, language, motor coordination, executive functions). In 2016, we established a secure computerized database of genetic data for these cases. Between 2017 and 2019, we diagnosed MDI in 637 patients of whom 133 had a genetic assessment, using DNA microarrays to

detect Copy Number Variants, and sometimes other methods such as whole genome or exome sequencing..

Results: We found a genetic abnormality in 82 (61%) of the 133 patients that can be classified as follows: (1) 49 known genetic abnormalities associated with complex NDD (e.g. del22q11.2); (2) 12 genetic abnormalities associated with severe ASD/DI (e.g. a class 4 mutation in GRIA3 on Xq25); (3) 19 genetic abnormalities not previously described in NDD (e.g. duplication Xq21.1 spanning chrX:82,161,602-82,795,961).

Conclusion: Our referral center sample may be biased toward more severe and rare cases, but the high frequency of genetic abnormalities found in MDI suggests that more systematic genetic evaluation is warranted in these cases.

References: Weisbrot DM, Carlson GA. Child Adolesc Psychiatric Clin N Am. 2021;30:445-457.

Xavier J, Cohen D. Handbook of Clinical Neurology: neurodevelopmental and cognitive disabilities 2020;174:159-169.

Grants: None.

Conflict of Interest: Cyril Hanin: None declared, Paloma Torres: None declared, Isabelle Millet: None declared, Joana Matos: None declared, Marianna Giannitelli: None declared, Cora Cravero: None declared, Anne Sophie Pellen: None declared, DELPHINE HERON: None declared, Boris Keren: None declared, Cyril Mignot: None declared, Charline Grossard: None declared, Ingrid Zammouri: None declared, Astrid De Foucaud: None declared, Angele Consoli: None declared, Claudine LAURENT-LEVINSON: None declared, David Cohen Dr Cohen has served as a consultant to Janssen, Otsuka, Lundbeck and Nestle.

P10.094.A Investigation of causal association between ALS and Autoimmune disorders

Paria Alipour[1;2], *Konstantin Senkevich*[2;3], *Despoina Manousaki*[4;5;6], *Dan Spiegelman*[2;3], *Patrick Dion*[1;2], *Guy Rouleau*[1;2;3]

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Background/Objectives: Amyotrophic Lateral Sclerosis (ALS) is reported to be associated with different autoimmune disorders according to epidemiological studies. This study aims to explore the causal relationship between ALS and these various traits.

Methods: Following selection of 11 different traits, we used largest available GWAS summary statistics of autoimmune disorders with European ancestry participants. Applying two-sample Mendelian randomization (MR) we investigated the causal association between exposures (autoimmune disorders) and an outcome (ALS in our case). MR uses the SNPs strongly associated with exposure to establish the causal association between exposure and outcome. The MR analysis was performed using R package TwoSampleMR.

Results: After applying Bonferroni correction for multiple testing, MR analyses shows no causal relationship between autoimmune disorders and ALS. The variance in the exposure variables explained by SNPs ranged from 8.1E-04 to 3.1E-01. All instruments had F-statistics of >10, which is above the standard cut-off indicating sufficient instrumental strength. The SNPs within the human leukocyte antigen (HLA) region were excluded in the analyses. We also performed reverse MR between ALS and autoimmune disorders and did not find any causal relationship.

Conclusion: Our MR study did not support the direct causal relationship between ALS and autoimmune disorders in European population. The associations observed in epidemiological studies could be, in part, attributed to shared biology, confounders and bias.

References: Turner, M.R., et al., Neurology, 2013. 2. Hemani, G., et al., elife, 2018.

Grants: Canada's federal funding agency for health research.

Conflict of Interest: None declared.

P10.095.B A novel Adnp mutant mouse model reveals involvement of the WNT signaling pathway in the Helsmoortel-Van der Aa syndrome

Claudio D'Incal[1;2], *Elisa Cappuyns*[1], *Jolien Huyghebaert*[1], *Ellen Elinck*[3], *Debby Van Dam*[4], *Elke Callus*[4], *Peter De Deyn*[4], *Takuro Horii*[5], *Izuho Hatada*[5], *Wim Vanden Berghe*[4], *Frank Kooy*[4]

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Background/Objectives: Loss-function mutations in the Activity-Dependent Neuroprotective Protein Homeobox (*ADNP*) gene have been linked to the Helsmoortel – Van der Aa Syndrome (HVDAS), a complex neurological disorder characterized by autism, intellectual disability amongst many additional abnormalities. Mutations in the *ADNP* gene are almost exclusively de novo with the p.Tyr719* being the two most frequent mutation.

Methods: We introduced a frameshift mutation in the murine *Adnp* gene with CRISPR/Cas9 technology to create a mouse model for the human disorder and we studied the effect of the Leu822Hisfs*6 mutation in a heterozygous model. Mutant mice underwent a behavioural testing battery including Morris water maze, elevated plus maze test, and the marble burying test. RNA sequencing and differential proteome analysis were performed on the frontal cortex of mutant and wild-type littermate controls.

Results: *Adnp* mutant mice carry a 14 base pair deletion, resulting in a frameshift in exon 5, a mutation akin to that observed in patients. A 30% reduction of *Adnp* mRNA and protein levels were observed in the mutant mice. Behavioural testing showed an increased anxiety, cognitive impairments together and increased repetitive behaviour, a hall mark of autism. A combination of RNA sequencing and differential proteome analysis of the frontal cortex of *Adnp* mutant mice identified a down-regulation of the WNT signalling pathway, impacting neuronal development and development.

Conclusion: Our findings constitute the baseline behavioural and molecular characterization of a new mouse model for the HVDAS, and identify the WNT signalling pathway as a potential target for future therapy of the disorder.

References:.

Grants:.

Conflict of Interest: Claudio D'Incal PhD student and Academic Assistant, Elisa Cappuyns: None declared, Jolien Huyghebaert: None declared, Ellen Elinck: None declared, Debby Van Dam: None declared, Elke Callus: None declared, Peter De Deyn: None declared, Takuro Horii: None declared, Izuho Hatada: None declared, Wim Vanden Berghe: None declared, Frank Kooy: None declared.

P10.096.C Constitutional thinness and anorexia nervosa differ on a genomic level

Christopher Hübel[1;2], *Mohamed Abdulkadir*[3], *Moritz Herle*[1], *Alish Palmos*[1], *Gerome Breen*[1], *Ruth Loos*[4], *Nadia Micali*[3], *Cynthia M Bulik*[5;6]

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Background/Objectives: Constitutional thinness and anorexia nervosa both present with low weight (e.g., BMI <18.5 kg/m^2). However, individuals with anorexia nervosa additionally fear gaining weight and engage in weight loss behaviours not seen in constitutional thinness. Body composition regulation and anorexia nervosa are both heritable. It is unclear how constitutional thinness differs from anorexia nervosa on a genomic level.

Methods: We calculated genetic correlations among constitutional thinness, anorexia nervosa and traits genetically correlated with anorexia nervosa, to investigate similarities and differences. Additionally, we identified individuals with constitutional thinness in the Avon Longitudinal Study of Parents and Children (ALSPAC) and performed polygenic score analyses.

Results: Our results suggest that, in contrast to anorexia nervosa, attention deficit hyperactivity disorder ($r_{gAN} = 0.02$ vs. $r_{gCT} = -0.24$) and alcohol dependence ($r_{gAN} = 0.07$ vs. $r_{gCT} = -0.44$) are significantly genetically correlated with constitutional thinness. A higher polygenic score for posttraumatic stress disorder is associated with an increased risk of constitutional thinness in the ALSPAC cohort (OR = 1.27; $Q = 0.03$) whereas posttraumatic stress disorder shows no genetic correlation with anorexia nervosa ($r_g = -0.02$).

Conclusion: Even though both anorexia nervosa and constitutional thinness are marked by low BMI, they differ on a genomic level.

References:.

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Conflict of Interest: Christopher Hübel: None declared, Mohamed Abdulkadir: None declared, Moritz Herle: None declared, Alish Palmos: None declared, Gerome Breen NIHR UK, Maudsley Charity, Guy's and St. Thomas's Trust, Compass Pathways Ltd, Otsuka Ltd, Ruth Loos NIH US, Nadia Micali NIH, Cynthia M Bulik Shire, Lundbeckfonden, NIH USA, Shire, Idorsia, Pearson (author, royalty recipient).

P11 NEUROMUSCULAR DISORDERS

P11.001.D Gene variant and neuromuscular findings from a Long-Chain Fatty Acid Oxidation Disorder gene panel program

Vanessa Rangel Miller[1], *Peter Baker II*[2], *Omid K. Japalaghi*[1], *Nicola Longo*[3], *Deborah Marsden*[1], *Heather McLaughlin*[4], *Tom Pulles*[5], *Kate Simmons*[1], *Jillian Yong*[4], *Nicole Miller*[1]

[1]Ultragenyx Pharmaceutical Inc., Novato, United States; [2]University of Colorado, Anschutz Medical Campus, Aurora, United States; [3]University of Utah, Salt Lake City, United States; [4]Invitae Corporation, San Francisco, United States; [5]Ultragenyx Europe GmbH, Allschwil, Switzerland.

Background/Objectives: Long-chain fatty acid oxidation disorders (LC-FAOD) are rare, life-threatening, autosomal recessive conditions that can be diagnosed clinically with plasma acylcarnitine analysis and molecular testing. Undiagnosed LC-FAOD may present with hypoglycaemia, cardiomyopathy, cardiac arrhythmias, and neuromuscular symptoms.

Methods: Patients with a clinical diagnosis or suspicion of LC-FAOD are eligible for this no-charge NGS gene panel which includes 6 genes associated with LC-FAOD plus 18 genes associated with disorders that cause abnormal acylcarnitine profiles.

Results: As of 28 October 2021, LC-FAOD gene variants were identified in 153 (37%) of 417 patients tested, including 83 variants of uncertain significance (VUS), 8 likely pathogenic (LP), and 102 pathogenic (P) variants. Twenty-three patients had positive (2 P/LP) LC-FAOD results and 19 had potential positive (2 variants, at least 1 VUS) results. VUS resolution analysis led to the reclassification of 6 variants from VUS to LP or P. Five patients had variants in two or more LC-FAOD genes and 22 had variants in one LC-FAOD gene and one or more non-LC-FAOD genes. Fifty-one patients had only one LC-FAOD gene variant identified.

The most common neuromuscular symptoms among patients ages ≥13 (76 reported) were myopathy (42), elevated creatine kinase (36), and rhabdomyolysis (30), and among patients <13 years (83 reported) were elevated creatine kinase (22), and myopathy (12).

Conclusion: Program results demonstrate the diverse composition of gene variants in patients referred for LC-FAOD genetic testing. Approaches to resolve VUS and identify previously undetected variants in patients with suspected LC-FAOD are important and necessary.

References:.

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Conflict of Interest: Vanessa Rangel Miller Ultragenyx Pharmaceutical Inc., Ultragenyx Pharmaceutical Inc., Peter Baker II Ultragenyx (ended 2021, unspent), Omid K. Japalaghi Ultragenyx, Nicola Longo Clinical Trial Support: Aeglea, Amicus Therapeutics, Audentes/Astellas, AvroBio, BioMarin, Chiesi/Protalix, Genzyme/Sanofi, Hemoshear, Homology, Horizon Pharma, Moderna, Nestle' Pharma, Pfizer, PTC Ther./CENSA, Reneo, Retrophin/Travere Ther, Shire/Takeda, Synlogic, Ultragenyx, Speaker: Cycle Pharmaceuticals, Alnylam, Amicus Therapeutics, BioMarin, BridgeBio/CoA Ther, Chiesi/Protalix, Genzyme/Sanofi, Hemoshear, Horizon Pharma, Jaguar Gene Therapy, Leadiant Biosciences, Moderna, Nestle' Pharma, PTC Ther./CENSA, Recordati, Reneo, Shire/Takeda Synlogic, Ultragenyx, Data Safety Monnitoring Board: ACI Clinical (Applied Ther, Taysha), CTI-Clinical Trial (Vtesse), Deborah Marsden Ultragenyx, Ultragenyx, Heather McLaughlin Invitae, Invitae, Tom Pulles Ultragenyx, Ultragenyx, Kate Simmons Ultragenyx, Ultragenyx, Jillian Yong Invitae, Invitae, Nicole Miller Ultragenyx, Ultragenyx.

P11.002.A Extended clinical spectrum associated with de novo variant in CLTC gene

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Background/Objectives: De novo heterozygous variants in CLTC, which encode the clathrin heavy chain protein, are associated with a variable phenotype from mild to severe intellectual disability (ID), microcephaly, hypoplasia of the corpus callosum, and epilepsy. Congenital heart defects and kidney anomalies have also been described. No thorough muscle investigations are in published CLTC patients so far.

Methods: The boy is a second child of non-consanguineous parents born after 10 years infertility from in vitro fertilization. He was born at term by caesarean section, with normal growth parameters and Apgar scores (8/9). At 13 months, he was investigated due to developmental delay and muscular hypotonia, which was more prominent in proximal muscles. There was more subcutaneous tissue in the thighs and upper arms compared to other parts of the body. His motor development corresponded to 6 months and communication skills to 4 months. Magnetic resonance imaging (MRI) of the leg and pelvis muscles showed massive general muscular hypotrophy without clear dystrophic replacement and a peculiar amyoplasia of the medial gastrocnemius. Electroneuro-myography and muscular biopsy indicated a possible congenital myopathy or congenital myotonic dystrophy. MRI of the brain was normal.

Results: Trio exome sequencing revealed a pathogenic de novo missense variant NM_004859.4(CLTC):c.2669C>T p.(Pro890-Leu), and was confirmed by Sanger sequencing.

Conclusion: Muscular hypotonia is a constant clinical finding in previously reported patients with CLTC gene variant. We present a patient with marked general muscular hypotonia, which may cause more pronounced delay in motor development.

References:.

Grants: Estonian Research Council grant PRG471.

Conflict of Interest: None declared.

P11.004.C Three novel MTM1 pathogenic variants identified in patients with X-linked myotubular myopathy presented by severe neonatal hypotonia: diagnostic algorithm, genotype-phenotype correlations

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Background/Objectives: X-linked myotubular myopathy (X-MTM, OMIM: 310400) is a rare neuromuscular disorder (prevalence 1:50,000 males), caused by mutations in the *MTM1* gene (OMIM: 300415). The most severe is the classic form manifested in neonates as floppy infant, areflexia, and early ventilatory support dependence. Due to the severity of clinical manifestations with early mortality, and potential therapy options (current clinical trials for X-MTM[1]), early diagnosis is essential. According to previously published data it seems that severe genotype is related to the truncating mutation while non-truncating mutations are responsible for moderate or mild phenotype. However, an exact genotype-phenotype correlation still remains unclear[2].

Methods: We present 3 detailed case reports of patients (2 Austrian, 1 Slovakian) with severe neonatal hypotonia, where X-MTM was diagnosed according to diagnostic algorithm (in-house approach presented) using exact clinical evaluation, histopathological findings in muscle biopsy sample and molecular genetics approach using whole exome sequencing.

Results: 3 novel hemizygous pathogenic variants were found in *MTM1* gene: (NM_000252.2): c.438_439delCA (p.His146Glnfs*10), 2) (NM_000252.3): c.(342+1_343-1)_(444+1_445-1)del, and 3) (NM_000252.3): c.(1053+1_1054-1)_(1467+1_1486-1)del (p.Leu352_Gln489). Histopathological findings in biopsied muscle (n = 2) were consistent with X-MTM.

Conclusion: A comprehensive diagnostic approach is essential for the early diagnosis of neonatal forms of NMDs. The easiest and time-saving approach is an immediate muscle biopsy in case of postnatal severe muscular hypotonia. Subsequent molecular examinations serve to confirm the diagnoses, render a prognosis and potential treatment.

References: 1. Lawor MW, Dowling, JJ. Neuromuscular Disorders, 2021.

2. Oliveira J et al. European Journal of Human Genetics, 2013.

Grants: APVV-17-0296.

Conflict of Interest: None declared.

P11.005.D Interactome-based multi-omics approach to study disease activity in the early phases of Multiple Sclerosis

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Background/Objectives: Multiple Sclerosis (MS) is an autoimmune disease of the Central Nervous System (CNS), characterized by high clinical heterogeneity. Here, we analysed multi-omics data using molecular networks to disentangle the biological basis of MS inflammatory activity by identifying relevant gene networks and pathways implicated in disease activity [1].

Methods: Relapsing remitting MS patients were characterized as EDA (Evidence of Disease Activity) or NEDA (No-EDA) at 2 years of follow-up. GWAS (n = 1533): PLINK and MAGMA for gene-wise statistics. Differential methylation analysis (n = 243): minfi; differential expression analysis (mRNA and miRNA) (n = 243): Deseq2. Gene-gene interactions: STRING and iReflndex. Tissue-specific gene-gene interactions: HumanBase. miRNA-mRNA interactions: mirTarbase and miRNet. Identification of gene networks supported by multi-omics evidences: dmfind and mND. Gene-pathway associations: MSigDB. Enrichment analysis and pathway cross talk analysis: Ulisse.

Results: We mapped the results of EDA-NEDA GWAS and EDA-NEDA differential methylation/expression analysis onto genome-wide molecular networks. We found brain-specific and lymphocyte-specific networks of genes that carry variants associated with EDA, and interact with "core" (recurring) network regions. The integrative analysis revealed gene networks that are supported by genomics, epigenomics and transcriptomics. Network analysis shed light on key genes involved in multiple connections and/or connectors between diverse pathways.

Conclusion: Our network-based multi-omics analysis provides an in-depth knowledge of genes and pathways which appear to be associated with inflammatory activity in MS, including novel candidates that require further study.

References: [1] https://www.findingms.com.

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Conflict of Interest: Valentina Nale: None declared, Alice Chiodi: None declared, Antonino Giordano: None declared,

Béatrice Pignolet: None declared, Ferdinando Clarelli: None declared, Elisabetta Mascia: None declared, Silvia Santoro: None declared, Laura Ferrè: None declared, Miryam Cannizzaro: None declared, Matteo Gnocchi: None declared, Marco Moscatelli: None declared, Kaalindi Misra: None declared, Melissa Sorosina: None declared, Luciano Milanesi: None declared, Massimo Filippi Significant; Roche, Biogen Idec, Merck-Serono, Novartis, Teva Pharmaceutical Industries, Bayer, Biogen Idec, Merck-Serono, Novartis, Roche, Sanofi Genzyme, Takeda, Teva Pharmaceutical Industries., Modest; Bayer, Biogen Idec, Merck-Serono, Novartis, Roche, Sanofi Genzyme, Takeda, Teva Pharmaceutical Industries., Roland Liblau Significant. GlaxoSmithKline and BMS, Modest. Biogen, Merck, Roche, Novartis, and Sanofi-Genzyme., FEDERICA ESPOSITO Modest; Merck Serono and Novartis, Modest. Merck Serono, Ettore Mosca: None declared.

P11.006.A Late onset neuropathy caused by a heterozygous variant in the SCL12A6 gene

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Background/Objectives: The SLC12A6 gene encodes the potassium chloride cotransporter, KCC3, which is involved in maintaining cell volume and intracellular chloride levels in neurons [1]. Pathogenic variants in SLC12A6 are known to cause autosomal recessive motor and sensory neuropathy with corpus callosum agenesis and mental retardation (HMSN/ACC). In recent years, heterozygous de novo SLC12A6 variants have been described in patients with early onset predominantly motor neuropathy [2-4].

Methods: At two Norwegian centres performing genetic analysis of peripheral neuropathies, the SLC12A6 gene was analysed by next-generation sequencing as part of the diagnostic routine. Family members were examined by Sanger sequencing. For functional characterization, the mutant KCC3 cotransporter was modelled in Xenopus oocytes.

Results: In five families presenting with late onset peripheral neuropathy, the heterozygous SLC12A6 variant, NM_001365088.1 c.1655G>A (p.(Gly552Asp) was found to segregate with the disease in ten affected patients and to be absent in two unaffected family members. Clinical and neurophysiological evaluations revealed a predominant sensory axonal polyneuropathy with slight to moderate motor components. Functional characterization of the p.(Gly552Asp) variant showed a significant reduction in function, demonstrated through reduction of potassium influx.

Conclusion: Our findings further expand the spectrum of SLC12A6 disease to involve late onset autosomal dominant axonal neuropathy with predominant sensory deficits.

References: 1. Flores, et al., Neurochem Int, 2019. 123.
2. Kahle, et al., Sci Signal, 2016. 9(439).
3. Park, et al., J Med Genet, 2020. 57(4).
4. Shi, et al., Neuromuscul Disord, 2021. 31(2).

Grants: HH received funds from the South Eastern Norway Regional Health Authority (HSØ).

Conflict of Interest: None declared.

P11.007.B Audiologic and vestibular findings in patients and asymptomatic carriers with DM1 and its association with CTG repeats in the DMPK gene. Case-control study

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Background/Objectives: Myotonic dystrophy type 1 (DM1) is a neuromuscular disease, caused by an unstable mutation of CTG repeats in the *DMPK* gene. The present work shows the results from audio vestibular findings in patients and pre-symptomatic carriers, and their correlation with a CTG repeats in *DPMK* gene.

Methods: 58 patients and 10 pre-symptomatic carriers and their controls were analyzed. All of them had an audiologic and vestibular evaluation. A correlation index between the repeats number and each one of the findings was made.

Results: 69% of the patients had familial background, 48% had sensorial hearing loss, ($p = 0.0001$); falls in high frequencies ($p = 0.001$). The vestibular manifestations were present in 21 patients (36.2%), $p = 0.00001$. Nistagmus was present in 2 patients, none of the controls, the DHI test, in its 3 scales showed some degree of disability in 53.4% ($p = 0.00001$). The 3 correlations that show p statistically significant were DGI and DHI with CTG number repeats (-0.3215/$p = 0.0313$ and 0.3143/$p = 0.0355$ respectively) and high frequency audiometry (0.3422/ $p = 0.0230$), with evolution time.

Classic DM1 group showed Muscular Impairment and CTG expansion tract between 121 and 1021, while pre-symptomatic group registered no muscular impairment, with a CTG tract between 50 and 110.

Conclusion: The falls in high frequencies and superficial hearing loss were the most frequent findings in both, classical and pre-sympotomatic DM1 patients. correlation between CTG repeat number with ocular saccades test and in the DGI were low in patients with classical DM1; With the time of evolution association was only with high frequency audiometry.

References:.

Grants:.

Conflict of Interest: None declared.

P11.008.C Mosaic collagen 6-related myopathy mimicking a hemihypotrophy syndrome

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Background/Objectives: Somatic mosaicism as a cause of genetic-related disorders is well known but the detection in routine diagnostic workup of monogenic disorders is challenging.

Methods: We present a patient with hemihypotrophy and relevant leg/arm length and circumference difference (right > left). Mildly elevated CK levels, progressing muscular weakness and pain led to MRI examination which showed marked reduction and fatty degeneration of the muscles of the left side. Muscle biopsy in the affected area revealed a chronic myopathy with connective tissue and fat proliferation combined with a moderately chronic neurogenic muscle atrophy. Electron microscopy revealed fine filamentous, dense deposits in the extracellular matrix in close proximity to the sarcolemma, consistent with altered collagen synthesis.

Results: Exome sequencing of blood DNA showed no pathogenic variant using standard filter settings, but in exome sequencing of the affected muscle DNA the variant NM_004369.3:c.6210+1G>A in the *COL6A3* gene was identified in approximately 18% of the reads. This canonical splice-site variant has already been described as pathogenic in the literature and in the ClinVar database (Bethlem myopathy, Ulrich congenital muscular dystrophy, OMIMG: 120250). Further investigation of the exome data from the blood DNA revealed that the variant was present in about 13% of the reads, being slightly below the internal cut-off value. Sanger sequencing confirmed somatic mosaicism in both tissues.

Conclusion: Our findings show that mosaicism for a structural myopathy may not only manifest as milder generalized disorder, but may mimic a hemihypotrophy syndrome. Careful evaluation of NGS data with regard to low-grade and sometimes tissue-specific mosaicism is needed.

References:.

Grants:.

Conflict of Interest: None declared.

P11.009.D Exome sequencing in understanding the etiology of hypotonia

Arya Shambhavi[1], *Amita Moirangthem*[2], *Haseena Sait*[2], *Somya Srivastava*[2], *Renu Suthar*[3], *Shubha Phadke*[2]

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Background/Objectives: Hypotonia is a common feature in neuromuscular disorders having varied etiologies. NGS has a good diagnostic yield (upto 59%) given the phenotypic and genotypic variability seen in Hypotonia[1,2]. This study aims to provide information about the diagnostic yield and expand the genotypic spectrum in patients presenting with hypotonia in Indian population.

Methods: Patients with hypotonia with unknown etiology based on preliminary evaluation were enrolled. Exome sequencing was done followed by genotype-phenotype correlation.

Results: Forty cases (10 consanguineous) were recruited and exome sequencing was performed where causative variant(s) were identified in 34 cases, suggesting a diagnostic yield of 85%. Of these 34 cases, pathogenic/likely pathogenic variants were identified in 21 cases. Of the 34 cases, 22 novel variants were identified in 18 genes. Biallelic variations were identified in 17 autosomal recessive genes, heterozygous variations in 8 autosomal dominant genes and hemizygous variation in one X-linked gene. One patient was homozygous for two genes (PYCR1 and FBLN5) and the variants segregated in the family suggesting blended phenotype.

Conclusion: The diagnostic yield in our cohort is 85%. We have identified 22 novel variants in 18 genes. Additionally, our study expands the genotypic and phenotypic spectrum of the genetic forms of hypotonia.

References: 1. AlBanji, Mohammed H et al. "Utility of Hypotonia Diagnostic Investigations: A 12-year Single Center Study." *Molecular genetics and metabolism reports* vol. 25 100665 (2020). 2. Sharma, Sonal et al. "Diagnostic yield of genetic testing in 324 infants with hypotonia." *Clinical genetics* vol. 100,6 (2021): 752-757.

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Conflict of Interest: None declared.

P11.010.A FSHD analysis pipeline by Bionano optical genome mapping - a field report

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Background/Objectives: The autosomal-dominantly inherited facioscapulohumeral muscular dystrophy-1 (FSHD1) is caused by contractions of D4Z4 repeats to less than 10 on the permissive 4qA-haplotype on chromosome 4q35. We tested the practicability of the Bionano EnFocus™ FSHD Analysis pipeline.

Methods: Optical genomic mapping data were generated on Bionano Genomics Saphyr with data quality recommended (minimum 80x with total DNA >=150 kb, output 1300 Gb, map rate 70,7-92,4%) from 80 patients with clinical indication not implicating FSHD diagnosis and two positive FSHD-controls, and were analysed by FSHD1.

Results: In our FSHD-negative patient dataset, the FSHD pipeline analysis was successful in 64 cases and gave normal results with repeats ranging from 11 to 61 on 4qA-alleles. Eleven cases had low coverage (<20 locus-spanning reads per allele), and five were not analysable, e.g. due to merged data.

The reads were discriminated between chromosome 4 and the homologous region on chromosome 10, the respective haplotypes A or B were assigned, and for each allele the number of D4Z4 repeats was analysed.

In 14% of the cases, shorter/incomplete reads were called as additional alleles (+/-1-2 repeats) and were removed manually, whereas a third allele in two patients (3%) indicated a mosaicism of repeat length. Alleles with >30 repeats were underrepresented (65%).

In two commercial FSHD1-positive samples, the respective repeat contractions on one 4qA-allele were correctly detected and distinguishable from chromosome 10.

Conclusion: A validated Bionano optical genomic mapping method allows a reliable analysis for FSHD1 with the Bionano EnFocus™ FSHD Analysis pipeline integrated in the software.

References: -.

Grants: -.

Conflict of Interest: Monika Morak: None declared, Constanze Kurschat: None declared, Jenny Schiller: None declared, Soheyla Chahrokh-zadeh: None declared, Julia Philippou-Massier: None declared, Imma Rost: None declared, Hanns-Georg Klein partner of IMGM laboratory, Uwe Heinrich: None declared.

P11.011.B Functional characterization of mutations causal to rare muscle disorders in genes encoding intermediate filament proteins

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Background/Objectives: Mutations in the DES gene encoding cytoplasmic desmin proteins and LMNA gene encoding A-type lamins, are a common cause of familial desminopathy and laminopathy. Here, we provide evidence of the pathological role of two novel desmin and lamin mutations occurring in two patients of Indian origin.

Methods: Exome sequencing was performed to identify pathogenic or likely pathogenic mutations in DES and LMNA gene in two Indian origin patients with muscle disorder. Two novel mutations, NM_001927.4:c.448C>T;p.(Arg150*) in the DES gene leading to formation of truncated desmin protein and the missense mutation NM_001282625.1:c.590T>C;p.(Leu197Pro) in the LMNA gene were identified. The variants were validated by sanger sequencing and segregation analysis was also performed. The pathological role of these mutations were determined in cultured primary human skeletal muscle cells (HSkMC). Confocal imaging was performed to observe any defect in the localization of these two proteins.

Results: Analysis of the confocal micrographs elucidated the formation of aggregates and migration of truncated desmin proteins into the nucleus of the cells transfected with mutant desmin constructs. On the other hand, c.590T>C change in the LMNA gene also showed formation of aggregates and disappearance of laminar rims in the nuclei of the muscle cells.

Conclusion: The formation of desmin and lamin aggregates and absence of laminar rim in the nuclei of the muscle cells provides a clue to explain pathogenicity of c.448C>T and c.590T>C in desminopathies and laminopathies, respectively. The aggregates might disturb the cellular homeostasis and organelle positioning thereby impacting the mechano-transduction signalling.

References:.

Grants: NIBMG intramural funds to M.A.

Conflict of Interest: None declared.

P11.013.D Delineating genetic heterogeneity behind a disease: dystonia

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Background/Objectives: Dystonia is characterized by muscle contractions leading to abnormal movements and/or postures. Genetic variants associated with dystonia may be inherited autosomal, X-linked or mitochondrial. Penetrance of the variants might be reduced. In our study we aimed to investigate the role of variants in genes previously associated with focal dystonic disorders. This is the first comprehensive genetic study in a Hungarian cohort.

Methods: 110 unrelated patients diagnosed with cervical dystonia or blepharospasm were recruited for this study. Next generation panel sequencing was used to assess 24 dystonia related genes, selected based on literature. Variants of interest were validated via bidirectional Sanger sequencing. Deep phenotyping and family screening was also performed in some cases.

Results: 37 variants of interest in 13 genes were uncovered. We identified 3 likely pathogenic variants in the *LRRK2* gene and one novel stop gain variant in the *COL6A3* gene. 31 variants may be classified as variants of uncertain significance (VUS), including one novel variant in the *KMT2B* and two in the *CACNA1B* gene. In the *TOR1A* gene a VUS associated with spasmodic dysphonia was uncovered. A variant associated with mitochondrial membrane protein-associated neurodegeneration was also detected.

Conclusion: We identified several known dystonia related genetic variants and putative novel ones. With our study we aim to provide genotype-phenotype correlations from a population-specific aspect.

References:.

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Conflict of Interest: None declared.

P11.014.A Non-invasive biomarker discovery in amyotrophic lateral sclerosis

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Background/Objectives: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease with complex pathogenesis. Although there are several candidates[1], there is no clinical biomarker for ALS. A biomarker, however, could expedite diagnosis, monitor disease progression, or prioritize patients for clinical trials[2]. Here, we propose using cell-free DNA (cfDNA) as a non-invasive biomarker for ALS. CfDNA is an ideal candidate because it is enriched in ALS patients, is informative about tissue-specific cell death, and can be extracted from a standard blood draw[3].

Methods: To study cfDNA in ALS with the potential to be clinically relevant, we developed a capture panel technology that has a projected cost of under $300 per sample. The panel was designed to capture methylation sites that are informative for tissue status. Using this technology, we captured over four thousand regions and performed next-generation sequencing of 48 cases and 48 controls. Sequencing data was combined with supervised machine learning to predict ALS disease status using the methylation status of the reads and clinical covariates.

Results: With this approach, we replicated past findings of higher skeletal muscle cfDNA in ALS patients (logit p-value: 0.026). Furthermore, our model could accurately predict ALS disease status (cross-validated AUC: 0.95, AUPRC: 0.95), which may reach the level of clinical utility.

Conclusion: Together, these results demonstrate that cfDNA may be a valuable predictive biomarker in ALS.

References: 1. Bowser et al. (2011) 2. Taga & Maragakis (2018), 3. Caggiano et al. (2021).

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Conflict of Interest: None declared.

P11.015.B SMN1 and SMN2 status in Georgian SMA patients

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Background/Objectives: Spinal muscular atrophy is an autosomal recessive inherited disorder affecting 1:10000 individuals, causing the progressive degeneration, muscle weakness and atrophy. It is considered to be one of the most life limiting condition in infants. The aim of the study was to determine the genotype of the Georgian patients diagnosed with SMA.

Methods: 51 patients clinically diagnosed with SMA were tested for SMN1 and SMN2 status. Quantitative RT- PCR was performed. Amplification of SMN1 exon 7/8 and SMN2 exon 7 was used to detect the number of copies.

Results: From tested 51 individuals only in 41 (80%) diagnose was confirmed, 1 (2%) was a carrier. 35 (85%) of confirmed cases were homozygous for SMN1 exon 7 and 8 deletion, 6 (15%) revealed 1 copy of exon 8. Patients with homozygous deletion showed heterogeneity in SMN2 copy number. From 41 patients 23 (56%) have 3 copies of SMN2 gene, 15 (36.5%) 2 copies and 3 (7.5%) 4 copies. From 11 patients who need palliative care, 100% are homozygous for SMN1 exon7/8 deletion and do not have more than 2 copies of SMN2 gene.

Conclusion: As well as in most other populations SMA phenotype in Georgian patients is highly dependent on the disease genotype. There is a strong correlation between the clinical features of the disorder and the number of SMN1 and SMN2 genes copies. Type 1 SMA is affecting nearly 30% of the patients. Implementation of National SMA screening program is highly recommended.

References:.

Grants:.

Conflict of Interest: None declared.

P11.016.C An unusual case of TPI1 deficiency causing early-onset dystonia without hemolytic anemia

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Background/Objectives: Triosephosphate isomerase deficiency (TPID) is a rare, severe autosomal recessive enzymopathy, characterized by congenital hemolytic anemia, progressive neuromuscular dysfunction beginning in early childhood, cardiomyopathy, increased susceptibility to infections, and neurodegeneration (OMIM 615512).

Methods: We used trio exome sequencing to study DNA from a 10 years old girl affected with early-onset dystonia and epilepsy.

Results: Exome sequencing revealed two compound heterozygous mutation in the Triosephosphate isomerase gene, TPI1. The pathogenicity of these two novel mutations (a frameshift variation and a missense in the first exon) was confirmed by an enzyme activity assay, showing a strongly decreased TPI activity. However, this patient's phenotype is unusual, as repeated exams showed the absence of nonspherical hemolytic anemia, which is considered a hallmark of TPID. Some phenotypic variability has been described, including patients with anemia without neurologic involvement, or severe forms with death in childhood due to respiratory failure, but an isolated dystonia without anemia was never reported two our knowledge.

Conclusion: The identification of these two novel variations, in association with an unusual phenotype, provides the opportunity to enhance the current knowledge about this rare and poorly understood disorder. We would like to gather data from more patients with classic or atypical TPID, which will pave the way towards a better comprehension of the disease mechanism.

References: None.

Grants: None.

Conflict of Interest: None declared.

P11.017.D Primary hypogonadism in a patient with CONDSIAS: coincidence or spectrum?

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Background/Objectives: ADP-ribosylation is a posttranslational modification of proteins in response to cellular stressors, with excessive poly-ADP-ribose accumulation triggering the cell-death cascade. Two genes (*ADPRHL2, PARG*) encode specific PAR-degrading enzymes and bi-allelic pathogenic *ADPRHL2* variants are reported to cause the ultra-rare autosomal recessive *Childhood-onset, neurodegeneration, stress-induced, with variable ataxia and seizures* (CONDSIAS, OMIM 618170).

Methods: We present a 34-year old male patient with a 20-year long history of progressive generalized hypotonia and hypotrophy, ataxia, reduced superficial and deep sensation, mild intellectual disability, hypogonadism, and normal brain MRI scan. Laboratory examination detected very low testosterone and high FSH levels and testicular MRI demonstrated profound testicular hypoplasia and DEXA scan confirmed significant osteoporosis.

Results: Trio whole-exome sequencing using Illumina NGS platform, identified a homozygous pathogenic missense *ADPRHL2* variant c.1004T>G; p. Val335Gly. This variant has been previously reported in several unrelated patients with CONDSIAS and was shown to influence enzyme activity and reduce cell survival in the setting of oxidative stress. The variant is present within the gnomAD database with an extremely low frequency (0.00009544).

Conclusion: To our knowledge this is the first case of CONDSIAS associated with primary hypogonadism, raising the interesting question whether the hypogonadism is part of the underling genetic spectrum, or whether it is a coincidental finding. This case also raises the question about the potential role of testosterone/steroid hormones in *ADPRHL2* deficiency, given the milder phenotype, no history of seizures, normal brain MRI and the repeated history of adverse muscular effect of testosterone supplementation in our patient.

References:.

Grants:.

Conflict of Interest: None declared.

P11.018.A HINT1-Neuropathy in France: Let's expand genotypic and phenotypic spectrum

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Background/Objectives: Inherited peripheral neuropathy (IPN) is a heterogeneous group of disorders due to mutations in more than 90 genes. In 2012, the first cases of IPN associated with *HINT1* variants were described in 33 families sharing the same phenotype[1]. A novel disease was discovered: **Autosomal Recessive Axonal Neuropathy with Neuromyotonia (ARAN-NM OMIM #137200)**. HINT1 is involved in the regulation of transcription, cell-cycle control and possibly in neuropsychiatric pathophysiology [2]. To date, only 3/127 patients have been described with neuropsychiatric symptomatology or neurodevelopmental disorder.

Methods: Herein, we report 6 French patients with ARAN-NM identified by Next Generation Sequencing. We conducted a literature review and compared phenotypic and genotypic features with our cohort.

Results: We identified a **novel *HINT1* mutation involved in ARAN-NM: c.310G>C (p.Gly104Arg)** and the first African patient. This cohort is comparable with literature data regarding age of onset (8,5yo), neuronal involvement (sensorimotor 3/6 and motor pure 3/6) and skeletal abnormalities (scoliosis 2/6, feet anomalies 5/6). However, we describe **neuropsychiatric features** in 5 out of 6 individuals.

Conclusion: We expand genotypic et phenotypic spectrum of ARAN-NM. Neuropsychiatric features could be part of HINT1-related disease and we should further study the clinical phenotype of the patients previously described.

References: 1. Zimoń, M., Baets, J., … Jordanova, A. (2012). Loss-of-function mutations in HINT1 cause axonal neuropathy with neuromyotonia. *Nature Genetics*, *44*(10), 1080–1083.

2. Varadarajulu, J., … Martins-de-Souza, D. (2012). Differential expression of HINT1 in schizophrenia brain tissue. *European Archives of Psychiatry and Clinical Neuroscience*, *262*(2), 167–172.

Grants:.

Conflict of Interest: None declared.

P11.019.B Targeted screening of the C9orf72 and ATXN2 gene in Bulgarian amyotrophic lateral sclerosis patients

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Background/Objectives: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterized by progressive degeneration of the upper and lower motor neurons, leading to muscle weakness, hypotrophy, swallowing and respiratory failure.

The cause of ALS is not fully elucidated, but there are 35 associated genes and 2 gene loci with an unidentified gene. The most common are C9orf72, SOD1, TARDBP and FUS found in approximately 10% of patients. It has been established that intermediate length polyglutamine (polyQ) expansions (~27–33 Qs) are a significant risk factor for ~4.7% of ALS cases.

Methods: Expansions in the C9orf72 and ATXN2 genes were analysed by in-house short PCR and triplet repeat-primed PCR assay (TP-PCR), followed by fragment analysis.

Results: From 200 ALS patients included in the current study, 7 (3,5%) were diagnosed with C9orf72 repeat expansion, carrying more than 145 GGGGCC repeats. In addition, 7 ATXN2 expansions ranging from 27 to 30 CAG repeats and 2 borderline expansions of 26 CAG repeats were identified. Furthermore, two asymptomatic family members were genetically proved to be ATXN2 expansion carriers.

Conclusion: Amyotrophic lateral sclerosis is a rare neurodegenerative disease for which there is a lack of genetic data for Bulgarian patients. The obtained results enrich the worldwide database and shed light onto genetically characterized Bulgarian ALS patients. Affected patients and their families can be offered adequate medical-genetic consultation and prenatal diagnostics.

References:.

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Conflict of Interest: None declared.

P11.021.D A recurrent variant in the SOD1 gene – is a frequent pathogenic or a rare benign variant?

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Background/Objectives: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the death of motor neurons in the brain, brainstem, and spinal cord, resulting in fatal paralysis. In this study, we report a recurrent variant c.401A>G (p.Glu134Gly) in the SOD1 gene is the most frequent genetic cause for ALS with incomplete penetrance.

Methods: DNA samples of more than 10000 patients were tested by using clinical and whole exome sequencing.

Results: Among Russian patients variant c.401A>G in the *SOD1* have been identified in 6 cases. In 3 of them (50%) the variant was identified in patients with ALS. Their ages ranged from 32 to 50 years. The variant was also identified in 3 patients with diagnosis unrelated to ALS. Two of them have not yet reached the middle age of manifestation of this disease. The mother of one of the probands with ALS, carrying the variant, had no clinical manifestations of the disease. A notable feature in our study is that three probands live in the same region.

Conclusion: The variant was classified as likely pathogenic according to the guidelines for massive parallel sequencing (ACMG) data interpretation (criteria PM1, PM2, PP3, PP5). Despite this the question of the pathogenicity of this variant remains

open, due to the fact that half of the cases were found in one region, including in patients without ALS. Interpreting such variants beyond the primary cause of the disease can be challenging and time-consuming for both clinicians and labs.

References:.

Grants:.

Conflict of Interest: None declared.

P11.022.A The Middle Eastern Genomes Project: Leveraging consanguinity in inherited diseases

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Background/Objectives: Consanguineous marriages are common in the Middle East and associated with an increased burden of autosomal recessive genetic conditions. We have characterised two thousand individuals with a variety of conditions including inherited neuromuscular disease. The variant frequency data, enriched for homozygous variants, and particularly homozygous loss of function variants, provides an invaluable opportunity to gain scientific and clinical insights into gene function, as well as providing support for candidate disease gene identification and variant interpretation.

Methods: Homozygosity mapping and whole exome/genome sequencing was carried out and analysed according to best practice and included single nucleotide, copy number and mitochondrial variant analysis. Candidate disease variants were assessed based on predicted effect on protein structure and function, amino acid conservation, and population frequency, and by family segregation using Sanger sequencing.

Results: We have detailed frequency and functional prediction data on 2.7 million coding variants, including approximately 100,000 putative loss-of-function variants and 1.5 million missense variants. Likely pathogenic/pathogenic variants were detected in 46% cases in different disease-causing genes, including novel genes first identified in this population.

Conclusion: Our results provide novel insights into genotype-phenotype relationships and identify high frequency disease variants in this unique population, suggesting the utility of population specific sequencing efforts. The availability of genetic data from the Middle East, currently an underrepresented group in large genomic databases, will support both local genetic diagnostics as well as facilitating the assignment of pathogenicity to variants found in other populations and provide opportunities for discovery of new disease-gene associations.

References:.

Grants: Muscular Dystrophy UK 19GRO-PG12-0395.

Conflict of Interest: None declared.

P11.023.B RYR1 variants in Czech patients with neuromuscular disorders and malignant hyperthermia

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Background/Objectives: The RYR1 gene encodes the skeletal muscle ryanodine receptor, which serves as a calcium release channel of the sarcoplasmic reticulum. The RYR1 pathogenic variants are involved in wide spectrum of neuromuscular disorders with both dominant and recessive inheritance and are the main cause of malignant hyperthermia (MH).

Methods: We use panel sequencing with 300 genes related with neuromuscular disorders for variant detection and CNV analysis in patients with neuromuscular disease or suspected malignant hyperthermia. For structural analysis, we used both an open channel structure (PDB code: 5gL1 and 5t9v) and a closed structure (PDB code: 5gky).

Results: The aim of our study was to get an overview of the occurrence of the RYR1 mutations in Czech population. We detected pathogenic / likely pathogenic variants in 17 patients with recessive neuromuscular disease and 10 patients with dominant type. MH susceptibility was confirmed in 31 patients. We performed the structural analysis of the 48 described diagnostics MH variants. Based on this analysis, we suggested the rules associated with pathogenicity: i) about 80% of variants are associated with a change in charge, ii) about 10% of variants are present at the subunit interface, iii) about 10% of the variants represent substitutions on methionine, threonine, serine or tyrosine, which can be oxidized/phosphorylated.

Conclusion: Detection of pathogenic variants in RYR1 gene is important for understanding the phenotypic variability of RYR1 –related disorders. Structural analysis allow us to capture variants potentially related with MH.

References:.

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Conflict of Interest: None declared.

P11.024.C Severe neonatal respiratory distress is part of the clinical spectrum of congenital myopathy with tremors associated with dominant MYBPC1 variants

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Background/Objectives: Congenital myopathies are phenotypically and genetically heterogeneous. While *MYBPC1* variants were previously described in Distal Arthrogryposis type 1B, Complex Congenital Arthrogryposis or Lethal Congenital Contractural Syndrome, heterozygous missense variants in the *MYBPC1* gene have been recently identified in 11 patients from five families with congenital myopathy with tremor (MYOTREM, MIM 618524)[1]. All variants are located at highly conserved residues in the M-motif. We report one new patient with MYOTREM expanding the clinical spectrum to severe neonatal respiratory distress.

Methods: Trio Whole Genome Sequencing has been carried out as part of the France Genomic Medicine 2025 Plan.

Results: The patient presented with neonatal respiratory distress due to stridor, severe axial and peripheral hypotonia, weak spontaneous movements and myogenic tremors of the jaw and four limbs on stimulation. We identified a de novo heterozygous missense variant in *MYBPC1*: c.742G>A; p.(Glu248Lys) in a 16 month-old girl. This variant has been previously described as pathogenic in MYOTREM. Motor skill abilities improved over time as previously reported. She sat up unaided at 15 months and started crawling and standing at 16 months. Gastrostomy supplementation could be discontinued at 13 months. Despite gradual improvement, she remained highly dependent on non-invasive ventilation (12-hour daily dependence at 16 months).

Conclusion: This observation extends the clinical spectrum of MYOTREM, which should be considered in the differential diagnosis of neonatal hypotonia associated with severe respiratory distress. Early diagnosis is essential to reassure parents about good motor prognosis in adulthood.

References: [1] Stavusis et al, *Ann Neurol*, 2019.

Grants: None.

Conflict of Interest: None declared.

P11.025.D Deep intronic mutations, alternative splicing and complex chromosomal rearrangements in the DMD gene: genetic diagnosis of dystrophinopathies through mRNA analysis

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Background/Objectives: Becker and Duchenne Muscular Dystrophy (BMD/DMD) are X-linked muscular dystrophies caused by alterations in the *DMD* gene. An early genetic diagnosis of the disease allows anticipatory care, an optimal management of the disease, be eligible for mutation-specific therapies and genetic and reproductive counselling. It has been estimated that 5-7% of the patients remain genetically undiagnosed despite an extensive study in the genomic DNA (1). Here, we evaluate the diagnostic utility of mRNA sequencing of the *DMD* gene in dystrophinopathy patients.

Methods: In eight patients with a clear clinical suspicion of dystrophinopathy supported by the presence of alterations in muscle biopsy and without a precise genetic diagnosis after MLPA and NGS, we extracted RNA from muscle biopsy and evaluated the whole mRNA of the *DMD* gene. Droplet Digital PCR was performed to quantify transcript isoforms.

Results: Through cDNA sequencing, we have identified alterations in *DMD* mRNA in all the patients. We have detected the presence of two novel pseudoexons, transcript isoforms related with mild BMD and chromosomal rearrangements.

Conclusion: The mRNA analysis of the *DMD* gene is a useful technique to fulfil the genetic diagnosis of dystrophinopathy patients. It enables the identification of alterations in mRNA processing that cannot be detected through MLPA and NGS.

References: 1. Dent KM, Dunn DM, Von Niederhausern AC, et al. Improved molecular diagnosis of dystrophinopathies in an unselected clinical cohort. Am J Med Genet. 2005;134A(3):295–8.

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Conflict of Interest: None declared.

P11.026.A Evaluation of molecular-genetic testing results of myotonic dystrophies in the last 30 years in Slovakia

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Background/Objectives: Myotonic dystrophies (DM) are genetic disorders with two known genetic forms and overlapping multiorgan symptomatology. DM1 and DM2 are caused by tandem repeat expansions in the *DMPK* and in the *CNBP* genes, respectively. We report the statistical results of molecular-genetic testing of DM suspected patients.

Methods: We retrospectively evaluated the findings of 739 individuals tested for DM1 and DM2 belonging to 610 families. They consisted of approved DM1 and DM2 patients and patients with no DM expansions detected. Symptomatology in all three groups was assessed; however, only the main complaints and clinical findings when ordering molecular-genetic testing, i.e., do not reveal the complex symptomatology of patients.

Results: DM1 expansion was confirmed in 69 families (102 patients). DM2 expansion was confirmed in 94 families (142 patients). 390 families remained without confirmed expansions. DM2 seems to be more common in Slovakia than DM1. The age of molecular diagnosis was a decade higher in the DM2 group (49.02 years) than in the DM1 group (40.97 years). DM1 and DM2 were identified in both sexes equally, while DM2 was more commonly identified among women. The most commonly reported symptoms, i.e., myogenic findings and myotonia on EMG, muscle weakness, and cataracts, were identified in all three groups, although slightly different frequencies.

Conclusion: No single symptom or combination of symptoms was identified that would differentiate between the DM1 and DM2 groups, or even patients suspected but not proved to have DM.

References:.

Grants: APPV-18-0319; *VEGA-2/0167/20*.

Conflict of Interest: None declared.

P11.027.B Neuromuscular disease diagnosis: the experience of a reference laboratory in Brazil

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Priscila Soares Rodrigues[1;2], Thiago Cavole[1], Cecilia Micheletti[1;4], Armando Fonseca[1]

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Background/Objectives: Neuromuscular diseases (NMD) are a group of disorders that involve injury or dysfunction of peripheral nerves or muscle. Many NMDs have a genetic origin, and around 600 genes related to more than a thousand neuromuscular diseases were described to date. Onset may occur in the neonatal period, childhood, or adult life, pointing to the importance of identifying these diseases' etiology. We aim to describe genetic findings in molecular analysis of patients with symptoms of NMDs.

Methods: We performed a descriptive, cross-sectional study, with retrospective collection of DNA sequencing results performed in a clinical reference laboratory from Brazil, from 1996 to 2021. Four gene panels were carried out: mitochondriopathies (196 genes); muscular dystrophies, myopathies and myasthenic syndrome (80 genes); myopathies and muscular dystrophies (200 genes) and neuromuscular diseases (82 genes).

Results: 79 patients (40 males/39 females) suspected of having NMDs with age ranging from 2 to 78 years old were evaluated. Pathogenic mutations were detected in 33 patients (41.8%), involving 27 different NMD-relates genes (*ACTA1, ATP2A1, CAPN3, CHRNE, COL6A1, DMD, DOK7, ECHS1, GAA, HEXA, ITGA7, LAMA2, LMNA, MT-TL1, PDHA1, PNPLA2, POMT2, POMT1, PYGM, RAPSN, RYR1, SGCB, SURF1, OPA1, TPM2, TTN, WFS1)*. 32 patients carried variants of unknown significance (40.5%), and 1 patient (1.2%) had a pseudodeficiency variant.

Conclusion: High-throughput sequencing enables the genetic diagnosis of NMD patients, supports evidence-based treatment and the familiar genetic counseling. The identification and classification of these genetic variants are essential to improve the knowledge of the etiology of the NMD diseases.

References:.

Grants: None.

Conflict of Interest: None declared.

P11.028.C GGPS1-associated muscular dystrophy with and without hearing loss

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Background/Objectives: Geranylgeranyl diphosphate synthase 1 (GGPS1) gene is a member of the prenyltransferase family encoding a protein with geranylgeranyl diphosphate (GGPP) synthase activity. Ultra-rare biallelic pathogenic variants in GGPS1 have been associated with Muscular Dystrophy/Hearing Loss/Ovarian Insufficiency Syndrome. We describe nine cases from two new families with defective GPPS1 gene and provide follow-up details from the previously reported family with two affected.

Methods: Two families were collected as part of the SYNaPS Study Group collaboration, and the third family was followed up from the report by Tucker et al. (2020). Exome sequencing and segregation analysis by Sanger sequencing were utilized to identify and confirm the GGPS1 variants.

Results: More than 70% of cases presented with delayed motor milestones, short stature, hypotonia, progressive generalized or proximal muscle weakness, and muscular dystrophy. Sensorineural hearing loss was reported in three cases, while hearing remains clinically intact in the remaining eight cases. One 30-year-old female case reached puberty and had no clinical signs of primary ovarian insufficiency. Ultra-rare likely pathogenic homozygous missense GGPS1 variants (NM_004837.4):c.269A>G; p.(Asn90Ser) and c.439A>G; p.(Met147Val) were identified, which segregated with the phenotype within both families.

Conclusion: This report consolidates the disease-causing role of GGPS1 biallelic variants and demonstrates that hearing loss and ovarian insufficiency might be variable features of the GGPS1-associated muscular dystrophy.

References: Tucker EJ, Rius R, Jaillard S, et al. Genomic sequencing highlights the diverse molecular causes of Perrault syndrome: a peroxisomal disorder (PEX6), metabolic disorders (CLPP, GGPS1), and mtDNA maintenance/translation disorders (LARS2, TFAM). Hum Genet 2020; 139(10):1325-1343.

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Conflict of Interest: None declared.

P11.029.D New phenotype of myopathy in ASCC1-related disorder

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Background/Objectives: Spinal muscular atrophy with congenital bone fractures 2 (SMABF2) is a severe neuromuscular disorder caused by homozygous or compound heterozygous variants in the ASCC1 gene. There are only eight families described with the disease. We are presenting a case of a patient with the myopathy phenotype and two novel variants in ASCC1 gene.

Methods: 3 y.o. boy underwent neurological examination and genetic investigation in the RCMG. WES of proband's DNA sample was performed on Illumina NextSeq500. The functional effect of splicing variant was investigated using RT-PCR analysis. The expression analysis was performed by qPCR.

Results: Proband was born with muscular hypotonia, ulna fracture. Early motor skills were delayed. Neurological examination at 3 y.o. revealed severe muscular hypotonia, hyporeflexia with muscular patter on the NCS. WES identified two novel variants in ASCC1 gene: splicing variant c.311-2A>G and decreased coverage of exons 2 and 3 (ΔEx2,3) seemed as deletion. The analysis of c.311-2A>G by RT-PCR showed the presence of two isoforms: with truncated exon 5 without frameshift and exon 5 skipping. Splicing variant validated on proband's and father's DNA. The deletion was validated in heterozygous state in proband's and mother's cDNA. The increased expression of the transcript with ΔEx2,3 in 1.8 times was shown. Also, we suggested that ΔEx2,3 transcript could

produce a new truncated protein p.Glu2_Met86del from new Kozak sequence in exon 4.

Conclusion: We believed that a detailed analysis of this case could explain new phenotype of the proband.

References:.

Grants:.

Conflict of Interest: None declared.

P11.030.A Mutational spectrum and clinical features of GNE myopathy in Russia

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Background/Objectives: GNE myopathy (MIM 605820) is the most common distal myopathy in several countries. However, the genetic and clinical spectrum of Russian patients is unknown. Here we summarized the mutational spectrum and clinical features of 30 patients with GNE myopathy from Russia.

Methods: Massive parallel sequencing was performed for all probands, followed by Sanger DNA sequencing for segregation analysis. Clinical data, including manual muscle testing and the GNE myopathy functional activity scale (GNEM-FAS), were collected for 24 patients from 20 families. Muscle MRI was performed in 8 patients.

Results: As a result of molecular genetic analysis, novel mutations were found: 2 frameshift mutations, 2 large deletions, and 4 missense variants. In other cases, 12 known missense mutations were identified. One novel missense mutation c.169_170delGCinsTT, p.Ala57Phe was identified in 4 families in a homozygous state and 3 unrelated patients in a compound heterozygous state. All families with this novel frequent mutation in our cohort were originally from the 3 neighboring areas of European Russia. GNEM-FAS scores were analyzed for 21 patients, and scores varied from 2 to 97 (mean (SD) 55 (31)). Two patients have atypical clinical features: one has severe quadriceps atrophy, and another patient has a prominent asymmetry of muscle involvement, both cases were supported by muscle MRI.

Conclusion: We reported the clinical and genetic spectrum of the largest Russian group of patients with GNE myopathy. The novel variant c.169_170delGCinsTT is a frequent mutation in the European part of Russian Federation.

References:.

Grants:.

Conflict of Interest: None declared.

P11.031.B RFC1 intronic repeat expansions on a Spanish late-onset neuromuscular disorder cohort

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Background/Objectives: CANVAS syndrome (Cerebellar Ataxia, Neuropathy, and Vestibular Areflexia Syndrome) is associated with biallelic AAGGG intronic expansions in RFC1. A pattern of progression from isolated sensory neuropathy to full CANVAS manifestations has been proposed.

Methods: We studied a cohort of 19 patients with late onset manifestations compatible with CANVAS. Conventional PCR (C-PCR) flanking the reference allele $(AAAAG)_{11}$, was used to assert the presence of the wild-type allele. The screening of expansions of the non-pathogenic (AAAAG and AAAGG) and pathogenic (AAGGG) alleles was performed by repeat-primed PCR (RP-PCR). We characterized the motif with Long-range-PCR (LR-PCR) and Sanger sequencing.

Results: C-PCR showed no product in 9 patients suggesting that they did not carry the wild-type allele, 3 patients had larger products, compatible with an expansion of the reference allele, and the remaining 6 had the reference allele $(AAAAG)_{11}$. RP-PCR showed biallelic expansions of the AAGGG repeat in the 9 patients with failed C-PCR and confirmed the normal result in the 6 previously normal patients. RP-PCR of the remaining 3 patients showed that 2 were heterozygous for the expanded wild-type allele, whereas one (patient 11) showed an expansion of the pathogenic AAGGG allele and of an AAAAG allele, suggesting compound heterozygosity. LR-PCR confirmed RP-PCR results in all patients, except in patient 11, who carried a pathogenic AAGGG allele and an expanded AAGAG allele of unknown significance, previously described in Canadian and Brazilian populations.

Conclusion: We confirmed the clinical diagnosis in 47% (9/19) patients: 4 showed full CANVAS, whereas the remaining 5 presented with different PNS involvement.

References:.

Grants:.

Conflict of Interest: None declared.

P11.032.C Biallelic founder mutation in PDE2A causes paroxysmal dyskinesia with Intellectual disability in Pakistani families

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Background/Objectives: Intellectual developmental disorder with paroxysmal dyskinesia or seizures (IDDPADS, OMIM# 619150) is an ultra-rare childhood-onset movement disorder manifesting paroxysmal dyskinesia slowly progressive global developmental delay, impaired cognitive development and seizures (variable). We investigated three unrelated Pakistani families with six affected individuals born to cousin marriages. Belonging to diverse age groups (13 - 60 years), patients presented with severe developmental delay, cognitive abnormalities, speech impairment and seizures with onset in early years of age.

Methods: Whole exome sequencing (WES) was used to detect potentially pathogenic variants and identify shared regions of homozygosity.

Results: We identified a rare homozygous missense variant c.1490T>C (NM_002599.5), (p.Phe497Ser) in Phosphodiestrerase2A *(PDE2A)* gene. The identified variant segregates with the phenotype in all three families. To assess the possibility of a founder mutation, we performed homozygosity mapping which revealed a shared 5.5 Mb homozygous region at Chr11: q13.4-q13.5 among all three families. PDE2A is involved in hydrolysis of second messengers, cAMP and cGMP. It is activated by binding of cGMP to

GAF-B domain of the protein. The function of PDE2A is very critical for temporal regulation of these second messengers consequently modulating critical cellular processes like proliferation, neuronal function, apoptosis and differentiation. So far, only five variants and five patients are reported with disrupted PDE2A.

Conclusion: Our findings add important details to the clinical and genetic spectrum of *PDE2A*, and highlight c.1490T>C, (p. Phe497Ser) as a founder mutation in Pakistani families.

References:.

Grants:.

Conflict of Interest: None declared.

P11.033.D The rare case of a patient with neurodevelopmental disorder with epilepsy and hypoplasia of the corpus callosum and a homozygous LNPK gene mutation

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Background/Objectives: The endoplasmic reticulum (ER) has a variety of cellular functions that require the acquisition of distinct states. It is well established that ER dictates a tight regulation through a wide number of proteins. Perturbation of these ER-shaping proteins are known to cause distinct phenotypes such as Alzheimer's and Hereditary Spastic Paraplegia. Lunapark (LNPK) is a recently discovered transmembrane protein which is involved in the regulation of ER-shaping. The protein consists of an N-terminal domain, two transmembrane domains, and a unique zinc-finger domain and has been shown to act as a stabilizer to the negative membrane curvature. However, even though the role of LNPK has been established, its association with disorders remains unexplored. A study by Breuss et al. found an association between homozygous mutations in LNPK with a neurodevelopmental disorder in three patients.

Methods: n/a.

Results: Here we report the fourth documented case in the literature of a Cypriot patient from a non-consanguineous family with clinical features similar to those described by Breuss et al. and a homozygous mutation in LNPK gene. The patient was presented to the clinic with generalized hypotonia, severe developmental delay, absent speech, corpus callosum hypoplasia and movement challenges. Whole exome analysis revealed a homozygous pathogenic, splice site variant c.258-2A>G in LNPK gene, in agreement with the previous study.

Conclusion: Further investigation using functional studies for the identified variant and molecular and clinical characterization of other patients with similar phenotype are a prerequisite in order to shed light into the mechanistic role of LNPK in human diseases.

References: n/a.

Grants: n/a.

Conflict of Interest: Ouranio Anastasiou full, Andri Miltiadous full, Petroula Gerasimou full, yiannos kyprianou full, Jason Chi full, Paul Costeas full, Violetta Christofidou Anastasiadou full.

P11.034.A Genetic defects causing Amyotrophic lateral sclerosis in Czech Republic

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Background/Objectives: Amyotrophic lateral sclerosis (ALS) is a rare progressive neurodegenerative disorder that affects motor neurons in the brain and spinal cord. ALS is a multifactorial disorder with genetic defects affecting various pathways, including RNA metabolism, cytoskelet, or protein degradation. Such diversity challenges both ALS treatment and diagnosis. There is no cure, only symptomatic treatment. Incidence rates of ALS change across the countries, creating a need for population-specific studies.[1] Our aim was to identify genetic defects causing ALS in Czech population and to compare the data with previous studies in Caucasian population.

Methods: We have collected blood samples from over 50 patients with familial and sporadic ALS of Czech origin. After DNA extraction, samples were analysed for the most common genes causing ALS, such as *C9ORF72* and *SOD1*.

Results: We found a typical distribution of about 10% of familial vs sporadic ALS in our cohort based on the patient's history, and confirmed the findings by molecular genetic analyses of selected genes.

Conclusion: Our study shows the first preliminary data for Czech Republic, with a typical distribution of common pathological variants causing ALS in Caucasians.[2] Next we plan on enlarging the sample size and collecting the clinical data, and further genetic analyses.

References: 1. Cronin, et al (2007), *Neurology*, *68*(13), 1002-1007. 2. Mejzini, et al (2019). *Frontiers in Neuroscience* 06 (2019).

Grants: PRIMUS UK, Alzheimer NF.

Conflict of Interest: None declared.

P12 MULTIPLE MALFORMATION/ANOMALIES SYNDROMES

P12.002.D A novel FLCN-related syndrome that leads to intellectual disability, developmental delay and immunodeficiency

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Background/Objectives: FLCN forms complexes with the FLCN-interacting proteins 1 and 2 (encoded by FNIP1/2) that modulate mechanistic target of rapamycin complex 1 (mTORC1) and AMP-activated protein kinase (AMPK) which have opposite roles in cells[1]. The balance between mTORC1 and AMPK depends on the FLCN/FNIP complex[2]. Here, we present a 14-year-old boy with intellectual disability, short stature, dysmorphic features, late psychomotor development, hearing impairment and immunodeficiency, where whole genome sequencing (WGS) detected a homozygous ultra-rare variant in FLCN.

Methods: WGS analysis, quantitative real-time PCR and western blot were performed to identify the variant and investigate its effect on expression. Immune parameters were evaluated with flow cytometry analysis (FCA).

Results: WGS identified a homozygous, ultra-rare missense (NM_144997.7:c.43G>A; p.(Gly15Ser)) variant in FLCN fitting to autosomal recessive inheritance. In the patient fibroblasts, the

expressions of FLCN and FNIP1 were increased while no significant change at the protein level was observed. FCA showed hypogammaglobinaemia (IgM, IgG) and low B and NK cells.

Conclusion: Overexpression of FLCN and FNIP1 signals dysregulation of the balance between mTORC1 and AMPK pathways, resulting in disruption of homeostasis in the cells. Patients with FNIP1 mutations had a remarkable phenotypic overlap with our patient[3]. Therefore, we conclude that the FLCN variant causes a novel FLCN-related syndrome with phenotypic overlap with the FNIP1 immunodeficiency.

References: 1. Takagi, Y., et al., Oncogene, 2008. 27(40): p. 5339-47. 2. Gonzalez, A., et al., Cell Metab, 2020. 31(3): p. 472-492. 3. Saettini, F., et al., Blood, 2021. 137(4): p. 493-499.

Grants: The Swedish Childhood Cancer Fund.

Conflict of Interest: None declared.

P12.003.A Familial cleft tongue caused by a unique translation initiation codon variant in TP63

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Background/Objectives: Variants in transcription factor p63 have been linked to several autosomal dominantly inherited malformation syndromes. These disorders show overlapping phenotypic characteristics with various combinations of the following features: ectodermal dysplasia, split-hand/foot malformation/syndactyly, lacrimal duct obstruction, hypoplastic breasts and/or nipples, ankyloblepharon filiforme adnatum, hypospadias and cleft lip/palate.

Methods/Results: We describe a family with six individuals presenting with a striking novel phenotype characterized by a furrowed or cleft tongue, a narrow face, reddish hair, freckles and various foot deformities. Whole-exome sequencing (WES) identified a novel heterozygous variant, c.3G>T, in *TP63* affecting the translation initiation codon (p.1Met?). Sanger sequencing confirmed dominant inheritance of this unique variant in all six affected family members.

Conclusion: In summary, our findings indicate that heterozygous variants in *TP63* affecting the first translation initiation codon result in a novel phenotype dominated by a cleft tongue, expanding the complex genotypic and phenotypic spectrum of *TP63*-associated disorders.

Conflict of Interest: None declared.

P12.004.B Incomplete penetrance and variable clinical expression of a Belgian TGFB3 founder variant suggests the presence of a genetic modifier

Melanie Perik[1], *Emmanuela Govaerts*[2], *Steven Laga*[3], *Inge Goovaerts*[1], *Josephina (Jeannette) Meester*[1], *Aline Verstraeten*[1], *Lut Van Laer*[1], *Bart Loeys*[1]

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Background/Objectives: Pathogenic TGFB3 variants cause Loeys-Dietz syndrome type 5, an aortic aneurysm-presenting connective tissue disorder.

Methods: We provide the results of an haplotype analysis as well as a medical record review of clinical features of 29 individuals from five families segregating an identical pathogenic TGFB3 variant, p.Asp263His, which affects a critical integrin-recognizing RGD motif.

Results: In five families with the p.Asp263His variant, we identified a shared haplotype (min 1.92Mb-max 4.14Mb), suggesting the presence of a founder originating ±400 years ago. Remarkably, only 4/29 patients presented with aortic aneurysms/dissections. One 31-years old male presented with a type A dissection, while another 66-years old male underwent a Bentall procedure because of severe insufficiency of a bicuspid aortic valve and a sinus of Valsalva aneurysm (50mm, z-score = 5.2). Two other male mutation carriers presented with a pathologically enlarged ascending aorta or aortic root aneurysm at older age (75 and 80 years). None of the 25 other TGFB3 founder mutation carriers (10-84 years, 14 males/11 females) had aortic aneurysms. Additional cardiovascular observations are ventricular septal defect, valve insufficiency and variable conduction abnormalities. We also observed minor systemic involvement such as easy bruising, inguinal hernia and ruptured ligaments and tendons.

Conclusion: The low penetrance for aortic involvement suggests that the pathogenic TGFB3 variant is not sufficient to cause the aneurysm phenotype. No aggravating cardiovascular risk factors were documented in the aneurysm-presenting patients. Comparative whole genome- and RNA-sequencing of iPSC-vascular smooth muscle cells of affected and unaffected variant carriers is currently being performed to pinpoint genetic modifiers.

References:.

Grants:.

Conflict of Interest: Melanie Perik full, Emmanuela Govaerts full, Steven Laga full, Inge Goovaerts full, Josephina (Jeannette) Meester full, Aline Verstraeten full, Lut Van Laer full, DOCPRO: FFB170247, Bart Loeys full, Genomia – ERC-COG-2017-771945.

P12.005.C Delineation of a KDM2B-related neurodevelopmental disorder and its associated DNA methylation signature

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Background/Objectives: Mutations in genes involved in the epigenetic machinery are an emerging cause of neurodevelopmental disorders (NDDs). Lysine-demethylase 2B (KDM2B) encodes an epigenetic regulator but has not been convincingly recognized as a NDD gene to date.

Methods: We assessed 24 heterozygous missense and loss-of-function variants in KDM2B in 33 individuals and reviewed their clinical data. We applied genome-wide methylation arrays on leukocyte-derived DNA samples to establish a KDM2B-specific epigenetic signature.

Results: For 21 individuals their variants were classified as pathogenic, in another 12 they remained variants of unknown significance. We observed a clustering of variants in the DNA-binding CXXC domain. Affected individuals presented with developmental delay and/or intellectual disability (20/21), autism (8/21), AD(H)D (7/21), cardiac anomalies (10/21), single kidney (4/21), ophthalmological abnormalities (6/21) and subtle facial dysmorphism. We established a KDM2B-specific epigenetic signature, characterized by hypermethylation of CpG-dinucleotides and with a stronger subsignature of the CXXC-domain variants. We identified two distinct episignatures in individuals with concurrent deletions of SHANK3 or SETD1B respectively, confirming their dual diagnosis.

Conclusion: Heterozygous KDM2B variants cause a NDD with frequent congenital anomalies and a distinct DNA methylation signature. Importantly, we were able to detect the KDM2B episignature in the context of a dual diagnosis, demonstrating the robustness of this assay.

References:.

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Conflict of Interest: None declared.

P12.006.D A novel missense variant in FLNC causes syndromic dominant cardiomyopathy in a large family

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Background/Objectives: Pathogenic variants in FLNC (filamin C) cause cardiomyopathies and myopathies with known genotype-phenotype correlations (1). A few cases have been described with accompanying manifestations, such as congenital cardiac defects. We present a large family with dominant cardiomyopathy and extra-cardiac manifestations caused by a novel missense variant in FLNC.

Methods: Eleven affected individuals (age 0-73 years) in four generations underwent clinical evaluation. We performed whole-exome sequencing in three affected individuals, followed by Sanger sequencing of a shared variant in all other family members. Functional effects of the identified genetic variant were experimentally explored by overexpression in C2C12 myoblasts.

Results: Clinical evaluation revealed hypertrophic cardiomyopathy in children and adults, congenital but transient myopathy with contractures, and congenital malformations: cardiac defects, micrognathia, cleft palate, small/absent uvulas, short stature and hernias. Exome sequencing identified a novel missense variant in FLNC (NM_001458.4: c.7118A>C: p.Tyr2373Ser) in a highly conserved position in Ig-like domain 21. Overexpression of the variant versus wild-type FLNC showed intracellular accumulation of

filamin C protein, suggesting misfolded protein aggregation as mechanism of disease.

Conclusion: We report a novel pathogenic missense variant in FLNC, causing syndromic cardiomyopathy and myopathy in a four-generation family. Our findings expand the clinical spectrum of FLNC related disease, and gives unique insight in the lifespan course of the disease.

References: 1) Verdonschot JAJ et al. A mutation update for the FLNC gene in myopathies and cardiomyopathies. Hum Mutat 2020 Jun;41(6):1091-1111.

Grants: The National Neuromuscular Centre of Norway, University Hospital of North Norway, Tromsø, Norway.

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P12.007.A Investigating Rho dysregulation in Adams-Oliver syndrome as a model of vascular development

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Background/Objectives: Adams-Oliver syndrome (AOS) is a genetically heterogeneous developmental disorder, predominantly characterised by scalp, limb and cardiovascular anomalies. Of the six known causal genes, DOCK6 and ARHGAP31 encode Rho GTPase regulators of CDC42 and RAC1. In AOS, DOCK6 depletion or ARHGAP31 gain-of-function have been shown to inactivate CDC42/RAC1, affecting cytoskeletal dynamics. Nonetheless, the molecular mechanisms of CDC42 and RAC1 dysregulation in disease progression remain largely unexplored. AOS is hypothesised to be a disorder of vasculogenesis, therefore we sought to model Rho GTPase dysregulation in zebrafish to examine embryonic vascular development.

Methods: A novel Tg(dock6:mCherry) zebrafish reporter line was developed to examine dock6 expression during embryogenesis. Models of Rho dysregulation were generated by morpholino and CRISPR technology. Whole-mount in situ hybridisation was used to assess the expression of known endothelial markers kdrl and fli1 in our disease models. Vascular analysis was conducted by microangiography.

Results: Depletion of dock6 and arhgap31 resulted in cardiovascular defects, with dock6 embryos additionally displaying eye abnormalities. Microangiography revealed asymmetric and truncated intersomitic vessels and impaired optic vessel formation in both models. In arhgap31 morphants, kdrl and fli1 were diminished throughout the vasculature. Conversely, dock6 knockdown induced aberrant patterning of the trunk vasculature and reduced marker expression in the caudal vein plexus (CVP).

Conclusion: The observed defects in models of Rho dysregulation indicate a possible vascular origin to AOS. Specifically, dock6 depletion leads to compromised CVP patterning and intersomitic vessel development. Future work to investigate arhgap31-mediated regulation of fli1 and kdrl may support the delineation of novel pathways driving AOS pathogenesis.

References:.

Grants:.

Conflict of Interest: None declared.

P12.008.B Luscan-Lumish syndrome: Nine new cases and review of the literature

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Background/Objectives: Luscan-Lumish syndrome (LLS) is a relative recent overgrowth disorder. Clinical features of patients with LLS also included neurodevelopmental disorders such as intellectual disability, autistic behavior and epilepsy. Since the initial description, only few cases have been reported in the literature, with the lack of deep phenotyping and molecular underlying mechanism. LLS is caused by pathogenic variants in SETD2, which encoded a methyltransferase (MTs) protein involved in histone regulation, playing an important role in gene expression regulation.

Methods: Analysis by custom NGS panel, from a cohort of >2000 cases with overgrowth disorders from the Spanish Consortium. This panel was designed in-house and included 214 genes. We have also reviewed the clinical and molecular features of the cases described in the literature so far.

Results: Here, we report nine additional individuals with LLS, in which pathogenic or likely pathogenic variants. Most common clinical features of the patients included overgrowth, intellectual disability, and neurodevelopmental disorders with variable degree of severity. We have also seen that autism behaviour in quite common, and can appear without overgrowth.

At molecular level, we have detected nine new variants not reported previously in the literature, expanding the causative variants in LLS. The majority of the variants detected were nonsense and frameshift, which is in line with low tolerance of SETD2 for this kind of changes according to the pLI score (pLI = 1).

Conclusion: In summary, we report nine additional cases with LLS and reviewed clinical and molecular features of all cases described.

References:.

Grants: FIS PI21/01053.

Conflict of Interest: None declared.

P12.009.C A large, ten-generation family with autosomal dominant preaxial polydactyly/triphalangeal thumb: Historical, clinical, genealogical and molecular studies

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Background/Objectives:.

Methods:.

Results: We present a large, ten-generation family of 273 individuals with 84 people having preaxial polydactyly/triphalangeal thumb due to a pathogenic variant in the zone of polarizing activity regulatory sequence (ZRS) within the exon 5 of LMBR1. The causative change maps to position 396 of the ZRS, located at position c.423+4909C>T (chr7:156791480; hg38; LMBR1 ENST00000353442.10; rs606231153 NG_009240.2 in the intron 5 of LMBR1. The first affected individual with the disorder was traced back to mid-1700, when some settlers and workers established in Cervera de Buitrago, a small village about 82 Km north to Madrid. Clinical and radiological studies of most of the affected members have been performed for 42 years (follow-up of the family by LFGA). Recently, molecular studies have confirmed a pathogenic variant in the ZRS that segregates in this family. Currently, four affected individuals at child-bearing age have been included in a pre-implantation genetic diagnosis program with the aim to avoid the transmission of the disease.

Conclusion: To the best of our knowledge, this is the largest family with preaxial polydactyly/triphalangeal thumb reported so far.

References:.

Grants:.

Conflict of Interest: None declared.

P12.010.D Biallelic mutations in TIE1 in a family with congenital lymphedema, intestinal lymphectasia and cutis aplasia

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Background/Objectives: Intestinal lymphangiectasis (IL) is a very rare condition; it occurs due to dilatation of the intestinal lymphatic vessels resulting in lymphatic leakage to the bowel lumen leading to protein loss and edema.

Congenital cutis aplasia (CAA) is also a rare phenotype, occurring mainly on the scalp; up to 90% of cases present as sporadic isolated lesions, but can rarely be associated with additional abnormalities as part of a genetic disorder.

We report co-occurrence of IL, CAA and lymphedema in two male siblings. One sibling died in infancy due to sagittal sinus bleeding.

Methods: Exome sequencing performed in the proband, parents and two healthy siblings.

Results: Sequencing revealed compound heterozygosity for c.1502G>A; p.Ser501Asn and c.2536G>A; p.Gly846Arg (NM_005424.5) in *TIE1* in the proband. Three asymptomatic family members were heterozygous for one or the other variant.

Conclusion: The *TIE1* gene encoding TIE1 orphan receptor is associated with autosomal dominant (AD) Lymphatic malformation 11 (MIM #619401) characterized by peripheral edema, with onset in the second-third decade of life. Biallelic variants in this gene have been reported to cause extensive edema in knock-out mice suggesting a loss of function mechanism. To the best of our knowledge, this is the first report of possible autosomal recessive inheritance of this disorder in humans with a more severe phenotype than the AD form. However, the possibility of AD inheritance with incomplete penetrance cannot be excluded.

To conclude, we suggest that biallelic deleterious variants in *TIE1* might lead to a more severe phenotype with previously unreported clinical features including CAA and IL.

References:.

Grants:.

Conflict of Interest: None declared.

P12.012.B Cardiac involvement and TBCK-related neurodevelopmental disorder: is it a new feature of this condition?

Gioia Mastromoro[1], *Daniele Guadagnolo*[1], *Nader Khaleghi Hashemian*[1], *Laura Bernardini*[2], *Antonella Giancotti*[3], *Antonio Novelli*[4], *Viviana Caputo*[1], *Cira di Gioia*[3], *Gerardo Piacentini*[5], *Antonio Pizzuti*[1]

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Background/Objectives: *TBCK* (TBC1 Domain-Containing Kinase;MIM*616899) encodes a protein that plays a role in actin organization, cell growth/proliferation by regulating the mTOR signaling pathway. Deleterious homozygous or compound heterozygous *TBCK* variants determine Hypotonia, infantile, with psychomotor retardation and characteristic facies 3 (MIM#616900). We report on three affected sibs, presenting also with cardiac malformations.

Methods: A couple of first cousins had previously been studied because their first child presented developmental delay, dysmorphisms, hyperthricosis and right-sided aortic arch, and a deceased daughter had showed similar features and Tetralogy of Fallot. Whole exome sequencing, performed on the infant detected the homozygous c.1532G>A;p.(Arg511His) pathogenic variant in the *TBCK* gene, resulting in a compatible clinical picture. The couple was referred again to perform prenatal diagnosis on their current pregnancy. The ultrasound scan had showed cystic hygroma and hypoplastic nasal bone, previously unreported in *TBCK*-related cases. We thereby requested chromosomal microarray analysis, whole exome sequencing and early fetal echocardiography.

Results: Chromosomal microarray analysis revealed 8.6% runs of homozygosity. The homozygous *TBCK* variant was detected by whole exome sequencing, without further pathogenic or candidate variants. Early fetal echocardiography identified hypoplasia of left ventricle and aortic arch. The couple opted for pregnancy termination. Fetopsy confirmed the sonographic findings and revealed a hypoplastic aorta arising from right ventricle and corpus callosum agenesis.

Conclusion: This is one of the very few families reported with *TBCK* mutations. Interestingly, the cardiac phenotype segregates with mutations and cardiac involvement could be considered a new feature of this variant causing Hypotonia, infantile, with psychomotor retardation and characteristic facies 3.

References:.

Grants:.

Conflict of Interest: None declared.

P12.013.C Mouse in vivo and in vitro models to understand neural pathobiology associated with ACTB loss of function

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Background/Objectives: Heterozygous loss of function variants in *ACTB* encoding β-actin cause a human syndrome featuring developmental delay. We hypothesised that mouse in vivo and in vitro models would further our understanding of the neural pathobiology of this human genetic disease.

Methods: We used two models: mice with a heterozygous *Actb* deletion in early embryogenesis, using Cre-Lox technology; and mouse N2a neuroblastoma cells with siRNA-mediated *Actb* knockdown. In each, *Actb* expression is predicted to be reduced but not absent, thus modelling *ACTB* haploinsufficiency.

Results: In wild type mouse brains, as assessed by immunohistochemistry, β-actin was widespread and was most prominent in microglial-like cells. Neonatal mice with heterozygous deletion of *Actb* had normal brain weights. Nevertheless, RNA sequencing identified significantly deregulated transcripts including, as expected, downregulated *Actb*, but with increased *Acta2* (encoding a smooth muscle actin) and transcripts encoding filamin (an actin binding protein) and vinculin (another cytoskeletal protein). We exposed N2a cells to *Actb* siRNA, resulting in decreased *Actb* mRNA and β-actin protein. *Versus* cells exposed to control siRNA, knockdown cells showed slower migration, with increases in neurite length and number as well as prominent secondary branches. There was no effect on proliferation. These cells showed changed levels of several of the transcripts altered in *Actb* mutant mouse brains.

Conclusion: *Actb* knock-down neural cells provide clues regarding altered cell biology and we hypothesise that similar changes occur in vivo. In heterozygous deleted *Actb* brains, an altered signature at the transcript level may suggest druggable targets to ameliorate the pathobiology.

References:.

Grants:.

Conflict of Interest: None declared.

P12.014.D Mildly skewed X-chromosome inactivation as a mechanism for the expression of Allan-Herndon-Dudley syndrome phenotype in a female patient

Bianca Pereira Favilla[1], *Caroline Olivati*[2;3], *Erika Lopes Freitas*[4], *Bibiana Santos*[4], *Maria Isabel Melaragno*[1], *Vera Ayres Meloni*[1;2], *Flavia Balbo Piazzon*[2;5;6]

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Background/Objectives: Allan-Herndon-Dudley syndrome (AHDS) is an X-linked condition characterized by neuropsychomotor development delay, intellectual disability, neurological impairment, and abnormal thyroid hormone profile. It mainly affects males, as female carriers are usually asymptomatic or present only abnormal thyroid blood tests. In rare cases of symptomatic female carriers, X-chromosome abnormalities and skewed X-chromosome inactivation (XCI) may play a role in the expression of the phenotype.

Methods: We describe a 16-year-old female patient with mild intellectual disability, dysmorphic facial features, marfanoid habitus, joint hypermobility, lumbar hyperlordosis, mild thoracic scoliosis, genu recurvatum, high serum levels of 3,3,5´-triiodothyronine (T3), and normal to low levels of thyroxine and thyroid-stimulating hormone (TSH). Karyotyping, whole-exome sequencing, Sanger sequencing, and XCI analysis through Human Androgen receptor assay (HUMARA) were performed.

Results: The proposita and her parents presented normal karyotypes. Whole-exome sequencing revealed a de novo rare missense variant in *SLC16A2*: c.1388C>T:p.Pro463Leu, confirmed by Sanger sequencing. Haplotype analysis using a single nucleotide polymorphism (rs5937843) in cis with the variant showed that the missense variant was in the X chromosome originated from the father. HUMARA revealed the inactivation of the maternal and paternal alleles in ratios of 71% and 29%, respectively. Thus, the chromosome carrying the normal *SLC16A2* allele was preferentially inactive.

Conclusion: Skewed XCI may have played a role in the expression of a mild AHDS phenotype in the patient. Together with *SLC16A2* variants, skewed XCI may be investigated in females with AHDS phenotypic features even when X-chromosome abnormalities are absent.

References:.

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Conflict of Interest: None declared.

P12.016.B In vitro effect of TRAF7 germline and somatic missense variants on its subcellular localization and cell shape

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Background/Objectives: TRAF7 syndrome is an ultra-rare neurodevelopmental disorder characterized by intellectual disability, motor delay, cardiac alterations and dysmorphic features. It is caused by germline mutations in *TRAF7*, which codes for an E3 ubiquitin ligase acting in different signalling pathways. Somatic mutations in this same gene have been associated with tumorigenic processes. Our aim was to explore the effects of germline and somatic missense variants on TRAF7 subcellular localization.

Methods: We selected five *TRAF7* variants (one benign (p.H478Y), 2 tumorigenic (p.L519P, p.N520S), 2 syndromic (p.L519F, p.R655Q)) and introduced them in a *TRAF7* expressing vector by site-directed mutagenesis. We then transfected these constructs into HeLa cells and performed immunocytochemistry to study their effects on TRAF7 subcellular localization. We also assessed colocalization with its partner MEKK3.

Results: Wild-type TRAF7 was found throughout the cytoplasm in aggregate-like structures. Upon transfection with *TRAF7*

pathogenic mutants, aggregate size was larger and cell shape was modified. *TRAF7* syndromic mutations presented an aberrant behaviour, with 86-95% of transfected cells being rounded (compared to 27-37% in WT/benign conditions). Additionally, tumorigenic and syndromic mutations showed higher levels of large protein aggregates in comparison to WT/benign conditions. Finally, TRAF7 and MEKK3 presented a high degree of colocalization, and no differences were noted between *TRAF7* variants.

Conclusion: The pathogenic mutations caused drastic cell shape and TRAF7 subcellular localization modifications after their transfection. These modifications could be used as biomarkers to test the pathogenicity of newly identified variants in patients with an unclear neurodevelopmental presentation.

References:.

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Conflict of Interest: None declared.

P12.017.C Two novel cases of IQSEC2 syndromic mental retardation revealed an intronic variant disrupting expression of different transcripts isoforms

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Background/Objectives: Pathogenic hemizygous or heterozygous mutations in the IQSEC2 gene cause X-linked mental retardation-1 (MRX1) characterized by a variable phenotype. It affects both males and females typically through loss of function in males and haploinsufficiency in heterozygous females. IQSEC2 gene is one of the described genes that scape X chromosome inactivation. It is expressed in neurons and participates in cytoskeletal organization, dendritic spine morphology, and excitatory synaptic organization.

Methods: The diagnostic activity of the SpainUDP (Undiagnosed Rare Disease Program) revealed two unrelated cases, one male and one female, with de novo IQSEC2 variants detected by trio-based whole exome sequencing. The female case had an undescribed frameshift mutation and the male case showed an intronic variant in intron 6, with unknown effect. IQSEC2 gene expression was analyzed by RT-PCR and QT-PCR specific for the three transcripts isoforms.

Results: Expression analysis revealed that the intronic variant created an alternative donor splicing site and an aberrant product, with the inclusion of 19bp leading to a frameshift and premature stop codon. Moreover, quantitative expression of the three different IQSEC2 transcripts in both cases comparing to their progenitors showed a higher reduction of all isoforms in the male, but expression was less reduced in the female case, with no decrease of the short IQSEC2 isoform expression.

Conclusion: Our study allowed us to establish an intronic variant in IQSEC2 as the cause of the disease leading to the diagnosis. Different expression level of the IQSEQ2 isoforms might be implicated in the disease manifestations.

References:.

Grants: SpainUDP Program (ISCIII).

Conflict of Interest: None declared.

P12.018.D Simpson-Golabi-Behmel syndrome type 1: How placental immunohistochemistry can rapidily predict the diagnosis

Giacomo Fiandrino[1], Alessia Arossa[2], Silvia Kalantari[3], Elisa Giorgio[4;5], Francesco Bassanese[6;7], Annachiara Scatigno[2;7], Anna Meroni[2;7], Chiara Melito[2], Stefania Cesari[1], Gioacchino D'Ambrosio[1], Stefania Longo[8], Stefano Ghirardello[8], Tiziana Angelica Figar[8], Marco Paulli[1;4], Arsenio Spinillo[2;7], Enza Maria Valente[4;5], ***Fabio Sirchia****[4;9]*

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Background/Objectives: Glypican-3 (GPC3) is an oncofetal protein involved in cellular signalling, strongly expressed in placenta and absent or diminished in postnatal life, but often increased in human malignancies (i.e. hepatocellular carcinoma and germinal tumours). Germline loss-of-function variants of *GPC3* gene are associated with Simpson-Golabi-Behmel syndrome type 1 (SGBS1), an exceedingly rare recessive X-linked overgrowth disease characterized by typical facial features, congenital abnormalities and an increased risk of tumours during childhood.

Methods: A clinical suspicion of SGBS1 was postulated for a newborn with prenatal history of overgrowth and polyhydramnios, presenting at birth with neonatal weight and length >99th centile, coarse face, iris coloboma, supernumerary nipples and splenomegaly. Prenatal genetic testing for Beckwith-Wiedemann syndrome (the most common genetic overgrowth syndrome) and SNP-arrays resulted normal. In order to support the diagnostic hypothesis, placental GPC3 immunohistochemical expression was investigated. In parallel, we assessed placental GPC3 expression in 20 probands representative of multiple disorders associated with fetal macrosomia and/or placentomegaly, including pregestational/gestational diabetes and placental mesenchymal dysplasia, as well as in healthy controls.

Results: Whole genome sequencing in the proband identified a likely pathogenic maternally inherited missense variant in *GPC3*: c.1645A>G; (p.Ile549Val).

GPC3 immunohistochemistry demonstrated full-thickness negativity on all parenchymal sections of the SGBS1 case. On the contrary, we demonstrated preservation of GPC3 placental antigenicity in all the other overgrowth conditions and in healthy controls.

Conclusion: Evaluation of GPC3 expression in the placenta may prove useful in the differential diagnosis of fetal macrosomia, allowing targeted genetic testing and earlier diagnosis both in prenatal and in neonatal setting.

References: https://doi.org/10.1186/s13023-014-0138-0, https://doi.org/10.14670/HH-16.71.

Grants:.

Conflict of Interest: None declared.

P12.019.A TSC1/TSC2 mosaicism accounts for ~10% of all diagnoses of tuberous sclerosis and genome sequencing fails to identify a third causative gene

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Maitz Silvia[12], Cristina Gervasini[13], Pierangelo Veggiotti[14], Aglaia Vignoli[1;15], Gaetano Bulfamante[1;3], John Carey[2], Maria Paola Canevini[1;15]

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Background/Objectives: Aims: 1. To investigate the molecular basis of tuberous sclerosis complex (TSC) in patients with no mutation identified (NMI); 2. To evaluate the rate of mosaicism in a real-world setting and delineate the phenotype of mosaic patients.

Methods: Pilot study: chromosomal microarray (8x60K), trio genome sequencing (60X), deep-coverage *TSC1/TSC2* NGS (>2000X), and HaloPlex custom capture array of the *TSC1/TSC2* genomic regions on 10 individuals with TSC and NMI. Complete study: deep-coverage *TSC1/TSC2* sequencing on 200 patients and deep phenotyping of those with mosaicism.

Results: Pilot study: we identified mosaic pathogenic variants in *TSC1/TSC2* in 8/10 patients and deep-intronic inherited VUSs in 2/10. Genome sequencing failed to identify causative variants in other relevant genes. Complete study: we identified 24 patients with mosaic pathogenic variants in *TSC1* (n = 2) or *TSC2* (n = 22), defining a rate of mosaicism of 12%. Mosaic variant allele frequency (VAF) was 1%-32% in blood, 2%-35% in saliva. Extensive phenotypic analysis showed that 82% of individuals with mosaic variants displayed normal cognitive level, and the number of several manifestations - although present - was often insufficient to meet diagnostic criteria. Notwithstanding, we observed a high frequency of pulmonary/renal manifestations, which were as severe as those seen in non-mosaic individuals.

Conclusion: We demonstrated for the first time that at least one in 10 individuals with TSC carries a mosaic pathogenic variant in *TSC1/TSC2* and that mosaic patients have a distinctive phenotypic severity. We did not obtain evidence for a third TSC locus. Our findings have implications for surveillance and counselling.

References:.

Grants:.

Conflict of Interest: Angela Peron Italfarmaco, Italfarmaco, Rosa Maria Alfano: None declared, Barry Moore: None declared, Mark Nellist: None declared, Brent Pedersen: None declared, Francesca La Briola Italfarmaco, GW, Italfarmaco, Luigina Spaccini: None declared, Federica Natacci: None declared, Maria Paola Recalcati: None declared, Valentina Chiesa: None declared, Rosangela Arancio: None declared, Ugo Cavallari: None declared, Chiara Vannicola: None declared, Graziella Cefalo: None declared, Maitz Silvia: None declared, Cristina Gervasini: None declared, Pierangelo Veggiotti: None declared, Aglaia Vignoli Italfarmaco, Italfarmaco, Gaetano Bulfamante: None declared, John Carey: None declared, Maria Paola Canevini Italfarmaco, Italfarmaco.

P12.020.B Further delineation of SMG9-related heart and brain malformation syndrome

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Background/Objectives: *SMG9*-deficiency syndrome, (MIM #616920), is a recently described autosomal recessive disorder associated with intellectual disability and multiple malformations. To date, seven affected individuals have been reported. Here, we describe two previously unpublished cases that expand the phenotypic spectrum of *SMG9*-related heart and brain malformation syndrome.

Methods: The proband born with midbrain atrophy, interrupted inferior vena cava. Baby suffered from hypotonia, chronic lung infections, was on continuous positive airway pressure, and died after respiratory cardiac arrest at 25 months of age, he underwent exome sequencing which revealed homozygous variant in *SMG9*. He had another sister diagnosed antenatally to have a banana-shaped cerebellum, scoliosis, a narrow chest, and ectopia cordis with an anterior abdominal wall defect, died soon after birth. Her carrier testing for *SMG9* showed the same homozygous mutation.

Results: Whole exome sequence for the boy reviled homozygous splicing variant in *SMG9* (NM_019108.4: exon7: c.701+4A>G), which was also found in his sister. This is the first time ectopia cordis, jejunal atresia and involvement of brainstem and basal ganglia have been documented.

Conclusion: Since the severe phenotypic presentation observed in the proband's sibling is the first to include ectopia cordis, a defect in the anterior abdominal wall, further characterization of the phenotypic spectrum of SMG9-related heart and brain malformation syndrome is imperative to conclude if a mutation in *SMG9* alone is responsible for these major abnormalities. These new features should inform the management and anticipatory health supervision of children as we learn more about this newly emerging syndrome.

References:.

Grants:.

Conflict of Interest: None declared.

P12.021.C Novel variant in OTUD5 detected in a patient with multiple congenital anomalies-neurodevelopmental syndrome and suggested in a fetus in the same family

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Background/Objectives: The *OTUD5* gene at Xp11.23 encodes ovarian tumor deubiquitinase 5 protein, a deubiquitinating enzyme member of the ovarian tumor family. Recently 3 families with 16 male patients were reported with a new X-linked recessive disorder (Multiple congenital anomalies-neurodevelopmental

syndrome; MCAND; OMIM 301056), arising from pathogenic missense *OTUD5* variants (Tripolszki et al. 2021; Saida et al. 2021). Here we report a 29-year-old male patient with MCAND and a truncating variant in *OTUD5* as well as a male fetus of the same family, a putative carrier of the same variant.

Here we report two additional patients with MCAND and a novel truncating variant in *OTUD5* in the index patient, suggesting that also truncating variants in *OTUD5* are responsible for MCAND and supporting the data on intrafamilial variability.

Methods:.

Results: The index patient presents with severe intellectual impairment, hypotonia, distinctive dysmorphic facial features, intrauterine growth retardation, scoliosis, strabismus, bifid tongue, tetralogy of Fallot, single transverse palmar crease, self-injurious behavior, hypoplasia of corpus callosum, cryptorchidism and short stature. Single exome analysis showed the hemizygous variant *OTUD5*: c.1492C>T; p.(Gln498*).

In 1990 the mother of the patient had a spontaneous abortion at 14 weeks of gestation. The male fetus showed clinical features consisting with MCAND. Unfortunately fetal DNA was no longer available for molecular analysis.

Conclusion: Here we report two additional patients with MCAND and a novel truncating variant in *OTUD5* in the index patient, suggesting that also truncating variants in *OTUD5* are responsible for MCAND and supporting the data on intrafamilial variability.

References:.

Grants:.

Conflict of Interest: None declared.

P12.022.D Fourth patient with metaphyseal chondromatosis with D-2-hydroxyglutaric aciduria caused by a recurrent IDH1 mosaic mutation

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Background/Objectives: Mutations in the isocitrate dehydrogenase (IDH) enzyme *IDH1* lead to elevated levels of D-2-hydroxyglutarate (D-2HG) by changing the enzymatic activity of the IDH enzyme. Mutations of the *IDH1* gene as somatic mosaics cause metaphyseal chondromatosis with urinary excretion of D-2-hydroxy-glutaric acid (MC-HGA). MC-HGA is a rare disorder characterized by metaphyseal disorganization, chondrodysplasia, urinary excretion of D-2HG and cerebral involvement.

Methods: We report on a 7-months-old girl with MC-HGA. Prenatal ultrasound revealed shortened long bones of the upper and lower extremities, intrauterine growth retardation and an enlargement of the subarachnoid space. Echocardiography showed a patent foramen ovale and tricuspid insufficiency. The patient had generalized hypotonia, motor delay and flexion contractures of the right 3rd and 4th fingers. She presented with short stature and a relatively large head circumference. Dysmorphic features included low-set ears, epicanthus, short nose with slightly anteverted nostrils, long philtrum, small upper lip, retrognathia and a prominent forehead. The girl suffered from hearing loss. Cerebral MRI showed enlarged subarachnoid space, immature and altered gyration, subdural hygroma, small pons, poorly developed tentorium cerebelli and falx cerebri as well as cerebral atrophy. Urine analysis showed an excessive excretion of hydroxy-glutaric-acid.

Results: Whole exome sequencing showed a de novo pathogenic variant c.395G>A, p.(Arg132His) in the *IDH1* gene in somatic mosaicism. The variant was found in 59 of 152 reads (38,8%). This missense mutation is predicted to be likely pathogenic.

Conclusion: The phenotype of our patient overlaps with the phenotype of the previously reported three patients in the literature and thus marks the fourth case.

References:.

Grants:.

Conflict of Interest: None declared.

P12.023.A Whole exome sequencing (WES) and functional analyses suggest synergistic effects of deleterious variants in two candidate genes for Poland Syndrome

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Background/Objectives: Poland Syndrome (PS, OMIM 173800) is a rare congenital condition characterized by pectoral muscle agenesis/hypoplasia variably associated with ipsilateral thoracic and/or upper limb anomalies. Most cases of PS are sporadic, familial recurrence has been observed with phenotypic heterogeneity. Different inheritance patterns have been reported and polygenic/multifactorial mechanisms have been hypothesized in some cases. The genetic etiology of PS remains unknown. In this study we aimed at investigating the genetic mechanisms underlying PS.

Methods: A cohort of 30 PS patients were analysed by WES, and potentially deleterious variants prioritized by custom filtering strategies including the use of Oligogenic Resource for Variant Analysis Platform (ORVAL). Functional analyses of identified variants included in vitro mutagenesis followed by cell imaging and gene reporter assays.

Results: In a familial case with pectoral muscle hypoplasia, thoracic and mammary gland anomalies deleterious missense variants were found in two genes: *MUSTN1*, expressed in the nucleus and involved in the development of pectoral muscle and cartilage, and *PARD3B* localized at the cell membrane and involved in cell polarity, and mammary gland development. Both mutant proteins displayed altered cell localization likely impairing their function. One further PS sporadic patient, with a similar complex phenotype, showed a 5'UTR variant of *MUSTN1* causing a reduced expression of *MUSTN1*.

Conclusion: These results suggest that PS may be due to a digenic inheritance mechanism with the *MUSTN1* variant responsible for the pectoral muscle defects and *PARD3B* substitution for the asymmetry and mammary gland anomalies.

References: Romanini et al., Semin Pediatr Surg. 2018.

Grants:.

Conflict of Interest: None declared.

P12.024.B A fatal progeroid syndrome caused by a recessive RAF1 loss-of-function mutation

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Background/Objectives: Somatic and germline gain-of-function point mutations in RAF, the first oncogene to be discovered in humans, delineate a group of tumor-prone syndromes known as RASopathies. In this study, we document the first human phenotype resulting from the germline loss-of-function of the proto-oncogene RAF1 (a.k.a. CRAF).

Methods: Whole exome sequencing followed by Sanger sequencing were performed. HEK293T wildtype and mutant were grown in Dulbecco's modified Eagle's medium. RAF1 mutant cells were generated with CRISPR/Cas9 method. Cell death was assessed by adding SYTOX® Green Nucleic Acid Stain. For western blotting, cells or Xenopus embryos were lysed in RIPA buffer supplemented with protease inhibitor cocktails and phosphatase inhibitors. RAF1 mutants mRNA were injected into 4-cell stage Xenopus laevis embryos, and harvested at various developmental stages for subsequent protein extraction or WISH analyses.

Results: In a consanguineous family, we uncovered a homozygous p.Thr543Met mutation segregating with a neonatal lethal progeroid syndrome with cutaneous, craniofacial, cardiac and limb anomalies. Structure-based prediction and functional tests using human knock-in cells showed that threonine 543 is essential to: 1) ensure RAF1's stability and phosphorylation, 2) maintain its kinase activity towards substrates of the MAPK pathway and 3) protect from stress-induced apoptosis. When injected in Xenopus embryos, unlike RAF1WT, mutant RAF1T543M failed to phenocopy the effects of overactive FGF/MAPK signaling confirming its hypomorphic activity.

Conclusion: Collectively, our data disclose the genetic and molecular etiology of a novel segmental progeroid syndrome, which highlights the importance of RAF1 for human development and homeostasis.

References:.

Grants:.

Conflict of Interest: None declared.

P12.025.C ATRIP-deficient patient expands molecular and clinical spectrum of Seckel syndrome

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Background/Objectives: We report the second ATRIP-deficient patient clinically diagnosed with Seckel Syndrome (SS). The ATRIP protein is required for ATR stabilization by complex formation and is crucial for localization of the ATR-ATRIP complex to regions of DNA damage and ATR activation. Besides the typical clinical SS characteristics (primary dwarfism, facial dysmorphia, skeletal abnormalities, microcephaly and mental retardation), our patient suffers from an immunodeficiency. However a link between ATRIP and the immune system was not previously reported.

Methods: Whole exome sequencing (WES), transcriptomics, western blot, micronucleus assays, flow cytometry and single cell RNA-Sequencing.

Results: The patient is homozygous for a splice variant (c.829+5G>T) in ATRIP leading to out-of-frame exon 5 skipping. Western blot showed absence of ATRIP protein and analysis of micronuclei in response to DNA damage by mitomycin C and ionizing radiation revealed defective DNA repair. Downstream substrates of the ATR-ATRIP complex are currently investigated. WES ruled out a pathogenic variant in 460 genes linked to inborn errors of the immune system. Immunophenotyping reveals low absolute B cell numbers, aberrant T cell subsets, decreased plasmacytoid dendritic cells, low CD56dimCD16+ natural killer cells and increased low density neutrophils. Additionally, a first look at scRNA-Seq data suggests a recombination deficiency during B and T cell development, as was published for ATR-deficient SS patients (1), and allows to further elaborate the immune phenotype.

Conclusion: We expanded the molecular and clinical spectrum of SS and further validations will provide insights into the link with the immune system and will contribute to the disease mechanism.

References: (1) https://doi.org/10.1084/jem.20050595.

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Conflict of Interest: None declared.

P12.026.D Differential alternative splicing analysis to link variation in ZRSR2 to a novel syndrome with oral, digital and brain anomalies

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Background/Objectives: ZRSR2, located on Xp22.2, is part of the minor spliceosome complex which recognises U12-type introns representing 0.35% of human introns. Minor spliceosome defects have been associated with developmental disorders. Somatic mutations in *ZRSR2* are found in myelodysplastic syndromes, germline variation in *ZRSR2* has not been implicated in impaired human development yet.

Methods: We describe a 24-months-old boy presenting upper limb bilateral postaxial polydactyly, doubled first ray of the feet, a tongue nodule, seizures, pituitary abnormalities and polymicrogyria. A maternal uncle died neonatally with holoprosencephaly, polydactyly and ambiguous genitalia. A son of a maternal aunt of the mother died prenatally with brain and limbs anomalies. Unfortunately DNA of these male relatives was not preserved. Whole exome sequencing and segregation analysis of 6

informative males was performed, followed by whole transcriptome RNA-SEQ on fibroblasts from the extra digit and tongue nodule, and on EBV cell lines of the index and mother.

Results: WES showed a maternally inherited frameshift c.1207_1208delAG (p.Arg403Glyfs*24) in the last exon of *ZRSR2*, compatible with X-linked recessive inheritance. This variant is absent in reference databases, but has been described as a variant of unknown significance in a family with 5 male foetuses with holoprosencephaly. Whole transcriptome differential expression and alternative splicing analysis of minor spliceosome gene targets showed a similar effect on U12-dependant splicing as seen in somatic *ZRSR2* mutations in myelodysplastic syndrome.

Conclusion: Genetic and functional data associate this *ZRSR2* variant to a novel syndrome with variable expression of oral, digital and brain (holoprosencephaly) anomalies.

References:.

Grants: KUL C24M/19/075.

Conflict of Interest: None declared.

P12.027.A Assessing the burden of rare CNVs on miRNA genes in CAKUT

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Background/Objectives: Rare copy number variants (rCNVs) are the common genetic cause of Congenital Anomalies of the Kidney and Urinary Tract (CAKUT).[1] miRNAs located in rCNVs represent well-founded functional variants for human CAKUT research. However, the impact of rCNVs on miRNA genes in CAKUT is unknown. Thus, burden assessment was performed to identify chromosomes with non-random representation of miRNA genes in rCNVs associated with CAKUT.

Methods: A comprehensive literature mining of rCNV regions associated with CAKUT was performed. The total cumulative length of rCNVs per chromosome was the sum of corresponding CNV-DNA regions, taking into account overlapping. Mapping of miRNAs onto cumulative rCNV regions gave counts of affected miRNA loci. The correlation analysis was performed between the number of miRNA genes overlapping rCNVs, and the fractional lengths of cumulative rCNVs regions in relation to the chromosome size.

Results: A statistically significant positive correlation was observed for duplications and deletions respectively (Spearman correlation $p < 0.0001$, $r = 0.9$, $r = 0.8$). However, a deviation from the best fit line for chromosome 16, for both rare duplications and deletions, was observed due to the high overrepresentation of miRNA genes in identified rCNVs.

Conclusion: The current finding of the high overall burden of rCNVs on miRNA genes in chromosome 16 suggests that miRNAs located on this chromosome could serve as candidates for the investigation of miRNA role in CAKUT development.

References: Verbitsky, M. et al.(2019) J.Nat Genet. 51,117-127.

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Conflict of Interest: None declared.

P12.028.B Heart defects, oral clefts, and polydactyly caused by novel compound heterozygous variants in WDPCP gene involved in ciliogenesis

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Background/Objectives: Ciliopathies are a group of disorders with overlapping phenotypes, caused by dysfunctional cilia. WDPCP is a planar cell polarity protein, which is involved in the recruitment of molecules essential for ciliogenesis. *Wdpcp* mutant mouse exhibited developmental defects including anophtalmia, polydactyly, kidney cysts, heart defects, and facial clefts. In humans, only four patients with biallelic pathogenic variants in *WDPCP* gene have been reported, presenting with congenital heart defects, hamartomas of tongue, and polysyndactyly (CHDTHP), or Bardet-Biedl Syndrome 15.

Methods: In trio-whole exome sequencing (WES) was performed to identify candidate variants.

Results: We describe a 2-year-old girl born to non-consanguineous parents. Pregnancy was achieved by ICSI due to male infertility. Prenatal ultrasound revealed multiple anomalies including heart defects, cleft lip/palate, and polydactyly. Chromosomal microarray analysis and an Ellis-van Creveld Syndrome gene panel were negative. Delivery occurred at term and the newborn presented left cleft lip and palate, postaxial polydactyly of the left hand and hallux duplication bilaterally. Echocardiography revealed a large atrial septal defect and left atrioventricular regurgitation. WES identified two novel compound heterozygous variants in *WDPCP* gene: NM_001354044.1:c.1486T>G, p.(Cys496Gly), and NM_001354044.1:c.852_860delinsG, p.(Asp285Alafs*4).

Conclusion: Only two patients with CHDTHP have been reported so far. Interestingly, our patient is the first one presenting oral clefts, resembling the phenotype of the *Wdpcp* knockout mice. This study further expands the molecular and phenotypic spectrum of this rare and still poorly known disorder, contributing to a deeper understanding of WDPCP function in ciliogenesis. To the patient´s family, molecular diagnosis allowed proper genetic counselling and informed reproductive choices.

References:.

Grants:.

Conflict of Interest: None declared.

P12.029.C C20orf24: a potential novel gene responsible for Cerebrofaciothoracic Dysplasia

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Background/Objectives: Cerebrofaciothoracic Dysplasia (CFTD) is a rare syndrome characterized by intellectual disability, dysmorphic facial features and skeletal anomalies. Biallelic loss of function variants in the *TMCO1* were found to be responsible for CFTD. However, due to no mutations in the coding region of

TMCO1 could be identified in some patients, it has been considered that CFTD could be a genetically heterogeneous disorder.

Methods: The molecular etiology of CFTD was investigated using whole exome sequencing in Illumina NextSeq 550 platform.

Results: A 3 years old boy was the first child of consanguineous healthy parents. He had global developmental delay and dysmorphic facial features. Skeletal survey revealed craniosynostosis and rib anomalies. Regarding clinical and radiological findings CFTD was considered in the patient. Any *TMCO1* variants could not be found via sequence analysis. WES revealed a homozygous variant (c.75G>A, p.Trp25Ter) in *C20orf24* gene. In *C20orf24* gene, the c.75G>A variant which causes a premature termination codon at 25th position of *C20orf24* mRNA, a highly conserved amino acid during evolution, has not been reported in public databases to date.

Conclusion: C20orf24 is a protein coding gene, and its function has not yet been fully elucidated. Recently, Lewis and Hegde suggested that TMCO1 and C20orf24 seemed to be interaction partners to form an Oxa1 superfamily insertase complex. Regarding studies analyzing structure of integral membrane protein biogenesis, we consider that biallelic variants in the *C20orf24* gene may cause similar phenotype with loss of function *TMCO1* variants. However, functional studies are needed to show the relationship between *C20orf24* variants and CFTD.

References:.

Grants:.

Conflict of Interest: None declared.

P12.030.D Pathogenicity assessment of DISP1 variants associated with midline anomalies spectrum

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Background/Objectives: Holoprosencephaly (HPE; MIM# 236100) results from incomplete midline division of the prosencephalon. The clinical spectrum is very wide, ranging from severe HPE with cyclopia to HPE microforms with midline craniofacial defects.

HPE genes belong to the Sonic Hedgehog (SHH) pathway, a complex molecular cascade allowing ventralization during early central nervous system development. DISP1 is a positive factor necessary for the efficient secretion of the SHH morphogen and the establishment of its concentration gradient along the midline of the neural tube. We describe the first cohort of HPE patients with *DISP1* variants.

Methods: We describe 22 individuals from 18 unrelated familes, regrouping *DISP1* variants retained during molecular diagnosis in our lab, along with collaborative and previously published findings.

Results: 13 patients have microform HPE or lobar HPE, 8/13 with orofacial cleft. 4/13 have single median incisor and piriform aperture stenosis, a rare association described previously in patients with SHH variants. 9 additionnal patients from 6 families present with severe HPE (alobar or semilobar), 2/9 with cyclopia, 3/9 with orofacial cleft.

We further delineate the clinical spectrum of *DISP1*-related HPE, provide insight into the underlying pathomechanism and describe various factors modulating the phenotypic output of *DISP1* variants.

Conclusion: DISP1 is major gene of holoprosencephaly. Autosomal recessive inheritance and haploinsufficiency lead to HPE microforms. Severe HPE can be caused by oligogenic transmission.

The accumulation of rare and pathogenic variants in different genes negatively impact SHH and lead to HPE, more or less severe depending on the early role of the genes involved: "SHH-disorder".

References:.

Grants:.

Conflict of Interest: None declared.

P12.031.A Losses and gains including PURA cause reciprocal ID syndromes: results of a large-scale study on 5q31.3 copy number variations

Margot Reijnders[1], *Laura Hamilton*[2;3], *Diana Baralle*[2;3], *David Hunt*[2;3], *Nicole de Leeuw*[4]

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Background/Objectives: Deletions in the 5q31.3 region have been occasionally reported to cause an intellectual disability (ID) syndrome. Previously, we reported a cohort of patients with PURA-syndrome caused by de novo nucleotide variants in *PURA*, located at 5q31.3.

Methods: To further investigate the contribution of *PURA* in copy number variants (CNVs) in the 5q31.3 region, we (1) re-analyzed available CNV data of ID patients available in Southampton (UK) and Nijmegen/Maastricht (Netherlands), (2) asked genetic centers in the UK and the Netherlands to re-evaluate diagnostic genetic data on 5q31.3 CNVs and (3) contacted clinicians of patients reported in the DECIPHER database.

Results: This led to the identification of eight patients with a de novo loss and eight patients with a de novo gain including *PURA*. Depending on the size and involvement of neighboring ID genes such as *KDM3B*, *PPP2CA* and *PITX1*, losses lead to severe ID, feeding difficulties with low weight, multiple congenital malformations and/or (prenatal) death. We show that gains cause a milder reciprocal syndrome with moderate ID, aggressiveness and obesity as common features. Severe laryngomalacia is present in 3/8 microdeletion patients and in one patient reported in literature, but has never been observed in patients with PURA-syndrome before. By analyzing the smallest region of overlap, we suggest *NRG2* and *UBE2D2* as candidate genes for the development of laryngomalacia.

Conclusion: Close international collaboration led to the clinical delineation of 5q31.3 losses and identification of a novel,

reciprocal ID syndrome caused by 5q31.3 gains, showing the power of data sharing for studies on rare syndromes.

References:.

Grants:.

Conflict of Interest: None declared.

P12.032.B Homozygous NRP1 truncating variant in a multiplex family with conotruncal heart defects, lymphatic malformations and genitourinary anomalies

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Background/Objectives: Neuropilins (NRP1 and NRP2) are cell surface proteins conserved across species, acting as coreceptors for signal proteins including members of semaphorin and VEGF families. They participate in a multitude of processes: development of the cardiovascular system, (lymph)angiogenesis, neuronal patterning and immune function. NRP1 deficient mice display embryonic lethality due to a severely abnormal cardiovascular phenotype, resembling those of Vegf and Vefgr2 knockouts. Shaheen et al. (2015) reported a homozygous null mutation in the proband of a multiplex family with truncus arteriosus.

Methods: Exome sequencing in the proband was performed in a consanguineous multiplex family with conotruncal anomalies. Affected and unaffected family members were screened for the candidate variant to reveal segregation.

Results: We present a 32-day-old girl born to 1st cousins once-removed, with a severe congenital heart defect comprising atrial and ventricular septal defects with patent ductus arteriosus, left microphthalmia with cystic lymphatic malformation, and renal anomalies. Weight was 3500 g (14p), height 52.5 cm (31p) and head circumference 37 cm (46p). Cranial MRI was unremarkable. The parents had a medical abortion due to a huge parapharyngeal lympangioma, and two children deceased due to severe coarctation of the aorta and tetralogy of Fallot, respectively. Chromosomal array was normal. WES revealed homozygous nonsense c.1213C>T; p.(Arg405*) in *NRP1* [NM_003873], segregating with the phenotype in unaffected parents and sister, and one deceased affected sibling. Parental echocardiograms were normal.

Conclusion: This is the second report of a family with homozygous truncating variants in NRP1, further supporting *NRP1* as a human disease gene.

References: https://doi.org/10.1016/s0092-8674(00)80534-6, https://doi.org/10.1016/s0092-8674(00)81402-6, https://doi.org/10.1073/pnas.022017899, https://doi.org/10.1136/jmedgenet-2015-102992.

Grants: None.

Conflict of Interest: None declared.

P13 CANCER GENETICS

P13.001.C Development of genomic instability score for ovarian cancers from a limited panel of genes

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Background/Objectives: High-grade serous ovarian cancers with a deficiency of homologous recombination DNA repair (HRD) are sensitive to the combination of bevacizumab and olaparib as maintenance therapy. HRD status is determined by mutational scars within the tumor genome. To date, only the method developed by the commercial company Myriad Genetics® (MG) has been clinically validated to identify HRD status. In this study, we developed a new method to identify HRD status and suitable with the most of molecular biology laboratory constraints.

Methods: We computed a score from the genomic scars, i.e. chromosomic breaks, genomic deletion/duplication and allelic imbalance of polymorphisms. We used sequencing data from limited panel of 127 genes to detect these events. The score training was performed on a collection of 146 samples from ovarian cancer with HRD status previously defined by MG. Among these samples, 32 samples were managed and sequenced by another laboratory to assess the robustness of our method.

Results: Our new score reached an accuracy of 92.46 % (Chi-Square p-value < 0.00001) compared to MG HRD status, with a sensitivity of 95.38 % and specificity of 90.12 %. The pearson coefficient between the two scores was 0.810 (p-value < 0.00001). We observed similar results for the 32 samples analyzed by the second platform.

Conclusion: Our score showed a significant correlation with MG data, confirmed on data from different platforms. Thus, our method is robust enough to be deployed in other molecular biology laboratories. The validation of the method on clinically characterized samples is in progress.

References: No references.

Grants: No grants.

Conflict of Interest: Leman Raphael Centre François Baclesse, Etienne Muller CERBA, Nicolas goardon Centre François Baclesse, Imène Chentli Centre François Baclesse, Aurore Tranchant Centre François Baclesse, Angelina Legros Centre François Baclesse, Laurent Castera Centre François Baclesse, Alain Morel Institut de Cancérologie de l'Ouest, Christel Brunet Hôpital Pitié-Salpêtrière, Véronique Bocly Hôpital Pitié-Salpêtrière, Eric Fernandez Hôpital Pitié-Salpêtrière, Florence Coulet Hôpital Pitié-Salpêtrière, Dominique Vaur Centre François Baclesse.

P13.002.D Imprinting relaxation of an ovarian dysgerminoma in a patient with Prader-Willi syndrome

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Background/Objectives: Prader-Willi syndrome (PWS) is a rare intellectual disability syndrome characterized by dysmorphic features, short stature, obesity, hypotonia, hyperphagia and hypogonadism. PWS is caused by lack of expression of paternally inherited imprinted genes on chromosome 15q11-q13. Loss-of-imprinting has previously been reported in a testicular seminoma of a patient with PWS caused by maternal UPD[1]. Here, we present the genetic and microscopic findings of an ovarian tumour in a 13-year-old girl with PWS due to a paternal 15q11.2-13 deletion.

Methods: Microscopic examination of tumour tissue including immunohistochemical staining was performed. Tumour and germline DNA were analysed with 30X whole genome sequencing and germline genome was evaluated for pathogenic variants in 153 known childhood cancer predisposition genes. Methylation sensitive MLPA was carried out to assess the methylation status in the tumour-DNA at the PWS locus compared to a control imprinted region (11p15).

Results: Tumour morphology and immunohistochemical staining were consistent with a dysgerminoma. Additionally, bilateral microscopic sex-cord stromal tumours were found. MLPA analysis revealed that methylation was reduced by 50% in the PWS locus in the tumor compared to blood, while preserved in other imprinted regions. A somatic pathogenic activating KIT mutation was detected in the tumor (NM_000222.3:c.1676T>G AF:20%). No additional germline aberrations were found.

Conclusion: We present a second case of locus-specific loss-of-imprinting in a germ-cell tumour from a patient with PWS and propose imprinting relaxation as a possible mechanism of carcinogenesis in PWS.

References: [1] Eldar-Geva T, et al. Molecular Genetics and Genomic Medicine. 2018;6(5).

Grants: The Swedish Childhood Cancer Fund.

Conflict of Interest: None declared.

P13.003.A Primary mediastinal large B-cell lymphoma is hallmarked by large-scale copy-neutral loss of heterozygosity

Stefania Tuveri[1], *Koen Debackere*[2], *Lukas Marcelis*[3], *Nicolas Dierckxsens*[1], *Jonas Demeulemeester*[1], *Eftychia Dimitriadou*[1], *Daan Dierickx*[4], *Pierre Lefesvre*[5], *Karen Deraedt*[6], *Carlos Graux*[7], *Lucienne Michaux*[1], *Jan Cools*[1], *Thomas Tousseyn*[3], *Joris Vermeesch*[1], *Iwona Wlodarska*[1]

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Background/Objectives: Development of primary mediastinal B-cell lymphoma (PMBL), an aggressive subtype of non-Hodgkin lymphoma, is driven by cumulative genomic aberrations. To identify the driver mutational events, we screened PMBL cases by SNP arrays.

Methods: PMBL samples were investigated by SNP arrays (Illumina HumanCytoSNP-12v2.1 BeadChip), fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), whole-exome sequencing (WES) and whole-genome sequencing (WGS) [NovaSeq 6000 (Illumina), Oxford Nanopore Technology sequencing].

Results: The screen uncovered an extreme burden of copy-neutral loss of heterozygosity (CN-LOH) in PMBL which distinguishes this tumour from other B-cell malignancies, including the biologically related diffuse large B-cell lymphoma (respectively on average per patient 4.04 and 1.8). We identified large-scale CN-LOH lesions in 90.9% (30/33) of diagnostic PMBLs and both investigated PMBL-derived cell lines. The cohort showed 133 extra-large (25.3-248.4 Mb) CN-LOH lesions affecting up to 14 chromosomes per case. Notably, CN-LOH stretches non-randomly clustered on chromosome 6p (60%), 15 (37.2%) and 17q (40%), and frequently co-occurred with homozygous mutations in MHC I (6p21), B2M (15q15) and GNA13 (17q23) genes, as yielded by preliminary whole-exome/genome sequencing data.

Conclusion: Altogether, our findings implicate large-scale CN-LOH as a novel mutational process contributing to the molecular pathogenesis of PMBL. The prevalent occurrence of segmental CN-LOH in a heterozygous diploid context, alongside the lack of common CNVs and/or recurrent scars in regions flanking CN-LOH regions revealed by long-read sequencing, points to a key role of mitotic homologous recombination. This mechanism usually follows DSB and likely acts as an errant DNA repair mechanism leading to CN-LOH.

References:.

Grants:.

Conflict of Interest: Stefania Tuveri Research Foundation-Flanders (FWO 1S74420N), Koen Debackere: None declared, Lukas Marcelis: None declared, Nicolas Dierckxsens: None declared, Jonas Demeulemeester Research Foundation-Flanders (FWO 12J6921N), Eftychia Dimitriadou: None declared, Daan Dierickx Kom op tegen Kanker, Pierre Lefesvre: None declared, Karen Deraedt: None declared, Carlos Graux: None declared, Lucienne Michaux: None declared, Jan Cools: None declared, Thomas Tousseyn Mandate for Fundamental and Translational Research from the "Stichting tegen Kanker" (2°14-083), Joris Vermeesch KULeuven grant C1/018, Iwona Wlodarska: None declared.

P13.004.B Overview of cancer predisposition syndromes in a national, unselected cohort of 836 children with a neoplasm

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Background/Objectives: The diagnostic approach of cancer predisposition syndromes (CPSs) in children with cancer is shifting from a phenotype-driven approach towards a genotype-first approach. To decide on best practice in CPS diagnostics, it is essential to evaluate the yield of universal germline sequencing and to compare it with targeted genetic testing based on clinical selection. However, a reliable comparison is difficult since recent reports on a phenotype-driven approach in large, unselected childhood cancer cohorts are lacking.

Methods: Medical records of newly diagnosed children with cancer in the Netherlands between 01/06/2018 and 31/12/2019 were screened for medical history and clinical genetic assessment. In this period, it was standard practice that pediatric oncologists checked for characteristics of CPSs and selected children for referral to clinical geneticists.

Results: In 72/836 patients (8.6%) a CPS was identified (26 different conditions), of which the majority (96%) was identified

by a phenotype-driven approach. Down syndrome and NF1 were the most common CPSs diagnosed. In 42/72 patients (58%) a CPS was identified after these children had developed a neoplasm. The specific type of neoplasm was the most frequent indicator for referral to a clinical geneticist and targeted genetic testing, whereas family history played a small role.

Conclusion: The mostly phenotype-driven diagnosis of CPSs in our unselected cohort revealed a CPS prevalence similar to that in earlier genotype-based studies, but the spectrum of CPS diagnosis is clearly different. This study can be used as a reference cohort for future genotype-driven studies.

References: NA.

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Conflict of Interest: Jette Bakhuizen JJB was funded by the KiKa Foundation (project number 355)., Saskia MJ Hopman: None declared, Machteld I Bosscha: None declared, Charlotte J Dommering: None declared, Marry M van den Heuvel-Eibrink: None declared, Janna A Hol: None declared, Lennart A Kester: None declared, Marco J Koudijs: None declared, Karin PS Langenberg: None declared, Jan LC Loeffen: None declared, Annette C Moll: None declared, Max M van Noesel: None declared, Stephanie E Smetsers: None declared, Johannes HM Merks: None declared, Roland Kuiper: None declared, Marjolijn Jongmans: None declared.

P13.005.C constitutional mismatch repair deficiency (cmmrd) presentation in two unrelated saudi patients with early onset malignancies

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Background/Objectives: Biallelic germline mismatch repair gene mutations are rare events that cause constitutional mismatch repair deficiency (CMMRD) syndrome. They frequently go undiagnosed and often presents with fatal pediatric malignant brain tumors, early onset colorectal cancer, leukemia, lymphoma, and other malignancies. CMMRD may also present with non-neoplastic features such as cafe au lait macules (CALMs).

Methods: Case series of two cases of CMMRD.

Results: We present two cases of CMMRD without prior established diagnosis. The cases were of two 13-year-old children who presented with Neurological and/or gastrointestinal symptoms, skin findings and suggestive family history of Lynch syndrome. Both cases were the product of consanguineous marriages. The first case of a 13-year-old boy, who has unfortunately passed away due to a high-grade brain glioma, and his testing revealed a homozygous pathogenic variant in MSH6 (c.2772_2773del). While the second case, is of a 13-year-old girl who has locally advanced rectal cancer has revealed a homozygous pathogenic variant in PMS2 (c.1376C>G), she has a brother with multiple CALMs and a sister that passed away of a brain tumor at the age of 3 but she was not tested beforehand.

Conclusion: We hope to raise awareness of CMMRD and its phenotype. Such awareness is needed especially in regions where consanguinity is prevalent, in aims to identify similar cases and to provide familial counseling, surveillance and timely intervention. Prompt recognition and prevention is crucial due to its early age of presentation and the aggressiveness of the disease.

References: The abstract does not contain references.

Grants: The abstract does not contain grants.

Conflict of Interest: None declared.

P13.006.D Constitutional mosaicism: a critical issue in the definition of BRCA inherited cancer risk

Elena Tenedini[1;2], *Simonetta Piana*[3], *Angela Toss*[1], *Marco Marino*[2], *Elena Barbieri*[4], *Lucia Artuso*[2], *Marta Venturelli*[4], *Elisa Gasparini*[5], *Vincenzo Dario Mandato*[6], *Isabella Marchi*[4], *Sara Castellano*[1], *Mario Luppi*[1;4], *Tommaso Trenti*[2], *Laura Cortesi*[4], *Enrico Tagliafico*[1;2]

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Background/Objectives: Low-frequency constitutional variants represent alterations that inform the personal and familiar inherited cancer risk definition and precision medicine cancer strategies.

Methods: Following the standard procedure for BRCA diagnostic testing, peripheral blood and ovarian cancer FFPE sections were collected from a woman with a diagnosis of high-grade serous carcinoma. The tissue's DNA was NGS-sequenced for BRCA genes and Sanger confirmation of the pathogenic variant was assessed in the blood. A second blood draw, a buccal swab along with sections from her previous triple negative breast carcinoma and from normal nasal mucosa were NGS-sequenced. Targeted Sanger sequencing was performed in parents and offspring.

Results: The DNA from the patient's ovarian carcinoma was sequenced and a BRCA1 nonsense pathogenic variant was identified. Its presence was confirmed in her peripheral blood, though with lower-than-expected heterozygous frequency. Further analyses in secondary normal tissues revealed the patient as a constitutional mosaic for this variant. In addition, they showed that both her breast and ovarian neoplastic tissues harbored this variant with high frequency. A cascade screening of family members revealed that both parents were negative, but one of the daughters was a previously undiagnosed heterozygous carrier.

Conclusion: Constitutional mosaicism is a renowned mechanism for multiple hereditary cancer-associated genes and enables access to personalized therapies and preventive cancer strategies. To improve the current standard of constitutional analysis, we propose a new algorithm for the BRCA diagnostic routine to increase the sensitivity of germinal assessment and decrease the number of false negatives when pathogenic or likely pathogenic variants occur at low frequencies.

References:.

Grants:.

Conflict of Interest: None declared.

P13.008.B Yield of cancer surveillance in PTEN Hamartoma Tumour Syndrome

Nicoline Hoogerbrugge[1], *Meggie Drissen*[1], *Kevin Kwinten*[2], *Alma Hoxhaj*[3], *Dorien Van der Biessen-van Beek*[4], *Tanya Bisseling*[4], *Anne van Altena*[2], *Joanne de Hullu*[2], *Iris Nagtegaal*[5], *Romana Teodora Netea-Maier*[6], *Martin Gotthardt*[3], *Ritse Mann*[3], *Janet Vos*[1]

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Background/Objectives: Patients with PTEN Hamartoma Tumour Syndrome (PHTS) are at high hereditary cancer risk and advised surveillance for breast (BC), endometrial (EC), thyroid (TC) and colorectal (CRC) cancer. Current PHTS guidelines are expert opinion-based only. We aimed to assess the yield of cancer surveillance.

Methods: A single-institution retrospective cohort study including adult PHTS patients between 2005-2021.

Results: BC: 39 women, median age first examination 38 yr, underwent 156 annual surveillance rounds with MRI and mammography. BC was diagnosed in 7/39 women (CDR: 45/1000 rounds) and benign breast lesions in 18/39 women.

EC: 25 women, median age first examination 39 yr, underwent 93 rounds with annual trans vaginal ultrasound, and endometrial biopsy. No EC was diagnosed. Endometrial hyperplasia with and without atypia was diagnosed in 28%.

TC: 85 patients, median age first examination 36 yr, underwent 324 annual surveillance rounds with ultrasound. TC was diagnosed in 2/85 patients (age 17, 22) and another 2/85 had a thyroid adenoma. Nodular progression occurred in 16%.

CRC: 35 patients, median age first examination 45 yr, underwent 58 rounds with max. 5 yearly colonoscopy. CRC was diagnosed in 1/35 patient (age 41), and no advanced adenomas were found. Polyps were detected in 30/35 (86%), including hamartomas (13/30), non-advanced adenomas (14/30) and ganglioneuromas (13/30).

Conclusion: Our unique data show that surveillance in PHTS contributes to early detection of cancer. Our findings support surveillance for BC, EC,TC and CRC, and offer guidance to optimize the age and frequency of current surveillance recommendations for this rare disease.

References: None.

Grants: None.

Conflict of Interest: None declared.

P13.009.C POLE and POLD1 germline variants in familial glioma

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Background/Objectives: Pathogenic variants in the DNA polymerase genes *POLE* and *POLD1* cause polymerase proofreading-associated polyposis, a dominantly inherited disorder with an increased risk of colorectal carcinomas and other tumors. Tumors with *POLE/POLD1* variants, particularly in the exonuclease domain, commonly show an accumulation of somatic mutations, making them susceptible to checkpoint blockade immunotherapy. Here, we explored the role of *POLE/POLD1* germline variants in glioma predisposition.

Methods: Whole-exome sequencing was performed on germline DNA of 53 tumor families with at least one glioma case each. For genotype-phenotype correlations, gliomas from patients with *POLE/POLD1* germline variants are being characterized with respect to histology, immunophenotype, mutational burden and signature. Cellular assays are being performed to investigate the mutational rate in *POLE/POLD1*-deficient versus wildtype cells, and upon stable expression of *POLE/POLD1* variants versus wildtype.

Results: Rare heterozygous *POLE/POLD1* missense variants predicted to be deleterious were detected in eight patients diagnosed with glioblastoma, astrocytoma, or oligodendroglioma from 7/53 (13%) families, co-segregating with the tumor phenotype in both families with available DNA from two tumor patients. The other tumor types diagnosed in these families were breast, colorectal, lung, prostate and uterus cancer, and meningioma. In primary gliomas of *POLE/POLD1* variant carriers, enlarged nuclei or multinucleated cells were observed in 3/8 cases, and evidence for hypermutation in 3/7 cases. Immunophenotyping is ongoing. An increased mutation rate in *POLE*-deficient versus *POLE*-wildtype LN-229 glioblastoma cells was observed in an *HPRT1* mutation assay. The effects of the *POLE/POLD1* variants are being investigated.

Conclusion: *POLE/POLD1* variants may predispose to glioma and render patients susceptible to immunotherapy.

References:.

Grants:.

Conflict of Interest: None declared.

P13.010.D Investigating genetic susceptibility to male breast cancer by multigene panel testing: an Italian population-based case-control study

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Background/Objectives: About 20% of male breast cancer (MBC) patients report BC family history (FH) and personal history (PH) of cancer other than BC, suggesting a role of genetic susceptibility. BRCA1/2 pathogenic variants (PVs) are responsible for about 15% of MBCs. The aims of this study were to evaluate the role of PVs in

non-BRCA1/2 genes in MBC susceptibility and to identify possible distinctive clinical-pathologic features in PV carriers.

Methods: A population-based, case-control study including 725 BRCA1/2 PV negative MBCs and 917 healthy male controls, enrolled in the frame of the Italian multicenter study on MBC, was performed using a custom 50-gene panel in NGS. Statistical analyses were performed using chi-square test and logistic regression model.

Results: Overall, MBCs were more likely to carry PVs in cancer genes compared with controls (5% vs 2.3%, $p = 0.003$). PALB2 PVs were identified in 1% of MBCs and were significantly associated with increased MBC risk (OR: 4.6, 95% CI: 1.00-22.3; $p = 0.04$). BLM and FANCM emerged as possible genes associated with MBC risk in the case-control analysis ($p = 0.02$, each). PV carriers were more likely to have a PH of cancer ($p = 0.02$) and FH of cancer ($p = 0.007$), not limited to BC.

Conclusion: Our results support a central role of PALB2 in MBC susceptibility in the Italian population. Overall, our data indicate that a multigene testing approach may benefit from appropriately selected patients, particularly those with family and personal history of multiple cancers, with implications for clinical management of MBC patients and their relatives.

References:.

Grants: Study supported by AIRC (IG21389) to LO.

Conflict of Interest: None declared.

P13.011.A Germline NGS gene panel testing in routine care of Breast and Ovarian cancer patients in Estonia 2016-2020

Mikk Tooming[1;2], *Kadri Rekker*[2], *Kadri Toome*[2], *Olga Fjodorova*[2], *Hanno Roomere*[2], *Piret Laidre*[2], *Ülle Murumets*[2], *Katrin Ounap*[1;2], *Tiina Kahre*[1;2]

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Background/Objectives: Targeted NGS gene panels have replaced the standard single-gene testing for hereditary cancer diagnostics of Breast cancer (BC) and Ovarian cancer (OC) in Estonia. The aim of this study was to investigate the prevalence of pathogenic variants (PV) in hereditary BC and OC patients and healthy individuals with positive family history (HI) from Estonia during the years 2016-2020.

Methods: The study included 2200 patients referred to the Department of Clinical Genetics at Tartu University Hospital for analysis of suspected hereditary BC and OC or HI carrier screening. Individuals were analyzed using Illumina TruSight Cancer or TruSight Hereditary Cancer gene panel.

Results: Analyzed group consisted of 1080 (49.1%) BC patients, 366 (16.6%) OC patients, 27 (1.3%) BC/OC patients and 727 (33%) HI group. NGS revealed PV findings in 363/2200 (16.5%) cases. Altogether, PVs were found in 195/1080 (18.1%) BC patients, 98/366 (26.7%) OC patients, 7/27 (26%) BC/OC patients and 63/727 (8.7%) HI group. Respectively, BRCA1/2 PVs were in found 113/1080 (10.4%) BC patients, 69/366 (18.9%) OC patients, 6/27 (22.2%) BC/OC patients and 39/727 (5.4%) HI. Furthermore, nonBRCA1/2 (ATM, BARD1, BRIP1, CDH1, CHEK2, NF1, PALB2, RAD51C, RAD51D, TP53) PVs were found in 93/1080 (8.6%) BC patients, 29/366 (7.9%) OC patients, 1/27 (3.7%) BC/OC patients and 29/727 (4%) HI.

Conclusion: The diagnostic efficacy of NGS gene panels is in between 18-26% in BC and OC cases, which is similar to the literature data. Detection rate with NGS panel is ~7% higher compared to BRCA1/2 gene testing.

References:.

Grants: Estonian Research Council grant PRG471.

Conflict of Interest: None declared.

P13.012.B MLH1 variant c.836T>G, a Tyrolean hypomorphic founder mutation?

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Background/Objectives: Correct classification of mismatch repair gene variants is of utmost important for appropriate counseling, surveillance, and treatment of suspected Lynch syndrome patients and their entire families.

Methods: We used multiple lines of evidence to classify MLH1 variant NM_000249.3:c.836T>G, reported twice in ClinVar, as likely benign and of uncertain significance, respectively.

Results: NM_000249.3(MLH1):c.836T>G, found in five Tyrolean patients with cancers showing MLH1 and/or PMS2 expression loss and no MLH1 hypermethylation (PP4_Strong), is absent in GnomAD (PM2_Supporting). Full-length transcripts with c.836G predicted to cause p.(Val279Gly) with a MAPP+PolyPhen-2 prior probability of 0.9 (PP3_Moderate) represented only ~25% of transcripts in blood lymphocytes of four carriers. Use of a novel 5′ splice site created by the variant is observed in ~20% and skipping of exon 10 in <10% of transcripts. Minigene experiments confirmed both splice effects, which are predicted to cause loss of 16 amino acids, p.(Val279_Ser295delinsGly), in the MLH1 N-terminal domain and p.(His264fs*2), respectively. Ignoring that they are leaky, both splice effects render a very strong argument for pathogenicity (PVS1).

Conclusion: Considering its missense and newly characterized splice effects, we classify c.836T>G according to the draft InSiGHT ACMG criteria as (likely) pathogenic. Ongoing studies explore whether this variant has an attenuated penetrance, which is suggested by the lack of a strong Lynch syndrome family history of the five patients and its accidental finding in one patient with a concomitant MSH6 pathogenic variant and two different cancers with MSH6 expression loss.

References:.

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Conflict of Interest: None declared.

P13.013.C Molecular characterization of an embryonal rhabdomyosarcoma occurring in a patient with Kabuki syndrome: report and literature review in the light of tumor predisposition syndromes

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Background/Objectives: Kabuki syndrome (KS) is a MCA/ID syndrome in which most patients have a KMT2D germline variant.

With the publication of several case reports of patients with KS and a concomitant malignancy the topic of tumor predisposition in KS has received increased attention. We describe a patient with KS who developed an ERMS. To get more insight into a potential tumor predisposition in KS we performed molecular (epi)genetic analyses on tumor tissue and a literature review.

Methods: Exome sequencing and DNA-methylation profiling (EPIC-array) was performed on tumor DNA. For DNA methylation-based sarcoma classification we used the DKFZ-Sarcoma classifier(1). We conducted a literature search for reports of patients with KS and a malignancy.

Results: DNA-methylation profiling mapped the case to ERMS. Exome sequencing revealed variants in ERCC5 and TP53. Copy number variant analysis revealed (partial) gains of chromosomes 2,3,7,8,12,15, and 20, and focal deletions in 11p. Sanger re-sequencing of the germline variant suggested a gain of the wild-type KMT2D allele in the trisomy 12. Including our patient literature review identified 18 patients with KS and a malignancy. Overall, the landscape of malignancies in patients with KS was reminiscent of that of the pediatric population in general. No secondary malignancy, bilateral-/multifocal or meta-synchronous malignancies were reported. Histopathological and molecular data were infrequently reported and did not include next generation sequencing and/or DNA-methylation profiling.

Conclusion: Although, based on our (molecular) analyses and literature review, a tumor predisposition cannot be confirmed or ruled out we found no strong arguments pointing towards KS as a tumor predisposition syndrome.

References: (1) Koelsche,Nat.Commun.2021.

Grants:.

Conflict of Interest: None declared.

P13.014.D Severe neurovascular manifestations in PTEN-related hamartoma tumour syndrome

Anna Gerasimenko[1], *Patrick Benusiglio*[1], *Florence Coulet*[1], *Olivier Naggara*[2;3], *Mélanie Eyries*[1], *Cyril Mignot*[1;4], *Annouk Bisdorff*[5]

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Background/Objectives: PTEN-related hamartoma tumor syndrome (PHTS) is an overgrowth and cancer susceptibility syndrome associated with germline pathogenic variants (PV) in the *PTEN* gene. Brain anomalies such as dysplastic ganglioma of the cerebellum are common, although there are no central nervous system surveillance recommendations. Brain vascular anomalies in the context of PHTS were scarcely studied. The aim of our study was to systematically search for cerebral vascular anomalies in a series of PHTS patients, with a focus on cranial dural arteriovenous fistulae (dAVF) and their clinical consequences.

Methods: We retrospectively studied brain imaging in 58 adult and pediatric PHTS patient s (MRI in 57 patients) who underwent germline testing at the AP-HP.Sorbonne-University neurodevelopmental, vascular and cancer genetics laboratory.

Results: A benign developmental venous anomaly was observed in 6 patients and DAVF in two (3.4%) patients. The first patient was a 36 yrs old man with severe epilepsy with a fatal evolution after 6 years of repetitive endovascular embolizations. The second was a 21-year-old woman who presented with intracranial hypertension as a consequence of the dAVF, subsequently cured by embolization.

Conclusion: In the present observational study, occurrence of cranial dAVF, a condition that may be associated with debilitating or life-threatening evolution, seems to be higher than in the general population. A screening brain imaging may be discussed at diagnosis in PHTS, ideally using MRI with dynamic MR angiography and perfusion imaging. In parallel, clinicians should be wary of neurological symptoms in their PHTS patients.

References:.

Grants:.

Conflict of Interest: None declared.

P13.015.A Searching for germinal variants of TET2, IDH1, SETD2, CHD1 and ASXL1 epigenetic genes in Polish prostate cancer patients - preliminary results

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Background/Objectives: The epigenetic variants are present in all human cancers and associated with genetic alterations to drive a cancer phenotype. Thus, we searched for germinal variants of five epigenetic genes in Polish prostate cancer patients.

Methods: The material of investigation was DNA from 97 men with prostate cancer (PC) from all over Poland. NGS and Sanger sequencing.

Results: In 11/97 (11,3%) PC patients 13 variants of analyzed genes were detected. These were eight missense variants of TET2 (2), IDH1 (1), SETD2 (3), CHD1 (1) and ASXL1 (1), four silent variants of TET2 (3) and CHD1 (1) and one duplication of ASXL1. Bioinformatic analysis of variants was performed using VarSome database. TET2 c.2218C>T and ASXL1 c.1934dupG were predicted as pathogenic, ASXL1 c.3623C>T, SETD2 c.3383C>G, TET2 c.2370G>A, c.4161C>T and IDH1 c.565A>G as VUS (variants of uncertain significance), CHD1 c.2321A>T and c.1434C>T, SETD2 c.1643C>A and c.3229A>G and TET2 c.972A>G were predicted as benign and TET2 c.3251A>G as likely benign. Two prostate cancer patients were both carriers of two variants, one of them was a carrier of ASXL1 c.1934dupG and TET2 c.2370G>A and the second one a carrier of CHD1 c.2321A>T and TET2 c.972A>G.

Conclusion: The results of the preliminary investigation point at the need to study germinal variants of epigenetic genes to help fully understand the pathogenesis of prostate cancer, identify men at PC high risk and predict the disease recurrence risk after radical prostatectomy.

References: Soo You J, Jones PA: Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell. 2012, 22: 9-20.

Grants: MN-1/WL/2020.

Conflict of Interest: None declared.

P13.017.C Rapid progression to Richter's syndrome in patient with chronic lymphocytic leukemia and near triploid karyotype

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Background/Objectives: The presence of aneuploidy in patients with chronic lymphocytic leukemia (CLL), except for trisomy 12, is quite uncommon. The frequency of hyperdiploidy or near-tetraploidy ocurrs in 1-3% of the CLL patients and usually confer poor prognosis.

Methods: Cytogenetic, Fluorescent in situ hybridisation (FISH) and Polymerase chain reaction analyses were performed and complemented with immunophenotyping by flow cytometry and pathohistological examination.

Results: Our patient showed splenomegaly, more than three enlarged lymph node regions and elevated serum LDH and β2 microglobulin levels, at presentation. Highly progressive disease was proven by other diagnostic methods, including the surface expression of CD38 and CD49d in the immunophenotype. The FISH result revealed a trisomy of 13q14 region and the C-MYC gene as the most prevalent aberrations, followed by a trisomy of the 11q, CEP12, IGH gene disruption and a tetrasomy of chromosome 12. In the patient's karyotype, multiple structural and numerical changes of chromosomes not covered with the FISH probes, were observed, as well. In addition, the mutational analysis of the TP53 gene was positive for the frameshift mutation in exon 6.

Conclusion: In our report, the presence of the near-triploid karyotype was designated as an uncommon and very rare event in both CLL and RS. Utilization of comprehensive diagnostic techniques is highly recommended in patients with a progressive phase of CLL, primarily due to adequate choice of management strategy. The current case confirms poor prognosis of the previously reported CLL patients with aneuploidy.

References:.

Grants:.

Conflict of Interest: None declared.

P13.018.D MS-MLPA reliably detects promoter methylation as well as deletion of MGMT gene in patients with glioblastoma

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Background/Objectives: Glioblastoma (GBM) is the most common primary malignant brain tumor with very poor prognosis. The good response of the patients with GBM to temozolomide treatment is associated with low expression of *MGMT* gene (localized on 10q26.3), which may be affected by methylation or deletion. However, the appropriate methods of analysis and the cut-off levels for the detection of *MGMT* gene promoter methylation remain to be discussed.

Methods: We examined the brain tissue and peripheral blood of 70 patients with GBM, IDH-wildtype, WHO grade 4 using MS-MLPA method, ME012 kit (MRC Holland). We evaluated the specificity and sensitivity of individual MS-MLPA kit probes via ROC curve analysis and heatmap function in R software. The deletion of *MGMT* gene was confirmed by I-FISH with *MGMT* probe (Empire Genomics).

Results: We found two MS-MLPA probes to be the most specific. The first one localized -263nt upstream and the second +165nt downstream of the *MGMT* gene transcription start. We confirmed methylation of the *MGMT* gene promoter in 38% of patients. In 87.5% of them, we found deletion of 10q26.3 region along with the promoter methylation. In 14% of patients, only deletion of 10q26.3 was found.

Conclusion: The results of our study show that the MS-MLPA method can reliably detect not only methylation but also deletion of the *MGMT* gene. The comprehensive analysis of the *MGMT* gene may lead to more precise and personalized treatment of the patients with GBM.

References:.

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Conflict of Interest: None declared.

P13.019.A Germline mutations in WNK2 are associated with serrated polyposis syndrome

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Background/Objectives: Serrated polyposis syndrome (SPS) is characterized by multiple serrated polyps and colorectal cancer (CRC) predisposition. WNK2 was previously suggested as a new candidate gene to SPS (1). We aimed at supporting WNK2 as a new genetic predisposition gene to SPS with functional evidence and replication in additional SPS cohorts.

Methods: We performed a gene-panel sequencing of 211 SPS patients and assessed sequencing data of 297 external SPS cases. We produced a cellular model for WNK2 using CRISPR/Cas9 and lentiviral delivery system. Functional assays monitored the MAPK pathway, cell cycle progression, survival and adhesion.

Results: Five germline potentially pathogenic variants among our cohort and 10 among the external cohort were identified in

WNK2. Genetic variants c.2105C>T (p.Pro702Leu), c.4820C>T (p.Ala1607Val) and c.6157G>A (p.Val2053Ile) were functionally characterized, displaying higher phospho-PAK1/2 and phospho-ERK1/2 levels, increased CCDN1 expression, higher clonogenic capacity and upregulated MMP2 levels.

Conclusion: Potentially pathogenic variants in WNK2 were identified in 3.24% of SPS patients (17/524). WNK2 genetic variants seems to disrupt ERK1/2 MAPK pathway, affecting cellular functions as cell cycle progression, survival and adhesion capacity, supporting the role of WNK2 as a new germline predisposition gene to SPS.

References: 1- Soares de Lima Y et al. Germline and Somatic Whole-exome Sequencing Identifies New Candidate Genes Involved in Familial Predisposition to Serrated Polyposis Syndrome. Cancers 2021; 13(4):1–21.

Grants: LCF/BQ/DI18/11660058 (ID100010434) Marie Skłodowska-Curie no. 713673, Fundació La Marató 2019-202008-10, Fondo de Investigación Sanitaria/FEDER (17/00878, 20/00113), AECC (PRYGN211085CAST), Generalitat de Catalunya (GRPRE 2017SGR21), COST Action CA17118, CIBEREHD, CIBERONC and CIBERER.

Conflict of Interest: None declared.

P13.020.B Evaluating the potential of polygenic risk score to improve colorectal cancer screening

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Background/Objectives: Colorectal cancer (CRC) has a high incidence and associated mortality worldwide. Screening programs are recommended for men and women over 50 y.o.. Intermediate screens such as fecal immunochemical testing (FIT) select patients for colonoscopy with suboptimal sensitivity. Additional biomarkers could improve the current scenario.

Methods: We included almost 3,000 individuals with a positive FIT test. They were classified as cases when a high-risk lesion for CRC was detected after colonoscopy, whereas the control group comprised individuals with low-risk or no lesions. Sixty-five CRC risk genetic variants were genotyped. Polygenic risk score (PRS) and additive models for risk prediction incorporating sex, age, FIT value and PRS were generated.

Results: Risk score was higher in cases compared to controls (per allele OR = 1.04; 95%CI 1.02-1.06; P-value < 0.0001). We also observed a 2-fold increase in CRC risk for subjects in the highest decile of risk alleles (≥65), compared to those in the first decile (≤54) (OR = 2.22, 95%CI 1.59-3.12, P-value < 0.0001). The model combining sex, age, FIT value and PRS reached the highest accuracy for identifying patients with a high-risk lesion (cross-validated AUROC: 0.639; 95%CI 0.619-0.660).

Conclusion: This is the first investigation analyzing PRS in a two-step CRC screening program. PRS could improve current CRC screening. However, its capacity is limited and should be complemented by additional biomarkers such as microbiome or environmental factors.

References:.

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Conflict of Interest: None declared.

P13.022.D Diagnostic uncertainties in hereditary leiomyomatosis: complementary role of genetic analysis and immunohistochemical staining

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Background/Objectives: Multiple cutaneous and/or uterine leiomyomas are associated with hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome but also occur with other disorders or are sporadic. HLRCC is caused by heterozygous germline variants in FH and is an important diagnosis to make because of the RCC risk. Though mutation analysis of FH may detect a pathogenic variant, detection of a rare variant of unknown clinical significance (VUS) or negative testing can leave diagnostic uncertainty. Here we report our findings from a patient series with multiple leiomyomas in which genetic findings were inconclusive and the role of immunohistochemistry in resolving diagnostic uncertainty.

Methods: Eight individuals with multiple cutaneous and/or uterine leiomyomas were evaluated. Genetic analysis identified an FH variant (initially classified as a VUS) in 6 cases. Immunohistochemical (IHC) staining for FH expression and detection of S-(2-succino)-cysteine (2-SC) was performed [1].

Results: IHC on leiomyoma and normal tissue revealed 2-SC positivity in 100% (6/6) and FH protein loss in 67% (4/6) of cases with a germline FH variant. All variants with 2-SC tumour positivity were upgraded to likely pathogenic/pathogenic. Two individuals with multiple leiomyomas and a family history of leiomyomatosis but no detectable germline FH variant demonstrated retention of FH expression and negative staining for 2-SC and a diagnosis of HLRCC was excluded.

Conclusion: We propose a management pathway for the investigation of suspected HLRCC in individuals with multiple

leiomyomas that incorporates genetic testing and IHC for 2-SC and FH in a complementary fashion to resolve diagnostic uncertainty.

References: 1. Bardella et al. 2011.

Grants: CRUK.

Conflict of Interest: Avgi Andreou: None declared, Anne Warren: None declared, Saba Sharif: None declared, Beverley. Speight: None declared, Laura Butland: None declared, Abhijit Dixit: None declared, Marc Tischkowitz: None declared, Eamonn R Maher Invited speaker fees from MSD.

P13.024.B Incidence of Pediatric Cancer Predisposition in Czechia

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Background/Objectives: Genetic predisposition plays important role in cancer development especially when enviromental factors couldn´t be the key player. However the field of pediatric cancer predisposition is not yet fully understood (Ripperger, 2017). Accurate incidence need to be validated under new findings. Our main objective is determine the incidence of predisposing genetic variants in Czechia.

Methods: NGS analyses using targeted gene panels (226 genes) were redesigned for pediatric cohort (add 64 genes). The study of the variants was carried out with the help of the ClinVar, professional HGMD, as well as in silico predictions. The variants were classified as benign, likely benign, VUS (variant of uncertain significance), likely pathogenic, pathogenic. All patients enrolled in the study were reffered to genetic counseling under hematooncology department between January 2020 and December 2021. 217 patients (M 111, F 104) fulfilled the criteria and agreed to participate in the study, all parents and/or patients signed informed consent.

Results: A pathogenic variant connected to cancer predisposition was detected in 31 patients (31/217;14%), in following genes TP53, NF1, RB1, PALB2, EXT2, BRCA1, WNR, ABRAXAS1, STK11, HOXB13, NBN, MUTYH, ATM, CHEK2, FANCA, SBDS, FANCG, FANCI, remarkable enrichment in neurooncology group for variants in MUTYH, CHEK2, WNR. Likely pathogenic variant was found in 14 patients (14/217;6,%). Reckon up the incidence to 20,7%. Interestingly variant of uncertain signifikance was discovered in 55 cases and needed to be futher validated (55/217;25%).

Conclusion: For the first time accurate incidence for cancer predisposition in Czech pediatric cohort was determined (20,7%).

References: Ripperger, 2017, AJMG, https://doi.org/10.1002/ajmg.a.38142.

Grants:.

Conflict of Interest: None declared.

P13.025.C The Manchester Scoring System in 2022: performances before and after raking in breast and ovarian cancer patients undergoing multigene panel testing

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Background/Objectives: Recent recommendations from the USA state that most breast and ovarian cancer patients (BC/OC) should be offered germline testing. However, European oncogeneticists still need to select a subset of suggestive cases, as resources would not allow for near-universal testing. Clinical criteria, prediction models or scores are used in this context. We have adapted the Manchester Scoring System (MSS-P) to French practice and multigene panels, and report its performances in a large series of cases.

Methods: We included 1219 BC/OC patients referred between 2016 and 2020 to the AP-HP.Sorbonne University Oncogenetics laboratory, Paris, and who underwent germline panel testing (BRCA1, BRCA2, PALB2, TP53, RAD51C, RAD51D, ATM, CHEK2, PTEN, CDH1, MMR). MSS-P correlation with the identification of pathogenic variants was assessed using AUC/ROC curves. For a subset of 210 patients, MSS-P was compared to BOADICEA. Curves were also re-calculated using the raking method as a correction, matching cases statistics to those of unselected BC/OC cases.

Results: MMS-P performances were average, with an AUC of 0.61. Sensitivity was 0.95, 0.75 and 0.55 at the 9, 12 and 15-point thresholds. Specificity was 0.14, 0.36 and 0.63, respectively. Negative and positive predictive values varied very little with thresholds, with estimations of 0.93-0.96 and 0.10-0.13, respectively. The BOADICEA AUC on a subset of cases was similar to MSS-P (0.61). MSS-P performances, though, were improved after raking, with an AUC of 0.74.

Conclusion: Improved performances after raking suggest that MSS-P should actually be used by gynaecologists and oncologists to select BC/OC patients for Oncogenetics referral.

References:.

Grants:.

Conflict of Interest: Patrick Benusiglio AstraZeneca, Lucas Ducrot: None declared, Jasmine Hasnaoui: None declared, Florence Coulet: None declared, Camille Desseignes: None declared, Geoffroy Canlorbe: None declared, Diarietou Gueye: None declared, Catherine Uzan: None declared, Erell Guillerm: None declared, Gregory Nuel: None declared.

P13.026.D ENIGMA effort to standardize the classification of variants disrupting splicing of in-frame exons using BRCA1 exon 18 as an example

Joanna Domènech-Vivó[1], *Hélène Tubeuf*[2;3], *Romy L.S. Mesman*[4], *Aurelie Drouet*[2], *Mélanie Girardi*[2], *María Concepción Alonso-Cerezo*[5], *Diana Baralle*[6;7], *Nadia Boutry-Kryza*[8], *Dave Bunyan*[6;9], *Helen J Byers*[10;11], *Kathleen Claes*[12], *D Gareth Evans*[13], *Miguel de la Hoya*[14], *Sophie krieger*[15;16;17], *Conxi Lázaro*[18;19], *Mélanie Leone*[8], *Eva Machackova*[20], *Mireia Menéndez*[18], *Alejandro Moles-Fernández*[1], *Gemma Montalban*[21], *Elke van Veen*[13], *JUDITH BALMAÑA*[1;22], *Amanda B. Spurdle*[23], *Orland Diez*[1;24], *Maaike Vreeswijk*[4], *Alexandra Martins*[2], *Sara Gutiérrez-Enríquez*[1]

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Background/Objectives: *BRCA1* germline pathogenic variants are associated with high risk of breast/ovarian cancer. Variants inducing complete skipping of in-frame exons or causing partial/leaky splicing defects are challenging to classify, due to lack of knowledge about the functionality of the generated in-frame proteins or the minimal level of full-length transcripts (FL) required for normal function. In the ENIGMA consortium, we performed a comprehensive study of variants affecting *BRCA1* exon 18 (*BRCA1*e18) to identify those spliceogenic and characterize their severity to estimate the threshold of FL levels for sufficient normal BRCA1 function.

Methods: Patients' RNA (n = 30), minigene (n = 160), and mouse embryonic stem cell (mESC) complementation assays were combined to determine the impact of *BRCA1*e18 variants on RNA splicing and the minimal level of *BRCA1* FL required for function.

Results: Variants in which we detected a complete skipping of *BRCA1*e18(Δ18) were not functional by the saturation genome editing assay[1], and neither were those that induced 50% Δ18 in minigenes (25% expected in patients). In contrast, synonymous and intronic variants causing up to 10% of Δ18 were considered functional. Complete deletion of *BRCA1*e18 in mESC leads to a non-functional protein supporting that Δ18 is not a rescue transcript.

Conclusion: This data evidences that the leakiness rate of *BRCA1*e18 skipping and residual FL levels are critical to determine the pathogenicity of variants affecting this exon.

References: [1] G.Findlay et al.,"Accurate classification of BRCA1 variants with saturation genome editing", Nature,2018,v562,pp217-222.

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Conflict of Interest: Joanna Domènech-Vivó: None declared, Hélène Tubeuf H. Tubeuf was employed by Interactive Biosoftware for the time period October 2015-September 2018 in the context of a public-private PhD project partnership between INSERM and Interactive Biosoftware (CIFRE fellowship #2015/0335), Romy L.S. Mesman: None declared, Aurelie Drouet: None declared, Mélanie Girardi: None declared, María Concepción Alonso-Cerezo: None declared, Diana Baralle: None declared, Nadia Boutry-Kryza: None declared, Dave Bunyan: None declared, Helen J Byers: None declared, Kathleen Claes: None declared, D Gareth Evans: None declared, Miguel de la Hoya: None declared, Sophie krieger: None declared, Conxi Lázaro: None declared, Mélanie Leone: None declared, Eva Machackova: None declared, Mireia Menéndez: None declared, Alejandro Moles-Fernández: None declared, Gemma Montalban: None declared, Elke van Veen: None declared, JUDITH BALMAÑA: None declared, Amanda B. Spurdle: None declared, Orland Diez: None declared, Maaike Vreeswijk: None declared, Alexandra Martins: None declared, Sara Gutiérrez-Enríquez: None declared.

P13.027.A In vivo cancer co-driver identification and dependency mapping via evaluation of CRISPR-induced repair outcomes in Xenopus tropicalis cancer models

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Background/Objectives: With the emergence of CRISPR/Cas9 techniques, *Xenopus* is receiving increased attention with regard to modeling of human genetic diseases. *X. tropicalis* is especially uniquely placed for these applications because it combines the general amphibian features of large externally developing embryos, which are straightforward to inject with genome editing reagents, a relatively short life cycle and a true diploid genome.

Methods: We use targeted microinjections of multiplexed genome editing reagents in early developing embryos and rapid F_0 screening of such mosaic mutant animals. This allows rapid identification of cooperative driver genes in specific cancer contexts. Additionally, we use an in-house developed methodology to identify cancer dependencies built on principles of Darwinian cell-specific clonal selection, opening possibilities for development of new treatments.

Results: Over the last couple of years, we built multiple genetically engineered *Xenopus* models (GEXMs) for several human cancers. Furthermore, in a model for Gardner syndrome, we validated an in vivo negative-selection screen that identified the epigenetic regulator *EZH2* as a druggable dependency factor needed for desmoid tumor growth. A similar strategy is followed to identify vulnerabilities in other GEXM tumor models.

Conclusion: We provide findings and perspectives important for current and future (*Xenopus*) cancer research. The generation of GEXMs even more closely mimicking the patient situation, will be an extra valuable tool for a reliable and time-efficient screening of novel genetic dependencies and the discovery of extra potential inhibiting compounds.

References:.

Grants:.

Conflict of Interest: None declared.

P13.028.B Lung metastases and subsequent malignant transformation of a fumarate hydratase -deficient uterine leiomyoma

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Background/Objectives: Uterine leiomyomas are common benign smooth muscle tumors. Their potential to metastasize or transform into leiomyosarcomas is extremely low. Here, we report a patient who was diagnosed with a uterine leiomyoma and seven and nine years later with pulmonary leiomyosarcomas.

Methods: Histopathological examination, whole-exome sequencing and 3'RNA sequencing.

Results: Histopathological re-evaluation confirmed the cellular leiomyoma diagnosis for the uterine tumor, whereas the pulmonary tumors met the criteria of a leiomyosarcoma. Somatic copy number analysis showed similar mutational profiles in all three tumors, including a homozygous deletion in a rare leiomyoma driver gene *FH*. Tumor evolution analysis confirmed their clonality. Pulmonary tumors harbored additional alterations affecting e.g. the cancer-associated genes *NRG1*, *MYOCD*, and *FGFR1*. The uterine tumor showed similar gene expression patterns as other FH-deficient leiomyomas.

Conclusion: This data supports the occasional metastatic capability and malignant transformation of uterine leiomyomas with FH-deficiency and/or cellular histopathology. Identification of such tumors may provide early diagnosis and even cancer prevention.

References:.

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Conflict of Interest: None declared.

P13.029.C Significance of ARID1A mutations in colorectal cancer

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Background/Objectives: Colorectal cancer (CRC) is one of the most common neoplasms and the leading cause of death worldwide. It is a complex heterogeneous disease, arising from the accumulation of many different genetic mutations.

Methods: The study included 15 patients with CRC. Circulating tumor DNA was extracted from blood plasma and a target sequencing of a panel of 484 target genes was performed.

Results: A somatic variant ARID1A/c.3999_4001delGCA was identified in 13out of 15 patients. ARID1A is a tumor suppressor gene that encodes a protein involved in the SWI/SNF protein complex.Its function is to regulate the transcription of certain genes by changing the structure of the chromatin around them. The SWI/SNF protein complex acts as a tumor suppressor, preventing uncontrolled cell growth and division.The majority of mutations in the ARID1A gene are frame shifts or nonsense mutations that contribute to mRNA breakdown and loss of protein expression, 5% of ARID1A mutations are in-frame insertions or deletions (indels).Recent studies show that variants in the ARID1A gene have been found in many types of cancer, including colorectal cancer recent functional analysis revealed, that no or low expression of ARID1A is observed in CRC patients.

Conclusion: Clearly mutations in ARID1A are involved in CRC, but more research is needed to clarify their role in tumorigenesis.

References: Erfani M, et al., Altered ARID1A expression in colorectal cancer. BMC Cancer. 2020 Apr 25;20(1):350.

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Conflict of Interest: None declared.

P13.031.A Multi-gene panel testing of melanoma patients

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Background/Objectives: Melanoma is characterized by a complex and heterogeneous etiology, which involves environmental, phenotypic and genetic risk factors. Approximately 5-10% of melanoma cases occurs in a familial context. Carriers of mutations in high-penetrance genes show a lifetime risk of 52%; however, in the majority of families no causative mutations are identified. The aim of our study is to find novel genetic alterations that could be useful to identify pathways involved in the development of the disease.

Methods: We tested 306 patients with a diagnosis of familial or multiple or juvenile malignant melanoma and belonging to cases referred for testing at the Genetic Unit of *Città della Scienza e della Salute* of Turin (Prof. B. Pasini).

Results: Patients firstly underwent screening of four established familial melanoma susceptibility genes: *CDKN2A, CDK4, MITF*, and *TERT*. Twenty-four out of 306 patients (7.8%) presented 24 pathogenic mutations in *CDKN2A* (19 cases) and *MITF* (4 cases) genes. As expected, the most frequent mutation was the missense variant *CDKN2A* c.301G>T present in 10 out of 19 patients. To understand the genetic etiology of wild-type cases, we will screen 21 low to high-risk genes plus 256 melanoma-associated SNPs through Next Generation Sequencing. Analysis of the first 101 cases revealed pathogenic variants in *MC1R, OCA2, PTEN, TYR* and *SLC45A2* genes.

Conclusion: Extended testing beyond main high-risk genes could identify new potentially actionable deleterious mutation. Moreover, it allows investigating the combination effect of low to moderate variants.

References:.

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Conflict of Interest: None declared.

P13.033.C BRG1-dependent chromatin remodeling at the promoters of lysosome-localized ABC transporters confers multidrug insensitivity in paclitaxel-resistant non-small cell lung cancer cells

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Background/Objectives: Paclitaxel (PTX) is an important first-line drug for the treatment of non-small cell lung cancer (NSCLC), but it leads to the development of resistance to many other structurally unrelated compounds. This phenomenon that is known as Multidrug Resistance (MDR) is frequently conferred by the overexpression of membrane-anchored ABC transporters capable of transfer of xenobiotics across membranes. Furthermore, enhanced lysosomal function has been shown to be characteristic of paclitaxel resistance in cancer cells. ATP-dependent chromatin remodeling complexes are an important part of the epigenetic mechanism of transcriptional regulation. Knowing that ATPase BRG1 is involved in the expression of ABC transporters in breast cancer cells, we examined the role of BRG1 in the expression of genes responsible for MDR, paying particular attention to those that occur in the lysosomal membrane and may contribute to increased drug accumulation in lysosomes.

Methods: The expression of ABC transporters was analysed by qPCR and Western Blot. Enrichment of ABC genes promoters in BRG1 was measured by ChIP-qPCR. Drug accumulation was analysed by confocal microscopy.

Results: PTX exposure has been shown to promote the expression of MDR-associated ABC transporters and BRG1. Furthermore, BRG1 has been shown to interact with promoter sequences of genes for ABC proteins. The silencing of BRG1 expression decreases ABC transporter expression and reduces drug accumulation in lysosomes.

Conclusion: Our results suggest that specific BRG1 inhibitors may have potential applications in overcoming resistance acquired after paclitaxel therapy by reducing drug accumulation in lysosomes.

References:.

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Conflict of Interest: None declared.

P13.034.D Development of APC-specific ACMG/AMP variant classification guidelines

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Background/Objectives: To resolve the interpretative challenges of constitutional variants in colorectal cancer predisposing genes, a Hereditary Colon Cancer/ Polyposis Variant Curation Expert Panel (VCEP) was established as a collaborative effort between ClinGen and the International Society for Gastrointestinal Hereditary Tumours (https://clinicalgenome.org/affiliation/50099/). Under oversight of the ClinGen Sequence Variant Interpretation working group, our subcommittee reviews APC variants underlying Familial adenomatous polyposis and aims to improve variant classification through the development of gene-specific specifications and the implementation of expert-reviewed pathogenicity assertions.

Methods: APC-specific specifications of the variant guidelines by the American College of Medical Genetics and the Association of Molecular Pathology (ACMG/AMP) were cross-examined in teleconferences based on database analyses, literature review and expert opinions. A balanced selection of 57 variants underwent pilot testing to further refine and finalise the APC-specific classification criteria.

Results: Three of the 28 original criteria were left unchanged (BS4, BP1, BP5), whilst eight were removed (PM1, PM3, PM4, PP2, PP4, PP5, BP3, BP6). Gene, disease-based and/or evidence strength modification were applied to the remaining 17 criteria. Major specifications were noted for PVS1 (including variants at the 5' and 3' of the gene and splice variants) and allele frequency criteria (BA1, BS1 and PM2). Moreover, a point system for the phenotypic description of variant carriers was developed.

Conclusion: A list of prioritised APC variants will be classified by the VCEP regularly, whose consensus will represent the most authoritative classification of pathogenicity for widespread clinical use. The reclassifications will be publicly available through both ClinVar and the InSiGHT APC databases (www.lovd.nl/APC).

References:.

Grants:.

Conflict of Interest: Xiaoyu Yin: None declared, Isabel Spier: None declared, Marcy Richardson: None declared, Marta Pineda: None declared, Deborah Ritter: None declared, Tina Pesaran: None declared, Gabriel Capellá: None declared, Sean V. Tavtigian: None declared, John-Paul Plazzer: None declared, Andreas Laner: None declared, Andrew Latchford: None declared, Ian M. Frayling I'm a member of CanVIG-UK https://www.ncbi.nlm.nih.gov/clinvar/submitters/506655/, Sharon E Plon Scientific Advisory Panel for Baylor Genetics, Marc Greenblatt: None declared, Finlay A. Macrae Councillor for InSiGHT, Stefan Aretz: None declared.

P13.035.A The interplay between pathology, molecular alterations and outcomes in esophageal adenocarcinoma

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Background/Objectives: Esophageal adenocarcinoma (EAC) is a severe malignancy with increasing incidence in Western countries over the past few decades. EAC pathogenesis is still poorly understood and the survival rate is low1. Therefore, there is a need to better clarify EAC risk factors to improve patient identification and treatment.

Methods: 207 EAC cases classified according to EACGSE2 were included. DNA was extracted from formalin fixed paraffin embedded surgical specimens and sequenced for 26 cancer-related genes (#226722257; IDT) with high coverage on NextSeq 500 (Illumina). Data analysis was performed using an in-house pipeline3.

Results: A total of 353 variants were identified across the whole cohort. TP53 was the most frequently altered gene (134/207 cases carrying at least one mutation; 64.73%). TP53 missense variants correlated with a worse cancer-specific survival in the histological glandular poorly differentiated group (Log Rank P = 0.0047) and, globally, in the Higher Risk group2 (Log Rank P = 0.0005). Loss-of-function variants in HNF1A, tumor suppressor gene not previously associated with EAC, were found in 7 cases, together with other gene alterations. HNF1A mutant samples showed a decreased immunostaining in the tumor, correlated with an increase in the number of variant alleles.

Conclusion: A specific type of mutations (missense changes in TP53) negatively affect cancer-specific survival of EAC. HNF1A was identified as a new EAC-mutated gene and its loss might contribute to EAC development and progression.

References: 1. Coleman et al., Gastroenterology 2018;154:390–405. 2. Fiocca et al., Cancers 2021;13:5211. 3. Isidori et al., CTG 2020;11:e00202.

Grants:.

Conflict of Interest: Arianna Orsini PhD student, Isotta Bozzarelli post-doc, Federica Isidori post-doctoral fellow, Roberto Fiocca MD, Luca Mastracci MD, Marialuisa Lugaresi MD, Maria Antonietta D'Errico MD, Deborah Malvi PhD, Paola Spaggiari MD, Anna Tomezzoli MD, Luca Albarello MD, Ari Ristimäki MD, Luca Bottiglieri MD, Sheila Krishnadath MD, riccardo rosati MD, Uberto Fumagalli Romario MD, Giovanni de Manzoni MD, Jari Räsänen MD, Sandro Mattioli MD, Elena Bonora PhD.

P13.036.B Joint effects of the CHEK2 c.1100delC mutation and treatment on contralateral breast cancer risk

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Background/Objectives: Breast cancer (BC) patients with a CHEK2 c.1100delC germline mutation have an increased risk of contralateral breast cancer (CBC). Studies in general population-based BC cohorts showed that radiation treatment for the first BC increases CBC risk, while systemic therapy decreases CBC risk. We aimed to assess, in the largest dataset available to date, the joint effects of CHEK2 c.1100delC status and adjuvant therapy on CBC risk.

Methods: The study dataset derived from the international Breast Cancer Association Consortium consisted of 69,345 women (including 748 CHEK2 c.1100delC carriers) from European ancestry diagnosed with invasive, stage I-III BC between 1980 and 2018. Delayed entry Cox regression models, stratified by country, were used to estimate the association of adjuvant therapy with CHEK2 mutation status and time to CBC. Analyses were adjusted for age at diagnosis, ER-status, nodal status, size and grade of first BC. Potential differential effects of adjuvant therapy by CHEK2 c.1100delC status were tested by including interaction terms in the multivariable model. Multiple imputation was used to handle missing values.

Results: Within this dataset, chemotherapy (HR = 0.81; 95% CI = 0.68-0.95) and endocrine therapy (HR = 0.71; 95%CI = 0.59-0.84) reduce CBC risk, while there was no effect of radiation therapy on CBC risk found (HR = 1.02; 95%CI = 0.87-1.18). Furthermore, there was no evidence of differential effects of chemotherapy (Pinteraction =0.45), endocrine therapy (Pinteraction =0.91) or radiation (Pinteraction = 0.20) by CHEK2 c.1100delC status on CBC risk.

Conclusion: Preliminary results showed no evidence that CHEK2 c.1100delC carriers may respond differently to any adjuvant treatment than non-carriers.

References:.

Grants:.

Conflict of Interest: None declared.

P13.037.C Protein expression analysis in NOMO1 knockout cells, a recurrent alteration in early-onset colorectal cancer

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Background/Objectives: Early-onset colorectal cancer (EOCRC; age younger than 50 years) incidence has been steadily increasing over the last decades worldwide. Recently, we have detected a homozygous deletion of the *NOMO1* gene in more than 70% of EOCRC patients, suggesting that it could be a molecular marker associated with EOCRC. However, the mechanisms of NOMO1 in EOCRC carcinogenesis are currently unknown. In this work, we analyzed the changes in protein expression between NOMO1 Knockout and NOMO1 wild type cell lines by liquid chromatography-mass spectrometry (LC-IMS/MS).

Methods: CRISPR/Cas9 technology was used to generate NOMO1-KO HCT116 (EOCRC) and HS-5 (bone marrow) cell lines. 0.5 µg of total protein was digested with trypsin and used for LC-IMS/MS analysis. Raw files were analyzed by MaxQuant software using Andromeda. Data search was against the Human UniProt Reference Proteome and WebGestalt tool was used for proteome data analysis.

Results: A total of 12 overexpressed (such as HMGA1) and 16 infraexpressed proteins (such as NOMO, NCLN, CTND1, HMGB1 and LMNB1) showed up in common between the two cell lines. Interestingly, CTND1, HMGA1 and LMNB1 alterations are associated with a modification to migration capability, which may explain the increased migratory ability that we observed in NOMO1 KOs.

Conclusion: NOMO1 loss leads to protein expression changes that could explain its implication in the development of EOCRC. Further studies are being performed to explore other signaling pathways deregulated by the loss of NOMO1 that might play a relevant role in the pathogenesis of the disease.

References:.

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Conflict of Interest: None declared.

P13.038.D Combined conventional cytogenetic and genomic testing in hematological malignancies: the added value of the old and the new

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Background/Objectives: Conventional karyotyping and FISH are the gold standards for the genetic diagnosis, monitoring and prognosis of hematological malignancies. Genomics have introduced new approaches to these clinical questions. We aim to show the synergistic value of traditional and exome sequencing approaches in hematological-patient management.

Methods: In 20 prospective cases, karyotype/FISH/exome analyses were requested to investigate hematological malignancies. Bone marrow or peripheral blood samples were processed with karyotype/FISH with standard procedures. Exome sequencing was performed on DNA extracted from the same samples: libraries preparation was performed with Clinical Exome (Sophia Genetics) or Whole Exome (Twist Bioscience) and sequenced on Illumina NextSeq-550 (Illumina). Bioinformatics analyses were conducted by SOPHiA DDM® bioinformatics pipelines.

Results: Exome sequencing single-nucleotide-variation (SNV) analysis revealed actionable variants in 17 cases, while copy number variation (CNV) analysis detected large deletions/duplications in 11 cases. Cytogenetic analyses revealed clinically significant findings in 13 cases. Discrepant results between exome and cytogenetic analysis were either due to balanced aberrations, limits of detection, the chromosomal constitution complexity, test failure or test not performed.

Conclusion: Chromosomal analysis is superior to exome sequencing CNV analysis due to its ability to detect clinically significant balanced abnormalities and complex clones. Nevertheless, exome CNV analysis may detect critical deletions/duplications thus complementing chromosomal analysis or even reveal crucial information where chromosome analysis is unavailable. On the other hand, incorporating exome sequencing in routine hematologic diagnosis, actionable gene variants can be detected allowing diagnosis/prognosis confirmation and most importantly, consist eligibility criteria for targeted therapies or inclusion in clinical trials.

References:.

Grants:.

Conflict of Interest: TARA KELLY Full time employee: Genotypos M.S.A, Despoina Iakovaki Full time employee: Genotypos M.S.A., Pavlos Pollakis Full time employee: Genotypos M.S.A., Vasiliki Katsini Full time employee: Genotypos M.S.A., Stavroula Samara Full time employee: Genotypos M.S.A., Aikaterini Oikonomaki Full time employee: Genotypos M.S.A., Georgia Christopoulou Full time employee: Genotypos M.S.A., Pantelis Constantoulakis Full time employee: Genotypos M.S.A.

P13.039.A Characterization of MRTFB loss in colorectal cancer cell lines

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Background/Objectives: Colorectal cancer (CRC) is one of the most frequent and deadliest tumours worldwide. The early-onset subtype (EOCRC, cases diagnosed under 50 years of age) has become especially relevant due to its poor prognosis along with its rapid incidence increase during the last decades. In the search for new EOCRC biomarkers, we found the region including *MRTFB* (myocardin-related transcription factor B) to be more frequently lost in patients below the age of 50. Thus, *MRTFB* is proposed as an EOCRC biomarker candidate.

Methods: *MRTFB* knockout (KO) colorectal cancer cell line HCT-116 was generated using CRISPR-Cas9 technology. KO clones were validated by Sanger and qPCR and MRTFB depletion was tested by Western blot. Characterization of the *MRTFB*-KO cell line was then carried out. MTT viability assays were performed to assess cell proliferation and cell cycle assays to check cell cycle status. Apoptosis assays were used to evaluate potential differences in cell death. Cell migration capability was evaluated by wound healing and transwell assays.

Results: Viable *MRTFB* clones were generated and validated, still no differences were found between the KO and the wild type (WT) cell lines during the characterization studies involving proliferation, apoptosis and cell cycle. Preliminary results show a small increase in cell migration capability in the *MRTFB*-KO cell line.

Conclusion: Preliminary results show no differences between the studied KO and WT cell lines phenotypes regarding cell viability. Further studies will be carried out to confirm the increased migratory capability seen in *MRTFB*-KO clones.

References:.

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Conflict of Interest: None declared.

P13.040.B Androgen receptor-independent pathways in the response to enzalutamide in breast cancer cell lines

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Background/Objectives: Breast cancer (BC) is the leading female cancer worldwide. Some patients develop resistance to traditional treatments after a variable period of time and some subtypes of breast cancer, like triple negative breast cancer, lack targeted therapies. The androgen receptor (AR) is emerging as a novel factor in breast cancer. It has been demonstrated that both AR and its inducible proteins are expressed in 60-80% of BCs. Thus, we evaluated BC cell line response to enzalutamide, an AR inhibitor, as a potential therapeutical alternative.

Methods: AR+ BC cell lines (BCCLs): BT549, BT474 and MCF7 and AR- HCC1937 and HCC1569 were used. MTT and apoptosis assays were performed to test enzalutamide and dihydrotestosterone sensitivity. Stable AR+ cell lines (HCC1937, HCC1569) were constructed using lentiviral vectors. Western blot and RT-qPCR were used to validate AR status.

Results: Our results show that enzalutamide inhibits cell growth and induces apoptosis in both AR+ and AR- BCCLs. Additionally, dihydrotestosterone stimulation decreases enzalutamide-mediated cell viability inhibition regardless of AR status. Response to enzalutamide treatment is independent of AR status in AR- and AR+ HCC1937 and HCC1569.

Conclusion: Our study suggests that enzalutamide could mediate its action through AR-independent pathways.

References: Anestis, A., Zoi, I., Papavassiliou, A. G., & Karamouzis, M. V. (2020). Androgen receptor in breast cancer—clinical and preclinical research insights. Molecules, 25(2), 358.

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Conflict of Interest: None declared.

P13.041.C Di-genically inherited highly penetrant adenomatous polyposis- and colorectal cancer syndrome

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Background/Objectives: Most cases of adenomatous hereditary polyposis- and colorectal cancer (CRC) syndrome (HPCS) result from heterozygous *APC* or bi-allelic *MUTYH* germline pathogenic variants (PVs). Rare monogenic recessive or dominant adenomatous HPCS resulting from different constitutional DNA repair defects have also been uncovered. Nevertheless, many HPCS cases remain unexplained.

Methods: To explore genetic aetiology in two teenage siblings with multiple adenomas and CRC, we performed comprehensive *PMS2*-mutation analysis including transcript analysis, massive-parallel sequencing of a polyposis gene panel, germline microsatellite instability (gMSI)-testing, and tumour mutational profiling.

Results: Absence of polyposis in both parents and PMS2-expression loss in the neoplastic and surrounding mucosa cells of both siblings' CRCs initially suggested constitutional mismatch-repair deficiency (CMMRD) as the underlying condition. However, we identified only a maternally inherited heterozygous *PMS2*-exon 12 deletion, NM_000535.7:c.2007-786_2174+493del1447, in both siblings, and excluded CMMRD by showing absence of gMSI, its pathognomonic feature. A paternally inherited POLD1 variant NP_002682.2:p.Asp316Asn, previously associated once with classical polymerase proofreading (PP-)associated polyposis (PPAP), was identified in both siblings. The patients' extreme phenotypes suggest that their constitutional PP-defect is compounded by, and may even increase their propensity for, somatic MMR-deficiency from second hits in *PMS2*. Indeed, both siblings' CRCs were ultramutated (>100Mut/Mb) with mutational signature SBS20 rendering strong evidence for concurrent Pol δ-associated PP- and MMR-deficiency.

Conclusion: These siblings represent the first cases of a digenic severe adenomatous HPCS, which may escape detection due to the nature of the underlying *PMS2*-PV and the combined somatic MMR- and PP-deficiency, which is occasionally seen in related monogenic HPCS.

References:.

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Conflict of Interest: None declared.

P13.042.D Tumour radio-sensibility by PX478, a hypoxia-inducible factor 1-alpha inhibitor

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Background/Objectives: Hypoxia is a common situation in the solid tumours' microenvironment. It is associated with resistance to radiotherapy and with poor clinical results. The activation of Hypoxia-inducible Factor (HIF) signalling pathway plays a pivotal role in the tumour adaptation to hypoxia. HIF proteins regulate the transcription of several genes implicated in angiogenesis, apoptosis, metastasis or tumoral growth and their overexpression correlates with a poor prognosis after radiotherapy. So, HIF inhibitors are used to improve the cell response to radiotherapy. Here, we study the effects of PX478, a first-generation HIF-1-alpha inhibitor, on tumoral cell lines under hypoxic and normoxic conditions.

Methods: HT29 (Colon), MCF7 (Breast), HCC1937 (Breast), VCAP (Prostate), CAL33 (Head and Neck) cell lines were used to test PX478 radio-sensibility through MTT, cell cycle and apoptosis assays. Western blot was used to analyse the expression of HIF-1-alpha.

Results: Western blot analysis showed that the expression of HIF-1-alpha was partially inhibited by PX478 in a dose-dependent manner. PX478 inhibited cell proliferation and decreased cell survival in normoxia but especially in hypoxic conditions. In addition, the compound increased the radio-sensibility of HT29 and results in other cell lines are pending.

Conclusion: PX478 inhibited the expression of HIF-1-alpha in a dose-dependent manner. This drug had a strong effect on cell survival and increased radio-sensibility, especially in hypoxic conditions.

References: Albadari N; Deng S, Li W. Expert Opin. Drug Discov. 2019. https://doi.org/10.1080/17460441.2019.1613370.

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Conflict of Interest: None declared.

P13.043.A Second-hit in ATM in a case of gallbladder cancer: hints at driver mechanism, therapeutic targetand cancer susceptibility redefinition

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Background/Objectives: Invasive biliary cancers are severe neoplasms with limited therapeutic options. The molecular bases are not fully elucidated, and targeted therapies are lacking.

Methods: A 65-year-old man presented with metastatic gallbladder cancer, biliary type. His father and his uncle had prostate cancer at age 70 and 63. Next Generation Sequencing (NGS) analysis of 324 genes implied in cancer development and progression was performed on tumor sample to identify potential molecular therapeutic targets. Variants with possible germ-line origin were confirmed on DNA extracted from peripheral blood lymphocytes with Sanger sequencing.

Results: NGS on tumoral DNA identified the pathogenic c.4611_4611+9del, p.(Pro1480Tyrfs5*), rs80969040 and c.901+1G>A, variants in the ATM gene (MIM*607585;NM_000051;NP_000042.2). The c.4611_4611+9del was demonstrated to be of germ-line origin in heterozygosity. It has a frequency of 8/1000000 in GnomAD v.2.1.1. The presence of a germ-line mutation and a concurrent somatic pathogenic variant suggest a second-hit mechanism for ATM in this case. In accordance with recent cases published in the literature, this means that heterozygous ATM variants might lead to biliary/gallbladder cancer susceptibility and that ATM loss-of-function might be implied in biliary/gallbladder cancer tumorigenesis. The finding lead to the proposal a PARP-inhibitor drug in conjunction with standard chemotherapy protocols. This class of drugs might be considered even in sporadic cases with loss of ATM.

Conclusion: ATM variants might lead to biliary/gallbladder cancer susceptibility, and the gene might be a driver in biliary cancer development. This can lead to diagnostic and therapeutic changes.

References:.

Grants:.

Conflict of Interest: None declared.

P13.044.B Clinical and histopathological features predictive of BRCA1/2 pathogenic variants in ovarian cancer patients: single-center experience and meta-analysis

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Background/Objectives: BRCA1/2 testing is currently recommended in almost-all ovarian cancer patients to inform treatment and prevention. In order to identify prior predictors of BRCA1/2 germline variants, we compared features of ovarian cancer patients carrying BRCA1/2 pathogenic variants versus non-carriers among our patient series and in the literature.

Methods: Features of ovarian cancer patients tested for BRCA1/2 at our laboratory were registered and analysed. A systematic literature review, performed according to PRISMA guidelines, was synthesized using meta-analysis (PROSPERO 2021: CRD42021271815).

Results: Among 869 ovarian cancer patients, 188 carried germline BRCA1/2 pathogenic variants (21.6%); young age at diagnosis (56.6±11.1 vs 60.4±12.1 years, $p < 0.001$), personal breast cancer history (23.0% vs 8.6%, $p < 0.001$), family history of breast/ovarian cancer (53.5%/29.3% vs 30.3%/5.6%, $p < 0.001$), serous histotype, (87.1% vs 68.1%, $p < 0.001$), high grade (97.6% vs 81.6%, $p < 0.001$) and advanced stage (76.3% vs 66.6%, $p = 0.04$) were significantly more frequent in germline BRCA1/2 carriers than in non-carriers, while there were no statistically-significant differences regarding personal history of non-breast/ovarian cancers. According to the meta-analysis of 34 papers, predictors of BRCA1/2 alterations were: personal breast cancer history (OR 4.45, 95% CI: 3.58-5.52, compared to no personal breast cancer history), serous histotype (OR 2.49, 95% CI: 2.20-2.82, compared to other histotypes), grade 3 (OR 2.43, 95% CI: 2.01-2.93, compared to grade 1-2) and stage III/IV (OR 1.87, 95% CI: 1.65-2.11, compared to stage I/II).

Conclusion: Our results reinforce previous evidence on features associated to BRCA variants, providing data on the prior probability of finding alterations that may prove helpful in counselling patients and prioritizing testing.

References:.

Grants:.

Conflict of Interest: None declared.

P13.045.C Barrett's esophagus after esophageal atresia corrective surgery

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Background/Objectives: The surgical correction of Esophageal Atresia (EA) can impact the natural reflux barrier many patients suffer from motility problems, chronic gastroesophageal reflux and reflux esophagitis. These are risk factors for the development of Barrett's Esophagus (BE), a metaplastic lesion in which esophageal squamous epithelium is replaced with gastric columnar epithelium. EA patients have an increased population risk and earlier age of onset of BE. Recent advances have identified specific BE patient subclusters associations to the risk of developing esophageal adenocarcinoma (EAC). Interestingly, BE in EA patients seems to progress into EAC as well as esophageal squamous cell carcinoma and could represent a distinct subpopulation.

Methods: We compared the transcriptomes of mucosal esophageal biopsies of adults born with EA who developed BE to those of BE patients who did not have EA/TEF in their medical history. Differential expression analysis was done using DESeq2 after deconvolution using Granulator. To evaluate differences in isoforms-exon usage we used DEXSeq, aberrant splicing was evaluated using FRASER (Find Rare Splicing Events in RNA-seq), Chromosomal stability using SuperFreq and mutational signatures

and driver gene variation were determined with VarScan and FreeBayes.

Results: Using this experimental set-up, we created genomic signatures. Initial analysis revealed differences in some of these signatures as well as increased inflammatory, stress response and activation of oncological processes.

Conclusion: Results hint at differences in tissue homeostasis between BE patients with and without EA in their medical history. It is worthwhile to investigate these differences more systematically in a larger study population.

References:.

Grants:.

Conflict of Interest: None declared.

P13.046.D MiRNA expression in non-small cell lung cancer

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Background/Objectives: MicroRNAs (miRNAs) are small non-coding RNAs expressed in various tissues and cell types.They can help understanding the carcinogenesis of lung cancer and serve as potential diagnostic biomarkers for differentiating lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC).The aim of the present study is to analyse and compare the expression patterns of miRNAs in LUAD and LUSC samples.

Methods: The expression of 24 NSCLC patients(12LUAD,12LUSC) was evaluated by SurePrint human miRNA microarrays (Agilent Technologies).The validation of the selected miRNAs was performed on enlarged cohort(50LUAD,50LUSC) by qRT-PCR.The normalization of data, statistical and target prediction analyses were performed using R version 3.0.2 and GSEA with the Python package,GSEApy(version0.9.12).

Results: We assessed the expression levels of 2549 human mature miRNAs and found 107 to be significantly differentially expressed($FC>2.0; p < 0.05$) between the LUAD and adjacent normal tissues. 240 miRNAs were significantly differentially expressed($FC>2.0; p < 0.05$) between the LUSC and adjacent normal tissues. 26 miRNAs were common for LUAD and LUSC but showed different expression pattern.After bioinformatics analyses we chose the most suitable miRNAs and validated them in an extended cohort by qRT-PCR.GO and pathway enrichment analysis was performed to investigate relationship between miRNA and targeted mRNA.

Conclusion: Our results were in agreement with previous observations that different panels of miRNAs including miR-375&miR-21 have better potential to discriminate between LUAD and LUSC.The expression patterns of validated miRNAs and their target genes revealed both common and subtype specific signal pathways for LUAD and LUSC.However further analysis in enlarged samples and validation is necessary to ascertain their diagnostic potential in NSCLC.

References: None.

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Conflict of Interest: None declared.

P13.047.A Characterization of driver mutations in the erb-b2 receptor tyrosine kinase 2 (ERBB2) gene

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Background/Objectives: We adapted duplex sequencing to screen a unique type of mutagenesis that confers a selective growth advantage in *ERBB2* gene [1-3]. We hypothesize that a series of driver mutations in this oncogene are expanding in sperm and testis. Some of these mutations lead to aberrant ERBB signaling that could induce the clonal expansion of mutant germ cells with paternal age and testis specific-mosaicism.

Methods: Further, we characterized the clonal expansion of candidate hotspot mutations in the testis by droplet digital PCR (ddPCR), along with the analysis of the functional changes in the downstream activation of the ERBB2 signaling pathway by biophysical methods [4-7].

Results: Some of the mutations were identified as hotspot pathogenic variants that could be expanding at low, sub-clonal levels in the male germline. Further, the functional analysis showed that the selected variants have a significant activation increase compared to the wild-type constructs [6-8].

Conclusion: The identified variants found at low levels might contribute to tumor development creating a microenvironment that promotes malignancy.

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Conflict of Interest: None declared.

P13.048.B Genomic landscape of endometrial polyps

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Background/Objectives: Endometrial polyps are common gynecological lesions affecting approximately 10% of women. While polyps are non-cancerous, some may undergo malignant transformation. The pathogenesis of polyps is largely unknown. The aim of this study was to characterize the genomic landscape of endometrial polyps.

Methods: Twenty-four fresh frozen endometrial polyps from 24 patients and corresponding blood samples from 18 of the patients were whole-genome (WGS) and whole-exome sequenced (WES). WGS and WES data were combined to increase the sequencing coverage. Presence of somatic single nucleotide (SNV), copy number, and structural variants were analyzed and filtered using the corresponding blood samples and a panel of normals.

Results: We identified *HMGA1* and *HMGA2* rearrangements in 18/24 (75%) polyps. These rearrangements recurrently involved

7p15.1, *RAD51B, LRMDA* and *TRAF3IP2* as translocation partners. The SNV analysis revealed a high mutational burden comparable to cancers. Polyps carry recurrent low-allelic fraction mutations in cancer genes, including well-established cancer driver mutations in *KRAS, PIK3CA, PIK3R1*, and *PTEN*.

Conclusion: Most polyps harbor chromosomal alterations affecting *HMGA1* and *HMGA2*. Cancer-associated driver mutations indicate that endometrial polyps may act as precursor lesions to cancer. These findings contribute to our understanding on polyp pathogenesis, and provide tools for the development of personalized, non-invasive treatment options.

References:.

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Conflict of Interest: None declared.

P13.049.C Digenic contribution of Cancer driver genes and TNFRSF13B rare variants in malignancies predisposition

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Background/Objectives: Mutations in the *TNFRSF13B* gene encoding TACI (Transmembrane Activator and CAML Interactor) were previously associated with common variable immunodeficiency (CVID). A normal function of immunity-related genes and their signaling pathways safe-guards against the development of tumor, whereas its impairment might increment the likelihood of their recurrence.

Methods: By Exome Sequencing (ES) we analyzed a series of 60 individuals divided into a control group (30 patients) and an affected cohort from different forms of cancer (30 patients).

Results: We focused on cancer patients in whom at least one pathogenic or likely pathogenic variants in cancer genes belonging to ERN-GENTURIS panel have been detected. Simultaneously, data analysis in the same patients identified recurrent heterozygous stop-variants (c.431C>G, p.Ser144*; c.706G>T, p.Glu236*; c.198C>A, p.Cys66*; c.579C>A, p.Cys193*) and a missense-variant (c.310T>C, p.Cys104Arg) (CADD 25.7) in the *TNFRSF13B* gene. The significant association ($p < 0.005$) of heterozygous rare pathogenic or likely pathogenic variants in *TNFRSF13B* in cancer patients suggests a possible digenic inheritance of cancer driver genes and *TNFRSF13B*.

Conclusion: In conclusion, this study supports a major role of the immune system (and especially the cell-mediated system) in the malignancies predisposition. Analysis of additional cases and families will be necessary to more precisely assess the risk associated with *TNFRSF13B* variants.

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Grants:.

Conflict of Interest: None declared.

P13.050.D Identification and characterisation of BMPR1A and SMAD4 germline variants in patients with colorectal adenomatous polyposis

Anna Sommer[1], *Iris te Paske*[2], *J. Garcia-Pelaez**[3;4;5], *Leslie Matalonga*[6], *Dieter Wolf*[7], *Rita Katharina Knaust*[7], *Sophia Peters*[1], *Verena Steinke-Lange*[7;8], *Elke Holinski-Feder*[7;8], *Andreas Laner*[7], *Carla Oliveira*[3;4;5], *Nicoline Hoogerbrugge*[2], *Richarda De Voer*[2], *Isabel Spier*[1;9], *Stefan Aretz*[1;9]

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Background/Objectives: Colorectal polyposis syndromes are rare inherited disorders with a high colorectal cancer lifetime risk. The European Reference Network GENTURIS aims to uncover causal germline variants for unexplained polyposis in a large multicentre cohort. Therefore, whole-exome sequencing (WES) data from unsolved patients are re-analysed within the European Solve-RD project.

Methods: Germline-WES data from 211 unrelated patients with adenomatous or serrated polyposis from multiple centres in the Netherlands and Germany were re-evaluated to identify rare, (likely) pathogenic variants. Transcript analyses were performed to evaluate potential splice variants.

Results: In a first analysis of known polyposis genes, one truncating, pathogenic variant in *SMAD4* c.1231_1232delAG;p.(Ser411Leufs*17), and two presumed splice variants c.67G>C and c.868+2dup in *BMPR1A* were identified. Aberrant splicing could be confirmed for c.868+2dup by RNA-Seq, and is likely for c.67G>C based on conventional transcript analysis. Surprisingly, the variants were found in three unrelated patients with an adenomatous polyposis phenotype. Histologic reclassification by an experienced gastrointestinal pathologist confirmed the adenomas, just the polyps in the *SMAD4* variant carrier seemed likely juvenile when being aware of the affected gene.

Conclusion: Pathogenic germline variants in the *BMPR1A* and *SMAD4* genes cause juvenile polyposis. In casuistic reports, single pathogenic *BMPR1A* variants were described as causative of a mixed or untypical polyposis. The observed genotype-phenotype discrepancy supports a broader disease spectrum of *BMPR1A* and *SMAD4* germline variants and challenges to classify juvenile polyps correctly. Given the phenotypic variability, germline mutation screening in patients with a suspected adenomatous polyposis should be extended by other polyposis genes.

References:.

Grants:.

Conflict of Interest: None declared.

P13.051.A Somatic variant PARP4 c.3509C>T identified in ctDNA in patients with colorectal cancer, preliminary results

Hristo Ivanov[1;2], *Gabriela Raycheva*[3;4], *Janet Grudeva-Popova*[3;4], *Peter Shopov*[5], *Nelly Miteva-Marcheva*[1], *Ivan Zheliazkov*[1], *Aleksandar Linev*[1], *Dimitar Dimitrov*[1], *Momchil Topalov*[1], *Vili Stoyanova*[1;2]

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Background/Objectives: Colorectal cancer (CRC) is the third most common cancer worldwide and affects both genders equally. Tumors arising from the colorectal tract are heterogeneous diseases that result from the accumulation of distinctive genetic mutations. The aim of this study is to identify genetic alterations in patients with colorectal cancer by liquid biopsy.

Methods: ctDNAs were isolated from 15 patients with colorectal cancer and a target sequencing of a panel of 484 target genes was performed.

Results: A somatic variant PARP4(c.3509C>T, p.Thr1170Ile) was identified in 10 (66%) out of 15 patients. A recent study found different PARP4 variants in 13,46% patients with colorectal cancer. The c.3509C>T variant was also detected at significant high frequency in patients with primary thyroid and breast cancers. A gene set meta-analysis identified PARP4 as a candidate prognostic factor for CRC metastasis. Gene expression and proteomic analysis showed a trend of PARP4 in down-regulation in more aggressive CRC cell lines, implying its potential as a prognostic candidate.

Conclusion: The results of our study and these exploratory analyses need to be confirmed, and functional experiments demonstrating the role of PARP4 in CRC need to be performed.

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Conflict of Interest: None declared.

P13.052.B Synergistic effect of Chloroquine and Non-Homologous End Joining inhibitors through induction of DNA damage and inhibition of DNA repair in ovarian cancer

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Background/Objectives: Ovarian cancer (OC) is the deadliest gynecologic malignancy; therefore, new therapeutic strategies are needed. We have previously shown that Chloroquine (CQ) induced DNA double strand breaks (DSBs) that are repaired by homologous recombination (HR) (*1*). The aim of this study was to analyze whether Non-Homologous End Joining (NHEJ), the other main pathway that repairs DSBs, was also involved in the repair of CQ-induced lesions and then, if the combination of CQ with NHEJ inhibitors (NHEJi) could be effective against OC.

Methods: OC cell lines (A2780, IGROV-1, OVCAR-8 and SK-OV-3) were used. DSBs were detected monitoring phosphorylation of H2AX by immunofluorescence. MTT, cell cycle and apoptosis assays were performed to test NHEJi (KU-57788, NU-7026, SCR7 pyrazine) anticancer activity. Synergistic interaction between Chloroquine and the three NHEJi was assessed using Chou-Talalay method.

Results: CQ induced DSBs that were completely repaired after the removal of this compound but persisted in the presence of NHEJ inhibitors. These results revealed that CQ-induced lesions are also repaired by this DNA repair mechanism. The combination of CQ with NHEJi caused a synergistic effect in all cell lines analyzed. The triple combination Chloroquine, NHEJi and an HR inhibitor, Panobinostat, exerted a stronger synergistic effect than the double combinations.

Conclusion: Our study suggests that the Chloroquine-NHEJi combination could be a novel therapeutic strategy against OC.

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Grants: This project was funded by FIS-FEDER PI20/01569 and project CSI264P20.

Conflict of Interest: None declared.

P13.053.C Whole genome and transcriptome sequencing reveals a complex rearrangement affecting MLH1 and LRRFIP2 in a family with Lynch syndrome

Ulrike Faust[1], *Dennis Witt*[1], *Kristin Bosse*[1], *Rebecca Buchert*[1], *Gertrud Strobl-Wildemann*[2], *Marc Sturm*[1], *Jakob Admard*[1], *Nicolas Casadei*[1], *Olaf Riess*[1], *Tobias Haack*[1], *Christopher Schroeder*[1]

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Background/Objectives: Lynch syndrome is a highly penetrant autosomal dominant cancer predisposition syndrome associated with a high risk of colorectal, gynecological and other cancers. The purpose of this study was to use combined genome and transcriptome sequencing (GS, TS) to search for a potential causative genomic rearrangement in a family with suspected Lynch syndrome and no pathogenic germline variant in all four MMR genes *MLH1, MSH2, MSH6*, and *PMS2* by conventional sequencing.

Methods: Genomic DNA and RNA from blood was used for GS and TS. Sequencing data was analyzed using an open-source bioinformatic data analysis pipeline (megSAP). An *in-house* software (*GSvar*) was used to further prioritize potentially clinically relevant DNA variants.

Results: We performed GS and TS in the 56-year-old index patient, diagnosed with colon cancer and cholangiocarcinoma and identified a complex structural variant containing exons 16-19 of *MLH1* as well as a deletion of neighboring exons 28-4 in *LRRFIP2*. Subsequent TS revealed two fusion transcripts: *MLH1* exon 1-15 and *LRRFIP2* exon 29, and *LRRFIP2* exon 1-3 fused with *MLH1* exon 16-19, confirming our initial finding. Meanwhile additional family members were found to be carriers of the pathogenic variant by direct sequencing of the inversion breakpoints in *MLH1* intron 15 and *LRRFIP2* intron 3.

Conclusion: Genomic rearrangements in the *MLH1* gene have been reported to account for an important proportion of the mutation spectrum in HNPCC. We demonstrate that GS and TS are an effective strategy for the characterization of genomic rearrangements in patients showing no mutation by conventional screening methods.

References:.

Grants:.

Conflict of Interest: None declared.

P13.054.D Pathogenic germline variants of TP53 gene are rare in patients with chronic lymphocytic leukemia

Šárka Pavlová[1;2], *Jltka Malcikova*[1;2], *Johana Gombíková*[1], *Jana Šmardová*[3], *Karla Plevová*[1;2;4], *Jana Kotašková*[1;2], *Barbara Dvořáčková*[1],

Barbara Kunt Vonková[1], Kristýna Zavacká[1;2], Veronika Navrkalová[1;2], Jakub Trizuljak[1;2;4], Michael Doubek[1;2;4], Sarka Pospisilova[1;2;4]

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Background/Objectives: In chronic lymphocytic leukaemia (CLL), B-cell malignancy with the median age at diagnosis >65 years, TP53 defects are routinely examined. We analysed TP53 gene variants and their germline/somatic origin in 706 CLL patients.

Methods: Tumour and non-tumour gDNA were subjected to amplicon-based NGS. Variants were interpreted according to TP53Website and TP53Database; functional yeast assay (FASAY) was performed for germline variants.

Results: In 102/706 (15%) patients, exonic or splice site TP53 variants >10% VAF other than common population variants (Pro72Arg, Pro36=, Arg213=) were identified. Six of them (6%) were non-pathogenic variants of germline origin (~50% VAF; Asp49=; Ile254Val; Arg283Cys; Arg290His; Gly360Ala). None was accompanied by a second allele defect, and the patients were free of personal or family history suggesting Li-Fraumeni syndrome. In a patient with Gly360Ala, another pathogenic TP53 variant expanded after treatment.

Of the remaining 96 patients, the variant origin could be assessed in 81. Germline origin was excluded in 80 cases, while it was confirmed in one patient carrying the heterozygous variant Arg158Cys. This variant has been reported with conflicting interpretations of pathogenicity due to contradictory results of functional assays. Our FASAY results showed intermediate functionality. CLL was the first tumour of the patient (aged 56) without a family history of cancer.

Conclusion: Germline (likely) pathogenic TP53 variants are infrequent in CLL, which corresponds to late disease onset but should be considered, especially if the activity of mutated protein is partially retained. Rare population variants should not be misinterpreted as pathogenic.

References:.

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Conflict of Interest: None declared.

P13.055.A Investigating the effect of estradiol levels on the risk of breast, endometrial and ovarian cancer: a mendelian randomization study

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Background/Objectives: High levels of estrogen are associated with increased risk of breast and endometrial cancer and have been suggested to also play a role in the development of ovarian cancer. Cancerogenic effects of estradiol, the most prominent form of estrogen, has been highlighted as a side effect of estrogen replacement therapy. However, whether high levels of endogenous estrogens, produced within the body, promote cancer development, has not been fully established.

Methods: Here we performed both two and one-sample Mendelian randomization (MR), using hitherto unexploited genetic instruments, to estimate the effect of endogenous estradiol on the risk of developing breast, endometrial and ovarian cancer.

Results: We showed that higher estradiol levels increase the risk of ER-positive breast cancer (OR = 2.43 [95% CI 1.07-5.51]), as well as ER-positive and negative breast cancers combined (OR = 1.99 [95% CI 1.02-3.87]). A significant effect was also identified for ovarian cancer (OR = 2.26 [95% CI 1.09-4.71]). However, we could not establish a clear link to the risk of endometrial cancer (OR = 1.93 [95% CI 0.77-4.80]).

Conclusion: Our results suggest that high production of estradiol in the body triggers the development of breast and ovarian cancer.

References:.

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Conflict of Interest: None declared.

P13.056.B In-frame deletions and insertions in TP53 gene identified in leukemia patients result in p53 protein inactivation

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Background/Objectives: In tumours, in-frame deletion/insertions in the TP53 gene are generally presumed to have a deleterious effect on protein function due to disruption of protein structure. However, functional data are missing for most of them. This fact causes controversy during their interpretation.

Methods: TP53 variants resulting in an in-frame deletion and/or insertion of amino acids identified during routine TP53 screening in chronic lymphocytic leukaemia (CLL) were subjected to functional analysis of separated alleles in yeast (FASAY) [PMID:7732013]. FASAY assesses the ability of the p53 protein to bind to RGC promoter sequences and induce transcription of the reporter gene in yeast cells.

Results: We have identified 12 in-frame TP53 variants among 1465 patients examined with NGS (Table 1) and three via functional screening of an independent CLL cohort (Table 2). In FASAY, all these variants clearly showed the loss of ability to activate transcription of the reporter gene.

Table 1:

c.320_331del	p.(Tyr107_Arg110del)
c.329_337del	p.(Arg110_Phe113delinsLeu)
c.339_341del	p.(Phe113del)
c.480_485dup	p.(Met160_Ala161dup)
c.513_516delinsC	p.(Glu171_Val172delinsAsp)
c.516_584del	p.(Val173_Ser185del)
c.652_654dup	p.(Val218dup)

c.745_747del	p.(Arg249del)
c.764_766del	p.(Ile255del)
c.834_836del	p.(Gly279del)
c.845_846insTGG	p.(Arg282_Arg283insGly)
c.846_847insTGGAAAAGG	p.(Arg282_Arg283insTrpLysArg)

Table 2:

c.472_477del	p.(Arg158_Ala159del)
c.754_756del	p.(Leu252del)
c.754_762del	p.(Leu252_Ile254del)

Conclusion: Although we cannot exclude that functional in-frame deletions/insertions may occur, we showed that all tested in-frame deletions/insertions identified in patients with CLL resulted in the loss of the transactivation function of p53 protein.

References:.

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Conflict of Interest: None declared.

P13.057.C Identification of new pathogenic mutations in BRCA1 through homologous recombination assays

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Background/Objectives: *BRCA1* is a tumor suppressor frequently mutated in breast and ovarian cancer syndrome that plays an essential role in homologous recombination (HR), a DNA double-strand break repair mechanism necessary to maintain genome integrity. Hundreds of mutations in *BRCA1* are classified as variants of unknown significance (VUS), since their effect on protein function has not been determined. In this study, we use different functional assays to quantify the efficiency of HR of VUS identified in the Genetic Counselling Unit of Salamanca in the last 20 years.

Methods: VUS were re-analysed using ClinVar, VEP and Varsome databases. QuickChange II Site-directed mutagenesis kit was used to generate plasmids containing the studied VUS. HeLa and HCC1937-derived cell lines carrying HR reporter cassettes were transfected with the BRCA1 variants and HR proficiency, determined by the reconstitution of the green fluorescent protein, was analyzed by flow cytometry.

Results: 39 previously identified VUS were re-analysed and 16 were still classified as such. Seven variants (~43%) decreased *BRCA1*-mediated HR in the HeLa system. c.32T>G and c.5202T>G, located in the RING and BRCT domains, respectively, exhibited the strongest HR deficiency and were also tested and validated in the HCC1937 system. The identified pathogenic mutations are being introduced in the genome of HCC1937 (*BRCA1* -/-) for further functional characterization.

Conclusion: The HR-based functional assays are effective methods to analyze VUS in BRCA1 and have allowed the re-classification of 16 VUS in benign or pathogenic.

References:.

Grants: This project was funded by CSI264P20.

Conflict of Interest: None declared.

P13.058.D Next-Generation Sequencing of circulating DNA: an innovative approach to detect, monitor and treat head and neck cancer

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Background/Objectives: Head and neck cancers are very heterogeneous, aggressive and difficult to treat. Existing screenings are mainly limited to clinical and radiological examinations. We previously demonstrated that liquid biopsy is able to detect key mutations characterizing tumors and monitor cancer progression [1]. The aim of this study is to molecularly profile patients with head and neck cancer using this novel approach to improve treatment options and post-treatment surveillance.

Methods: NGS-liquid biopsy using cell-free DNA (cfDNA) was performed in a cohort of 41 patients with head and neck cancer.

Results: Pathogenic variants were identified in 34 out 41 patients. The most frequently mutated genes were *TP53*, *MET*, *PIK3CA, SMAD4* and *BRCA1/2*. We found a total of 91 pathogenic variants in 34 different genes. Our approach was also successful to identify somatic mutations with allele frequencies as low as 0.05% in patients who were not yet in an advanced clinical stage allowing a prompt therapeutic response against actionable genes.

Conclusion: Our results demonstrated that NGS of cfDNA in head and neck cancers is able to identify key mutations involved in the disease progression mechanism in 83% of cases in a non-invasive way revealing the genetic tumor profile of head and neck cancers in real-time detecting potential therapeutic targets.

References: 1. Palmieri M et al. J Cancer Metastasis Treat. 2020;6:55.

Grants:.

Conflict of Interest: None declared.

P13.059.A Overview of the genetic predisposition from a large cohort of medulloblastoma

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Background/Objectives: Medulloblastoma is among the most frequent brain neoplasia in the paediatric population and represents 60% of childhood intracranial embryonal tumours. In 2016, the World Health Organization defined four subgroups of medulloblastoma: WNT, SHH, Group 3 and Group 4. The establishment of risk factors is uncompleted, the genetic factors being the only one partially elucidated.

The aim of this study was to establish a review of the genetic predisposition in medulloblastoma.

Methods: From our large cohort of 610 grouped medulloblastomas received at the Genetic Somatic laboratory since 2000, 404 benefited from a tumour genomic screening including the common medulloblastoma predisposition genes *SUFU*, *PTCH1* and *TP53*.

Results: 36.6% (148/404) of our patients presented somatic alterations in those genes and the SHH subgroup was confirmed as the most frequent one (72.3%; 133/184). Among the 73 cases for which a germline analysis was possible, we identified nine cases of germline alterations of *SUFU*, four of *PTCH1* and two of *TP53*.

In addition, we described germline alterations in the following genes: *APC*, *ELP1*, *GPR161*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NF1*, *NF2*, *SMO* and *TCF4*.

Conclusion: Our study performed on a large panel of medulloblastomas confirms the previously published data regarding well characterized even though rare predisposition genes and highlights the need to orientate the patient in a genetic counselling, especially if presenting a SHH medulloblastoma. We also describe new predisposition genes that will be crucial to identify before deciding the treatment, to help the clinicians in their decision-making.

References: None.

Grants: None.

Conflict of Interest: None declared.

P13.060.B Microsatellite instability is common in non-colorectal and -endometrial malignant tumors of Lynch syndrome patients

Lisa Elze[1], *Rachel van der Post*[2], *Janet Vos*[3], *Arjen Mensenkamp*[1], *Mirjam de Hullu*[1], *Iris Nagtegaal*[2], *Nicoline Hoogerbrugge*[1], *Richarda De Voer*[1], *Marjolijn Ligtenberg*[1]

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Background/Objectives: Lynch syndrome (LS) is caused by germline pathogenic variants (PVs) in the mismatch repair (MMR) genes. LS patients have a high risk to develop MMR deficient (dMMR) colorectal and endometrial cancers, which also present with microsatellite instability (MSI). Currently, in LS patients the incidence of MSI in other cancer types (hereafter 'non-canonical' cancers) is largely unknown. MSI presence in these non-canonical cancers is important as MSI may predict response to immune checkpoint inhibitors. Here we aim to identify the frequency of MSI.

Methods: Full tumor history was retrieved from a complete, historical clinic-based cohort (1990-2020) of 1,751 confirmed LS patients from the Dutch pathology registry. Out of 1,751 LS patients, 746 patients (43%) had developed cancer of which 284 (38%) developed non-canonical cancers. In total, 206 non-canonical cancers were available for further investigation. MSI, somatic second hit alterations and dMMR were analyzed using targeted sequencing and immunohistochemistry of MMR genes in each non-canonical cancer.

Results: MSI was present in 58% (119/206) of cancers, which was higher in LS-associated cancers (small bowel, stomach, renal pelvis, ureter, brain and ovary) compared to non-associated cancers (82% vs. 41%). LS patients with MSH2 and MLH1 PVs more often presented with MSI non-canonical cancers compared to patients with MSH6 or PMS2 PVs (MLH1: 74%, MSH2: 77%, MSH6: 40%, PMS2: 18%, $P < 0.1$).

Conclusion: Non-canonical cancers in LS patients are also frequently MSI, especially LS-associated cancers. LS patients may benefit from optimized treatment if MSI testing is considered for all cancer types they develop.

References:.

Grants:.

Conflict of Interest: None declared.

P13.061.C The highest frequency of BRCA1 c.3700_3704del detected among Albanians from Kosovo

Ivana Maleva Kostovska[1], ***Simona Jakovchevska***[1], *Milena Jakimovska Özdemir*[1], *Sanja Kiprijanovska*[1], *Katerina Kubelka-Sabit*[2;3], *Dzengis Jasar*[2;3], *Marina Iljovska*[4], *Emilija Lazareva*[4], *Snezhana Smichkoska*[4], *Dijana Plaseska-Karanfilska*[1]

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Background/Objectives: The spectrum of BRCA1/2 mutations varies among populations; however some mutations may be frequent in particular ethnic groups due to the "founder" effect. The c.3700_3704del mutation was previously described as a recurrent BRCA1 variant in Eastern European countries. This study aimed to investigate the frequency of c.3700_3704del BRCA1 mutation in Albanian breast and ovarian cancer patients from North Macedonia and Kosovo.

Methods: A total of 327 patients with invasive breast and/or ovarian cancer (111 Albanian women from North Macedonia and 216 from Kosovo) were screened for 13 recurrent BRCA1/2 mutations. Targeted NGS with a panel of 94 cancer-associated genes including BRCA1 and BRCA2 was performed in a selected group of 118 patients.

Results: We have identified 21 BRCA1/2 pathogenic variants, 17 (14 BRCA1 and 3 BRCA2) in patients from Kosovo (7.9%) and 4 (1 BRCA1 and 3 BRCA2) in patients from North Macedonia (3.6%). All BRCA1/2 mutations were found in one patient each, except for c.3700_3704del BRCA1 mutation which was observed in 14 unrelated families, all except one originating from Kosovo. The c.3700_3704del mutation accounts for 93% of BRCA1 mutation positive cases and is present with a frequency of 6% among breast cancer patients from Kosovo.

Conclusion: This is the first report of BRCA1/2 mutations among breast and ovarian cancer patients from Kosovo. The finding that BRCA1 c.3700_3704del represents a founder mutation in Kosovo with the highest worldwide reported frequency supports the implementation of fast and low-cost screening protocol, regardless of the family history and even a pilot population-based screening in at-risk population.

References:.

Grants:.

Conflict of Interest: None declared.

P13.062.D Cytogenetic evaluation in myelodysplastic syndrome

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Background/Objectives: Myelodysplastic syndrome (MDS) is a hematological malignancy that is characterized by prolonged cytopenia in the bone marrow (BM) and has a risk of conversion to acute myeloid leukemia. Morphological changes in the bone marrow are often required for diagnosis, this also needs to be correlated with other laboratory tests such as cytogenetic analysis. Cytogenetic risk grouping according to IPSS-R (Revised International Prognostic Scoring System) is important in patient follow-up. In this study, conventional cytogenetic(CC) and fluorescent in situ hybridization(FISH) results of MDS are presented.

Methods: The results of cytogenetic analysis of BM materials of patients with MDS were evaluated retrospectively referred to our department, in the period of 2019-2021.

Results: A total of 152 patients were included in the study. Of these, 89 (58.5%) were male and 63 (41.5%) were female. The age range of the patients was 19-89 (mean age 65.2). CC and FISH results are summarized in the table below.

	First Diagnosis		Follow-up			
			2.time		3.time	
	CC (n = 137)	FISH (n = 143)	CC (n = 48)	FISH (n = 46)	CC (n = 25)	FISH (n = 18)
Normal	88	89	29	24	22	12
Pathological	49	54	19	22	3	6
One change	29	36	3	14	1	4
Two changes	11	8	9	1	0	0
Three or more changes	9	10	7	7	2	2

Table 1: Outline of the cytogenetics results.

Conclusion: CC findings provide important data for the diagnosis and prognosis of MDS, and additional cytogenetic methods, especially FISH, are clearly beneficial in the diagnosis and follow-up of disease.

References: 1- https://doi.org/10.5772/intechopen.97112.

Grants:.

Conflict of Interest: Can Berk Leblebici Ankara University Faculty of Medicine, Medical Genetics, Sule Altıner Ankara University Faculty of Medicine, Medical Genetics, Arzu Vicdan Ankara University Faculty of Medicine, Medical Genetics, Nuket Y Kutlay Ankara University Faculty of Medicine, Medical Genetics, TIMUR TUNCALI Ankara University Faculty of Medicine, Medical Genetics, Halil Gürhan Karabulut Ankara University Faculty of Medicine, Medical Genetics, Sadiye Ekinci Ankara University Faculty of Medicine, Medical Genetics, Hatice Ilgın Ruhi Ankara University Faculty of Medicine, Medical Genetics.

P13.063.A Targeted gene panel investigations in chronic lymphocytic leukemia patients

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Background/Objectives: Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease with various genetical abnormalities that can affect the treatment and survival of the patients. Next-generation sequencing (NGS) studies have described recurrently mutated genes in CLL. The aim of this study was to detect pathogenic variants of commonly mutated genes in CLL and to explore their associations with IGHV mutational status and cytogenetic abnormalities.

Methods: NGS analysis was performed on 75 blood samples from CLL patients using Twist Custom Panel kit and Illumina NextSeq according to the manufacturers' instructions.

Results: Overall, 42 of 75 patients (56%) harbored at least one mutation, the majority of variants (63%) was clonal. The most frequently mutated gene was TP53, followed by NOTCH1, BRIC3 and SF3B1. Subclonal variants (5-10% VAF) were also detected in 17/75 patients (22%), particularly in BIRC3 and NOTCH1 genes. TP53 and NOTCH1 variants occured mostly in unmutated-IGHV CLL cases. Patients with mutated-IGHV harbored mainly BIRC3 and FBXW7 variants. 17p deletions co-occured with TP53 clonal mutations in 6 of 8 patients. SF3B1 and TP53 variants occured with 11q deletions in 6 of 8 cases. BIRC3 clonal and subclonal variants were observed in 4 of 7 patients with favourable 13q deletions.

Conclusion: This study confirmed the presence of high number of clonal and subclonal variants in the most frequently mutated genes (TP53, NOTCH1, BRIC3, SF3B1) in CLL cases. These NGS results complementing the IGHV mutational status and cytogenetic data can contribute to better prognostic workup and management of patients with CLL.

References:.

Grants:.

Conflict of Interest: None declared.

P13.064.B Analysis of circulating miRNA profile in plasma sapmles of glioblastoma patients

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Background/Objectives: Glioblastoma multiforme (GBM) is among the most aggressive cancers with a poor prognosis. Treatment options are limited, clinicians lack efficient prognostic and predictive markers. Liquid biopsy represents a great advance in this field because it can be useful to monitor the evolution and recurrence of the disease as well as the response of patients to therapy. Circulating miRNAs – besides being important regulators of cancer development – may have potential as diagnostic biomarkers of GBM.

Methods: In this study, profiling of 798 human miRNAs was performed on blood plasma samples from 6 healthy individuals and 6 patients with GBM, using a NanoString nCounter Analysis System. To validate our results, five miRNAs (hsa-miR-433-3p, hsa-miR-362-3p, hsa-miR-195-5p, hsa-miR-133a-3p and hsa-miR-29a-3p) were randomly chosen for RT-qPCR detection.

Results: 53 miRNAs were significantly differentially expressed in plasma samples of GBM patients when data were filtered for FC < 1 and FDR < 0,1. Target genes of the top 39 differentially expressed miRNAs were identified, and we carried out functional annotation and pathway enrichment analysis of target genes via GO and KEGG-based tools. General and cortex-specific

protein–protein interaction networks were constructed from the target genes of top miRNAs to assess their functional connections.

Conclusion: We demonstrated that plasma microRNA profiles are promising diagnostic and prognostic molecular biomarkers that may find an actual application in the clinical practice of GBM, although more studies are needed to validate our results.

References:.

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Conflict of Interest: None declared.

P13.065.C Study the significance of miR-30 family members in ovarian cancer

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Background/Objectives: Estrogens are able to trigger cell proliferation and cell death in a dose dependent manner. MicroRNAs are considered to play an important role in estrogen response. Our aim was to characterize the function of miR-30s (tumor suppressors) in the response of ovarian cells to estradiol (E2).

Methods: The applied human ovarian cell lines were PEO1 (expressing Estrogen Receptor α and β) and A2780 (ERβ). The effect of high dose E2 was studied by determining cell proliferation, apoptosis, cellular lysis, mRNA and miR-30s expression.

Results: The basal expression of miR-30a-3p, miR-30a-5p, miR-30d-5p and miR-30e-5p proved to be higher in PEO1 than in A2780. The expression of miR-30a-5p, miR-30d-5p and miR-30e-5p was induced to high dose E2 treatment (>50μM) where intensive cell death was observed according to the induction of apoptosis (*TP53*) and autophagy (*BAG3, ATG2B*) related genes. ERα-expressing PEO1 cells had higher tolerance to high dose E2 than A2780 what might be caused by the induction of ERα mediated estrogen response (*GREB1*, *CA12*). Bioinformatic analysis of miR-30s revealed that several targets are shared by miR-30a-5p, miR-30d-5p and miR-30e-5p that are involved in the regulation of cell proliferation and cell death related pathways. The application of miR-30d-5p mimic reduced cell proliferation and decreased the tolerance of PEO1 cells to high dose E2.

Conclusion: MiR-30a-5p, miR-30d-5p and miR-30e-5p might mediate the stress-response induced by high dose E2 in ovarian cells. MiR-30d-5p might be a promising therapeutic target in ovarian cancer.

References:.

Grants: FK138021 (National Research Development and Innovation Office, Hungary) and EFOP-3.6.3-VEKOP-16-2017-00009 projects.

Conflict of Interest: None declared.

P13.066.D Comparative analysis of transcriptomic changes including mRNA and microRNA expression induced by xenoestrogens in ovarian cells

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Background/Objectives: Xenoestrogens are natural (e.g. zearalenone; ZEA) or synthetic compounds (e.g. bisphenol A; BPA), which mimic the effect of physiological estrogens. The exposure to xenoestrogens increases the risk for ovarian cancer. The aim of our study was to compare the transcriptomic changes induced by physiological estradiol (E2) and xenoestrogens in ovarian cells.

Methods: PEO1 human ovarian cell line was treated with E2, ZEA and BPA. Transcriptomic changes were studied by mRNA and miRNA sequencing (Illumina NextSeq 500). Validation of data was made by qPCR.

Results: Estrogen exposure induced remarkable transcriptomic changes: 304, 283 and 62 genes were up-regulated (log2FC>1); 288, 255 and 44 genes were down-regulated (log2FC<-1) in response to E2, ZEA and BPA. Furthermore, the expression of 12 (E2), 11 (ZEA) and 10 (BPA) miRNAs changed significantly (log2FC>1, or log2FC<-1). Functional enrichment analysis revealed several pathways related to the regulation of cell proliferation, apoptosis and migration. The effect of xenoestrogens was highly comparable to E2: 319 genes were co-regulated in response to E2 and ZEA and the expression of 81 genes changed in response to all the three estrogens tested. Furthermore the expression of miR-197-5p, miR-5008-5p and miR-501-5p were down-regulated in response to at least to two treatments. Correlation proved to be high (r>0.9) between the data obtained from RNA sequencing and qPCR.

Conclusion: Xenoestrogens induce relevant transcriptomic changes that are comparable with E2. We identified key genes and miRNAs that might contribute to the carcinogenic effect of xenoestrogens.

References:.

Grants: FK138021 project, National Research Development and Innovation Office (Hungary).

Conflict of Interest: None declared.

P13.067.A Disequilibrium between BRCA1 and BRCA2 circular and messenger RNAs plays a role in breast cancer

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Background/Objectives: Circular RNAs (circRNAs) are single-stranded covalent structures in closed loops produced by back-splicing. As backsplicing and linear splicing compete against each other, we hypothesize that a balance disruption between circRNAs and messenger RNAs (mRNAs) could promote tumorigenesis and used breast cancer and BRCA1 and BRCA2 genes as a model.

Methods: We develop a novel gene-targeted technique for simultaneous quantitative and qualitative analyses of circRNAs and mRNAs. A total of 114 probes, located at exon extremities, were designed to explore all reported alternative transcripts for BRCA1 and BRCA2. Following reverse transcription and probing on cDNA, nearby probes were ligated and the number of ligations quantified using unique molecular identifiers and

sequencing on a MiSeq. Hence, circRNAs and mRNAs were identified and quantified.

This method was first validated on a set of 169 formalin-fixed, paraffin-embedded tumour samples harbouring various BRCA mutations (including splice mutations). Next, our hypothesis was tested using another test set of 95 pairs of tumour and adjacent normal breast tissues.

Results: On the validation set, all known circRNAs and mRNAs and those resulting from splice mutations were correctly identified. Four novel circRNAs were identified. On the test set, the ratio of BRCA1 and BRCA2 circRNAs/mRNAs were significantly lower in tumour breast tissue compared to normal tissue ($p = 6.9e-09$ and $p = 1.5e-06$ for BRCA1 and BRCA2, respectively).

Conclusion: We have validated an innovative method to study linear and backsplicing and showed for the first time that disequilibrium between BRCA1 and BRCA2 circRNAs and mRNAs plays a role in breast cancer.

References:.

Grants: Cancéropôle Nord-Ouest.

Conflict of Interest: None declared.

P13.068.B Comprehensive RNA and protein functional assessments contribute to the clinical interpretation of MSH2 variants causing in-frame splicing alterations

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Background/Objectives: Spliceogenic variants in disease-causing genes are often presumed pathogenic since most induce frameshifts resulting in loss-of-function. However, it was recently shown in cancer predisposition genes that some may trigger in-frame anomalies that preserve function[1]. Here, we addressed this question by using *MSH2*, a DNA mismatch repair gene implicated in Lynch syndrome, as a model system.

Methods: Eighteen *MSH2* variants, mostly localized within canonical splice sites, were analyzed by using minigene splicing assays. Then, the biological impact of the resulting protein alterations was assessed in a methylation tolerance-based assay. Clinicopathological characteristics of variant carriers were collected.

Results: Three in-frame RNA biotypes were identified based on variant-induced spliceogenic outcomes: exon skipping (E3/E4/E5/E12), segmental exonic deletions (E7/E15) and intronic retentions (I3/I6/I12/I13). The 10 corresponding protein isoforms exhibit either large deletions (49-93aa), small deletions (12/16aa) or insertions (3-10aa) within different functional domains. We showed that all these modifications abrogate MSH2 function, in agreement with the clinicopathological features of variant carriers.

Conclusion: Altogether, these data demonstrate that MSH2 function is intolerant to in-frame indels caused by the spliceogenic variants analyzed in this study, supporting their pathogenic nature. This work stresses the importance of combining complementary RNA- and protein-based approaches to ensure accurate clinical interpretation of in-frame spliceogenic variants.

References: [1]Meulemans et al, Skipping Nonsense to Maintain Function: The Paradigm of *BRCA2* Exon 12. Cancer Research, 2020.

Grants: FHU-NGP, Gefluc, (#R18064EE), INCa/DGOS (AAP/CFB/CI, FASDEC), the European Union/Région Normandie (ERDF), CNO and EDnBISE.

Conflict of Interest: None declared.

P13.069.C Whole exome sequencing reveals new candidate genes involved in non-MEN2 familial medullary thyroid cancer

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Background/Objectives: Medullary thyroid cancer (MTC) is a rare neuroendocrine tumor (2-5% of all thyroid cancer) derived from the parafollicular C-cells. About 75% of all MTCs are sporadic; the remaining 25% correspond to inherited cancer syndromes known as multiple endocrine neoplasia type 2 (MEN2), attributed to gain-of-function mutations of the RET proto-oncogene in >98% of cases. MEN2 includes three clinically differentiable types: MEN2A, MEN2B, and familial MTC (FMTC). However, there are FMTC cases in which RET is not affected and whose genetic cause remains unknown (non-MEN2 FMTC). In this study, we aimed at identifying possible causal variants involved in these rare familial forms.

Methods: We performed WES in nine non-MEN2 FMTC patients belonging to five different families, using an Illumina NextSeq550 sequencer. Multi-sample analyses were launched and annotated using the Varsome Clinical platform to study SNVs and CNVs. After variant filtering and prioritization, based on pathogenicity predictors, functional evaluation, and expression profiles, candidate variants were segregated and evaluated by Sanger sequencing in additional family members.

Results: We have identified new pathogenic variants in heterozygosis, in genes not previously associated with the disease. They are involved in diverse molecular functions, such as DNA repair, post-transcriptional regulation and a phosphatase receptor that regulates cell growth and differentiation. Further studies are on-going to assess the role of the candidate variants in the onset of the disease.

Conclusion: The determination of new genes involved in non-MEN2 FMTC forms offers new pathways for further elucidation of the molecular mechanisms that lead to the disease.

References: PMIDs:31717449;33407723.

Grants: ISCIII-ERDF/ESF(PI19/01550;FI20/00192;CD20/00171); Andalusian Government(PEER-0470-2019); I+D+i Funding-PAIDI2020(P20_00887).

Conflict of Interest: None declared.

P13.070.D Mutation Profile of Metastatic Castration-Resistant Prostate Cancer Patients Prior to Olaparib Treatment - EMA vs. FDA

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Background/Objectives: Olaparib has been approved by EMA and FDA for the treatment of patients with metastatic castration-resistant prostate cancer (mCRPC). However, the indication for its clinical use in the USA (FDA) and in the EU (EMA) differs

significantly. Patients with mCRPC in the USA are eligible for treatment if they harbor a somatic/germline mutation in ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, or RAD54L gene, whereas in the EU only patients with BRCA1 or BRCA2 gene mutations are eligible.

Methods: During the year 2021, 98 consecutive prostate tumor samples have been routinely genotyped for the presence of the mutations in ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54L genes, using Illumina's TruSightTumor 170 panel.

Results: Sequencing quality of 82% of samples was sufficient for data analysis. The most commonly mutated genes were BRCA2 (11%) and ATM (8%), followed by BRCA1 (3%), CHEK2 (2%) whereas RAD51B, RAD51C, and CDK12 were mutated in 1% of patients. More than half of patients (55%) did not harbor a mutation in any of the investigated genes.

Conclusion: Based on the tumor genotype, 14% of patients would be eligible for treatment with olaparib in the EU in comparison to 26% of patients in the USA.

References:.

Grants:.

Conflict of Interest: None declared.

P13.071.A Biallelic Pathogenic Variants in CHEK2 Predispose to Multiple Primary Tumours

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Background/Objectives: Biallelic germline pathogenic variants (PV) in CHEK2 are associated with an increased risk to develop breast cancers (BC) and suggested to predispose to other cancer types. Moreover, CHEK2-deficiency has been postulated to lead to homologous DNA repair deficiency (HRD). We aimed to elucidate the role of CHEK2-deficiency in the development of multiple primary tumours (MPT).

Methods: We collected individuals with a CHEK2-deficiency: i) from individuals who developed two or more tumours before 65 years (n = 94) who underwent germline whole-exome sequencing (WES), ii) by genotyping the CHEK2 c.1100delC PV in individuals who developed MPTs before the age of 60 (n = 787) and iii) identified through routine genetic testing, ERN GENTURIS centres and literature (n = 35 CHEK2-deficient cases). WES was performed on DNA from six tumour types (n = 16 tumours) and the somatic mutational signatures and cancer driver genes were analysed.

Results: We identified 3/94 (3%) and 3/787 (0.4%) MPT cases with CHEK2-deficiency in cohorts i and ii, respectively. In total, we collected 41 CHEK2-deficient cases, 85% were women (n = 35) and 76% developed at least one BC (n = 31). Next to BC, 51% of the cohort (n = 21) developed at least one benign (n = 9) and/or malignant (n = 19) tumour. CHEK2-deficient tumours harboured on average 3.14 variants/Mb (range 0.3–19.65). All tumours presented with clock-like SBS1 and SBS5 signatures and somatic PVs in cancer driver genes.

Conclusion: Individuals with CHEK2-deficiency are predisposed to develop MPTs. CHEK2-deficient tumours do not show HRD-associated mutational signatures. Our results suggest that individuals with MPTs should undergo germline genetic testing for CHEK2.

References:.

Grants: KWF 12174.

Conflict of Interest: None declared.

P13.072.B MassArray-based diagnostics of hot spot mutations in IDH1/2 and TERT genes and allelic losses of 1p/19q co-deletions in gliomas

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Background/Objectives: Multiplex genetic analysis, for example Next Generation Sequencing (NGS), is increasingly utilized for the routine identification of clinically significant biomarkers in cancer because it provides diagnostic, prognostic, and therapeutic guidance in a single workflow. However, NGS is expensive, laborious, and requires complicated bioinformatics analysis. The Agena MassARRAY system provides accurate, low cost, multiplexed analysis of hundreds of clinically relevant mutations with relatively simple analytics. In this study, we evaluated the utility of this system for the detection of somatic hotspot variants in the *IDH1, IDH2* and *TERT* genes and the identification of deletions of chromosomes 1p and 19q in a cohort of glial tumors.

Methods: Our custom panel was designed to identify 65 genetic alleles important for the diagnosis of glial tumors. Purified DNAs from 60 routine formalin-fixed, paraffin embedded tissue specimens was analyzed. Results were compared to orthogonal testing (FISH, IHC and/or NGS). Visualization of the genetic data was achieved using custom develop scripts in the R programming environment.

Results: Results obtained using the MassArray were >95% concordant to orthogonal FISH and NGS analysis. Testing required less DNA, was simpler to performer, and turnaround time was faster. A custom pipeline developed in R allowed visualization of the data and faciliated accurate interpretation.

Conclusion: In the current study, we described an alternative, multiplex method to NGS that is robust, fast, and inexpensive for the identification of 1p/19q co-deletion and variants of *IDH1/2* and *TERT* genes.

References:.

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stock options, Shuko Harada: None declared, Alexander Mackinnon: None declared.

P13.073.C Systematic functional analysis by hybrid minigenes of CHEK2 splice-site variants detected in the BRIDGES project

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Background/Objectives: *CHEK2* germline inactivating variants moderately increase the risk of developing breast cancer. Our goal was to functionally analyse *CHEK2* candidate spliceogenic variants identified in ~113,000 women in the large-scale sequencing project BRIDGES (https://bridges-research.eu/).

Methods: A total of 128 *CHEK2* variants of the intron-exon boundaries were bioinformatically analysed. Fifty-two potentially spliceogenic variants were found with splicing prediction programs, incorporated by site-directed mutagenesis into the minigenes mgChk2_ ex1-7 (exons 1 to 7), mgChk2_ex6-10 (exons 6 to 10) and mgChk2_e11-15 (exons 11 to 15), and functionally assayed in MCF-7 and HeLa cells.

Results: The three wild type minigenes produced the full-length transcripts of the expected size and sequence, with several alternative transcripts, all of which had been previously reported as physiological alternative events of *CHEK2*. Forty-nine variants (94.2%) impaired splicing and 34 out of them produced severe splicing anomalies. At least 113 transcripts were detected, 95 of which could be characterized (68 protein-truncating isoforms).

Conclusion: According to the biological indicators of pathogenicity (proportion of aberrant transcripts and predicted protein impact), 32 *CHEK2* variants could be classified as pathogenic or likely pathogenic variants. Splicing functional assays with minigenes have been proven to be a straightforward and robust method for the initial characterization of variant-splicing outcomes and the clinical interpretation of variants of any disease-gene.

References:.

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Conflict of Interest: None declared.

P13.075.A First estimates of diffuse gastric cancer risks for carriers of CTNNA1 germline pathogenic variants

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Background/Objectives: Pathogenic variants (PV) of *CTNNA1* are found in families evocative of hereditary diffuse gastric cancer (DGC) but no risk estimates were available until now. The aim of this study is to evaluate DGC risks for carriers of germline *CTNNA1* PV.

Methods: Authors of published *CTNNA1* families updated their data and unpublished families were identified through international collaborations. The cumulative risk of DGC by age for PV carriers was estimated with the Genotype Restricted Likelihood (GRL) method, taking into account non-genotyped individuals and conditioning on all observed phenotypes and genotype of the index case to obtain unbiased estimates. A non-parametric and the Weibull functions were used to model the shape of penetrance function with the GRL. Kaplan-Meier incidence curve and Standardized Incidence Ratios (SIR) were also computed. A "leave-one-out" strategy was used to evaluate estimate uncertainty.

Results: Thirteen families with 46 carriers of PV were included. The cumulative risks of DGC at 80 years for carriers of *CTNNA1* PV are 49%, 57% and 77% respectively with the Weibull GRL, NP GRL and Kaplan Meier methods. Risk ratios to population incidence reach particularly high values at early ages and decrease with age. At 40 years, they are equal to 65, 833 and 21,574 respectively with the Weibull GRL, NP GRL and SIR methods.

Conclusion: This large series of *CTNNA1* families provides the first risk estimates of GC. It will help to improve management and

surveillance for these patients and support inclusion of CTNNA1 in germline testing panels.

References:.

Grants:.

Conflict of Interest: None declared.

P13.076.B Can genetic testing in healthy individuals prevent inherited cancer? Genetic data analysis of a Spanish cohort

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Background/Objectives: A hereditary cancer risk assessment is the key to identifying patients and families who may be at increased risk of developing certain types of cancer. Recent studies show that more than 50% of individuals at risk have not received genetic nor genetic counselling and have not been identified. Improving the knowledge and access to testing by individuals and families at high-risk for cancer provides significant improvements for prevention and early treatment of inherited cancer.

Methods: We analysed the carrier rate of pathogenic/likely-pathogenic variants in 31 genes associated to high risk of inherited cancer in 30582 Spanish healthy individuals between September 2015 to August 2021. Genetic data was obtained by WES and variants were pre-categorized using an in-house algorithm according to ACMG criteria.

Results: 867 individuals out of 30582 (2,83%) presented pathogenic/likely-pathogenic variants in 26 out of the 31 genes related with cancer.

148 likely pathogenic and 295 pathogenic different variants were identified in our cohort, being the most frequent genes BRCA2 (76 variants) and ATM (71 variants). Genes with the most presence in the population were ATM (111 carriers), BRCA2 (105 carriers) and CHEK2 (100 carriers).

Conclusion: We identified a moderate amount of healthy individuals carrying variants in cancer related genes. These individuals have a high-risk of developing cancer and can benefit from a preventive cancer detection, have a regular screening or preventive treatment.

Awareness of the result may reduce any stress and anxiety that comes from not knowing through an appropriate genetic counselling programme.

References:.

Grants:.

Conflict of Interest: Cristian Perez-Garcia IGENOMIX, Jordi Perez-Lopez IGENOMIX, Daniel Sanchez-Valero IGENOMIX, Rocio Garcia-Jimenez IGENOMIX, Gemma Cartagena IGENOMIX, Eva Barroso IGENOMIX, Marina Martinez-Matilla IGENOMIX, Sandra Garcia-Herrero IGENOMIX, Javier Garcia Planells IGENOMIX.

P13.077.C Triple-negative breast cancer in association with RAD51C pathogenic variants

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Background/Objectives: *RAD51C* pathogenic variants (PV) are associated with high risk of ovarian cancer (OC) in female carriers. The association with breast cancer (BC) is less well established, but there is some evidence triple-negative disease is more common in *RAD51C* positive patients. The aim of our study was to investigate the tumour and mutation spectrum in Slovenian *RAD51C* carriers, with an emphasis on a possible association with specific BC subtypes.

Methods: In order to identify carriers of *RAD51C* PV tested at the Institute of Oncology Ljubljana, we analysed records for 5170 individuals who underwent genetic testing due to personal or family history of cancer using TruSight Cancer/TruSight Hereditary Cancer panels between January 2016 and December 2021. We then analysed personal and family history data obtained from medical records and the National registry of tested individuals from cancer families for our *RAD51C* PV carriers.

Results: We identified 22 *RAD51C* PV carriers from 17 different families, 20 female and 2 male. The majority of our probands (13/17) had a positive personal or family history of BC. Of the 20 female carriers, 10 had OC (aged 46-80, median 59.5) and 10 had BC (aged 34-65, median 50), with two carriers developing both BC and OC. Five carriers developed triple-negative BC (aged 36-65, median 53). Of our 17 families, 14 carried the c.572-1G>C p.? PV, which appears to be a founder variant in the Slovenian population.

Conclusion: Triple-negative BC is disproportionally frequent in our *RAD51C* carriers and OC appears to be rare before age 50.

References:.

Grants:.

Conflict of Interest: None declared.

P13.078.D Alternative classification of pediatric oncology patients based on gut microbiota biomarker prediction with machine learning

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Background/Objectives: Pediatric oncology patients suffer not only from the disease itself but also from additional complications (febrile neutropenia, GvHD, gastrointestinal problems) associated with changes in gut microbiome composition. However, it is still not elucidated whether individual bacterial genera or higher taxonomical level group is responsible for functional differences.

Methods: *16S rRNA* gene sequencing (V1-V9) of gut microbiome from feces was carried out on Illumina MiSeq (2x300bp). Healthy controls ($n = 14$) and pediatric oncology patients ($n = 30$), with prevailing ALL were involved. Pre-processing, taxonomic classification and diversity analysis were performed with QIIME2 Core 2020.8./DADA2/BLAST+ (Silva_132). Functional profiles were predicted in R with Tax4Fun2 package. Statistical analysis was carried out using SPSS_21.0; machine-learning (ML) using

randomForest SRC analysis and elastic network ML for biomarker discovery and obtaining ROC curve.

Results: The ML analysis discriminated healthy individuals from oncology patients receiving chemotherapy using joint predictors *Blautia, Oscilibacter, Parabacteroides, Eubacterium_halii_group, Enterococcus*, *Streptococcus* (AUC 0,962). We have identified significantly discriminant *Firmicutes* to *Bacteroidetes* ratio in oncology patients in conditioning regime before transplantation (allo-HSCT). The dominant effect of the therapy was supported by the best joint predictors – *Enterococcaceae, Lachnospiraceae, Clostridiaceae* and *Lactobacillaceae* (AUC 0,86) discriminating between children with ALL from oncology department and from Transplantation Unit, associated mainly but not exclusively with metabolism of cofactors and vitamins, glycan biosynthesis and genetic information processing.

Conclusion: We have shown the highest importance of family level gut microbiota structure in pediatric oncology patients as biomarkers that can predict the loss of gut microbiota function.

References:.

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Conflict of Interest: None declared.

P13.079.A Clinical, splicing and functional analysis of a predicted truncating BRCA1 variant that alters splicing, increasing an in-frame Δ11q naturally occurring isoform

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Background/Objectives: Protein truncating variants are expected to produce loss of function by nonsense-mediated decay of the transcript and/or absence of critical domains. However, spliceogenic variants can rescue some function by causing in-frame deletions. Here we studied clinical, splicing, and functional effects of BRCA1 variant c.791_794del.

Methods: Cancer history was retrieved from available carrier families. Carrier RNAs underwent splicing analysis by Sanger sequencing and single-nucleotide primer extension. Homologous recombination repair (HRR) was determined by immunofluorescence-based detection of RAD51 and BRCA1 nuclear foci in tumours from carriers and a patient-derived xenograft (PDX).

Results: The variant was detected in 12 families with one or more cases of breast/ovarian cancer (BC/OC), most carrying also pathogenic variants in other BC/OC genes or showing poor variant segregation with disease or milder-than-expected phenotype. According to splicing in-silico predictions, c.791_794del increases the strength of a cryptic donor site used to generate the Δ11q, a BRCA1 naturally occurring in-frame splicing event. RNA analysis in blood of three carriers and one PDX's tumour revealed that carrier alleles generate almost exclusively Δ11q transcripts. Tumour samples from one carrier and its PDX presented a RAD51 score compatible with a proficient HRR, supported by the presence of BRCA1 foci.

Conclusion: These results suggest that BRCA1 c.791_794del confers a lower cancer risk than classical BRCA1 truncating variants, driven by an increased Δ11q in-frame transcript. Further studies will help to fully ascertain its associated cancer risk.

References:.

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Conflict of Interest: None declared.

P13.081.C Driver genes for chronic lymphocytic leukemia can be affected not only by mutations but also by complex genomic rearrangements

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Background/Objectives: In chronic lymphocytic leukemia (CLL), genomic complexity (GC) serves as an important prognostic marker and a high number of chromosomal defects (≥5; high-GC) is associated with an unfavorable prognosis. High-GC can be induced by chromothripsis and/or related mechanisms (high-GC-cth) (PMID: 34900721). This study aimed to characterize in detail high-GC-cth in CLL.

Methods: We analyzed 327 samples from 201 CLL patients using genomic arrays (CytoScanHD, ThermoFisher Sci.), 97 patients (48%) were tested at multiple time-points. iFISH for the most recurrent chromosomal changes in CLL and/or stimulated cytogenetics data were available for the cohort. Selected samples were subjected to WGS analysis.

Results: High-GC-cth was identified in 52 samples (16%) from 38 patients (19%). High-GC-cth occurred in initial samples of 20 cases and persisted during the follow-up in all nine patients tested repeatedly. In contrast, high-GC-cth newly developed at later time-points in 18 cases due to clonal evolution. Consistent with published data, high-GC-cth associated with *TP53* gene aberrations (deletions/mutations; 30 cases). In five of the remaining eight *TP53*-intact patients, high-GC-cth was linked to *ATM* gene aberrations. Chr6 was most frequently affected by high-GC-cth (10 cases), followed by chr8 and chr9 (eight cases each). Cytogenetics and WGS revealed additional chromosomes and loci involved in genomic rearrangements indicated by arrays. Genomic breakpoints recurred in the vicinity of genes known to be mutated in CLL, suggesting additional mechanisms of their activation or inactivation.

Conclusion: Our detailed analysis reveals another layer of genomic alterations shaping functions of genes essential for CLL phenotypes.

References:.

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Conflict of Interest: None declared.

P13.082.D Omic data integration for hereditary susceptibility to colorectal cancer

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Background/Objectives: The identification of new colorectal cancer (CRC) susceptibility genes through whole exome sequencing (WES) has not been as successful as expected, possibly due to the genetic heterogeneity of CRC. Our aim is to identify novel candidate susceptibility genes by integrating WES and RNA-seq data.

Methods: WES and RNA-seq were performed on paired normal-tumor tissue from a phenotypically homogeneous cohort of 19 MMR-proficient CRC patients. Germline calling (HaplotypeCaller) and annotation (ANNOVAR) were carried out on WES, RNA-seq data, as well as GTEx data as a control group. Somatic calling (MuTect2) and annotation (ANNOVAR) were also carried out on WES data. Aberrant expression, aberrant splicing and monoallelic expression analysis were performed following DROP pipeline (1). SKATO were carried out on our WES data and using 267 non-cancer individuals from the MGP Spanish (EGAC00001000222) as controls.

Results: None of the patients harbor germline pathogenic variants in already described Mendelian genes associated to hereditary CRC syndromes. We did not find rare variant (gnomAD <1%) recurrence in genes in our CRC cohort. At the individual level, RNA-seq expression analysis together with WES results reported some candidate genes previously involved in different types of cancers, metabolism of different metabolites or gastrointestinal physiology.

Conclusion: These results show the molecular heterogeneity underling CRC in a phenotypically homogeneous cohort. The use of RNA-seq together with WES allows prioritizing variants at the individual level, increasing the probabilities to identify new CRC predisposition genes. As a result, a more personalized genetic diagnostic could be achieved.

References: 1. PMID: 33462443.

Grants: ISCIII and FEDER funds PI17/00509.

Conflict of Interest: None declared.

P13.083.A Somatic variant landscapes of solid papillary and comedo subtypes of canine mammary tumors from a single dog

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Background/Objectives: Breast cancer (BC) is still the most common cancer reported in women and mammary tumor (MT) is also the most common malignancy in intact female dogs. The key goal of this research is to study two rare and less explored subtypes of canine mammary tumor, i) solid papillary subtype and ii) comedo subtype and compare the findings with human discoveries.

Methods: An intact female dog diagnosed with simple invasive carcinoma of these two sub-type was selected and cell populations from respective tumor sites and normal lobular cells were isolated from the using the method of laser pressure catapulting microdissection. Libraries of the cells isolated were prepared with PicoPLEX tool kit and whole genome sequenced with about 15X coverage. Data was aligned to latest canFam4, somatic variants were called and analyzed.

Results: The number of non-silent mutations is sixfold higher in comedo sub-type than in solid papillary sub-type. Genes related to MAPK, mTOR and NF-kappaB signaling pathways and Adherens junctions were enriched in Solid papillary. Genes related to ECM receptor interaction, focal and cell adhesion, PI3K-AKT and cGMP-PKG signaling pathways were enriched in Comedo.

Conclusion: The tumor mutation burden co-relates with the already known fact in humans that comedo carcinoma is more pathogenic than solid papillary sub-type of carcinoma. The presence of variants in cell proliferation signaling pathways and junction is supporting the invasive nature of the tumor which was identified as HER+ve and E Cadherin positivity.

References: PMs: 32680987; 33262909.

Grants: Partially supported by Cancer Foundation Finland.

Conflict of Interest: None declared.

P13.084.B Pan-cancer identification of signatures of survival-associated prognostic long non-coding RNAs

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Background/Objectives: Long non-coding RNAs (lncRNAs), which play key roles in modulating gene expression, are frequently

dysregulated in cancers. Their prognostic and therapeutic value currently remains unclear. Here, we aim to identify potentially actionable, survival-associated (PAS) lncRNAs as possible prognostic biomarkers in multiple cancers or therapeutic targets.

Methods: Survival-associated (SA) lncRNAs that are correlated with at least one SA-gene (|R|≥0.8) in the same cancer and survival-endpoint represent PAS-lncRNAs. Profiles of lncRNAs, miRNAs and mRNAs of 33 cancer types from The Cancer Genome Atlas were associated with four survival endpoints (Overall survival (OS), Disease-free survival (DFS), Progression-free interval (PFI), Diseases free interval (DFI)). PAS-lncRNAs that are associated with ≥1 survival-endpoint in ≥3 different cancer-types represent potential pan-cancer prognostic biomarkers, while nodal PAS-lncRNAs in PAS-(lncRNA-miRNA-mRNA) co-expression network associated with ≥100 SA-mRNAs represent promising therapeutic targets. Pathways modulated by the SA-genes of these PAS-lncRNAs are then determined.

Results: Two prognostic pan-cancer PAS-lncRNAs capable of distinguishing between patients with good and bad prognosis in ≥3 cancers were identified. Two nodal PAS-lncRNAs that potentially modulate the expression of ≥100 SA-mRNAs in cancer-associated pathways through miRNAs in Uveal Melanoma were also identified.

Conclusion: These prognostic PAS-lncRNAs may facilitate the design of potential clinically useful biomarkers and therapeutic targets.

References:.

Grants:.

Conflict of Interest: None declared.

P13.085.C Insights from multigene panel testing in non-Ashkenazi breast cancer patients – carrier rates and variant classification in a genetically diverse population

sari Lieberman[1], *Omer Murik*[2], *Fouad Zahdeh*[2], *Rachel Beeri*[1], *Pinhas Renbaum*[1], *Michal Barzily*[1], *tehila klopstock*[1], *Orit Freireich*[1], *Malka BenUziyahu*[1], *ariela tomer*[1], *David Zeevi*[2], *Tzvia Mann*[2], *Oded Olsha*[3], *Hadar Goldvaser*[4;5], *Tal Hadar*[6;7], *Shani paluch-shimon*[7;8], *Shelley Shoval*[9], *Eitan Friedman*[10;11], *amnon lahad*[12;13], *Rinat Bernstein-Molho*[11;14], *ephrat lahad*[1;7]

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Background/Objectives: There is limited information on the landscape of inherited breast cancer (BC) in non-Ashkenazi Jews. This heterogeneous, little tested population can offer insights into novel variants in known BC-predisposition genes.

Methods: In 2015-2021 consecutively diagnosed non-Ashkenazi BC patients underwent multigene panel testing (MGPT). MGPT was also offered to a control group of unaffected non-Ashkenazi participants.

Results: Genetic testing was performed in 751 affected and 810 unaffected women. BRCA1/2 pathogenic variants (PV) were identified in 23 (3.3%) affected vs. 5 (0.6%) unaffected women ($P < 0.0002$). Only 3 BRCA1 PVs were recurrent. PVs in other genes were found in 30 (4.8%) affected vs. 8 (1%) unaffected women. This included PVs in ATM, CHEK2, BRIP1, TP53, MRE11, FANCM, NBN, PALB2 and PTEN.

9490 rare variants (<0.01 MAF) were observed in 28 genes: 695 (7.3%) exonic, 8789 (92.7%) non-coding. 541 exonic variants were previously in ClinVar. Using frequencies from this cohort enabled downgrading of 15 ClinVar variants of uncertain significance (VUSs) to Benign, based on MAF >0.01 among unaffected low-family history (FH) women. MAF by sub-ethnicity downgraded another 28 VUSs. Among non-coding variants, 68 were significantly more common in affecteds with significant FH, suggesting possible pathogenicity. Conversely, 52 non-coding variants were classified as benign based on higher frequency in controls.

Conclusion: Non-Ashkenazi women exhibit expected rates of PVs in known breast cancer genes, but low rates in affected with substantial FH. Genetic analysis in diverse populations can contribute to variant classification, especially of non-coding variants whose interpretation by standard tools is limited.

References:.

Grants: BCRF-095.

Conflict of Interest: sari Lieberman Modest, AstraZenca Israel, Omer Murik: None declared, Fouad Zahdeh: None declared, Rachel Beeri: None declared, Pinhas Renbaum: None declared, Michal Barzily: None declared, tehila klopstock: None declared, Orit Freireich: None declared, Malka BenUziyahu: None declared, ariela tomer: None declared, David Zeevi: None declared, Tzvia Mann: None declared, Oded Olsha: None declared, Hadar Goldvaser: None declared, Tal Hadar: None declared, Shani paluch-shimon: None declared, Shelley Shoval: None declared, Eitan Friedman: None declared, amnon lahad: None declared, Rinat Bernstein-Molho: None declared, ephrat lahad: None declared.

P13.086.D Expression of ST6GAL1 protein in thyroid cancers

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Background/Objectives: ST6 β-galactoside α2,6-sialyltransferase 1 (ST6GAL1) is known as a cancer-associated glycosyltransferase. It plays important role in tumor progression where elevated expression of ST6GAL1 has been observed in several cancers, but not in thyroid cancers. Previously, our GWA studies identified that gene encoding ST6GAL1 was associated with plasma thyroglobulin (Tg) levels. In thyroid pathologies, Tg levels are altered, so the aim of the study was to analyze the expression of ST6GAL1 protein in papillary thyroid cancer (including follicular variant and microcarcinoma) and follicular thyroid cancer.

Methods: We performed immunohistochemical analysis using human thyroid tissue and analysed expression levels of ST6GAL1 protein in well-differentiated thyroid cancers in comparison to normal thyroid tissue. We examined 9 papillary thyroid carcinomas (PTC), 5 with classical papillary architecture and 4 follicular variants of papillary thyroid carcinoma (FVPTC), 3 microcarcinoma, and 9 follicular thyroid carcinomas (FTC).

Results: In human thyroid tumors, ST6GAL1 protein levels were higher in malignant thyroid tumors in comparison to normal thyroid tissue. The analysis showed high ST6GAL1 expression level in FVPTC (fold increase = 14.98, $p = 0.0086$) compared to normal

thyroid tissue, followed by classic PTC (fold in-crease = 7.55, p = 0.0093) and FTC (fold increase = 3.37, p = 0.0008). Micro-carcinomas had the expression levels closest to control (fold increase = 2.38, p = 0.0055).

Conclusion: The results of this study showed that ST6GAL1 expression, compared to normal thyroid tissue, was increased in all examined thyroid tumors, with the highest expression in FVPTC.

References:.

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Conflict of Interest: None declared.

P13.087.A Germline intergenic duplications at Xq26.1 cause an inherited basal cell carcinoma susceptibility syndrome

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Background/Objectives: Bazex-Dupré-Christol syndrome (BDCS; MIM301845) is a rare X-linked dominant genodermatosis characterized by follicular atrophoderma, congenital hypotrichosis and multiple basal cell carcinomas (BCCs). Previous studies have linked BDCS to an 11.4 Mb interval on chromosome Xq25-27.1 and suggested that variants in ACTRT1 are responsible.

Methods: Exome/genome sequencing with array comparative hybridisation with qPCR was used to define copy number variants. Immunohistochemistry determined staining of candidate proteins in skin and tumour tissue. Hi-C determined chromosomal TADs.

Results: In eight families with BDCS, we identified overlapping 18-135kb duplications (six in-herited and two de novo) at Xq26.1, flanked by ARHGAP36 and IGSF1. We detected ARHGAP36 expression near the control hair follicular stem cells compartment, and found increased ARHGAP36 levels in hair follicles, BCCs and trichoepitheliomas from patients with BDCS.

Conclusion: We provide compelling evidence for intergenic Xq26.1 duplications as the first exam-ple of inherited non-coding copy number variants causing a cancer-predisposition syndrome. Our proposed mechanism of BDCS-duplications likely impacting control of ARHGAP36 in the follicular stem cell compartment reconciles the genetic etiology and inheritance pattern of BDCS and reveals 'dysregulation' as a mechanism for he-reditary cancer-predisposition syndromes.

References: Br. J. Dermatol. 165, 201–203 (2011). Nature Med 23, 1226–33 (2017).

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Conflict of Interest: None declared.

P13.088.B Identification of new hereditary colorectal cancer candidate genes

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Background/Objectives: Despite the multiple initiatives to identify new hereditary non-polyposis colorectal cancer (CRC) genes, their yield has been extremely low. In the past decade, only *RPS20*, which explains less than 0.1% of familial CRC cases, has been unequivocally linked to the disease. To maximize success, we applied to exome sequencing data a specific strategy for variant prioritization, followed by gene burden tests for the selected genes.

Methods: Our variant prioritization strategy for the identification of hereditary CRC genes from exome sequencing data was based on different parameters, including, among standard criteria, the selection of predicted pathogenic variants affecting genes involved in pathways relevant in hereditary cancer, colorectal carcinogenesis, and/or acting as cancer drivers. The strategy was applied to exome sequencing data from 24 CRC-affected individuals from 15 families with CRC aggregation and/or early-onset CRC diagnoses. The selected genes were next studied in 465 additional familial CRC patients. The results, together with germline sequencing data from publicly available datasets of CRC and population controls, were included in gene-based burden analyses.

Results: We identified 20 candidate genes, all affecting TGF-beta or Wnt-pathways, six of which showed positive association with CRC in gene burden tests. We are currently performing the characterization of the candidate genes and carrier families, including the analysis of tumor molecular features.

Conclusion: The application of a well-designed variant prioritization strategy to exome sequencing data from familial/early-onset CRC patients, followed by gene burden tests including data from open-access repositories, facilitated the identification of candidate genes potentially involved in CRC predisposition.

References:.

Grants:.

Conflict of Interest: None declared.

P13.091.A Validation of genotype array analysis for the assessment of homologous recombination deficiency (HRD) in epithelial ovarian cancer

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Background/Objectives: Genomic instability caused by homologous recombination deficiency (HRD) - in 40-50% of cases due to BRCA1/2 pathogenic variants (PVs) - is a predictive biomarker for the response of different tumors to PARP-inhibitor therapy. In epithelial ovarian cancer (EOC) it is also considered predictive for sensitivity of platinum-based therapies. Currently, diagnostic genomic instability testing is mostly based on the HRD-score by Myriad Genetics.

Methods: To determine HRD positivity we examined genome-wide copy number variation and loss of heterozygosity (LOH) by genotyping 89 ovarian cancers, 26 of which contained a BRCA1/2 PV, using the Global Screening Array (GSA-24 v3.0+Multi-Disease Content; Illumina). Data analysis was performed with Illumina GenomeStudio 1.6.3 (Genotyping Analysis Module) and NxClinical (Biodiscovery, SNP-FASST2-Segmentation Algorithm) software. For quantification of HRD a LOH-score based on Swisher et al (2017; PMID: 27908594) and an Aneuploidy Normalized Telomeric Imbalance-Score (ANTI-Score, unpublished) were defined.

Results: The group of BRCA1/2-PV samples had significantly higher median scores than BRCA1/2-wildtype samples. LOH-score and ANTI-scores were concordant ($r = 0.83$) with each other and with the HRD-Score by Myriad (rLOH/MYRIAD = 0,82; rANTI/MYRIAD = 0,86). Based on the lowest scores determined in the BRCA1/2-PV samples, we defined the threshold for HRD-positivity as LOH-score ≥14 and/or ANTI-score ≥6. Current investigations focus on the correlation of the scores with the clinical outcome and in depth sequence and epigenetic analysis of BRCA-wildtype samples with positive LOH/ANTI-scores. Applicability of our scores in other tumor entities are tested.

Conclusion: Rapid and reliable HRD analysis is possible with a standard genotyping array on DNA from native tumor tissue.

References:.

Grants:.

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P13.093.C Study the role of miR200s in the estrogen response of ovarian cells

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Background/Objectives: Ovarian cancer is the fifth most common form of cancer death among women. Exposure to physiological estrogens or xenoestrogens (zearalenone or bisphenol A) increases the risk for cancer. We present a comprehensive study about the effect of estradiol (E2), zearalenone (ZEA) and bisphenol A (BPA) of human epithelial ovarian cell lines.

Methods: We applied the PEO1 (expressing Estrogen Receptor α and β) and A2780 (ERβ) human ovarian cell lines. We studied the effect of estrogens to cell proliferation, migration, mRNA and miR200s expression.

Results: E2, ZEA and BPA induced cell proliferation and migration in physiologically relevant doses in the case of the PEO1 cell line, that was accompanied with the induction of estrogen-responsive genes (*GREB1, CA12, DEPTOR, RBBP8*). However, A2780 did not respond to estrogens. The basal intracellular and cell-free expression of miR200s was higher in PEO1, which was accompanied with low *ZEB1* and high E-cadherin expression. These miRNAs showed a rapid but intermittent upregulation in response to estrogens that was diminished by an ERα-specific antagonist (MPP). MiRNA expression of cell lysates correlated well with cell-free miRNA expression. The role of ERα in the regulation of the MIR200B-MIR200A-MIR429 locus was further supported by publicly available ChIP-seq data. MiRNA expression of cell lysates correlated well with cell-free miRNA expression.

Conclusion: MiR200s might be regulated by ERα. Cell-free miR200s might be applicable biomarkers for estrogen sensitivity of ovarian cells that support therapy selection in ovarian cancer (e.g. the application of estrogen blocking agents).

References:.

Grants:.

Conflict of Interest: None declared.

P13.094.D Small non-coding RNA profile in esophageal squamous cell carcinoma patients

Nazerke Satvaldina[1], *Aigul Sharip*[2], *Mukhtar Tuleutaev*[3], *Ulan Kozhamkulov*[1], *Yuri Zhukov*[3], *Marat Omarov*[3], *Askhat Molkenov*[2], *Ainur Akilzhanova*[1], *Ulykbek Kairov*[2], *Baktybai Orazbekov*[3], ***Saule Rakhimova***[1]

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Background/Objectives: Esophageal cancer (EC) is the sixth among number of new deaths for 36 cancer types worldwide according to GLOBOCAN 2020 statistics. Among two main types of EC esophageal squamous cell carcinoma (ESCC) is the most frequent type. Lack of significant biomarkers complicates ESCC differentiation in the early stages. Mounting evidence indicates that small non-coding RNAs, especially microRNAs may serve as a potential biomarker.

Methods: Two total RNA samples (#36 and #38) extracted from tumor tissue of the patients with pathologically confirmed T3N0M0 ESCC. Small RNA libraries were prepared using NEXTFLEX Small RNA-Seq kit v3 protocol (Perkin Elmer). The microRNA control was included.

Results: Analysis and identification of small types of RNAs on COMPRSA pipeline has been implemented.The results of the further sequences annotation analysis of the absolute values distribution of small RNA types in #36 and #38 samples are pmiRNA -538,473 piRNA – 2335, 1938, tRNA – 386, 364, snoRNA – 444, 420, snRNA – 318, 322 and circRNA – 62733, 39748 respectively.

Conclusion: Recent findings reveal that differently expressed small RNA levels have correlation with clinicopathological characteristics of ESCC patients, may affect to proliferation of ESCC cells and advanced progression of cancer. Sequencing of non-coding regions of the genome may shed a light to distinguish new significant diagnostic markers for esophageal squamous cell carcinoma.

References:.

Grants: This work was supported by NU CRP grant 021220CRP2222 and grant MES RK #AP09058660.

Conflict of Interest: None declared.

P13.096.B Two different pathogenic variants affecting the translational initiation of the BMPR1A gene result in different phenotypes in patients with hereditary polyposis syndromes

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Background/Objectives: Juvenile polyposis syndrome (JPS) and hereditary mixed polyposis syndrome (HMPS) share common pathogenesis related to the disruption of the BMP pathway.

Methods:.

Results: Using NGS we detected two germline pathogenic variants located in the 5'region of the BMPR1A gene in two unrelated patients with familial history of polyposis; one novel splice variant (c.-152-2A>G, p = ?) and one rare, start loss variant (c.1A>G, p.Met1Val). The carrier of the splice variant presented a more severe phenotype reminiscent of JPS (>150 juvenile polyps at 9yrs of age) compared to the other patient which had a phenotype resembling the HMPS (~10 polyps of variable type and size at age of 35). The RNA analysis of the splice site variant confirmed the presence of an aberrant transcript lacking whole exon 3, resulting in a loss of the normal initiation codon. Additionally, this transcript was expressed at a reduced level, probably due to decreased stability. While both transcripts can be expected to result in an absent protein product, in silico analyses showed that a possible identical protein can be constructed via a rescue of translational initiation by the downstream in-frame methionine at codon 29 in exon 4. This truncated protein would lead to the loss of the signal peptide (residues 1-23), which is necessary for normal membrane localization of the BMPR1A.

Conclusion: Since both variants potentially result in the same truncated protein, the different clinical presentation of the disease in these two families might be due to the differences in the BMPR1A levels produced by the two different mutant alleles.

References:.

Grants:.

Conflict of Interest: None declared.

P13.098.D Molecular and infectious profiling of prostate cancer

Elena Todorova[1;2], *Anita Kavrakova*[1;2], *Bilyana Georgieva*[1], *Kremena Mesechkova*[1;2], *Kamen Anachkov*[2], *Krasimir Yanev*[3], *Georgi Ivanov*[3], *Goran Derimachkovski*[4], *Georgi Georgiev*[5], *Vanyo Mitev*[1], *Albena Todorova*[1;2]

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Background/Objectives: The heterogeneous nature and clinical behavior of prostate cancer (PCa) requires intensive investigations. Bulgarian patients were tested for *PCA3*, *TMPRSS2-ERG* fusions and *GSTP1* promoter hypermethylation. The role of low, intermediate and high-risk (hr) human papilloma viruses (HPVs) in PCa was assessed.

Methods: Sixty urine specimens obtained after digital rectal investigation (DRI) from suspected PCa patients and 55 "tru-cut" PCa biopsies were analyzed. DNA isolation, DNA sequencing, bisulfite conversion of DNA, PCR-based hybridization method, cytological preparations and staining were applied.

Results: Fluctuations in the molecular profile were registered in most of the patients: neoplastic *GSTP1* allele, *PCA3* strongly elevated expression/hyperexpression. A positive TMPRSS2-ERG fusion status was detected in 4 cases.

hrHPVs were detected in 34% of urine specimens, where 96% of hrHPVs are: 16, 33, 35, 31, distributed in the subgroup with highest oncogenic potential. The frequency of hrHPVs in the control group is significantly lower (11%). The cytological examination on hrHPVs positive urine specimens showed inflammation, atrophy, partially viral cytopathic effect and neoplastic findings (high-grade prostatic intraepithelial neoplasia (PIN), nuclear margination, tumor diathesis).

HPVs were detected in 9% of PCa "tru-cut" biopsies: low-risk HPVs 42, 54/55; hrHPVs 18, 51. Histological examination showed a presence of acinar adenocarcinoma in combination with benign prostatic hyperplasia (BPH), PIN and inflammation.

Conclusion: The selected molecular panel is useful for early diagnostics and molecular profiling of PCa. Long-term active infection with hrHPVs in prostate contributes to intraprostatic inflammation, precancerous lesions and BPH, later to malignancy.

References:.

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Conflict of Interest: None declared.

P13.099.A Our experience with von Hippel-Lindau syndrome in Bulgarian patients

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Background/Objectives: Von Hippel-Lindau syndrome (VHL) is an inherited disorder characterized by hemangioblastoms (HBs) which can develop in the brain and spinal cord and provoke headaches, vomiting, weakness and ataxia. Retinal angiomas can occur in the light-sensitive tissue and may cause vision loss.

In total 17 Bulgarian VHL cases were genetically screened for mutations in the VHL gene. One prenatal diagnosis was performed.

Methods: DNA was isolated from blood for all patients and from chorionic villus sampling of the pregnant woman for prenatal testing. The molecular genetic testing included direct Sanger sequencing and MLPA.

Results: Eight of the patients turned out to be positive for mutations in the VHL gene (47%). The detected mutations are four missense, a nonsense mutation, an indel, a small deletion and a large deletion. Prenatal testing was performed in a twenty-eight year old patient with multiple retinal HBs and vision loss. Her father had the same phenotype but milder clinical presentation. The detected mutation was a deletion of exon 3 of the VHL gene. The fetus did not carry the maternal mutation. All patients have clinical symptoms typical for VHL.

Conclusion: In total 47% of the VHL Bulgarian patients were genetically verified. The detected mutations in our patients are already known in the literature. The genetic testing in VHL gene

provides the possibility for adequate genetic counselling, family planning and prenatal diagnostics in affected families.

References: Glushkova M. et al. 2018 Int J Neurosci. 128(2):117-124.

Grants:.

Conflict of Interest: None declared.

P13.100.B microRNA profile in Bulgarian laryngeal squamous cell carcinoma

Gergana Stancheva[1], *Silva Kyurkchiyan*[1], *Veronika Petkova*[1], *Stiliana Panova*[1], *Diana Popova*[2], *Radka Kaneva*[1], *Todor Popov*[2]

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Background/Objectives: Laryngeal squamous cell carcinoma (LSCC) is an aggressive malignancy with poor prognosis, which despite modern treatment protocols, novel molecular markers are required to improve survival. Deregulation of miRNA expression plays key role in various pathological processes, as well as angiogenesis and hypoxia.

Methods: Expression of 2549 miRNA in fresh-frozen tumour materials and adjacent normal tissue was performed in 12 patients with advanced primary LSCC, by SurePrint G3 Human MiRNA r21 Microarray Kit, 8×60K(Agilent Technologies). The expression of miR-21-3p and miR-210-3p was evaluated in a group of 38 fresh frozen LSCC and adjacent normal samples by qRT-PCR analysis.

Results: Expression levels of 2549 miRNA were assessed and 242 of those were significantly dysregulated (cut-off>2.0(FC); BH-FDR<0.05). After the analysis, a subset of 14 miRNAs was selected-8 upregulated(miR-18a-5p, miR-181a-5p, miR-181b-5p, miR-21-3p, miR-24-3p, miR-93-5p, miR-210-3p, miR-1246) and 6 down-regulated (miR-140a-3p, miR-145-5p, miR-148a-5p, miR-204-5p, miR-497, miR-874-3p). miR-21-3p and miR-210-3p revealed to be an important and related to pathways of tumour angiogenesis and hypoxia so they were investigated in a validation group of patients. It was confirmed increased expression levels of miR-21-3p and miR-210-3p, respectively 78.94% and 39.47%. The ROC curve analysis showed that miR-21-3p can distinguish laryngeal tumour from normal tissue ($AUC = 0.816$; 95% CI:0.720-0.917;$p = 1.76.10^{-6}$) with sensitivity of 84.2% and specificity of 73.7% whereas miR-210-3p did not.

Conclusion: Our study revealed the subset of 14 miRNAs significantly deregulated in our LSCC group. miR-21-3p and miR-210-3p were significantly overexpressed in validation group but miR-21-3p only showed power to distinguish tumour from normal tissue.

References: None.

Grants: D-77/04.06.2021,DN13/12/20.12.2017;D01-285/17.12.2019,D01-395/18.12.2020,D01-302/17.12.2021.

Conflict of Interest: Gergana Stancheva Contracts D-77/04.06.2021/MU/Bulgaria; DN13/12/20.12.2017/NSF; D01-285/17.12.2019/MES/Bulgaria, D01-395/18.12.2020MES/Bulgaria, D01-302/17.12.2021/MES/Bulgaria -in all collaborator, Silva Kyurkchiyan Contracts D-77/04.06.2021/MU/Bulgaria; DN13/12/20.12.2017/NSF; D01-285/17.12.2019/MES/Bulgaria, D01-395/18.12.2020MES/Bulgaria, D01-302/17.12.2021/MES/Bulgaria -in all collaborator, Veronika Petkova DN13/12/20.12.2017/NSF; D01-285/17.12.2019/MES/Bulgaria, D01-395/18.12.2020MES/Bulgaria, D01-302/17.12.2021/MES/Bulgaria -in all collaborator, Stiliana Panova Contracts: D01-285/17.12.2019/MES/Bulgaria, D01-395/18.12.2020MES/Bulgaria, D01-302/17.12.2021/MES/Bulgaria -in all collaborator, Diana Popova Contracts: D-77/04.06.2021/MU/Bulgaria - principal investigator; collaborator - DN13/12/20.12.2017/NSF/MES/Bulgaria, Radka Kaneva Contracts DN13/12/20.12.2017/NSF; D01-285/17.12.2019/MES/Bulgaria, D01-395/18.12.2020MES/Bulgaria, D01-302/17.12.2021/MES/Bulgaria -in all collaborator, Todor Popov Contracts: collaborator - D-77/04.06.2021/MU/Bulgaria; principal investigator - DN13/12/20.12.2017/NSF/MES/Bulgaria.

P13.101.C Country-specific calibration of Polygenic Risk Scores for Breast Cancer in European ancestry populations

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Background/Objectives: The 313-SNP breast cancer polygenic risk score (PRS313), developed for women of European ancestry by the Breast Cancer Association Consortium (BCAC), can be used in predicting a woman's risk of developing the disease. However, calibration of the PRS is important for providing valid risk estimates. Here we evaluated the distribution of PRS313 in different countries. Analyses were based on 111,814 breast cancer cases and 94,718 controls of European ancestry from studies participating in the BCAC, from 17 countries in Europe, together with Australia, Canada, Israel, and the USA.

Methods: All samples were genotyped using either the Oncoarray or iCOGS platforms. We computed the mean and standard deviation (SD) of PRS313 and evaluated the association between breast cancer risk and PRS313 in each country separately. Fixed-effect meta-analysis was performed to calculate the overall estimates for each country and across all countries. The country-specific estimates were compared to the overall values.

Results: Relative to the overall mean, the mean standardized PRS313 differed significantly across Europe (p-value for heterogeneity <0.01), being highest in Greece (0.22), and Italy (0.10), and lowest in Ireland (-0.12). The mean estimates for Australia, Canada, Israel, and the USA are close to the overall mean.

Conclusion: The results indicate that the implementation of the PRS313 for breast cancer risk prediction will require country-specific calibration, which can be achieved by adjusting for the population-specific mean.

References:.

Grants: Telethon.

Conflict of Interest: None declared.

P13.102.D Development of combined somatic copy number alteration and fragmentation analysis for monitoring in colorectal cancer patients using liquid biopsy

Ariane Hallermayr[1;2;3], *Verena Steinke-Lange*[1;4], *Anna Benet-Pagès*[1;5], *Tobias Wohlfrom*[1], *Florentine Scharf*[1], *Ellen Heitzer*[6;7;8], *Christopher Haberl*[9], *Maike de Wit*[10;11], *Holger Vogelsang*[12], *Markus Rentsch*[13;14], *Elke Holinski-Feder*[1;4], *Julia Romic-Pickl*[1;4]

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Background/Objectives: Liquid biopsy (LB) for non-invasive disease monitoring of cancer patients is progressing towards routine clinical practice. Whole-genome sequencing (WGS) of ctDNA provides a promising tool for real-time monitoring of treatment response, as well as early diagnosis for all cancer patients.

Methods: For clinical validation of LIquid biopsy Fragmentation, Epigenetic signature and Copy Number Alteration analysis (LIFE-CNA), we performed WGS in 208 plasma samples collected from healthy individuals and colorectal cancer (CRC) patients.

Results: Analysis of downsampled data of a small cohort of 11 samples identified 5x coverage to be the minimal required coverage to reliably distinguish healthy controls from CRC patients based on SCNAs, global fragmentation profiles and epigenetic signatures. Although sensitivity increased with higher coverage, we show that 5x coverage is acceptable for sensitive analysis of ctDNA for routine clinical practice, by keeping costs per sample in the range of targeted hotspot assays. To determine the clinical validity of LIFE-CNA, ctDNA specific features were compared with clinical data of CRC patients over all disease stages which were treated with surgery and / or chemotherapy. We were able to show that changes in ctDNA signals can be associated with treatment response or disease progression.

Conclusion: We developed a cost-effective and sensitive method for untargeted ctDNA analysis, which may expand the detection of residual disease and recurrence, as well as treatment monitoring to all cancer patients. The cost-effectiveness and sensitivity of our approach form the basis to enable implementation of LIFE-CNA into routine clinical practice.

References:.

Grants:.

Conflict of Interest: None declared.

P13.103.A Hereditary cancer panels, beyond the expected

Jesús del Valle[1;2], *Lidia Feliubadaló*[1;2], *Roser Lleuger-Pujol*[1;2], *Xavier Muñoz*[1;2], *Eva Tornero*[1;2], *Mònica Salinas*[1;2], *Mireia Menéndez*[1;2], *Noelia Torres*[1;2], *Eva Montes*[1;2], *Marta Pineda*[1;2], *Àngela Velasco*[1;2], *Sara González*[1;2], *Joan Brunet*[1;2], *Conxi Lázaro*[1;2]

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Background/Objectives: The advent of NGS has drastically changed the paradigm of genetic testing, nowadays a large proportion of cases with hereditary cancer (HC) suspicion are analyzed using gene panels that include most known genes associated with cancer predisposition. However, only genes related to the phenotype and family history of the patient are usually analyzed. In this work we set out to analyze the percentage of pathogenic mutations identified in all clinically actionable genes outside the initial suspected clinical gene set.

Methods: Germline variant analysis was performed using NGS custom panel I2HCP (Castellanos 2017), which comprises 135-168 HC genes. A total of 1,748 HC-index patients previously analyzed using a phenotype-driven gene panel (Feliubadaló 2019) were reanalyzed with the aim of including all clinically-actionable genes in our panel.

Results: A total of 23 new variants classified as (probably)-pathogenic (PV) were identified. Our results also identified three patients with PV in two different genes.

Gene	PV
ATM	3
BRIP1	3
CHEK2	3
CDKN2A	2
TP53	2
BARD1	1
CDH1	1
Biallelic MSH3	1
NF1 mosaic	1
PALB2	1
PMS2	1
PTEN	1
RB1	1
RET1	1
SDHC	1

Conclusion: These variants increased the mutational yield in 1.8% in our cohort of patients. Although many variants are in genes of moderate/low penetrance, others are not, representing incidental findings or putative new genotype-phenotype correlations. In some instances, these findings could be used to improve clinical follow-up of carriers and may prevent unexpected cancers in these families.

References:.

Grants: PI19/00553.

Conflict of Interest: None declared.

P13.105.C Variability in the size of del(5q) and its clinical implication in myelodysplastic syndromes (MDS)

Zuzana Zemanova[1], *Nikola Sejgunovova*[1], *Karla Svobodova*[1], *Halka Lhotska*[1], *Denisa Vidlakova*[1], *Tatiana Aghova*[1], *Libuse Lizcova*[1], *Silvia Izakova*[1], *Iveta Mendlikova*[2], *Sarka Ransdorfova*[2], *Lucie Hodanova*[1], *Lenka Pavlistova*[1], *Monika Belickova*[2], *Jana Brezinova*[2], *Jaroslav Cermak*[2], *Tomas Stopka*[3], *Anna Jonasova*[3]

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Republic; [2]Institute of Hematology and Blood Transfusion, Prague, Czech Republic; [3]General University Hospital and First Faculty of Medicine, Charles University in Prague, 1st Medical Department, Prague, Czech Republic.

Background/Objectives: The most common cytogenetic abnormality in MDS is del(5q) occurring either as a sole aberration or as a part of complex karyotypes (CK). Isolated del(5q) is associated with favorable outcome, MDS with CK relate to poor prognosis. It remains unclear whether deletion size matters for different MDS phenotypes. The aim was to assess the relationship between del(5q) extent and *TP53* gene mutations, and to evaluate the effect of deletion size on disease prognosis.

Methods: We performed a detailed analysis of the del(5q) extent in a large cohort of 348 newly diagnosed MDS patients using mBAND (MetaSystems) and aCGH/SNP (Illumina, Agilent). Sequence analysis of *TP53* gene was performed using NGS on a 454 GS Junior system (Roche) or MiSeq sequencing instruments (Illumina).

Results: In the group of 175 cases with isolated del(5q), we observed a smaller extent of deleted segment. The deletion ranged between 5q14-5q33.3. *TP53* mutations were found in 19.4% of cases. In the group of 173 cases with CK, deletion often involved entire long arm. Mutations of *TP53* or LOH17p were detected in 49% of them. When both the prevalence of CK and the del(5q) extent were considered, only karyotype complexity had a significant effect on patient´s overall survival.

Conclusion: More extensive deletions were associated with CK, higher frequency of *TP53* aberrations/mutations and poor prognosis. Karyotype complexity is the main factor that negatively affects the prognosis. However, we cannot exclude that isolated large del(5q) may pose a higher risk of clonal evolution and complex karyotype formation during the disease.

References:.

Grants:.
MH CZ-DRO-VFN64165, DRO-UHKT00023736..

Conflict of Interest: None declared.

P13.106.D miR-210 reveals strong association with nodal metastasis and HIF2a, but not HIF1a in advanced LSCC

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Background/Objectives: Hypoxia has role in invasion and metastasis pathways. Advanced laryngeal squamous cell carcinoma (LSCC) is a disease with poor prognosis and presence of locoregional metastases. Our aim was to study hypoxic-master miR-210 its association with major hypoxia markers and nodal metastasis status.

Methods: In the study 30 advanced tumour and corresponding normal laryngeal samples were enrolled. Total RNA isolation, reverse transcription and real-time qPCR were performed with miRNeasy kit, miScript IIRT kit, and SYBR based detection (Qiagen). SPSS v19 (IBM) was used in statistical analysis, and p-value less than 0.05 was taken as significant.

Results: Most of the patients were with positive nodal metastasis status (67.5%) and overexpression of miR-210 (75%). Elevated levels of miR-210 demonstrated strong association with the presence of nodal metastasis ($p < 0.01$). ROC analysis distinguish patients developed nodal metastasis from those who are N-stage negative with 75% sensitivity and 65% specificity at RQ = 2.001 (AUC = 0.694, $p = 0.012$). Moreover, Spearman correlation analysis reveal potential co-expression and significant correlation between miR-210 and HIF2a ($r = 0.409$, $p = 0.001$), but not HIF1a, which is most commonly expressed during hypoxia.

Conclusion: Our results reveal that one of best-studied hypoxic-miRNA, miR-210, potentially could take a key role in nodal metastasis development in advanced LSCC. Correlation analysis reveal, that miR-210 may participates in latter stage during hypoxia via HIF2 transcription factor, which molecule is accumulated in prolonged hypoxia.

References: None.

Grants: D-77/04.06.2021,DN13/12/20.12.2017; D01-285/17.12.2019,D01-395/18.12.2020,D01-302/17.12.2021.

Conflict of Interest: Silva Kyurkchiyan DN13/12/20.12.2017 - collaborator.

D01-285/17.12.2019 - collaborator, Stiliana Panova D01-285/17.12.2019,D01-395/18.12.2020,D01-302/17.12.2021- collaborator, Gergana Stancheva D-77/04.06.2021- collaborator.

DN13/12/20.12.2017 - collaborator.

D01-285/17.12.2019,D01-395/18.12.2020,D01-302/17.12.2021- collaborator, Veronika Petkova DN13/12/20.12.2017 - collaborator, Diana Popova D-77/04.06.2021- principal investigator.

DN13/12/20.12.2017 - collaborator, Radka Kaneva D-77/04.06.2021- collaborator.

DN13/12/20.12.2017 - collaborator.

D01-285/17.12.2019,D01-395/18.12.2020,D01-302/17.12.2021 - collaborator, Todor Popov D-77/04.06.2021 - collaborator.

DN13/12/20.12.2017 - principal investigator.

P13.107.A An insertion in the MSH2 gene detected by Bionano optical mapping and confirmed by Nanopore sequencing in a family with suspected Lynch Syndrome

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Background/Objectives: Structural variants, also called SV's, are defined as a lesion of DNA approximately 1kb or larger in size. Humans harbour approximately 18 Mbp of structural variants per diploid genome, and these variants are an important underlying cause of genetic diseases. Despite the importance of SV's, they are difficult to detect by sequencing-based technologies. Bionano is a unique technology for detecting lesions by combining Nano-Channel arrays with optical mapping to image high-molecular weight DNA.

Methods: In this study, we investigated blood sampled from a patient with a personal and family history of cancer that gave a strong suspicion of Lynch Syndrome, a hereditary cancer syndrome caused by germline mutations in one of the four Mis-Match Repair genes *MLH1*, *MSH2, MSH6* and *PMS2*. However, no diagnostic tests, including sequencing, RNA-analyses and analysis of MSH2 inversion, could confirm the diagnosis.

Results: We therefore used Bionano technology to look for structural lesions in the patient's genome. The Bionano results showed a large (about 40 kb) heterozygous insertion in the *MSH2* gene. This finding was further confirmed using Oxford Nanopore sequencing, which produced reads spanning the entire insertion.

Conclusion:.

References:.

Grants:.

Conflict of Interest: None declared.

P13.108.B Beyond BRCA Genes: Frequency of genes with breast cancer-associated variants in a single center

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Background/Objectives: Breast cancer is a very common disease accounting for 11.6% of all new cancer cases. There is a hereditary predisposition in 5-10% of all breast cancer patients. Advances in next-generation sequencing technology have enabled the discovery of different genes other than *BRCA1* and *BRCA2*, which are the most common breast cancer susceptibility genes. In this study, germline multigene panel results in breast cancer patients are presented.

Methods: A hereditary cancer panel including 27 genes was performed in 449 patients with breast cancer only and 18 patients with multiple cancer, aged 19-77 years.

Results: A total of 69 pathogenic (P)/likely pathogenic (LP) variants in 14 genes were detected in 67 (14.9%) patients with breast cancer only and 9 (50%) patients with multiple cancer history. Variants of unknown clinical significance were detected in 37 (8.2%) patients. *BRCA1/2* mutations, the most common cause of hereditary breast cancer, were detected in 29 (6.5%) patients, constituting 42% of all P/LP variants while the P/LP variants in other genes were predominant. All the mutant genes in addition to *BRCA1/2* could be listed according to number of mutations as *PALB2, CHEK2, ATM, MUTYH, RAD50, NBN, BARD1, MRE11, TP53, BRIP1, PMS2* and *PIK3CA*.

Conclusion: The use of multigene panels promotes the rapid identification of individuals with cancer. In breast cancer, other genes in addition to BRCA genes should be scanned to explore the genetic background of the disease.

References: Clinical Cancer Research, 23(20), 6113-6119.

Grants:.

Conflict of Interest: None declared.

P13.109.C Preliminary evaluation of highly sensitive assessment of microsatellite instability in endometrial aspirates as a tool for cancer risk individualization in Lynch syndrome

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Background/Objectives: Lynch syndrome (LS) women are at increased risk of endometrial cancer (EC), characterized by mismatch repair deficiency (MMRd) and microsatellite instability (MSI). While risk-reducing gynaecological surgeries are effective there is a need to empower carriers in their decision regarding surgery timing. We aim at exploring the usefulness of highly sensitive-MSI (hs-MSI) assessment in endometrial aspirates for the individualization of gynaecological surveillance in LS carriers.

Methods: Hs-MSI was assessed in prospectively collected endometrial aspirates from 67 LS carriers, 30 controls with benign lesions and 26 sporadic-EC cases. MMR, PTEN and ARID1A expression patterns were evaluated in LS samples.

Results: High hs-MSI levels were detected in 20 aspirates from MMRd EC cases (4 of 4 LS and 16 of 16 sporadic) being negative in aspirates from controls and MMR-proficient EC cases. Interestingly, elevated hs-MSI scores were also detected in aspirates from LS women with complex hyperplasia (3 of 3). In addition, high hs-MSI was present in 10 of 49 aspirates from LS carriers showing histologically normal endometrium. In normal endometrium hs-MSI score positively correlated with density of MMRd-glands, presence of MMRd-clusters and PTEN and ARID1A loss. Hs-MSI levels increased in follow-up aspirates from 5 LS carriers.

Conclusion: Elevated hs-MSI scores were detected in aspirates from pre/malignant lesions and normal endometrium in LS carriers, correlating with MMR protein loss. Further analyses of sequential aspirates are needed to elucidate the predictive value of hs-MSI in the identification of LS carriers at higher risk of developing EC.

References:.

Grants: PID2019-111254RB-I00; PIE16/00049.

Conflict of Interest: None declared.

P13.110.D Cancer cell line variant knowledge resource for facilitated cell line identification

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Background/Objectives: One of the known features of cancer is the instability of its genome. Vast changes in the genome lead to duplications and deletions in the chromosome, that can be detected in most types of cancer. These chromosomal aberrations are also called copy number variants. Cancer cell lines are necessary tools for understanding the disease mechanisms as well as the development of new cancer treatments. However, they may not always be the best representation of their neoplasm of origin, due to cell line contamination and misidentification, as well as higher numbers of accumulated mutations in the cancer cell line genome. The analysis and comparison of cancer cell line and their origin's profiles provide information on how similar different instances of cancer and/or cancer cell lines are to each other.

Methods:.

Results: Here we build a cancer cell line variant resource for easier cell line identification and to enable finding the best representation for the cell line. This resource is open-source and uses the Beacon protocol for facilitated data sharing. To make this cell line variant knowledge resource complete we have also included cell line metadata and known single nucleotide variants to our database.

Conclusion:.

References:.

Grants:.

Conflict of Interest: None declared.

P13.111.A Development of a pan-cancer comprehensive genomic profiling assay for solid tumor cancers with integration of MSI and TMB immunotherapy biomarkers

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Background/Objectives: Over the last few years, there is a significant increase in the availability of targeted therapies. As the field of precision therapy expands, there's an increasing need for a highly accurate comprehensive assay for testing different types of genomic alterations such as single nucleotide variants (SNVs), small insertions and deletion (Indels), copy number alterations (CNAs) and rearrangements. In addition, new complex biomarkers such as microsatellite instability (MSI) and tumor mutational burden (TMB) can inform on eligibility to immunotherapy treatment. Herein, we describe the development of a 221-gene assay that can accurately detect all these therapy-associated biomarkers in a single assay.

Methods: DNA extracted from a set of FFPE and contrived samples was subjected to library preparation and hybrid capture enrichment with a 221-gene panel. Enriched libraries were then subjected to next generation sequencing (NGS) on a NovaSeq platform and analysed using in-house bioinformatics pipelines. A subset of FFPE samples were also subjected to WES for TMB assessment.

Results: A combination of FFPE tumour samples, reference material and contrived samples with known alterations and known MSI status were used to evaluate the performance characteristics for SNVs, Indels, CNAs and translocations. The assay demonstrated 100% sensitivity and 100% specificity for SNVs and Indels, CNAs, rearrangements and MSI. TMB assessment showed high concordance when compared with whole exome sequencing (WES).

Conclusion: Validation results demonstrate that the assay provides a sensitive and accurate tissue-based NGS method for assessing genomic alterations and immunotherapy biomarkers in a single assay.

References:.

Grants:.

Conflict of Interest: Alexia Eliades NIPD Genetics, Irene Hadjidemetriou NIPD Genetics, Marilena Elpidorou NIPD Genetics, Achilleas Achilleos NIPD Genetics, Charalambos Loizides NIPD Genetics, Christos Lemesios NIPD Genetics, Kyriakos Tsangaras NIPD Genetics, Elena Kypri NIPD Genetics, Marios Ioannides NIPD Genetics, George Koumbaris NIPD Genetics, Philippos Patsalis NIPD Genetics.

P13.113.C Clinical and molecular characterization of individuals with neurofibromatosis type 1 and breast cancer: genotype-phenotype relationships

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Background/Objectives: Women with NF1 have a moderately elevated risk for breast cancer, especially under age 50; specific NF1 mutation seem to predispose to BC. Aim of the study was to better elucidate the relationship between NF1 and BC.

Methods: Retrospective analysis of 719 NF1 consecutive subjects (311 males, 408 females) was performed in order to identify BC cases. The NF1-BC cohort was stratified according to age at diagnosis, histopathology, stage at diagnosis, other neoplasias, and *NF1* variation type and localization.

Results: Analysis identified 41 (5.7%) females NF1-BC patients. BC was the most common tumor behind glioma. Median age at diagnosis was 47.5 y; 44% of patients had BC diagnosis <50 y. The most frequent hystotype and molecular subtypes of BC were invasive ductal carcinoma and luminal B subtype, respectively. 33% of patients presented with stage II and and 21% with stage III BC. 10% had tumor relapse between 4 and 14 years from diagnosis. 27% had concomitant neoplasias. Mutation analysis was conducted on 28 NF1-BC cases. 86% of variants mapped in the first half and 14% in the second half of the *NF1* gene. 3/4 missense variants mapped at the N-term of the protein. 64% of *NF1* variants localized within the CSRD or TBD-GRD domains.

Conclusion: BC is one of the most common tumor in NF1. NF1-related BC shows an earlier age at onset and a more aggressive behavior compared with BC in the general population. The peculiar mutation spectrum of NF1 patients with BC is consistent with genotype-phenotype correlations.

References:.

Grants:.

Conflict of Interest: None declared.

P13.114.D The potential of high-throughput sequencing in the evaluation and monitoring of microsatellite instability by short homopolymer loci

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Background/Objectives: Evaluation of microsatellite instability (MSI) has broad clinical applications in some cancer patients' follow up treatment efficacy. Conventional methods for MSI screening have traditionally been low-throughput strategies for limited cancer types. Currently, in the era of massively parallel sequencing (MPS),

there is a great potential to improve capabilities of the methods, resulting in more reliable monitoring of MSI.

Methods: We designed a cost-effective PCR protocol to enrich a set of homopolymer loci ranging from 7 – 30 base pairs obtained from regions of the human genome associated with MSI across various malignancies. The proposed system was standardised for sequencing by Illumina MiSeq platforms. We annotated and genotyped the sequenced data using the tool Dante.

Results: Our initial validations have shown that optimized PCR conditions to enrich the homopolymer markers site can amplify FFPE samples, standardly problematic for similar purpose. We showed that biases resulting from the repetitive nature of homopolymers could be alleviated by pre-analytical steps, such as high fidelity DNA polymerases and reduced number of PCR cycles, and last but not least, by statistical and computational algorithms.

Conclusion: To sum it up, sequencing data can enable a more accurate evaluation of genomic MSI events. However, due to laboratory-induced biases caused by homopolymers, further laboratory and statistical improvements are required for MSI evaluation more precisely. With these promising results, we validate the proposed strategy also in the circulating cell-free tumour DNA.

References:.

Grants: The presented work was supported by the OP Integrated Infrastructure for the projects ITMS: 313011V578 co-financed by the European Regional Development Fund.

Conflict of Interest: None declared.

P13.115.A Beyond pathogenic RUNX1 germline variants: The spectrum of somatic mutations in RUNX1 familial platelet disorder with predisposition to hematologic malignancies

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Background/Objectives: Pathogenic germline variants in *RUNX1* cause familial platelet disorder with predisposition to hematologic malignancies (RUNX1-FPD). Accumulation of somatic alterations is presumed to drive clonal hematopoiesis and, finally, malignant transformation. The interplay of predisposing germline variants and acquired variants remain to be elucidated to better understand leukemic transformation.

Methods: We retrospectively reviewed 104 individuals with causal *RUNX1* germline variants who have been investigated for somatic gene variants, which were previously reported in the literature or the RUNX1 database.

Results: Out of 104 individuals, 29 (28%) were investigated in a non-malignant state (i.e., cytopenia). The majority of these patients had no or only a single variant (62%). Regarding malignant neoplasms, 45 (62%) were diagnosed with AML, 12 (16%) with MDS, 7 (10%) with MDS/AML, 6 (8%) with ALL, and 6 (8%) with other malignancies (e.g., CMML). On average, two somatic variants were identified per patient. No somatic variant was observed in only 10 (14%) patients with a hematologic malignancy. In MDS and/or AML, 26 (40%) samples displayed somatic *RUNX1* alterations. *BCOR, TET2, NRAS, PHF6* and *BCORL1* were most frequently mutated.

Conclusion: Retrospective analysis supports the theory of stepwise malignant transformation in RUNX1-FPD. It highlights the importance of somatic mutations in the development of frank leukemia. Different somatic variants may explain clinical heterogeneity. Detailed analyses will provide insights in synergistic effects of germline and somatic variants. This is key to enable better stratification during surveillance and may allow tailored chemoprevention studies to avoid malignant transformation in the future.

References:.

Grants: BMBF MyPred (01GM1911B).

Conflict of Interest: None declared.

P13.116.B Prognostic utility of targeted circulating cell-free DNA versus formalin-fixed paraffin-embedded DNA mutation analysis for advanced lung cancer

Madli Tamm[1], *Mart Kals*[1,2], *Tarmo Annilo*[1], *Kersti Oselin*[3], *Katrin Keerma*[1,3,4], *Paula Ann Kivistik*[1], *Miriam Nurm*[1,5], *Margot Saare*[1,6], *Jana Jaal*[7,8], *Neeme Tonisson*[1,9]

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Background/Objectives: We evaluated the performance of circulating cell-free DNA (cfDNA) and formalin-fixed paraffin-embedded (FFPE) tissue DNA analyses using a commonly employed targeted therapeutic pathway in predicting the outcomes of patients with lung cancer.

Methods: Patients (n = 106) donated blood samples at baseline and progression (n = 22), with matched FFPE biopsy samples being available for 75 patients. We set up a targeted sequencing workflow for the analysis of mutations in nine cancer-related genes. CfDNA concentration, number of mutations, and mutation occurrence in specific genes were analyzed. To identify factors associated with overall survival (OS), a multivariate analysis was performed using Cox regression.

Results: Higher cfDNA concentrations were associated with poorer OS (HR = 1.670, 95% CI 1.108-2.516, P = 0.014). OS was better among patients with at least one mutation in cfDNA (HR = 0.477, 95% CI 0.313-0.727, P = 0.0006). In baseline cfDNA, patients with mutations of VAFs < 5% had significantly better OS (HR = 3.510, 95% CI 1.672-7.370, P = 0.009). Patients with slowly progressing disease had significantly more cfDNA mutations than did those with rapid cancer progression (P = 0.045).

Conclusion: Our key finding was that cfDNA analysis performs better than FFPE tissue analysis in the prediction of patients' OS and disease progression.

References: Tamm M, et al. (2022) Prognostic Utility of Targeted Circulating Cell-Free DNA versus Formalin-Fixed Paraffin-Embedded DNA Mutation Analysis for Advanced Lung Cancer. Int J Oncol Res 5:033. https://doi.org/10.23937/2643-4563/1710033.

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Conflict of Interest: None declared.

P13.117.C Delineating Genotype and Parent of Origin Effect on the Phenotype in MSH6-Associated Lynch syndrome

Anne-Sophie van der Werf-'t Lam[1], *Mandy Villasmil*[1], *Msh6 study group*[1], *Diantha Terlouw*[1], *Mar Rodriguez Girondo*[2], *Manon Suerink*[1], ***Maartje Nielsen***[1]

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Background/Objectives: The reported severity in phenotype differs in Lynch syndrome is largely unexplained. Genotype and/or

parent of origin effect (POE) have been suggested to influence CRC or EC risk in Lynch syndrome (1). Previous studies have focused on MLH1/MSH2 and PMS2. In this study we analyze this for families with germline MSH6 variants.

Methods: Dutch MSH6 (likely) pathogenic variant carriers were grouped based on RNA expression and parental origin. Genotype-phenotype correlation and POE was estimated using hazard ratios (HR) with a (weighted) cox regression. In total 1615 variant carriers from 310 families were included.

Results: Variant carriers with retention of RNA expression (group 1) did not have a significantly different mean age of CRC diagnosis compared to group 2 (no RNA expression) (59.71 years vs. 58.83 years, $p = 0.209$) or EC diagnosis (54.7 years vs. 56.2 years, $p = 0.083$). There was no association between genotype and CRC risk (HR = 1.08, 95% CI 0.72-1.62). However, a significant lower risk for EC was found in group 2 compared to group 1 (HR = 0.43, 95% CI 0.27-0.69). There was no association between POE and CRC risk (HR = 0.93, 95% CI 0.62-1.37), or EC risk (HR = 0.87, 95% CI 0.55-1.37).

Conclusion: These data show that retained RNA expression is associated with a higher lifetime risk for EC, but not for CRC. No correlation was found for POE. Further research is needed to elucidate the mechanism behind higher EC risk before variant specific surveillance can be implemented.

References: 1.Ryan et al JAMA Oncol, 2017.**3**(12).

Grants: Dutch Digestive Disease Foundation (FP16-06).

Conflict of Interest: Anne-Sophie van der Werf-'t Lam MLDS (Maag Lever Darm Stichting, FP16-06)., Mandy Villasmil: None declared, Msh6 study group: None declared, Diantha Terlouw: None declared, Mar Rodriguez Girondo: None declared, Manon Suerink: None declared, Maartje Nielsen: None declared.

P13.118.D Predicting biology instead of disease risk by overlapping oncological polygenic risk scores

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Background/Objectives: Polygenic risk scores (PRS) have been constructed and validated for many cancer types. The most advanced, such as breast, lung or colon cancer PRS, are making its way into clinic and society. For example, through the "Genotyping on all patients" (GOALL) and "Societal impact of genetic science" (SENSE) personalized medicine and prevention programs in Rotterdam and the Netherlands, respectively. In this project, we combine PRS for different cancers and perform biological annotation and clustering to partition genetic risk into cancer-related biological pathways.

Methods: we extract PRS from literature for the most common cancers, and construct a network of disease-variant (GWAS results) and variant-variant (based on LD) associations. The network is soft clustered and visualized using R. All variants are annotated to genes using Ensemble's variant effect predictor and FUMA. Gene enrichment analyses are performed using GSEA in R.

Results: First results indicate between 5-10% overlap in genetic variants between cancer types, which varies per type. Tissue development, cell cycle, DNA metabolic process and immune response terms are enriched across all cancer types, while some biological processes appear specific to one cancer type, such as response to estradiol to breast cancer variants.

Conclusion: Preliminary results indicate shared biology between different cancer types, which can be ascertained by gene annotation and enrichment analysis. Identifying through which biological pathways an individual's risk is cause might have diagnostic and prognostic implications, e.g., altered treatment options for breast cancer caused primarily through DNA metabolism or hormone response variants.

References: NA.

Grants: NA.

Conflict of Interest: None declared.

P13.119.A A collaborative multicenter approach for the classification of mismatch repair gene variants in Spain: results of a pilot study

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Background/Objectives: Identification of germline pathogenic variants in mismatch repair (MMR) genes allows diagnosis of DNA MMR deficiency-associated syndromes. The identification of variants of unknown significance (VUS) precludes diagnosis. Recently, classification guidelines have improved reproducibility and transparency in variant classification. Our main aim is to accelerate and improve MMR variant classification based on the creation of a national registry of germline variants in Spain.

Methods: Twelve Spanish centers were invited to collaborate. Clinical and molecular data from MMR VUS carriers were submitted on a consensus template form. Collected data were curated. Monthly team conferences were held to update and review variant classification according to Insight v2.4 and Insight-ACMG v1 (pending to be approved by ClinGen) guidelines.

Results: A total of 255 VUS present in 324 index cases were collected. Fifty-two of them were identified in 2 or more individuals. Classification was revisited in a subset of 161 VUS (enriched for recurrent, silent and splicing suspected variants). Update of available information allowed the reclassification of 20 variants according to Insight guidelines (8 class 1-2; 12 class 4-5). When using Insight-ACMG criteria 60 VUS were reassigned (43 class 1-2; 17 class 4-5), increasing the reclassification yield from 12 to 37%.

Conclusion: The Spanish variant registry has accelerated the exchange of revised data, essential to improve classification. The use of Insight-ACMG MMR-specific guidelines further increases reclassification yield, mainly of silent/intronic VUS to likely neutral variants.

References:.

Grants:.

Conflict of Interest: None declared.

P13.121.C A novel variation in PTCH1 gene causing Gorlin-Goltz syndrome

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Background/Objectives: Gorlin-Goltz Syndrome (GGS) also known as basal cell nevus syndrome, is a rare multisystemic autosomal dominant disorder. It's estimated population frequency is 1/150.000 to 1/570.000 worldwide. Multiple basal cell carcinomas of the skin, palmar and plantar pits, odontogenic keratocysts and skeletal anomalies are the major presentation of GGS.Pathogenic variations in PTCH1, PTCH2 and SUFU genes encoding components of Hedgehog/ Patched signaling pathway, are associated with GSS.

Methods: The index case was evaluated with detailed anamnesis, pedigree, physical examination, laboratory and imaging methods. Following DNA extraction from peripheral blood lymphocytes all coding exons and exon-intron boundaries of PTCH1, PTCH2 and SUFU genes were analysed via next generation sequencing (NGS). Segregation analysis of the identified variant was performed using Sanger sequencing.

Results: A 11-year-old girl was referred to us because of odontogenic keratocysts of jaws, palmar and plantar pits, multiple basal cell carcinomas of the skin and trichoblastoma. She was born to nonconsanguineous parents after an uneventful pregnancy. Anthropometric measurements and neurodevelopmental characteristics were compatible with her age. Frontal bossing and congenital melanocytic nevus on forearm were present. Arachnoid cyst of the brain and bifid ribs were detected on imaging studies. Molecular genetic studies revealed a de novo novel heterozygous PTCH1 variant (c.3076dupC; p.His1026Profs*119), expected to cause a truncated protein.

Conclusion: GGS is a tumor prone multisystemic disorder in which asymptomatic carriers and families may benefit from early molecular diagnosis and preimplantation genetic diagnosis. Here we report a novel pathogenic variation related to GSS contributing genotype-phenotype correlation.

References:.

Grants:.

Conflict of Interest: None declared.

P13.122.D Faecal metagenomics and Whole Genome Sequencing of patients with somatic APC variants suiting the mutation signature caused by colibactin

Diantha Terlouw[1], *Arnoud Boot*[2], *Quinten Ducarmon*[3], *Sam Nooij*[3], *Manon Suerink*[4], *Alexandra Langers*[5], *Romy Daniëlle Zwittink*[3], *Tom van Wezel*[6], *Maartje Nielsen*[4], *Hans Morreau*[6]

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Background/Objectives: Our *APC* mosaicism analyses have revealed a possible additional explanation for the development of adenomas, namely colibactin produced by *pks+ E.coli* bacteria amongst others. More research into the impact of colibactin on colorectal mucosa and presence of putative carcinogenic bacteria in feces samples is required.

Methods: Twenty-one patients were selected for faecal shotgun metagenomics and somatic Whole Genome Sequencing (WGS). Two of these patients were negative controls, as Next Generation Sequencing did not show an *APC* variant suiting the colibactin mutational signatures (COSMIC: SBS88 or ID18) in any of their adenomas. The other patients included had at least one adenoma with an *APC* variant suiting SBS88 or ID18. WGS was mainly performed on adenomas without a colibactin *APC* variant while at least one other adenoma harbours such an *APC* variant.

Results: Fecal metagenomics shows genes involved in colibactin production (pks island) in 60% of patients with ≥1 adenoma with a colibactin *APC* variant. Although WGS of DNA isolated from Formalin-Fixed Paraffin-Embedded material of adenomas is challenging, the mutational signature (SBS88) was slightly enriched in patients with pks (4/8; 50%) compared to patients without pks in their feces samples (2/7; 29%). The negative controls did not show pks in their feces samples, nor the mutational signatures in their adenomas.

Conclusion: These results show subtle additional evidence for the carcinogenic influence of colibactin in patients with multiple adenomas. However, to draw firmer conclusions, additional negative controls will be analysed.

References:.

Grants: Dutch Cancer Society (11292).

Conflict of Interest: None declared.

P13.124.B Variants of uncertain significance in DNA repair genes: Clinical relevance and impact on therapeutic strategies in Tunisian breast and ovarian cancer patients

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Background/Objectives: Variants of uncertain significance (VUS) in cancer predisposing genes pose a critical challenge in clinical care since their association with disease remains unclear. Here we aim to evaluate the contribution of VUS in DNA repair genes to hereditary breast and ovarian cancer in Tunisian families.

Methods: A total of 78 breast and ovarian cancer cases investigated by NGS and negative for *BRCA* mutations were included in this study. The pathogenicity of variants was assessed using several in silico predictions tools, the stability predictor DynaMut and molecular dynamics simulation with NAMD. Segregation of variants with disease within the investigated families was also evaluated.

Results: A total of 36 VUS were identified among of which 8 are likely to be deleterious affecting *ATM, CHEK2, ERCC3, FANCC, FANCG* and *PMS2* genes. *ATM*_c.6115G>A and *CHEK2*_c.592+3A>T were the most clinically relevant variants identified in families with multiple breast cancer cases and segregation analysis has confirmed their cosegregation with disease. In addition, functional in silico analysis revealed that the *ATM* variant may lead to protein immobilization and rigidification decreasing hence its activity.

Conclusion: Our findings revealed that cancer predisposition may be linked to VUS that need to be reclassified as pathogenic for better disease management. This will help to improve the genetic diagnosis and therapeutic strategies of cancer patients not only in Tunisia but also in neighboring countries.

References: *Monteiro, et al. Journal of medical genetics (2020).*.

Grants: Tunisian Ministry of Health, Tunisian Ministry of Higher Education and Scientific Research.

Conflict of Interest: None declared.

P13.125.C Gene expression analysis on single cell level uncovers the subclonal architecture of chromosomes in a leukemia patient with multiple chromosomal aberrations

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Background/Objectives: In cancer cells, the chromosomal changes are often multiple/complex and can be present only in subclones prone to drive disease aggressiveness. Bulk analysis such as WGS, WES, or genomic array cannot precisely determine the co-occurrence of aberrations in individual cells. Therefore, we employed single-cell RNA sequencing (scRNAseq) technology to study subclonal changes in the malignant cells of a patient with relapsed/refractory chronic lymphocytic leukemia (CLL).

Methods: We identified a patient with multiple chromosomal changes based on the DNA analysis from separated CLL cells using a genomic array (ThermoFisher Scientific). Simultaneously, the transcriptome was analysed using scRNAseq (Chromium system, 10x Genomics) in 2330 cells. Detailed data analysis was carried out with Seurat R package. In addition, we used InferCNV tool to detect the chromosomal aberrations in every tested cell.

Results: We detected several changes with a variable proportion of affected cells using a genomic array. Unsupervised clustering of scRNAseq data according to gene expression defined major clones bearing aberrations (loss on chromosomes 1, 9, 18, del13q, del17p). Moreover, these clones were composed of cells with additional aberrations defining number of separate subclones. The proportion of subclones was typically under the detection limit of the genomic array and showed extreme intraclonal heterogeneity of the relapsed/refractory CLL case.

Conclusion: Genomic alterations influence the expression of affected genes; this fact can be considered to reconstruct chromosomal disruptions on a single cell level using scRNAseq and to uncover the subclonal architecture of the malignancy.

References:.

Grants: MH-CZ_AZV_NU20-08-00314, MH-CZ_AZV_NV19-03-00091, MEYS-CZ_MUNI/A/1330/2021, MH-CZ_RVO_65269705.

Conflict of Interest: None declared.

P13.126.D A Retrospective Cross-Sectional Analysis of Familial Adenomatous Polyposis Coli Cases

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Background/Objectives: Cancer is the second leading cause of death globally. 1.8 million people receive a new diagnosis each year. Colorectal cancers (CRC) constitute about 10% of all, and among them, 30% are familial. Among all familial cancer susceptibility syndromes, one of the best-known is Familial Adenomatous Polyposis Coli (FAP), inherited autosomal dominantly caused by the germline variants in the APC gene located on chr:5q22. The incidence of FAP is estimated as 1:8-15.000 and accounts for less than 1% of all CRC cases.

Methods: Between 2014-2020, 1024 patients have undergone colorectal surgery and diagnosed with CRC. In all the cases, only 24 were considered as FAP,female:male ratio was 3:7, and the mean age of diagnosis 31.6 (±7.1). Genetic testing and counseling were offered to all FAP cases;ten took APC and hereditary oncopanel testing.

Results: Pathogenic variants in APC were detected in six, and STK11 and SDHD variants of unknown significance(VUS) were detected in two. Cascade screening was offered to all the families, and only one with c.2510C>G(p.Ser837Ter) variant accepted the offer. In seven individuals tested, one was found to be the carrier for the pathogenic variant. Another patient with APC variant (c.4464_4468delATTAC/p.L1488fs), developed a desmoid tumor seven years after the colectomy operation, which is in correlation with the incidence of desmoid tumor occurrence (10-25%).

Conclusion: In conclusion, the risk of desmoid tumor development should be considered in families with hereditary cancer syndromes, especially in FAP. Furthermore, cascade screening should be supported worldwide for the effective follow-up of high-risk individuals in familial cancer syndromes.

References:.

Grants: None.

Conflict of Interest: None declared.

P13.129.C Comparative analyses of long non-coding RNA and associated microRNAs expression in plasma and ovarian tissue samples of patients with ovarian cancer

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Background/Objectives: Ovarian cancer is a leading cause of gynaecological cancer mortality among women, due to non-specific symptoms and late diagnosis. Early diagnosis should be essential in the effective treatment. Some non-coding RNAs, as long non-coding RNAs (lncRNA) and microRNAs seem to be promising biomarkers in non-invasive diagnosis. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is an lncRNA, which have a role in carcinogenesis and metastasis and cal also be a prognostic factor in different types of cancer.

Methods: Nineteen ovarian cancer patients and six healthy controls were involved in the study. Blood samples and ovarian tissue were collected; the RNA was isolated and then cDNA was synthesised. The expression of MALAT1 and microRNAs were determined by using RT^2 PCR and miRCURY system (Qiagen, Germany). The interactions between ovary-specific circulating microRNAs and MALAT1 were predicted using the miRNet tool.

Results: Shared interactions were predicted between MALAT1 lncRNA and hsa-miR-200b, hsa-miR-146a and hsa-miR-146b microRNAs. Significant difference was determined in the expression of MALAT1 ($p < 0.001$) in tissue samples and miR-146b ($p < 0.05$) in plasma samples, however no significant difference was observed in the expression of miR-146a, miR-200b and MALAT1 in plasma samples among the cancer patients and controls.

Conclusion: The expression of MALAT1 and three MALAT1-associated microRNAs were determined among Hungarian

women, our results are promising, but more samples and the extension of study are needed.

References: -.

Grants: -.

Conflict of Interest: None declared.

P13.130.B The effect of dietary habits on breast cancer development in patients with BRCA1/2 pathogenic/likely pathogenic variations

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Background/Objectives: The effect of nutrition on cancer development has been proven. However, there is no concrete data on the role of nutrition in the cancer development among the patients with *BRCA1/2* Pathogenic/Likely pathogenic variations (PVs). We evaluated distinctions in the dietary habits between breast cancer (BC) patients with *BRCA1/2* PVs and healthy individuals (HI) with *BRCA1-2* PVs.

Methods: We retrospectively screened 1000 patients that were referred to the Genetic Cancer Clinic at Umraniye Training and Research Hospital. Among these, 89 patients with germline PVs on *BRCA1/2* genes were included to the study. Sixty-one out of 89 were diagnosed with BC, whereas the remaining 28 were HI. Dietary habits and anthropometric measures (AM) of these two groups were compared by using the food consumption frequency form and the Mediterranean Diet Score.

Results: We compared the eating habits of the BC patients with germline *BRCA1/2* PVs and HIs with the germline *BRCA1/2* PVs and found that the ratio of individuals who adhere to the Mediterranean Diet was higher among the HI (60.7%) than BC group (39.3%). Besides, among the AM, body mass index of the cancer patients was significantly higher($p = 0.033$). Moreover, smokers were higher in BCs($p = 0.047$).

Conclusion: To our knowledge this is the first study evaluates the dietary habits and AM in individuals with germline *BRCA1/2* PVs. Our study suggests that there is a difference in nutritional habits between BC patients and HI. However, studies with larger groups are needed to clarify the impact of nutritional habits on cancer development in patients with germline cancer susceptibility.

References:.

Grants:.

Conflict of Interest: None declared.

P14 GENOME VARIATION AND ARCHITECTURE

P14.001.D LRFN5 locus structure is associated with autism and influenced by the sex of the individual and locus conversions

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Background/Objectives: LRFN5 is a regulator of synaptic development and the only gene in a 5.4 Mb topologically associating domain (TAD). The LRFN5 locus is highly conserved despite extensive copy number variation.

Methods: LRFN5 locus structure was studied by quantitative chIP-on-chip in fibroblasts, supplemented with capture-HiC determination of TAD structures. Locus interaction was indirectly studied by allele counting.

Results: An association between locus structural changes and developmental delay (DD) and/or autism was suggested by data from DECIPHER and own records. More significantly, we found that maternal inheritance of a specific LRFN5 locus haplotype segregated with an identical type of autism in distantly related males. This autism-susceptibility haplotype had a specific TAD pattern. We also found a male/female quantitative difference in the amount histone-3-lysine-9-associated chromatin around the LRFN5 gene itself ($p < 0.01$), possibly related to the male-restricted autism susceptibility.

To better understand locus behaviour, the prevalence of a 60 kb deletion polymorphism was investigated. Surprisingly, in three cohorts of individuals with DD ($n = 8757$), the number of deletion heterozygotes was 20-26% lower than expected from Hardy-Weinberg equilibrium. This suggests allelic interaction, also because the conversions from heterozygosity to wild-type or deletion homozygosity were of equal magnitudes. Remarkably, in a control group of medical students ($n = 1416$), such conversions were three times more common ($p = 0.00001$), suggesting a regulatory role of this allelic interaction.

Conclusion: LRFN5 regulation appears unusually complex and influenced by gender and inter-allelic interaction, and LRFN5 dysregulation could be an epigenetic cause of autism.

References: This work is in press in Autism Research (2022).

Grants: HelseVest grant #911459.

Conflict of Interest: None declared.

P14.002.A Combining cytogenetic and genomic technologies for deciphering challenging complex chromosomal rearrangements

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Background/Objectives: Complex chromosomal rearrangements (CCRs) are a class of structural variants (SVs) involving more than two chromosome breaks, classically thought to be extremely rare. As advanced technologies become more available, it has become apparent that CCRs are more common than formerly thought and are a substantial cause of human genetic disorders. Solving the mechanism of CCRs by precisely identifying sequence-level changes, and their order, are challenging, especially when repetitive sequences are involved.

Methods: Chromosomal microarray (CMA) and FISH analyses were used for interpretation of SVs detected by whole exome sequencing (WES). Breakpoint junctions were analyzed by

Nanopore sequencing, a novel long-read whole genome sequencing (WGS) tool.

Results: A large deletion identified by WES, encompassing the FOXF1 enhancer, was the cause of alveolar capillary dysplasia and respiratory insufficiency, resulting in perinatal death. CMA analysis of the deceased newborn's mother revealed two duplications encompassing the deleted region in the proband, raising our hypothesis that the deletion resulted from CCR in the mother. Breakpoint junctions of complex SVs were determined at the nucleotide level using Nanopore long-read sequencing. According to the sequencing results of breakpoint junctions, the CCR in the newborn was considered as the consequence of at least one double-strand break (DSB) during meiosis and reassembly of DNA fragments by intra-chromosomal homologous recombination.

Conclusion: The combination of CMA, FISH and long read NGS enabled delineation of the exact breakpoints and proposed a mechanism in which the CCR arises. We suggest an integrative approach combining cytogenetic and genomic technologies for deciphering challenging CCRs.

References:.
Grants:.
Conflict of Interest: None declared.

P14.003.B Hidden splice variants in stop-codons of the DMD gene in Duchenne/Becker muscular dystrophy

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Background/Objectives: The most common cause of Duchenne/Becker muscular dystrophy (DMD/BMD) is gross deletions and duplications. Frameshift deletion exons form Duchenne muscular dystrophy phenotype, while non-frameshift mutations are milder and result in the Becker muscular dystrophy phenotype.

One of the mechanisms of the molecular pathogenesis of DMD/BMD are variants affecting splicing in the DMD gene. It is known that not only mutations in canonical regions, but also missense, nonsense and intronic variants can affect splicing.

The aim of the study was to investigate the mechanism of the influence of stop-codons on the mRNA sequence.

Methods: We performed the bioinformatician analysis of 656 variants leading to the formation of a premature termination codon from the HGMD Professional database 2020.4. The splice site prediction programs Splice AI, SPiP, and NetGene2 were used.

Results: Out of 554 nonsense variants in DMD, 54 variants were predicted to affect splicing (9.7%). Of these, in 90% of cases a frameshift was predicted.

For variants associated with BMD 7 of 47 nonsense variants (14.8%) were predicted to affect splicing. For 100% splice variants the programs predicted inframe deletions part of exon or skipping of an exon entirely.

Conclusion: The results of the study are consistent with the concept of differences in the phenotypes of DMD/BMD and allow us to evaluate the mechanism of the influence of nonsense variants on DMD gene splicing. The lack of predictions about the effect of nonsense variants on splicing requires further study of the influence of these variants on the molecular mechanism of the pathogenesis of DMD/BMD.

References:.
Grants:.
Conflict of Interest: None declared.

P14.004.C Identification of alternative transcripts of nsd1 gene responsible for sotos syndrome

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Background/Objectives: NSD1 gene (nuclear-receptor-binding-set-domain-protein) encodes a methyltransferase [1,] implicated in the transcription and methylation of histone H3 at lysine 36 (H3-K36), but the molecular mechanisms involved in these processes remain largely unknown. Pathogenetic variants in NSD1 gene lead to Sotos syndrome (SoS), and they have been involved also in cell proliferation and drug resistance of some type of cancers [2].

Methods: Sequence analysis and array CGH were performed to verify NSD1 alteration in 14 Sotos patients and NSD1 mRNA expression by Real Time PCR was performed on fibroblast cell lines obtained from patients and from 8 controls. We sequence NSD1 cDNA isoforms observed in patients and controls.

Results: We describe the presence of two shorter NSD1 mRNA isoforms not yet reported: NSD1 Δ5Δ7 (isoform2) and NSD1 Δ19-23 (isoform 3) both in healthy subjects and in patients. We show that the NSD1 mutations in patients were associated to a decreased level of NSD1 mRNA. In addition, one SoS patient, bearing the NSD1 variant c.6010-10G>A, expressed an additional transcript derived from an aberrant splicing. Protein structure prediction of NSD1 isoform 2 indicates the presence of a shorter protein displaying only the PWWP1 domain that lacks the catalytic SET domain, while Isoform 3 lacks PHD3 and PHD4 C-terminal domains.

Conclusion: These results provide a basis to better elucidate the role of alternative transcripts both in SoS and in tumor progression.

References: 1. Douglas J et al. 2003 Am J Hum Genet.
4. Mohanty S et al 2020 Cancers.
Grants: AssiGulliver and Fondazione Sardegna.
Conflict of Interest: None declared.

P14.005.D Exploring the obscure clinical significance of uncommon uni-parental disomy (UPD)

Omri Weiss[1], *Sharon Zeligson*[1], *Orit Lobel*[1], *Malka BenUziyahu*[1], *Adi Ben Yehuda*[1], *Shachar Zuckerman*[1], *Shelly Lev-Hochberg*[1], *Rachel Beeri*[1], *Efrat Burak*[1], *Shira Shaviv*[1], *Tal Dror*[1], *Miriam Patt*[1], *Reeval Segel*[1,2]

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Background/Objectives: The use of next generation sequencing and single nucleotide polymorphism based chromosomal microarray analysis (CMA) leads to detection of uniparental disomy (UPD) in both pre- and post-natal settings. UPD may lead to disease by abnormal expression of imprinted genes or loss of heterozygosity (LOH) of mutations in recessive genes.

The unknown clinical significance of many UPD segments is challenging, especially in prenatal settings. Sharing UPD segments detected in healthy individuals can resolve such uncertainty.

Methods: We identified regions of homozygosity (ROH) suggestive of UPD in CMA samples in Shaare Zedek Medical Centre and correlated them to the clinical information.

Results: In our cohort, 14 samples had ROH suggestive of UPD. Four causing UPD-imprinting disease and ten were ROH of uncertain significance. Of the ten, three were found in healthy individuals tested for unrelated conditions and one was done due to LOH of a mutation in the CASR gene, causing autosomal recessive neonatal hyperparathyroidism.

We report four ROH indicative of UPD of uncertain significance: chr3 maternal (iso)UPD detected in the patient with hyperparathyroidism; 18q12.1q22.3 (unknown parental origin) in a healthy individual; maternal segmental UPD of 5p13.3q21.3 and paternal mosaic (iso)UPD of chr22 in amniocenteses referred for advanced maternal age. Pregnancy follow-up was normal and exome sequencing validated UPD.

Conclusion: Our data, together with previously published UPD segments, establish that UPD of chromosomes 3, 5, 18 and 22 are not expected to cause imprinting-related disease. Further reports of UPD from healthy individuals are necessary for mapping imprinting free regions, allowing more informative genetic counselling.

References:.

Grants:.

Conflict of Interest: None declared.

P14.006.A Hereditary Hemorrhagic Telangiectasia: first demonstration of a branch point causative variant

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Background/Objectives: Hereditary Hemorrhagic Telangiectasia (HHT) is a rare autosomal dominant disease leading to vascular dysplasia caused by a pathogenic variant in one of the HHT gene: *ENG*, *ACVRL1*, *SMAD4*, *GDF2*. We present a large HHT family without a definite pathogenic variant; linkage analysis suggested the *ACVRL1* involvement. The c.526-22 A>G VUS in this gene, located at a putative branch point, co-segregates with the disease in the family. This represents the first case of a branch point mutation in HHT.

By analyzing the *ACVRL1* transcript we aimed to demonstrate the variant pathogenicity.

Methods: We performed WES (filtering for the HHT genes) and MLPA analysis on a clinically confirmed case of the family. Co-segregation of c.526-22 A>G was confirmed by Sanger Sequencing. To proof the variant pathogenicity, RNA was extracted from peripheral blood of the patient and five healthy controls. After retrotranscription and PCR, semi-quantitative and qPCR analyses were performed. Sanger sequencing was carried out too.

Results: The variant is not present in GnomAD. Bioinformatics and semi-quantitative analyses suggested a quantitative change in the canonical coding RNA and the exon-5 skipping isoforms, changing the ratio between the two. These data were confirmed by quantitative and sequencing analyses.

Conclusion: We report the first case of a branch point pathogenic variant in HHT. This is, to the best of our knowledge, the first HHT-related variant modifying the quantity rather than quality of the transcript.

References:.

Grants: Italian Ministry of Education, University and Research to the DMM-University of Pavia "Dipartimenti di Eccellenza (2018-2022)".

Conflict of Interest: None declared.

P14.007.B Characterization of the mitochondrial genome landscape in induced MSCs (iMSCs) derived from patients with osteoarthritis and healthy donors

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Background/Objectives: Osteoarthritis (OA) is an age-related disease. Heteroplasmic mitochondrial variants accumulate with advancing age, impairing respiration and cell metabolism. The present study characterized the mtDNA landscape in OA and healthy BM-MSCs, iPSCs and iPSCs-derived-MSCs (iMSCs).

Methods: Linearization of mtDNA, ultra-deep NGS and mtDNA analysis were used to determined homoplasmic and heteroplasmic SNVs and short deletions.

Results: Homoplasmic variants were not different between donors and cell types, while heteroplasmic variants were higher in OA-MSCs compared to healthy-MSCs. Differences were observed between OA-MSCS, -iPSCs and -iMSCs. Unsupervised hierarchical clustering showed a "purifying effect" for heteroplasmic variants in OA patients during re-programming to iPSCs. The decreased variant number remained stable during iPSCs differentiation while ~58% of heteroplasmic variants in OA and (~48%) in healthy donors are coding. In OA donors, the variants with the highest heteroplasmic fraction (HF~10%) are located in the control region (DLOOP1, DLOOP2), whereas in healthy donors (HF>90%) in ATP6 and a t-RNA gene TQ. The majority of the heteroplasmic variants fall in the r-RNA gene followed by ND1, ND2 genes in both groups.

Conclusion: We characterized the mtDNA landscape during generation of iMSCs from OA and healthy MSCs-donors, suggesting a decreased heteroplasmic variant load in OA-iPSCs, which remains stable during differentiation to iMSCs. The findings indicate that iMSCs might be valuable alternative to cell-based OA treatment.

References: Wei et al, Nat Commun, 2021, 12:5241.

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Conflict of Interest: None declared.

P14.008.C Systematic characterisation of 5'untranslated regions reveals key difference between and across gene-sets

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Background/Objectives: Untranslated regions (UTRs) flank the coding sequence of a gene. 5' and 3' UTRs are not translated but mediate post-transcriptional regulation via linear and structural

elements. To interpret 5'UTR variants in a clinical setting, further understanding of these regions is required; what they normally 'look like' and how they vary between different categories of genes.

Methods: We analysed 5'UTRs across all genes to determine their overall landscape. 5'UTRs of disease genes (developmental disorder (DD), cancer (C) and dosage sensitive (DS) genes sets) were explored to establish enrichment for 5'UTR features as they may be under tighter regulatory control. 5'UTR features investigated were length, introns and the number and type of upstream open reading frames (uORFs; cis-regulatory elements that down-regulate protein translation).

Results: 5'UTR lengths range from one to over 2000 base pairs. 35% of genes have at least one intron. uORFs are present in 34% of genes. 5'UTRs of disease gene-sets were significantly longer (DD:P = 1.1x10-41, C:P = 1.8x10-05, DS:P = 1.1x10-98), have more uORFs (DD:P = 2.6x10-23, C:P = 0.0002, DS:P = 2.4X10-56) and some have more introns (C:P = 0.03) than all gene 5'UTRs. DD recessive genes reflected the inverse trend - significantly shorter 5'UTRs (P = 1.6x10-05), significantly fewer introns (P = 0.001) and fewer uORFs (P = 0.002).

Conclusion: These disease gene-sets have more complex 5'UTRs and are enriched for cis-acting regulatory elements. 5'UTR features may be important to subtly regulate translation levels. Well-characterised 5'UTRs will aid in understanding of UTRs role and interpreting variants.

References:.

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Conflict of Interest: Nechama Wieder: None declared, Alex Geary University of Oxford, Frederik Lassen: None declared, Elston D'Souza University of Oxford, Maria Fernandes University of Oxford, Robert Davies University of Oxford, Wellcome Trust, Nicola Whiffin University of Oxford, Sir Henry Dale Fellowship/Wellcome Trust.

P14.009.D Genomic analysis of germline and somatic lesions at the FCGR locus; from reference genomes to clinico-biological implications

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Background/Objectives: In cancer treatment, monoclonal antibody (mAb) immunotherapies educe their functional response by interacting with the Fc gamma receptors (FcγRs). The low-affinity locus (encoding FCGR2A, FCGR2B, FCGR2C, FCGR3A and FCGR3B) contains a 98% homologous 85kb segmental duplication and is highly polymorphic, harbouring multiple large copy number variations (CNVs) and single nucleotide polymorphisms (SNPs). Short sequencing reads often fail to map adequately, therefore we are generating accurate genomic maps of the FCGR locus using long-read Oxford Nanopore Technologies (ONT).

Methods: Utilising a healthy human cohort (n = 22) with different FCGR SNPs and CNV states, ONT adaptive sampling and cas9-mediated enrichment (tiling of 10 ROI) were employed to create comprehensive maps providing phased sequence, breakpoint and methylation information.

Results: Samples sequenced to an average of 200x (read N50 35kb) with some reads spanning >100kb. ONT adaptive sampling and cas9-targeting resulted in 6.2- and 500-fold increases in FCGR sequencing respectively, with enrichment as high as 967-fold. The bioinformatics pipeline of SNP and SV calling, phasing, de novo assembly and methylation calling has enabled the exact breakpoints of the CNVs to be identified, linkage disequilibrium relationships to be inferred and the connection between the FCGR (epi)genomic landscape and transcriptional regulation to be explored.

Conclusion: ONT transcriptomics for isoform quantification and expression analysis, and confirmation of large structural variants with BioNano optical mapping are both underway. ONT sequencing represents an exciting opportunity to overcome the difficulties of sequencing this complex region, facilitating greater understanding of FcγR regulation and how their function may be manipulated for the benefit of cancer patients.

References:.

Grants:.

Conflict of Interest: None declared.

P14.010.A Whole-genome sequencing data reveals higher number of structural variants in Chernobyl catastrophe clean-up workers from Lithuania

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Background/Objectives: Many of the Lithuanian Chernobyl catastrophe clean-up workers (LCCW) are already diseased, thus, we hypothesize that those, who experienced harsh exposures but survived and age relatively healthy, might have specific genomic variation which works in a protective way. Here we report initial structural variation (SV) analysis from whole-genome sequencing (WGS) data for 40 LCCW and 24 male control individuals of Lithuanian origin from the general population.

Methods: Short-read WGS data was of good quality (Q30 value: >90%) with the average coverage of ~38x. Structural variants were called on Illumina DRAGENv.3.6.4 using the same methods as Manta. SV (>49 bp) annotation was performed using *AnnotSV* with default parameters. Wilcoxon rank sum test with continuity correction (α = 0.05) was performed using R 4.1.2.

Results: Deletions and insertions were more abundant than duplications in both groups. There were significant differences in average numbers of deletions (p = 0.007), duplications (p < 0.001), and insertions (p < 0.001) among LCCW and control groups. The average number of SV in LCCW group was 9640 compared to 8987 in control group, and about 95% of SV was new in both groups.

Conclusion: It is the first WGS SV data analysis of the Chernobyl catastrophe clean-up workers' and Lithuanian origin genomes. Further detailed SV characterization and integrative analysis will provide useful information on local human population and unique sample group of LCCW genomic variation. Current results complementation with greater sample size data would be beneficial for analysis.

References:.

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Conflict of Interest: None declared.

P14.011.B Mobile element insertions as potential cancer predisposition in high-risk HBOC patients

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Background/Objectives: In more than 60% of patients at risk for hereditary breast and ovarian cancer (HBOC), current diagnostic tests fail to identify the causative variant. A currently underrated type of predisposing event is the disruption of gene structures by mobile element insertions (MEIs), since their systematic detection has not been possible before the implementation of high-throughput sequencing.

Methods: Panel sequencing data of 303 high-risk HBOC patients were reanalyzed using the bioinformatic tool *Mobster*. After filtering for rare, novel events proximal to HBOC-associated genes, one candidate MEI was further characterized by a minigene assay and fragment analysis.

Results: Of 55 novel MEI predictions, 13 located to HBOC-associated genes among which one predicted *Alu* element-insertion in intron 54 of *ATM* manifested as a true event and the most likely candidate to predispose for HBOC in two patients. Transcript analysis showed the expression of an alternative *ATM* transcript. Sanger sequencing revealed an exon skipping event resulting in the exclusion of exon 54 which was evident in up to 37% of total *ATM* mRNA in the patients. These results were reinforced by the minigene assay.

Conclusion: Since the aberrant transcript is unlikely to be translated into a functional protein due to a frameshift and subsequent premature stop codon in exon 55, the *Alu* element-insertion is a likely pathogenic variant associated with HBOC in two families. Our work has important implications for the treatment and surveillance of the patients and their families in addition to valuable insights into the detection and characterization of MEIs.

References:.

Grants:.

Conflict of Interest: None declared.

P14.012.C Multisite de novo mutations after paternal exposure to ionizing radiation

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Background/Objectives: In our ongoing study we evaluate the effects of ionizing radiation on the offspring of exposed soldiers.

Methods: We sequenced the whole genome of 310 individuals from 88 families. For 28 soldiers, we could obtain a retrospective dosage estimation ranging up to 325mSv during their service (Mean: 8.32mSv, Std: 48.51mSv). Other participants were not significantly exposed. The control cohort consists of 1275 families with no known exposure to ionizing radiation.

Results: Our focus lies on specific mutational patterns such as multisite de novo mutations (MSDNs; at least two de novo mutations within 20bp), and de novo SVs and CNVs which are linked to prolonged parental exposure to ionizing radiation.

After accounting for known confounders we found no significant difference ($p = 0.26$) in the mean number of (DNMs) between both cohorts. We found on average 5.4 MSDNs/offspring in the case cohort and 3.9 MSDNs/offspring in the control cohort. We detected 43% more MSDNs per DNM in the case cohort ($p < 0.00001$). The number of mutations in MSDN clusters is increased by 33% on average ($p = 0.018$) in the offspring of radar soldiers. Additionally, we identified ten candidates for large de novo SVs in the case cohort, including two translocations.

Conclusion: All MSDNs, and structural variants are undergoing extensive validation, which is also used to assert the parental origin of the mutation in question. Our efforts are now focused on statistical evaluations of the raw and validated datasets to draw final conclusions about the consequences of prolonged paternal exposure to ionizing radiation on the following generation.

References:.

Grants:.

Conflict of Interest: None declared.

P14.013.D Xq27.1 palindrome mediated interchromosomal insertion as the likely cause of familial congenital bilateral laryngeal abductor paralysis (Plott syndrome)

Felix Boschann[1], *Daniel Julian Acero-Moreno[2], Martin Atta Mensah[1], Henrike Sczakiel[1], Karolina Skipalova[1], Manuel Holtgrewe[3], Stefan Mundlos[1], Björn Fischer-Zirnsak[1]*

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Background/Objectives: Bilateral laryngeal abductor paralysis is a common cause of stridor in newborns. No convincing genetic aberration has been reported for Plott syndrome (X-linked isolated bilateral vocal cord paralysis, MIM: 308850). We aimed to study the underlying cause in a large family with eight affected males, of whom seven died shortly after birth.

Methods: We performed short read whole genome sequencing (WGS) in an affected boy and his unaffected brother. FISH analysis was conducted to confirm the detected genomic rearrangement. Breakpoint specific PCRs and qPCR were used for segregation analysis.

Results: In the affected boy, we identified a 404 kb large non-coding insertion into the well-known intergenic region Xq27.1, originating from chromosome 10q21.3. This genomic rearrangement was confirmed by FISH analysis. Breakpoint junction analysis identified patterns likely resulting from replication based mechanisms like MMBIR/FoSTeS. Segregation analysis confirmed strictly matrilineal inheritance of the derivative chromosome X.

Conclusion: Our findings add Plott syndrome as a further disease entity caused by interchromosomal insertions into the intergenic region Xq27.1 and show that phenotypically distinct diseases can result from the introduction of different regulatory elements into the same genomic region. Thus demonstrating the utility of WGS in the diagnosis of previously unsolved cases.

References: Boschann F, Moreno DA, Mensah MA, Sczakiel HL, Skipalova K, Holtgrewe M, Mundlos S, Fischer-Zirnsak B. Xq27.1 palindrome mediated interchromosomal insertion likely causes familial congenital bilateral laryngeal abductor paralysis (Plott syndrome). J Hum Genet. 2022 Jan 31. https://doi.org/10.1038/s10038-022-01018-z. Epub ahead of print. PMID: 35095096.

Grants:.

Conflict of Interest: None declared.

P14.014.A Unusual Suspects in hereditary melanoma: POT1, POLE and BAP1

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Background/Objectives: Three genes (*POT1*, *POLE* and *BAP1*), have been reported in association with familial melanoma in the past decade. Genotype-phenotype correlations of individuals with mutations in these genes have not been well described.

Methods: Systematic searches collated 273-variants pathogenic (*BAP1* n = 136, *POT1* n = 71, *POLE* n = 66) which were identified and annotated from 69-sources to identify genotype-phenotype correlations in these genes.

Results: Two high-cluster regions, predominantly associated with cutaneous melanoma (CM), were identified in *POT1* in the oligonucleotide/oligosaccharide binding region one (36 amino acids (aa)), and two (6aa). *POLE* had one high-cluster variant region (292aa) in the exonuclease domain associated with diverse cancer types. The highest proportion of variants in this cluster were associated with endometrial cancer; however, there was one hotspot (spanning 55aa) associated with CM. *BAP1* had six high-cluster regions with loss-of-function variants within 24aa associated exclusively with CM (between nuclear localisation signal domains). Two *BAP1* clusters (31aa and 12aa) were both associated with CM and uveal melanoma (UM) (located in unspecified regions between the BRCA-1 associated ring domain (BARD) and the BRCA1 binding domain); another two clusters had the predominant phenotype of UM (74aa and 21aa) located in the ubiquitin carboxyl-terminal hydrolase (UCH)/BARD crossover domain, and the BARD binding domain respectively. The remaining *BAP1* cluster was associated with diverse cancer phenotypes of CM, UM and malignant mesothelioma, and was located in the UCH domain (11aa).

Conclusion: Genotype-phenotype correlations captured from this research can be used to identify patient disease predisposition based on mutation position and cluster regions in *POT1*, *POLE* or *BAP1*.

References:.

Grants:.

Conflict of Interest: Ellie Maas: None declared, brigid betz-stablein: None declared, lauren aoude: None declared, Hans Peter Soyer HPS is a shareholder of MoleMap NZ Limited and e-derm consult GmbH and undertakes regular teledermatological reporting for both companies. HPS is a Medical Consultant for Canfield Scientific Inc., Blaze Bioscience Inc., MoleMap Australia Pty Limited, and a Medical Advisor for First Derm and Revenio Research Oy., Aideen McInerney-Leo: None declared.

P14.015.B Genetic and epigenetic effect of 22q12 large deletions in rare NF2 patients

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Background/Objectives: Neurofibromatosis type 2 is an autosomal dominant tumor-prone disorder, mainly due to point mutations or intragenic deletions of NF2 gene, encoding Merlin protein, that inhibits proliferation pathways. However, rare large 22q12 microdeletions have been found in patients with complex phenotype [1]. To identify the genetic and epigenetic mechanisms resulting from 22q12 microdeletions, we characterized three NF2 microdeletion patients, two with extended deletions and severe phenotype, including mental retardation, and one with a smaller deletion and milder phenotype.

Methods: We carried out MLPA, aCGH, in silico analysis, Long-Range PCR assays, and Sanger sequencing to characterize the deletions' breakpoints. We performed in silico analysis of 22q12 TADs and regulatory elements, together with gene expression analysis of 19 genes flanking patients' deletions by RT-PCR, to investigate a possible position effect generated by the chromatin 3D-structure alteration.

Results: Patient with milder phenotype shows 146 Kb deletion, involving three genes. In patients with more severe phenotype, deletions of 560 Kb and 1.8 Mb have been identified, counting 13 and 36 genes, respectively, and including AP1B1, a gene that could be linked to patients' mental retardation. Furthermore, patients' microdeletions alter from one to five TADs and the 22q12 regulatory landscape, and RT-PCR assays confirmed the position effect on some flanking genes. Interestingly, PIK3IP1 overexpression could be associated with the ischemic event occurred in patient with largest deletion.

Conclusion: Our results, providing new genotype-phenotype correlations in NF2 microdeletion patients, could have an impact on the identification of prognostic markers, useful for patient management, and potential therapeutic targets.

References: [1] PMID:10338003.

Grants: Academic fund.

Conflict of Interest: None declared.

P14.016.C A systematic annotation of the impact on splicing of all possible substitutions within the SPINK1 coding sequence

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Background/Objectives: In addition to mutations within splice junctions, both missense and silent variants within the coding region may affect pre-mRNA splicing. However, there are currently no human genes for which the consequences for splicing of all possible substitutions within the coding sequence have been systematically annotated. Herein, we attempted to achieve such a goal in the context of SPINK1.

Methods: All possible substitutions within the entire SPINK1 coding sequence were subjected to SpliceAI prediction [1]. The accuracy of SpliceAI prediction was first evaluated in the context of 27 known variants previously analyzed using a Full-Length Gene Splicing Assay (FLGSA) [2,3]. All three possible substitutions in a dozen selected coding nucleotide sites were further subjected to FLGSA as described [3].

Results: SpliceAI prediction and experimentally obtained findings concurred exactly with the previously analyzed 27 SPINK1 variants. As for the prospectively analyzed variants, there was an excellent correction between SpliceAI prediction and experimental validation. The use of FLGSA made it possible to correlate precisely the predicted and experimentally obtained aberrant splicing outcomes.

Conclusion: Having correlated SpliceAI-predicted and FLGSA-derived findings from a large number of known and prospectively generated variants, we were able to provide a systematic annotation of the splicing effects of all possible substitutions within the coding sequence of the SPINK1 gene with a high degree of accuracy.

References: [1]. Jaganathan et al. Cell. 2019;176:535. [2]. Zou et al. Gut. 2016;65:884. [3]. Wu et al. Genes. 2017;8:263.

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Conflict of Interest: Hao Wu Department of Gastroenterology, Changhai Hospital, the Secondary Military Medical University, Shanghai, China, The National Natural Science Foundation of China (no. 81800569); The Shanghai Pujiang Program, China (no. 18PJD057), Jin-Huan Lin Department of Gastroenterology, Changhai Hospital, the Secondary Military Medical University, Shanghai, China, The National Natural Science Foundation of China (no. 82000611); The Shanghai Pujiang Program (no. 2020PJD061), Wen-Bin Zou Department of Gastroenterology, Changhai Hospital, the Secondary Military Medical University, Shanghai, China, Emmanuelle Masson Service de Génétique Médicale et de Biologie de la Reproduction, CHRU Brest, F-29200 Brest, France, sacha schutz Service de Génétique Médicale et de Biologie de la Reproduction, CHRU Brest, F-29200 Brest, France, David N. Cooper Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom, Gerald Le Gac Univ Brest, Inserm, EFS, UMR 1078, GGB, F-29200 Brest, France, Claude Férec Univ Brest, Inserm, EFS, UMR 1078, GGB, F-29200 Brest, France, Zhao-Shen Li Department of Gastroenterology, Changhai Hospital, the Secondary Military Medical University, Shanghai, China, Zhuan Liao Department of Gastroenterology, Changhai Hospital, the Secondary Military Medical University, Shanghai, China, Jian-Min Chen Univ Brest, Inserm, EFS, UMR 1078, GGB, F-29200 Brest, France.

P14.017.D Identification of a novel physiological LRIG2 splicing variant associated with the development of Urofacial Syndrome

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Background/Objectives: Urofacial Syndrome 1 (UFS1) is a rare AR disorder characterized by congenital urinary bladder dysfunction caused by mutations in the *LRIG2* gene. LRIG2 is a transmembrane protein involved in the regulation of growth factor signaling. Although LRIG2 controls neuronal migration during embryonic development, its role in bladder innervation and in the pathogenesis of UFS is yet to be elucidated.

Methods: In two siblings with bladder dysfunction, by WES we found a novel *LRIG2* splicing variant c.1478-2A>G, that leads to skipping of exon 13 and the loss of the first Ig-like domain in the LRIG2 protein. We profiled the expression of the LRIG2 isoforms in patients and controls (normal and tumor CNS-derived tissues and blood).

Results: In the probands only the skipped isoform was expressed. Whereas we found the presence of the isoform with skipping of exon 13 in all the analyzed control tissues, together with the canonical one. Interestingly, no *LRIG2* mutations were found in controls, irrespective to the presence of the skipped isoform.

In silico protein modelling and binding dynamics allowed the identification of functional differences between the two LRIG2 isoforms (canonical and skipped). We found that the first Ig-like domain is fundamental for protein dimerization, suggesting a reduced activity or a different mechanism of action of the LRIG2 isoform lacking this domain.

Conclusion: We hypothesize that the identified mutation could force the skipping of exon 13, thus leading to the expression of the novel LRIG2 isoform, which is physiological when it is expressed at low levels compared to the canonical one.

References:.

Grants:.

Conflict of Interest: None declared.

P14.018.A BeSolveRD: The Belgian genome resource to resolve rare diseases

Mathilde Geysens[1], *Wouter Bossuyt*[1], *Elfride De Baere*[2], *Kim De Leeneer*[2], *Koenraad Devriendt*[1], *Annelies Dheedene*[2], *Aimé Lumaka*[3], *Jeroen Luyten*[4], *Gert Matthijs*[1], *Björn Menten*[2], *Marije Meuwissen*[5], *Olivier Monestier*[6], *Catharina Olsen*[7], *Leonor Palmeira*[3], *Nicole Revencu*[8;8], *Julie Soblet*[9], *Erika Souche*[1], *Yves Sznajer*[8], *Sonia Van Dooren*[10], *Catheline Vilain*[9], *Kris Van Den Bogaert*[1], *Joris Vermeesch*[1]

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Background/Objectives: Despite the diagnostic implementation of chromosomal microarrays/shallow whole genome sequencing and whole exome sequencing (WES) for patients with intellectual disabilities/developmental disorders (ID/DD), approximately half remain undiagnosed using this standard of care (SoC). To (1) technically validate whole genome sequencing (WGS) at different genetic centers in Belgium, (2) investigate the clinical utility of WGS for ID/DD diagnosis and (3) to assess the health economic impact, the Belgian genetic centers engaged in a multicentric prospective randomized control trial, called BeSolveRD.

Methods: A total of 800 patients and both parents will be recruited of which half will be processed by SoC and half by WGS. We have performed ring trials to compare different methods to generate WGS libraries, allowing the optimization and validation of WGS in all centers. The WGS pipelines for SNV detection of all centers and their performance have also been assessed. Most pipelines use the same tools and perform very similarly despite the different implementation. Finally, we have created a federated database to share variant and sequence information.

Results: We will present the process of collaboration, the challenges and the intermediate output data.

Conclusion: Intermediate conclusions will be deduced from the results gathered until May 2022.

References: /.

Grants: Illumina sponsored study.

Conflict of Interest: None declared.

P14.019.B Method optimisation for structural variant detection in the human genome

Oguzhan KALYON[1], *Bilal Alobaidi*[1], *Lois Batty*[1], *Miguel Xavier*[1], *Giles Holt*[1], *Aneta Mikulasova*[1], *Joris Veltman*[1]

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Background/Objectives: Structural variation (SV) has been traditionally difficult to identify and characterize. Now, whole genome sequencing (WGS) and optical genome mapping (OGM) are both available for more comprehensive SV detection. Here, we compared and optimized the accuracy of SV detection by combining different bioinformatic tools and platforms into an integrated workflow and applied this to both simulated and real genomic data.

Methods: Two short-read WGS BAM files with gains and losses (n = 841, size range 2kb–1Mb) were simulated. Different bioinformatics tools were tested for SV detection in these simulated WGS data. In addition, we performed both WGS and OGM in 9 samples and compared SV detection.

Results: Simulated WGS data showed that the combination of a depth of coverage method (CNVRobot) and a method based on discordant read pairs and split reads (dysgu-SV) provided the most efficient and accurate method for SV detection. This combination resulted in a true positive rate of SV detection of 99.3% and a false discovery rate of 0.02%. Next, we applied this integrated approach in WGS data from 9 samples and identified 96 SVs on average (mean size 8kb). In addition, we identified 135 SVs on average (mean size 31kb) by OGM. Only 34% of SVs detected by the integrated WGS pipeline were supported by OGM, which in general detected larger and more complex SVs.

Conclusion: Our study demonstrates that combined bioinformatic approaches provide highly accurate SV identification in WGS. By combining WGS with OGM we were able to obtain a highly reliable SV dataset.

References:.

Grants:.

Conflict of Interest: None declared.

P14.020.C Review of secondary findings reported in the qGenomics patient cohort, analyzed by exome sequencing

Marta Carreño[1], *Maria Segura-Puimedon*[1], *Raquel Garcia*[1], *Lidia Carreño*[1], *César Arjona*[1], *Hector San Nicolás*[1], *Cèlia Sintas*[1], *Mònica Vall*[1], *Olaya Villa Marcos*[1], *Marina Viñas-Jornet*[1], *LLuís Armengol*[1]

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Background/Objectives: A new update of the American College of Medical Genetics (ACMG) recommendations for the reporting of secondary findings (SF) in clinical exome and genome sequencing was published last year, along with the new list of 73 medically actionable genes ACMG SF v3.0. In it, an analysis of the impact of the use of the previous list (ACMG SF v2.0) is also exposed. The objective of our study was to calculate the frequencies of the SF report in our cohort and to compare it to previous studies in literature and with the frequencies obtained with the new list.

Methods: A retrospective investigation was carried out on 3638 patients, analyzed by whole exome sequencing during the period between the publication of both ACMG lists (2017-2021). Also, it was extended to 541 more patients, analyzed after the ACMG list update.

Results: In our cohort, SF have been reported in 98 cases (frequency of 2.69%), being *BRCA2* and *KCNQ1* genes the most frequently reported. As expected, this frequency has increased up to 3,51% with the use of the new list.

Conclusion: Although it is difficult to compare different studies, the frequencies obtained fit perfectly into the frequency ranges described in the update. Currently, the new expanded list has been implemented in our laboratory, continuing with the objective of preventing and reducing patient morbidity and mortality. Our purpose is to continue to analyze the diagnostic potential of SF with the new ACMG SF v3.0 list.

References:.

Grants:.

Conflict of Interest: Marta Carreño qGenomics, Maria Segura-Puimedon qGenomics, Raquel Garcia qGenomics, Lidia Carreño qGenomics, César Arjona qGenomics, Hector San Nicolás qGenomics, Cèlia Sintas qGenomics, Mònica Vall qGenomics, Olaya Villa Marcos qGenomics, Marina Viñas-Jornet qGenomics, LLuís Armengol qGenomics.

P14.022.A Genetic diagnosis can be missed by classical filtering criteria for WES in Mendelian Disorders – diagnostic strategies to approach challenging cases

Sarah L. Stenton[1;2], ***Christiane Neuhofer***[1;2;3], *Riccardo Berutti*[1;2], *Robert Kopajtich*[1;2], *Dmitrii Smirnov*[1;2], *Holger Prokisch*[1;2]

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Background/Objectives: More than 50% of patients with suspected hereditary disorders ultimately remain without molecular diagnosis. Variants with incomplete penetrance as well as heterozygous variants in autosomal recessive genes escape prioritization despite being potentially causative.

Methods: In case of reduced penetrance, segregation of pathogenic variants doesn't follow conventional Mendelian inheritance patterns. This required new filtering approaches, inclusion of gene burden tests in large cohorts and early integration of functional evidence.

Results: Variants of incomplete penetrance show higher allele frequency than expected from the incidence of the disease. We therefore eased the MAF filtering criteria, applied statistical analysis of gene burden and included functional evidence by transcriptome and proteome analysis to search for novel disease genes with incomplete penetrance[1].

In the absence of statistical evidence functional analysis is of paramount importance. In one family the unaffected grandfather was found to carry the same hemizygous variant in a complex I subunit gene as the index patient. Functional proteomics studies provided convincing evidence of the pathogenicity of the variant.

In another case a patient presented with a recognisable phenotype but unexpected inheritance pattern. Large scale proteome analysis strongly argued for a heterozygous variant to cause the clinical presentation of a disease considered to be recessive.

Conclusion: From a large body of data and long-standing experience in molecular genetic diagnostics we show strategies to approach cases unsolved in first line diagnostics. We derive general recommendations to address variation in reduced penetrance genes and following unexpected inheritance patterns.

References: 1. Stenton et al. J Clin Invest. 2021 https://doi.org/10.1172/JCI147734.

Grants: mitoNET, GENOMIT.

Conflict of Interest: None declared.

P15 CYTOGENETICS

P15.001.B International System for Human Cytogenomic nomenclature (ISCN) – the challenge of the nomenclature

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Background/Objectives: ISCN is an essential tool for reporting cytogenomic and some molecular analysis results (e.g. QF-PCR, MLPA) in a consistent and accurate manner to enable effective communication between clinicians and scientists and use of databases. Educational webinars plus a pilot online ISCN external quality assessment (EQA) highlighting some key ISCN principles were delivered to support laboratories reporting results, following publication of ISCN 2020(1).

Methods: Ideograms and/or images plus written descriptions of abnormalities were provided online. Participants were required to input the correct ISCN for the result described. Each case focussed on different aspects of ISCN: Constitutional karyotyping; Constitutional FISH; Neoplasia karyotyping; Oncology FISH; Microarrays; Region specific assays (rsa).

Results: There were numerous errors, with some particularly challenging case scenarios. Sixty-six participants answered at least one case. Recurrent errors included:.

-Using 'mat' instead of 'dmat' for a maternally derived unbalanced segregant;.

-Including the normal control probe and/or cell numbers in non-mosaic constitutional FISH;.

-Incorrect order for structural chromosome abnormalities;.

-Inappropriate omission of breakpoints for metaphase FISH;.

-Inconsistent use of terminology/punctuation.

Conclusion: This EQA highlighted the challenges of using ISCN. More centres gave the correct ISCN for rsa than the other cases. It was apparent from the submissions that FISH nomenclature was the most demanding and that the differences between constitutional and neoplastic ISCN were not fully understood.

This pilot demonstrated the continued need for training webinars and additional ISCN EQAs to educate and promote standardisation.

References: 1. McGowan-Jordan J., et al., (2020). ISCN 2020: An International System for Human Cytogenomic Nomenclature. S. Karger, Basel.

Grants:.

Conflict of Interest: None declared.

P15.003.D Comparative benchmarking of optical genome mapping and chromosomal microarray reveals high technological concordance in CNV identification and structural variant refinement

Dana Jaber[1], *Hayk Barseghyan[1], Andy Wing Chun Pang[1;2;3], Alka Chaubey[1], Yannick Delpu[1], Alex Hastie[1]*

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Background/Objectives: Children with developmental delays and/or congenital anomalies with suspected genetic etiology are often tested for SVs by chromosomal microarray (CMA), karyotype, fragile X testing, and sequencing [1]. Because CNV and SV are often found to be causative, CMA is recommended as first tier testing for many indications [2]. Optical genome mapping (OGM) is a technology that can detect not only CNVs, but also balanced rearrangements compared with CMA [3-5]. We evaluated the performance of OGM for reported genomic variants in comparison with CMA for 61 chromosomal abnormalities from 55 patients with variable phenotypes.

Methods: OGM was performed on samples from 55 patients with CNVs previously identified by CMA. SVs identified by OGM were filtered by a control database to remove polymorphic variants and a gene list to prioritize biologically relevant findings before comparison against CMA and FISH.

Results: OGM successfully identified CNVs, SVs, and absence of heterozygosity (AOH) in samples previously tested with CMA. OGM results showed high concordance with CMA while also characterizing the structure that CMA was unable to define without FISH.

Conclusion: OGM has sufficient analytical validity to detect biologically relevant variants identified by CMA. OGM can also identify rearrangements observed with FISH within the same single assay in a genome-wide scale that may provide additional biological utility compared to CMA.

References: 1. https://omim.org/.
2. Miller, Am. J. Hum. Genet. 2010.
3. Cope, Mol. Genet. Genomic Med. 2021.
4. Mantere, Am. J. Hum. Genet. 2021.
5. Mostovoy, Genetics. 2021.

Grants:.

Conflict of Interest: Dana Jaber Bionano Genomics, Hayk Barseghyan Bionano Genomics, Andy Wing Chun Pang Bionano Genomics, Alka Chaubey Bionano Genomics, Yannick Delpu Bionano Genomics, Alex Hastie Bionano Genomics.

P15.004.A Structural and copy number variant detection, filtering, annotation, and classification by Optical Genome Mapping in research of constitutional disorders

*Dana Jaber[1], Ben Clifford[1], Hayk Barseghyan[1;2;3], Andy Wing Chun Pang[1], Alka Chaubey[1], **Yannick Delpu**[1], Alex Hastie[1]*

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Medicine and Health Sciences, George Washington University, Washington, United States.

Background/Objectives: Identifying a breadth of variants and presenting actionable findings is key to disease research, classification and stratification. In contrast to methods like karyotyping, chromosomal microarray (CMA), FISH or sequencing, optical genome mapping (OGM) is sufficiently versatile to identify all classes of SVs with high sensitivity and specificity. Variant calls are managed within the Bionano Access software, where filters are applied to prioritize classification on variants likeliest to have a biological impact.

Here, we demonstrate a comprehensive and lab-ready workflow for whole genome OGM data analysis, alignment and annotation using GRCh38, variant evaluation, and classification.

Methods: For constitutional disorders, OGM data is analyzed with the Solve 3.7 De Novo Assembly pipeline., with sample data aligned to GRCh38 to resolve variants and annotate against a common reference.

Results: We apply a workflow to filter based on size (≥1.5 kbp), absence in assay-matched controls database (≤1%), and overlap with gene. The resulting prioritized variant list presents ~20-30 variants on average, added to a curation list. With the variant classifier visualization, we evaluate each variant captured as aligned to GRCh38, showing properties such as size and putative impact to genes. Multiple analysts may independently classify and supervisor can reconcile records to adjudicate classifications, record large-scale genomic irregularities, download a report.

Conclusion: OGM is suited to identify variants which are biologically relevant. The analytical approach annotates thousands of genome-wide variants accurately, and the filtering workflow directs focus to the most promising. The subsequent classification and reporting tools provide utility for communicating significance.

References:.

Grants:.

Conflict of Interest: Dana Jaber Bionano Genomics, Ben Clifford Bionano Genomics, Hayk Barseghyan Bionano Genomics, Andy Wing Chun Pang Bionano Genomics, Alka Chaubey Bionano Genomics, Yannick Delpu Bionano Genomics, Alex Hastie Bionano Genomics.

P15.005.B Genetic determinants of mosaic loss of the X chromosome in peripheral leukocytes of 800K women from 7 biobanks

Aoxing Liu[1], *giulio genovese*[2], *yajie zhao*[3], *po-ru loh*[2], *Andrea Ganna*[1], *john perry*[3], *mitchell machiela*[4]

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Background/Objectives: Mosaic loss of the X chromosome (mLOX) is the most frequently occurring age-related mosaic chromosomal alteration detected in peripheral leukocyte DNA of females, with a prevalence of 3.9% in women younger than 50 and reaching 33.3% after age 70. However, little is known about the genetic causes of mLOX and its epidemiological consequences.

Methods: We performed a GWAS meta-analysis of mLOX in 800K women of European ancestry (52K with mLOX) from 7 Biobanks to characterize the germline genetic architecture of mLOX.

Results: We identified 8 autosomal germline susceptibility loci, including one locus in the MHC region and multiple loci located in genes associated with blood cell counts (*SP140L*, *SCML4*, *KRI1*), blood protein level (*ADAMTS5*), and chronic lymphocytic leukemia (*SP110*). Rare variant analyses in UK Biobank exome sequencing data identified additional rare variation in the *BUB1B* cancer predisposition gene associated with increased risk of mLOX ($P = 5.4x10^{-7}$). Allelic shift analyses were performed on X chromosome data to identify germline variants for which one allele is preferentially lost in mLOX cases with heterozygous genotypes. Multiple such variants were identified over a large region spanning the centromere ($P < 10^{-250}$), and at or near *PLS3*, *ITM2A*, *SAGE1*, *P2RY8*, *WAS* and *PS3*.

Conclusion: Leveraging genotype data from 800k females, we detected multiple germline variants associated with mLOX suggesting susceptibility to mLOX exhibits relationships with blood cell traits and cancer predisposition genes. Allelic shift analyses further demonstrate the strong *cis* selection of specific X variants providing novel insights into the genetic etiology of mLOX.

References:.

Grants:.

Conflict of Interest: Aoxing Liu full employment, university of helsinki, giulio genovese broad institute of harvard and mit, yajie zhao university of cambridge, po-ru loh broad Institute of mit and harvard, Andrea Ganna institute for molecular medicine finland, john perry university of cambridge, mitchell machiela national cancer institute.

P15.006.C Multi-omics analysis of DNA and RNA identifies disruption of MINK1 in a balanced translocation carrier with congenital cataract and epilepsy

Jesper Eisfeldt[1;2;3], ***Jakob Schuy***[1], *Eva-Lena Stattin*[4], *Anna Lindstrand*[1;2]

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Background/Objectives: Genomic translocations are structural variants which are copy number neutral. Chromosomal segments are relocated forming fusing chromosomes which may result in genes disruption and/or fusion transcripts. The translocation breakpoints are commonly located in repetitive or insufficiently mapped regions of the genome complicating the process of resolving the rearrangement. In such cases, long-range information is required to precisely pinpoint the breakpoints. *MINK1* (Misshappen-like kinase 1) has been linked to skeletal and neuronal impairments and has also been mentioned in phenotypes like cancer, platelet formation, Alzheimer and Rheumatoid arthritis.

Methods: The here presented case has been investigated with a cascade of methods including karyotyping, paired-end and linked-read as well as long-read genome sequencing.

Results: The presented case contains a balanced translocation t(17,19) of which one breakpoint is located in the centromere of chromosome 19. Only by the addition of long-read Oxford nanopore technology, the centromeric breakpoint on chromosome 19 could be detected. The translocation disrupts *MINK1* leading to reduced levels of transcripts which is observed in patient-derived neuroepithelial stem cells.

Conclusion: Due to the unique translocation and the proband's phenotype matching the *MINK1* linked literature, we suggest *MINK1* as novel gene causing autism, epilepsy, and osteoporosis.

References: -.

Grants: Swedish Research Council (2019-02078), Swedish Rare Diseases Research foundation (Sällsyntafonden) and the Swedish Brain Foundation (FO2020-0351).

Conflict of Interest: None declared.

P15.007.D First Genome-first identification of a chromosomal balanced rearrangement responsible for a recessive disease: a case of renal tubular dysgenesis

Nicolas Chatron[1;2], *Claire Goursaud*[3], *Charlotte Dubucs*[4;5], *Jacqueline Aziza*[5], *Stéphane Decramer*[6], *Blandine Hougas*[7], *Dominique Chauveau*[8], *Julien Thevenon*[9;10], *Damien Sanlaville*[1;2], *Laurence Michel-Calemard*[3]

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Background/Objectives: Sequence resolution of balanced structural variation is now possible at scale through genome sequencing. Forty percent of karyotype visible de novo balanced rearrangements could be considered pathogenic once reached genic resolution and showing either direct disruption or a position effect. For recessive diseases, simultaneous detection of structural and nucleotide variants on both alleles makes genome sequencing the most comprehensive approach. Herein, we report the case of a stillborn male presenting with renal tubular dysgenesis (RTD). A heterozygous paternal single nucleotide variant of unknown significance in *AGTR1* was identified through panel sequencing. Biallelic variants of *AGTR1* are responsible for an autosomal recessive form of frequently lethal RTD.

Methods: Genome sequencing was used as a first-tier cytogenetics test also able to identify a differential diagnosis.

Results: A maternally inherited balanced reciprocal translocation disrupting *AGTR1* was identified by whole genome sequencing and confirmed through standard karyotyping, FISH techniques and breakpoint PCR followed by Sanger sequencing.

Conclusion: This is the first report of a compound heterozygosity for a nucleotide variant and a balanced rearrangement first identified through genome sequencing. We anticipate that such observation will now become possible. We also discuss the dual genetic counselling on RTD and the risk of unbalanced rearrangement of this balanced translocation. This case highlights the "compound expertise" required to maximize the diagnostic yield of genomic testing.

References:.

Grants:.

Conflict of Interest: None declared.

P16 NEW TECHNOLOGIES AND APPROACHES

P16.002.B Cost-effective, collaborative approach to prioritise variants of unknown significance from whole genome sequencing data: unexplained end stage renal disease exemplar

Katie Kerr[1;2], *Caoimhe McKenna*[2], *Diya Thomas*[1], *Jill Kilner*[1], *Cheryl Flanagan*[2], *Shane McKee*[2], *Amy Jayne McKnight*[1]

[1]*Queen's University Belfast, Belfast, United Kingdom;* [2]*Belfast Health & Social Care Trust, Northern Ireland Genomic Medicine Centre, Belfast, United Kingdom.*

Background/Objectives: The Northern Ireland Genomic Medicine Centre (NIGMC) recruited 444 rare disease probands to the 100,000 Genomes Project for whole genome sequencing. Forty-one probands had renal phenotypes, including nine participants with unexplained end-stage renal disease (ESRD) who remained undiagnosed following initial analysis by the NIGMC. We performed individual 'deep-dives' to prioritise variants of unknown significance (VUS) for follow-up analyses.

Methods: The top five ranked variants from Exomiser were prioritised for investigation. A custom-designed 'deep-dive' template was used to extract relevant information from diverse sources including: OMIM morbidity, gnomAD allele counts, REVEL pathogenicity, and gnomAD Z / probability loss-of-function scores. Findings were reviewed collaboratively with clinical geneticists and academic research scientists from the NIGMC to identify plausible VUS. The kidney specific public database NephroSeq was utilised to further explore the biological function of genes with VUS.

Results: Of the nine probands with unexplained ESRD, six phenotypically plausible VUS were identified within five individuals. This included splice donor variants within pseudohypoaldosteronism associated WNK1, missense / frameshift variants within the renal specific gene BBS9 associated with Bardet-Biedl syndrome, and a splice region variant within LAMC1 which has prior associations with increased ESRD risk.

Conclusion: This study highlights the potentials of deeply reviewing VUS from WGS data for patients with unexplained ESRD, as well as the benefits of clinical and academic collaboration for capitalising on VUS which could not feasibly within routine clinical services. Further functional analysis of these VUS is underway, including long read sequencing and functional follow-up.

References:.

Grants:.

Conflict of Interest: None declared.

P16.003.C CRISPR/Cas9-induced gene conversion between ATAD3 paralogs

Shira Yanovsky-Dagan[1], *Ayala Frumkin*[1], *James Lupski*[2;3;4], *Tamar Harel*[1;5]

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Background/Objectives: Pseudogenes are abundant within the human genome, and have been long considered non-functional. However, increasing evidence suggests that gene-pseudogene interactions can occur at the DNA, RNA, or protein level. Pseudogenes and paralogs can mediate non-allelic homologous recombination (NAHR) or gene conversion events. The ATAD3 locus on chromosome 1p36.33 contains three paralogs situated in tandem, and is therefore prone to NAHR-mediated deletions and duplications associated with severe neurological phenotypes.

Methods: To study the ATAD3 locus further, we aimed to generate biallelic loss-of-function variants by CRISPR/Cas9 genome editing in HEK293T cells, using a guide specific to ATAD3A. Following limiting dilution and clonal expansion, wells were Sanger-sequenced to determine the edited sequence. Primers specific to ATAD3A, which do not amplify ATAD3B or ATAD3C, were used to amplify the target locus for sequencing.

Results: Following limiting dilution and clonal expansion of CRISPR/Cas9-edited cells, four wells showed a single population. Unexpectedly, two of these were found to have an in-frame 13bp sequence alteration at the cut-site that resulted from gene conversion, with replacement of the targeted sequence of ATAD3A by a donor sequence from its paralog, ATAD3B. Chromosomal microarray did not show larger deletions or duplications at the locus.

Conclusion: We highlight the complexity of CRISPR/Cas9 design and recombination repair mechanisms when targeting genes that have paralogs or pseudogenes. Additionally, we suggest that endogenous gene conversion may be used to repair missense variants in genes with paralogs or pseudogenes.

References: Harel, T., et al. (2016). Am J Hum Genet 99, 831-845.

Grants: ISF 1663/17 to TH.

Conflict of Interest: Shira Yanovsky-Dagan: None declared, Ayala Frumkin: None declared, James Lupski Stock ownership in 23andMe, J.R.L. is a paid consultant for Regeneron Genetics Center, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted at Baylor Genetics (BG) Laboratories. J.R.L serves on the Scientific Advisory Board of BG., Tamar Harel: None declared.

P16.004.D Application of droplet digital PCR to the molecular characterization of recurrent deletions of the NF1 locus

Laurence PACOT[1;2], *Dominique Vidaud*[1;2], *Lucie Orhant*[1], *Nicolas Vaucouleur*[1], *Theodora Maillard*[1], *Ingrid Laurendeau*[2], *Juliette Nectoux*[1], *Eric Pasmant*[1;2]

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Background/Objectives: Neurofibromatosis type 1 (NF1) is caused by loss-of-function variants in the NF1 gene, among which ~5-10% are deletion of the whole NF1 locus. Three recurrent types of deletions have been described this far. They are caused by non-allelic homologous recombination (NAHR) mediated by low-copy repeats (LCR) at the NF1 locus. Here, we describe a new approach using droplet digital PCR (ddPCR) to quickly and easily distinguish the recurrent deletions of the NF1 locus from atypical deletions.

Methods: A total of 121 index cases with an NF1 deletion were included. All patients were phenotypically described and 109 had an MLPA typing (1). Seven ddPCR probe sets distributed along the NF1 locus were selected to closely delimitate the three recurrent deletion types.

Results: Among the 121 deletions analyzed: 74 were type-1 (61%), 22 type-2 (18%), 5 type-3 (4%), and 20 showed an atypical profile (17%). Among the 109 patients who had an MLPA analysis, 106 had perfectly matched results with ddPCR. Two of the discordant results might be due to an atypical breakpoint located between the telomeric ddPCR probe and the closest MLPA probe, located 223kb telomerically. The third discordant result showed a type-2 profile in MLPA, but the results in ddPCR clearly indicate no deletion of the SUZ12 gene, a profile that was previously named "group #2A" (2).

Conclusion: We developed a quantitative, sensitive, and efficient ddPCR approach to characterize recurrent deletions of the NF1 locus.

References: (1) Pacot et al. Cancers 2021.
(2) Kehrer-Sawatzki & Cooper. Hum Genet 2021.

Grants:.

Conflict of Interest: None declared.

P16.005.A Next generation sequencing analysis in clinical practice of cardiomyopathies diagnostics

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Background/Objectives: The aim of our study was application of NGS technology for evaluation of MYBPC3 gene sequence variants included in the pathogenesis of cardiomyopathies. We point out to advantages of NGS technologies and utilization of the modern methods of molecular diagnostics in clinical practice.

Methods: The next-generation sequencing technology by IlluminaHiSeq 2500 platform was used to detect sequence variants in 37 Slovak patients with diagnosed cardiomyopathy by whole exome sequencing. Detected sequence variants were evaluated using Polyphen-2, SIFT and MutationTaster algorithms.

Results: We identified 47 sequence variants of 27 genes. Our analyses were focused to evaluation of sequence variants of MYBPC3 gene. NGS analyses identified the novel MYBPC3 gene sequence variants: rs35078470, rs34580776, rs138753870 with minor allele frequency (MAF)<0.01.

Conclusion: The results of the study support the potential role of detected variants in Slovak patients with cardiomyopathy. These sequence variants may be susceptibility alleles determining clinical phenotype. The effect of this variant in pathogenesis of cardiomyopathies requires functional studies.

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TANJORE, R. R., et al., 2008. MYBPC3 gene variations in hypertrophic cardiomyopathy patients in India. Vol. 24, No. 2, p. 127-130. In: Canadian Journal of Cardiology. ISSN: 0828-282X.

Grants: KEGA 032PU-4/2021: "Innovation of the educational process - implementation of new knowledges of Virology and NGS technology in teaching of biological subjects".

Conflict of Interest: Iveta Boronova University of Prešov, Grant KEGA 032PU-4/2021, projec leader, Jarmila Bernasovská University of Prešov, Grant KEGA 032PU-4/2021, co-investigator of the project., Eva Petrejčíková University of Prešov, KEGA 032PU-4/2021, representative of the project manager, Michaela Zigová University of Prešov, KEGA 032PU-4/2021, co-investigator of the project.

P16.006.B Approaches for enhancing of HDR-based genome editing efficacy

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Background/Objectives: There are many types of genome editing enhancing approaches. They include cell cycle synchronization, activation of homology directed repair (HDR) and inhibition of non-homologous end joining (NHEJ), fusion of Cas9 with DNA repair participants, etc. In current study we decided to test

different methods for HDR enhancing: HDR activation by SCAI protein, NHEJ inhibition through MAD2L2 knockdown, NUDT16L1 knockdown/overexpression and SCR7 treatment.

Methods: Experiments were performed in HEK293T cells with stably integrated eGFP gene with mutation c.337delG (eGFPmut). Genome editing by CRISPR-Cas9 (eSpCas9(1.1)) in combination with ssODN should recover wt eGFP by insertion G nucleotide. SCAI was used as Cas9-SCAI fusion protein, knockdown of MAD2L2 and NUDT16L1 was performed by siRNAs transfected 24h before CRISPR-Cas9 plasmid. NUDT16L1 overexpression was achieved by adding additional plasmid coding this gene 24h before CRISPR-Cas9 plasmid. SCR7 were added with CRISPR-Cas9 plasmid. HDR was analyzed by counting GFP+ cells by flow cytometry and through TIDER analysis.

Results: MAD2L2 knockdown increased corrected alleles percentage 3,7 times, NUDT16L1 knockdown - 1,8 times, SCR7 treatment - 1,7 times. GFP+ cells percentage increase was 10.2-fold and 6-fold for MAD2L2 and NUDT16L1 knockdown, respectively, and SCR7 treatment didn't induce any differences in eGFP fluorescence. NUDT16L1 overexpression and Cas9-SCAI fusion did not affect percentage of corrected alleles, however, decreased the number of GFP-positive cells 2.5 times both.

Conclusion: Thus, we discovered that MAD2L2 and NUDT16L1 knockdown, and also SCR7 treatment enhance the efficiency of CRISPR-induced homology directed repair. These methods could possibly be combined in further experiments.

References:.

Grants:.

Conflict of Interest: None declared.

P16.007.C Solving a de-novo microdeletion PGT-M case by combining long and short range PCR with NGS

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Background/Objectives: A 34yo endometrial cancer patient was referred for PGT-M of a de-novo microdeletion in MSH2. It was not possible to diagnose the deletion and to construct a haplotype with standard molecular PGT techniques (informative markers surrounding the deletion), as the microdeletion breakpoints were unknown.

Methods: Precise deletion breakpoints were mapped by transposase-based library prep and next generation sequencing (NGS) of a long PCR amplicon derived from the patient's genomic DNA spanning exons 8-11 of MSH2. Forty single nucleotide polymorphic (SNPs) sites within the deletion were genotyped in the patient and spouse by high throughput short amplicon sequencing. Haplotype informative SNP sites were used for PGT-M of blastomere biopsies from the patient's previously frozen embryos.

Results: Long amplicon analysis revealed an 18kb sized heterozygous deletion in the patient with precise breakpoints in introns 8 and 10 of MSH2. Forty highly polymorphic SNPs within the deletion were then concurrently genotyped in both the patient and her spouse by targeted short amplicon sequencing resulting in the detection of 6 informative SNPs (i.e., hemizygous in patient vs homozygous opposite allele in spouse). Using these SNPs, together with informative deletion-flanking microsatellites, haplotype phasing of the de novo deletion was attained. PGT-M was performed and diagnosis was achieved all embryos.

Conclusion: We describe state-of-the-art NGS technologies in the detection of precise deletion breakpoints and in genotyping long segments of genomic DNA where traditional methodology made impossible to perform PGT-M. Accordingly, we highly recommend this approach as a general solution for de-novo PGT-M of microdeletions.

References:.

Grants:.

Conflict of Interest: None declared.

P16.008.D Assessing the digenic model in rare disorders using population whole-genome sequencing data

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Background/Objectives: An important fraction of patients with rare disorders remains with no clear genetic diagnostic, even after whole-exome or whole-genome sequencing. This poses a difficulty in giving adequate treatment and genetic counseling. The analysis of genomic data in rare disorders mostly considers the presence of single gene variants in coding regions that follow a concrete monogenic mode of inheritance. A digenic inheritance, with variants in two functionally-related genes in the same individual, is a plausible alternative that might explain the genetic basis of the disease in some cases. If this is the case, digenic disease combinations should be absent or underrepresented in healthy individuals.

Methods: In this work, we develop a method to evaluate the significance of digenic combinations by interrogating whole-genome data from the Genomics England 100,000 Genomes Project cohort.

Results: We apply the method to previously reported digenic combinations successfully validating one that has been suggested to cause a rare immune disorder.

Conclusion: Beyond the validation of possible pathogenic associations, we also suggest that this approach will be relevant with the advent of new sequencing efforts in projects including hundreds of thousands of samples.

References: This research was made possible through access to the data and findings generated by the 100,000 Genomes Project; http://www.genomicsengland.co.uk..

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Conflict of Interest: None declared.

P16.010.B Proximity extension assay in combination with Next-Generation Sequencing for high-throughput proteome-wide analysis in large population health studies

Cynthia Lawley[1], *Lotta Wik*[2], *Niklas Nordberg*[2], *John Broberg*[3], *Johan Björkesten*[4], *Erika Assarsson*[4], *Sara Henriksson*[2], *Ida Grundberg*[5], *Christina Westerberg*[2], *Elin Liljeroth*[2], *Adam Falck*[2], *Martin Lundberg*[4], *Sarantis Chlamydas*[5], *Tala Khosroheidari*[6], *Yan Chen*[7], *Anders Malarstig*[7]

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Background/Objectives: Understanding the dynamics of the human proteome is crucial for identifying biomarkers to be used as measurable indicators for disease severity and progression, patient stratification, and drug development. The Proximity Extension Assay (PEA) is a technology that translates protein information into actionable insights across large samples sizes in both healthy and disease samples. The high-throughput nature of the assay is enabled by linking protein-specific antibodies to DNA-encoded tags that can be read out on a next generation sequencer.

Methods: We have combined the PEA technology described above with automated sample preparation and a high-throughput sequencing readout for parallel measurement of ~3,000 proteins for up to 384 samples at a time, generating over 1 million data points per run.

Results: Characterizing the proteome alongside genetic and clinical data enables a pQTL framework to not only validate known clinical targets and identify new clinical targets but to also suggest repurposing opportunities of clinical candidates for new indications.

Conclusion: Here we will summarize results where proteomics is impacting large population health studies (e.g., UK Biobank, SCALLOP) to advance precision and personalized medicine.

References: Folkersen et al. 2020. Genomic and drug target evaluation of 90 cardiovascular proteins in 30,931 individuals. Nature Metabolism, 2, 1135-1148. https://www.nature.com/articles/s42255-020-00287-2.

Wik et al 2021. Proximity extension assay in combination with next-generation sequencing for high-throughput proteome-wide analysis. Molecular and Cellular Proteomics. Volume 20, 100168, January 01, 2021. https://doi.org/10.1016/j.mcpro.2021.100168.

Grants:.

Conflict of Interest: Cynthia Lawley Olink, Illumina, Lotta Wik Olink, Niklas Nordberg Olink, John Broberg Olink, Johan Björkesten Olink, Erika Assarsson Olink, Sara Henriksson Olink, Ida Grundberg Olink, Christina Westerberg Olink, Elin Liljeroth Olink, Adam Falck Olink, Martin Lundberg Olink, Sarantis Chlamydas Olink, Tala Khosroheidari Olink, Illumina, Yan Chen: None declared, Anders Malarstig Pfizer.

P16.011.C Blood RNA-seq in diagnostic genomic medicine

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Background/Objectives: RNA sequencing (RNA-seq) can identify changes in splicing not readily apparent through DNA analysis, allowing better functional interpretation of genetic variants of uncertain significance (VUSs). Blood is the most readily available clinical tissue for RNA analysis. However, the degree to which blood RNA-seq provides adequate coverage for analysing splicing in different genes has not been well studied. Here, we have investigated the sensitivity of RNA-seq for the detection of splicing and other abnormalities.

Methods: RNA was extracted from blood in 56 patients with likely genetic disorders, 33 with candidate VUSs. RNA-seq was performed in three batches at 70M 150-bp paired-end reads per sample with STAR alignment to GRCh38. Transcripts per million (TPM) values were calculated for all genes using stranded raw read counts. Splice junction and intronic reads were counted, filtered and annotated for skewed usage. UK Genomic Medicine Service (GMS) PanelApp gene panels were used to filter outputs.

Results: 72% of GMS genes had TPM>1 and 65% of observed annotated junctions had sufficient average coverage to detect alternative splicing occurring at a 0.5 usage level (55% at 0.25 usage, 42% at 0.1 usage). Genes with identified splicing abnormalities were not consistently down-regulated when compared across samples within the same RNA-seq batch. The RNA effects of previously identified chromosomal microdeletions were generally visible as contiguous regions of decreased gene expression. Skewed X-inactivation was identified in one case.

Conclusion: Blood RNA-seq can detect not only splicing abnormalities but also chromosomal microdeletions and skewed X-inactivation.

References:.

Grants: NIHR Research Professorship awarded to Diana Baralle (RP-2016-07-011).

Conflict of Interest: None declared.

P16.013.A Medical costs of children admitted to the neonatal intensive care unit: the role and possible economic impact of rapid exome sequencing in early diagnosis

Richelle Olde Keizer[1;2], *A. Marouane*[3], *A. Chantal Deden*[3], *Wendy Van Zelst-Stams*[3], *Willem de Boode*[4], *Willem Keusters*[1], *Lidewij Henneman*[5], *Johannes Kristian Ploos van Amstel*[6], *Gerardus Frederix*[1;6], *Lisenka Vissers**[2]

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Background/Objectives: Genetic disorders are likely underdiagnosed in neonates admitted to the neonatal intensive care unit (NICU) as testing is not always performed, not even for all neonates presenting with congenital anomalies (CA), despite their strong correlation with genetic defects. We modelled the cost impact of implementing of trio-based rapid exome sequencing (trio-rES) for neonates admitted at the NICU.

Methods: To determine baseline healthcare costs, we retrospectively collected postnatal healthcare data of neonates admitted to level IV NICU at Radboudumc (October 2013-October 2015), and linked these to unit costs. Next, scenarios were based on replacing current genetic tests by trio-rES, combined with clinical preselection of neonates based on the presence/absence of (multiple) CAs.

Results: Overall, on average €26,627 was spent per patient, of which 2.3% involved genetic testing. We next modelled four scenarios. First, implementing trio-rES for all neonates without clinical preselection will increase overall healthcare costs by 22.2%. Contrastingly, implementation of trio-rES for neonates with multiple CAs, but leaving neonates with an isolated CA untested, will be cost-neutral. We next modelled trio-rES for all patients with

CAs, increasing the average per patient healthcare costs by 5.3%, with a maximum of 5.5% when correcting for genetic diagnoses that are undetectable by rES.

Conclusion: Implementation of trio-based rES for all neonates with CAs will lead to a limited increase in overall healthcare budget, but maximizes the potential for diagnosing neonates with rare genetic disorders and personalized treatment options.

References: Not applicable.

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Conflict of Interest: Richelle Olde Keizer: None declared, A. Marouane: None declared, A. Chantal Deden: None declared, Wendy Van Zelst-Stams: None declared, Willem de Boode: None declared, Willem Keusters: None declared, Lidewij Henneman: None declared, Johannes Kristian Ploos van Amstel: None declared, Gerardus Frederix: None declared, Lisenka Vissers* ZonMw; No. 843002608 and No. 846002003. European Union's Horizon 2020; No. 779257.

P16.014.B Proteoform Detection in Deep Plasma Proteomics through Peptide Expression Correlation and Genomic Mapping

*Jian Huang[1], **Yingxiang Huang**[1], Margaret Donovan[1], Daniel Hornburg[1], Marwin Ko[1], Ryan Benz[1], Theo Platt[1], Asim Siddiqui[1], Serafim Batzoglou[1], Omid Farokhzad[1]*

[1]Seer Inc., Redwood City, United States.

Background/Objectives: Biological complexity exceeds what can be explained by the number of protein-coding genes, however, can be largely explained by alterative splicing and post-translational modifications of proteins yielding multiple molecular forms of a protein (proteoforms). The ability to perform quantitative proteomic experiments across a large cohort can improve proteoform inference by using peptide quantitative profiles. In this work, we demonstrate the power of the Proteograph™ Product Suite, which leverages physiochemically distinct nanoparticles for unbiased, deep, and rapid proteomics at scale to systematically infer proteoforms from plasma samples using peptide abundances and genomic mapping.

Methods: Proteomes of 141 non-small cell lung cancer and control plasma samples were profiled using Proteograph and LC-MS/MS. For all detected peptides within a given protein group, we calculated the Pearson pairwise correlation of abundances. To identify clusters of similarly abundant peptides, we then applied the silhouette method to obtain the optimal number of clusters and used K-means clustering on the correlation of peptide abundances, followed by filtering to ensure clusters were distinct. Next, peptides were mapped to known protein isoforms from the Ensembl database. We then inferred the presence of a proteoform if the known protein isoform explained the peptide clusters.

Results: Some identified proteoforms included BMP1, which has dual role in cancer possibly explained by short and long proteoforms, and Collagen XVIII, which has a short proteoform byproduct with anti-angiogenic effects.

Conclusion: Overall, this work demonstrates Proteograph utility to generate unbiased and deep plasma proteome profiles enabling the inference of biologically important proteoforms.

References: Blume et al. Nat. Comm. (2020).

Grants:.

Conflict of Interest: Jian Huang Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Yingxiang Huang Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Margaret Donovan Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Daniel Hornburg Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Marwin Ko Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Ryan Benz Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Theo Platt Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Asim Siddiqui Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Serafim Batzoglou Significant. Employee of Seer Inc., Modest. Own stocks at Seer Inc., Omid Farokhzad Significant. founder of Seer Inc., Modest. Own stocks at Seer Inc.

P16.015.C Genome-wide CpG methylation calling with standard HiFi whole genome sequencing

Justin Blethrow[1], *Daniel Portik[1], Kristofor Nyquist[1], Aaron Wenger[1], Richard Hall[1]*

[1]Pacific Biosciences, Menlo Park, United States.

Background/Objectives: Both the genome and epigenome contribute to inherited disease. While genome sequencing has been applied at large scale, epigenome sequencing remains more difficult and expensive and less frequently used. Here we extend PacBio HiFi sequencing to simultaneously generate accurate genomes and epigenomes with a single library prep and sequencing experiment.

Methods: In PacBio sequencing, the nucleotide incorporation rate is sensitive to epigenetic modifications like 5-methylcytosine, but the signal is spread over multiple positions and is challenging to detect in single sequencing passes. HiFi sequencing observes the same molecule across multiple serial passes, opening new approaches to detect 5mC. We implemented a multilayer convolution neural network to combine kinetics from multiple passes and assign a probability of methylation to each CpG. We trained the model on fully unmethylated (whole-genome amplification) and fully methylated (M.SssI-treated) reads.

Results: HiFi methylation calling accuracy for individual CpG sites in single reads (i.e. 1X coverage) is around 85%. At 30X coverage for Genome in a Bottle samples, HiFi CpG methylation correlates >95% with bisulfite sequencing, or >99% at the level of CpG islands. HiFi methylation calling also recapitulates biologically-relevant hypomethylation in the undifferentiated haploid CHM13 cell line, including unique patterns across chromosomes. In diploid samples – including rare disease samples – HiFi reads can be phased by sequence to reveal parental imprinting for genes like *GNAS* and *DIRAS3*.

Conclusion: HiFi sequencing provides an approach to generate more accurate genomes than short-read sequencing while simultaneously providing the epigenome, offering the opportunity to understand new aspects of evolution, disease, and diversity.

References:.

Grants:.

Conflict of Interest: Justin Blethrow Employed by Pacific Biosciences, Daniel Portik Employed by Pacific Biosciences, Kristofor Nyquist Employed by Pacific Biosciences, Aaron Wenger Employed by Pacific Biosciences, Richard Hall Employed by Pacific Biosciences.

P16.016.D By enabling same day synthesis of hydrolysis probes on the SYNTAX System, Enzymatic DNA Synthesis (EDS) promises to unleash the development of multiplexed qPCR assays

Benoit Derrien[1], *Nadège Tardieu[2], Henri Lachaize[2], Anaïs Vallette[2], Erwan Grolleau[2], Charles Rouillard[2], Gabriel De Crozals[3], Christine Peponnet[2], Florence Mahé[1;4], Xavier Godron[4]*

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Surface Chemistry, Le Kremlin-Bicêtre, France; [4]DNA Script, Le Kremlin-Bicêtre, France.

Background/Objectives: Quantitative PCR (qPCR) was first developed in the early 90's and has remained one of the gold standard techniques used in modern day gene expression studies. By implementing the synthesis of hydrolysis probes on the SYNTAX System, DNA Script opens access to the rapid on demand synthesis of good quality qPCR probes.

Methods: Among the various qPCR approaches developed, the most popular techniques utilize intercalating dyes and hydrolysis probes. Compared to intercalating dyes, hydrolysis probes provide several advantages including high specificity and the possibility of multiplexing, thus enabling the detection of multiple target genes within a single reaction.

Results: In this study, we demonstrate the performance of hydrolysis probes generated using DNA Script's proprietary enzymatic DNA synthesis (EDS) technology. By combining EDS and labelling chemistry within the SYNTAX System, we enable the fully automated synthesis of 32 hydrolysis probes and their associated primer pairs in less than 12 hours directly in the lab. The labelling reaction enables the user to position the labels inferentially at the 5′, 3′ or internally while blocking the 3′end of the probe. Our qPCR probes are available in 5 different colours and were tested within a multiplexed experimental design using two different gene expression models: 1) the validation of a shRNA cell line, 2) the analysis of the relative expression of two genes in breast cancer tumours.

Conclusion: By enabling same day synthesis of hydrolysis probes, the SYNTAX System alleviates one of the bottlenecks associated with hydrolysis probes, providing next-day results.

References:.

Grants:.

Conflict of Interest: None declared.

P16.017.A RNAseq from dried blood spots cards improves diagnosis in rare genetic diseases

Ruslan Al-Ali[1], *Mandy Radefeldt*[1], *Najim Ameziane*[1], *Sabrina Lemke*[1], *Christian Beetz*[1], *Peter Bauer*[1]

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Background/Objectives: A considerable fraction of genetic diagnostic analyses remains negative or ambiguous even when comprehensive approaches such es exome sequencing or genome sequencing are applied. This is largely due to non-coding causative variants escaping attention (false-negative reports) or misjudgement of in silico prediction tools (uncertain reports). Transcriptome analysis helps to solve these issues but faces challenges related to RNA stability in pre-analytical samples and logistics of those precarious samples. We established RNA-seq protocol that utilizes dried blood spots on easy-to-ship filter cards.

Methods: Several types of filter cards were probed with EDTA blood and stored under distinct conditions modelling typical shipment conditions for differing durations. Subsequently, compared protocols differed regarding RNA extraction, depletion of unwanted RNA species, library preparation and next-generation sequencing.

Results: A tentatively optimized protocol applies a proprietary filter card (CentoCard®), uses four blood spots covering a total area of ~3 cm^2, and involves a hemoglobin RNA depletion and polyA-tail capture. Up to 10 days from sampling preparation, we obtain >100 ng RNA with RIN score of 3-6, and could generate 5-12 million uniquely mapped RNA-seq reads on average, respectively. The sequence coverage for genes typically expressed in blood was sufficient to detect the pathological mutations and thereby confirm rare genetic disorders in our test cohort.

Conclusion: Further optimization will enable us to offer an innovative genetic diagnostic service that combines DNA-based and RNA-based data from the very same specimen, i.e. dried blood spot filtercard for achieving higher diagnostic yield and more definite genetic diagnoses.

References:.

Grants:.

Conflict of Interest: Ruslan Al-Ali Employed by Centogene, Mandy Radefeldt Employed by Centogene, Najim Ameziane Previously employed by Centogene, Sabrina Lemke Employed by Centogene, Christian Beetz Employed by Centogene, Peter Bauer Employed by Centogene.

P16.018.B Integrated heteroduplex correction in PacBio's circular consensus algorithm

Derek Barnett[1], *John Harting*[1], *Walter Lee*[1], *Armin Töpfer*[1], *Fritz Sedlazeck*[2], *Jenny Ekholm*[1], *Nina Gonzaludo*[1], *Justin Blethrow*[1], *James Drake*[1], ***Zev Kronenberg***[1]

[1]*Pacific Biosciences (PacBio), Menlo Park, United States;* [2]*Baylor College of Medicine, Human Genome Sequencing Center, Houston, United States.*

Background/Objectives: A heteroduplex is a double-stranded sequence comprised of two non-complementary strands that can form during PCR. These mixed-template artifacts produce misleading results in downstream analysis, e.g., false haplotypes during diplotyping. Unlike short-read technologies, PacBio Single-Molecule Real-Time sequencing produces strand-level base calls. Heteroduplex signatures can be directly observed and corrected using the stranded sub-read data. Our new method is integrated in the circular consensus sequence algorithm which generates accurate HiFi data from sub-reads.

Methods: The transformation of PacBio subreads into high accuracy HiFi reads is done by the circular consensus sequence (CCS) algorithm. During CCS, an intermediate draft sequence is generated, and subreads are mapped and aligned to the draft. The heteroduplex algorithm (hd-finder) takes the subread alignments and generates a read pileup whereby variants are identified. At each site, the bases are sorted and counted by strand. The 2x2 count data is subjected to a Fisher's exact test. The fraction of significant sites across the draft is used to determine if a read contains heteroduplex. Heteroduplex flagged reads are split by strand and reprocessed resulting in two HiFi reads, one for each strand.

Results: We demonstrate the accuracy of the hd-finder algorithm is >94% by using a heteroduplex enriched amplicon library. We also show that applying the hd-finder to amplified datasets improves the quality of downstream analysis of important human genes.

Conclusion: The heteroduplex algorithm is a powerful new method for improving HiFi amplicon targets. The method has been released (v6.3.) and is documented https://ccs.how/faq/mode-heteroduplex-filtering.html.

References:.

Grants:.

Conflict of Interest: Derek Barnett Pacific Biosciences, Pacific Biosciences, John Harting Pacific Biosciences, Pacific Biosciences, Walter Lee Pacific Biosciences, Pacific Biosciences, Armin Töpfer Pacific Biosciences, Pacific Biosciences, Fritz Sedlazeck Research funding from Pacific Biosciences and ONT, Jenny Ekholm Pacific Biosciences, Pacific Biosciences, Nina Gonzaludo Pacific Biosciences, Pacific Biosciences, Justin Blethrow Pacific Biosciences, Pacific Biosciences, James Drake Pacific Biosciences, Pacific

Biosciences, Zev Kronenberg Pacific Biosciences, Pacific Biosciences, Phase Genomics.

P16.019.C Characterizing genetic variants that affect splicing using CRISPR activation in easily accessible patient cells

Thorkild Terkelsen[1;2], *Nanna S Mikkelsen*[2], *Iben B Bentsen*[1;2], *Jenny Blechingberg*[1], *Henning Andersen*[3], *Rasmus O Bak*[2], *Uffe B Jensen*[1;2]

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Background/Objectives: Analysis of cDNA is the primary method to study the impact of rare genomic variants on mRNA splicing. Many disease genes are not expressed in easily accessible patient cells such as skin fibroblasts. We hypothesized that CRISPR activation (CRISPRa) would allow splicing analysis of the myelin protein zero gene (*MPZ*), where the full-length transcript is exclusively expressed in Schwann cells of the peripheral nervous system.

Methods: CRISPRa induces gene expression directed by guide RNAs and a catalytically inactivated Cas9 protein fused to transcriptional activators (e.g., dCas9-VPR). Thus, to activate full-length *MPZ* expression in skin fibroblasts, cells were electroporated with dCas9-VPR mRNA and synthetic guide RNAs that targeted the first transcriptional start site of *MPZ*. Normal fibroblasts and fibroblasts from a patient with a disruptive consensus donor splice site variant in intron 2 of *MPZ* were analyzed by RT-qPCR and RNA-Seq. Cells were cycloheximide treated before RNA extraction to protect the transcripts from nonsense-mediated decay.

Results: CRISPRa strongly upregulated *MPZ*, including its full-length isoforms, during the initial days after treatment, followed by a normalization of gene expression. In patient fibroblasts with the disruptive splice donor site variant, we detected multiple novel transcript isoforms that bypassed the splice site. The abnormal transcripts were only detectable in patient fibroblasts after CRISPRa and absent from normal fibroblasts induced to express *MPZ*.

Conclusion: CRISPRa expands the application of cDNA analysis to disease genes not expressed in easily accessible patient cells.

References:.

Grants: DFF grant number 9039-00337B.

Conflict of Interest: None declared.

P16.020.D Optical Genome Mapping in Routine Human Genetic Diagnostics - Lessons Learned

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Background/Objectives: Optical Genome Mapping (OGM) is an emerging method that promises to detect most classes of structural variants (SVs) of a nuclear genome in a single test. It holds unique potential where other current methods struggle, e.g. fine-mapping of translocations or covering repetitive regions. As OGM-systems are still in their infancy, their meaningful usage needs to be explored. Here, we present our experience with the Saphyr OGM-instrument (Bionano Genomics, CA, USA) in a human genetic setting.

Methods: We analyzed a panel of >100 diagnostic cases with the Saphyr OGM-instrument and other genetic methods (e.g. chromosomal analysis, microarray, long-range sequencing) with emphasis on chromosomal numerical aberrations, balanced and unbalanced SVs and mosaics of potential clinical significance.

Results: Comparison of the Saphyr-system to the other methods showed a high concordance of most variants. OGM characterized all SVs with a resolution of several kilobases and could localize rearranged genetic material in most instances. However, we also encountered several variants of potential clinical relevance that were systematically missed by the OGM-system, whereas they were detected by karyotyping or microarray. Among the reasons were insufficient coverage, incomplete calling and assembly artefacts.

Conclusion: While showcasing OGM's unique advantages as physical DNA imaging platform in clinical samples, we also provide documentation of typical pitfall scenarios. This is indispensable for a prior understanding to infer certain variants with detection biases. The results raise awareness for variation signatures that are challenging for OGM data analysis, relying on advanced interpretation beyond automated calling.

References:.

Grants:.

Conflict of Interest: None declared.

P16.021.A Performance equivalence of the newest generation SeqStudio Flex Capillary Electrophoresis system with established CE platforms

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Background/Objectives: Fluorescent capillary electrophoresis (CE) is a flexible genomic analysis method that separates fluorescently labeled DNA fragments based on size. It is the foundation of Sanger sequencing and DNA fragment analysis. The simple workflow, single-base resolution, rapid analysis time, small sample volume, and flexibility have resulted in widespread adoption for a variety of applications used in basic, translational, and clinical research. Applied Biosystems™ is the leader and continues to provide innovative solutions for CE analyses. We leveraged our experience in the field and incorporated many improvements and innovations into our newest addition to our CE instrument portfolio, the SeqStudio Flex.

Methods: In this poster, we demonstrate a broad spectrum of genetic analysis applications and workflows that can be run on this new instrument.

Results: These applications include cell line authentication (CLA) and human sample matching, microsatellite instability (MSI) analysis, multiplexed PCR analysis, genome editing efficiency analysis, double-stranded DNA and NGS library QC, rare allele confirmation, and Sanger sequencing plasmids and viral genomes. In all cases, we demonstrate that the data quality was equivalent to data generated on our existing, gold-standard Applied Biosystems™ genetic analyzers.

Conclusion: These results will give investigators of human genetic variation and function the confidence to transition their research to the new platform while taking full advantage of the newest innovations.

References: N/A.

Grants: N/A.

Conflict of Interest: Stephen Jackson Thermo Fisher Scientific, Archana Gupta Thermo Fisher Scientific, Edgar Schreiber Thermo Fisher Scientific.

P16.022.B Efficient, high sensitivity detection of oncogenic variants with UMIs and target enrichment

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[1]*Twist Bioscience, South San Francisco, United States.*

Background/Objectives: Early detection can significantly improve clinical outcomes for a number of cancers, but many of the best current screening methods require invasive procedures. A promising alternative approach is a liquid biopsy of cell-free DNA (cfDNA) from plasma. Because tumors generally shed relatively large amounts of DNA into the circulation, cancer can potentially be detected by identifying oncogenic variants in cfDNA. This process generally requires extremely deep sequencing, and in many cases is limited by the accuracy of next-generation sequencing (NGS).

Methods: One approach to overcoming this limitation is unique molecular identifiers (UMIs), short sequences that uniquely tag each input DNA molecule prior to preparing NGS libraries. The approach can further be improved by tagging each original strand of the DNA molecule, in a technique termed duplex sequencing, which can correct early PCR errors and/or single-strand DNA damage events. Here we describe a new library preparation system incorporating short, discrete UMI sequences to maximize sequence distances for error correction.

Results: We show that this system can determine the conversion efficiency of NGS libraries. Using the Twist cfDNA Pan-cancer Reference Standards to simulate a low fraction of tumor DNA in a healthy background, we demonstrate high sensitivity towards a variety of oncogenic substitutions, indels and structural variants. We demonstrate the baseline error rate using unmodified human cfDNA, and use the system to determine the mutation frequency in a synthetic biology application.

Conclusion: In summary, this study demonstrates the utility of UMIs for a variety of applications in NGS.

References:.

Grants:.

Conflict of Interest: Michael Bocek Currently employed with Twist Bioscience, Lydia Bonar Currently employed with Twist Bioscience, Jean Challacombe Currently employed with Twist Bioscience, Richard Gantt Former employee of Twist Bioscience, Derek Murphy Currently employed with Twist Bioscience, Esteban Toro Currently employed with Twist Bioscience.

P16.023.C Targeted nanopore sequencing ushers in the era of long-read sequencing in the clinic

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Background/Objectives: Nanopore sequencing offers attractive prospects (e.g., methylation analysis and production of long reads, allowing variant phasing and reliable structural variant (SV) detection, including gene fusions), but its integration in clinical laboratory workflows has been hampered by its low throughput. Adaptive sampling (AS)(1), a novel modular target enrichment method made possible by recent technological advances, can offset this limitation. The goal of this study was to evaluate the use of AS in a diagnostic laboratory setting.

Methods: We analysed 24 tumour samples and 2 germline samples with AS, targeting 570 cancer genes. The analytical performance of SV detection on AS data was compared to nanopore whole genome sequencing (nWGS) and Pacific Biosciences HiFi whole genome sequencing (pbWGS) on two samples.

Results: On-target median read depth was 12X (min = 6X, max = 30X) on a single flow cell, a four-fold improvement over baseline. Off-target reads were utilized to generate genome-wide copy number variant (CNV) profiles. Base-pair level characterization of causal SV was achieved for 24/26 samples. Relevant gene fusions (C11orf95-RELA, YAP1-MAMLD1, ETV6-NTRK3, MNI-BEND2) were detected in 5 samples. The analytical performance of AS was comparable to nWGS and pbWGS. Input DNA quantity (2µg), library preparation time (1h30), turnaround time (48h) and computational requirements were compatible with diagnostic workflows.

Conclusion: Targeted nanopore sequencing is an exciting addition to the clinical laboratory arsenal, offering single-assay reliable SV and CNV detection. Long-read sequencing can now be offered as a routine diagnostic tool, promising novel biological insights and improved diagnostic yield.

References: 1.Payne, A. et al. (Nat. Biotechnol, 2020).

Grants:.

Conflict of Interest: Abderaouf Hamza Recieved equipement used in this study from NVIDIA through its Academic Hardware Grant Program., Mathilde Filser: None declared, Kevin Merchadou: None declared, Laeititia Maillot: None declared, Éléonore Frouin: None declared, Elodie Girard: None declared, Albain Chansavang: None declared, Djihad Hadjhadj: None declared, Eric Pasmant: None declared, Franck Bourdeaut: None declared, Tina Alaeitabar: None declared, Sonia Lameiras: None declared, Sylvain Baulande: None declared, Nicolas Servant: None declared, Victor Renault: None declared, Olivier Delattre: None declared, Julien Masliah-Planchon: None declared.

P16.024.D Automated tissue dissociation system for single cell sequencing

Yang Ni[1], *Khalil Abou Elardat*[1]

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Background/Objectives: Getting high quality single cell suspensions is the first step and essential for all downstream sample processing procedures in single-cell, multi-omics studies today. An established tissue dissociation workflow should meet the following criteria: (1) high yield, (2) high cell viability, and (3) high reproducibility. These standards are particularly important for clinical researchers, as samples collected from patients are usually in low amounts.

Methods: The Singleron PythoN automated tissue dissociation system (abbreviated as PythoN) integrates tissue mincing, grinding, enzymatic dissociation and cell straining into one "click-of-button", and has huge advantages over the traditional manual protocol.

Results: First, PythoN has a broad input range of sample weights, spanning from 10 to 500 mg. Internal testing data

showed that 660,000 single cells were successfully collected from a mouse liver tissue as small as 14 mg. Thus, PythoN is well suited for biopsy samples commonly seen in the clinic. Second, PythoN combines both mechanical and enzymatic tissue dissociation, being able to finish the whole procedure within 15 minutes, while keeping cells at a high viability (>90%). Third, PythoN is fully automated and programmable, which ensures the reproducibility of sample preparation. Up to 30 programs can be saved in PythoN. Key parameters, such as the motor speed of the grinder and the number of strokes, can be customized, leaving users free space to optimize their own protocols for new types of samples.

Conclusion: PythoN enables users to obtain high quality single cell suspensions in a reproducible manner.

References: More information can be found on https://www.singleronbio.com/product/detail-19.html.

Grants:.

Conflict of Interest: Yang Ni Singleron Biotechnologies GmbH, Khalil Abou Elardat Singleron Biotechnologies GmbH.

P16.026.B Enablement of long-read targeted sequencing panels using Twist hybrid capture and PacBio HiFi sequencing

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Background/Objectives: Targeted resequencing allows for high-resolution characterization of gene panels at a scale and cost that is more accessible than whole genome sequencing. While long-read PacBio HiFi sequencing has been shown to accurately and comprehensively interrogate complex clinically actionable loci, such as pharmacogenomic targets, studies have been primarily focused on single genes using PCR amplicon-based methods. We describe a method to leverage Twist Bioscience target enrichment probes for the design of custom gene panels sequenced with HiFi reads.

Methods: PacBio partnered with Twist Bioscience to develop a long-read hybrid capture protocol for custom gene panels of up to 2 Mb in size. The protocol follows a standard hybrid capture workflow with DNA shearing, barcoding, and sample handling optimized for long-read sequencing. The resulting enriched material is used to construct a SMRTbell library sequenced on the Sequel II system from PacBio. Up to 24 samples may be multiplexed with HiFi read length of 5-10 kb.

Results: We demonstrate that this method efficiently and comprehensively covers gene targets using Coriell samples run with multiple gene panels of varying sizes, which include complex regions like pharmacogene *CYP2D6*.

Conclusion: This protocol can be utilized with custom gene panels from Twist to efficiently capture genes of interest using long-read HiFi sequencing. The demonstrated method allows for scalable and cost-efficient hybrid capture with long read lengths, unbiased coverage, and high accuracy to fully capture all variant types, including structural variation that can be inaccessible to short-read sequencing and non-NGS technology.

References:.

Grants:.

Conflict of Interest: Sarah Kingan Pacific Biosciences, Pacific Biosciences, John Harting Pacific Biosciences, Pacific Biosciences, Ting Hon Pacific Biosciences, Pacific Biosciences, Yu-Chih Tsai Pacific Biosciences, Pacific Biosciences, Ian McLaughlin Pacific Biosciences, Pacific Biosciences, Janet Ziegle Pacific Biosciences, Pacific Biosciences, Tina Han Twist Bioscience, Twist Bioscience, Leonardo Arbiza Twist Bioscience, Twist Bioscience, Susan Kloet: None declared, Loes Busscher: None declared, Geoff Henno Pacific Biosciences, Pacific Biosciences, Edd Lee Pacific Biosciences, Pacific Biosciences, Nina Gonzaludo Pacific Biosciences, Pacific Biosciences.

P16.027.C RMetSeq package for processing targeted MRE-seq

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Background/Objectives: Methylation-sensitive restriction enzyme sequencing or MRE-seq is one of the methods for determining the level of DNA methylation. It usually involves comparing the results of sequencing samples after exposure to methyl sensitive and insensitive enzymes targeting the same restriction sites. This approach has some limitations, which leads to the fact that this method has not found wide application. We propose a new approach to the design of experiments and analysis of MRE-seq data, which allows integration of this analysis with standard NGS sequencing of exome and target panels with probe enrichment.

Methods: DNA isolated from blood samples was sonicated in 300 and 500 bp modes. Thereafter, half of each sample was treated with the HpaII. All samples were enriched using SureSelect Custom DNA Target Enrichment Probes (which includes exomes of 37 genes of a total length of 168497 nucleotides) and sequenced using MiSeq. Genotyping analysis was performed using GATK4. The methylation level was assessed using the RMetSeq package published on github.com/alekseizarubin/RMetSeq.

Results: The sequencing result of the fraction of the target region with coverage greater than 50× was 95.9% and 95.7% for the untreated and treated with restriction enzyme. But at the same time, combining 50% of random reads from these samples gave a fraction of 96.4%. These regions accounted for 573 restriction sites, with >50 x coverage.

Conclusion: Targeted MRE-seq and the RMetSeq package make it possible to simultaneously determine the level of DNA methylation without compromising the quality of genotyping in standard sequencing using probe enrichment.

References:.

Grants:.

Conflict of Interest: None declared.

P16.029.A Molecular diagnostics of myotonic dystrophies from short-read whole genome sequencing data

Ingrid Lojová[1;2], *Marcel Kucharik[3;4], Zuzana Pös[1;3;4], Andrea Zatkova[1], Jaroslav Budiš[2;3;4;5], Ludevit Kadasi[1;2], Tomas Szemes[2;3;4], Jan Radvansky[1;2;3]*

[1]Institute of Clinical and Translational Research, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia; [2]Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia; [3]Comenius University Science Park, Bratislava, Slovakia; [4]Geneton Ltd., Bratislava, Slovakia; [5]Slovak Center of Scientific and Technical Information, Bratislava, Slovakia.

Background/Objectives: Recently we are witnessing the spread and diversification of bioinformatic tools allowing tandem repeats (TRs) characterisation from short-read massively parallel sequencing (srMPS) data. The complexity of TRs, however, represents specific challenges when considering the diagnostic possibilities of repeat expansion disorders (REDs). We are reporting on specific

aspects of TRs detection and characterisation using myotonic dystrophies (DM1/DM2) as model REDs.

Methods: Whole genome sequencing data, using srMPS, were generated for 52 individuals, including 8 DM1,5 DM2, and two patients having both DM1 and DM2 expansions. For TR characterisation we used the modified version of a TR dedicated tool Dante (Ref1). Validation of the results was performed using conventional PCR and repeat-primed PCR.

Results: Using Dante we found high genotyping accuracy in the DM1 simple repeat (when considering normal-range alleles), however, the whole DM2 complex motif (TG-TCTG-CCTG) was not inferable from srMPS. Despite this, we were able to identify all of the DM1-CTG and DM2-CCTG expansions. Moreover, expanded and permutation DM1 alleles harboring unexpected sequence interruptions were also detected, although their identification may require specific error-rate settings.

Conclusion: We found that TRs characterisation and expansion detection using srMPS are reliable when analyzing simple repeats or specific parts of complex repeats. TRs having expected or unexpected sequence interruptions may require, however, specific attention. Moreover, the characterisation of highly complex repeats, such as the whole CNBP-TG/TCTG/CCTG, may still present challenges because of read length limitations.

References: Budis et al. Bioinformatics,35(8),1310-1317.

Grants: APVV-18-0319; VEGA_2/0167/20.

Conflict of Interest: None declared.

P16.031.C Implementation of the GA4GH Beacon protocol for discovery and sharing of genomic copy number variation data

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Background/Objectives: Genomic copy number variations (CNV) are frequent, causative events in rare diseases and represent the majority of the mutational landscape in the most malignancies. However, the complexity of genomic CNV patterns requires large amounts of well-defined genomic profiles for meaningful meta-analyses.

Methods: The "Beacon" protocol of the Global Alliance for Genomics and Health (GA4GH) represents an emerging standard for an "Internet for Genomics". While the initial version of the protocol served as test bed for federated genomic query systems connecting hundreds of international resources, version 2 of the protocol provides extended, metadata-rich interactions various access scenarios. Here, the Progenetix cancer genomics resource (progenetix.org) - comprising the largest publicly accessible set of cancer CNV data - has served as a testbed for Beacon v2 features.

Results: With Beacon v2 API as backbone of Progenetix - serving genome-wide CNV profiling data from more than 130'000 - we demonstrate the use of the GA4GH Beacon v2 protocol for the sharing vast amounts of genomic profiles, using a documented, open API.

Conclusion: After the acceptance of the Beacon v2 protocol as GA4GH standard, work will focus on its implementation in a wide set of use cases, from clinical information systems to public research databases. Importantly, an ELIXIR-supported study by members of the ELIXIR h-CNV community (cnvar.org) explores Beacon v2 powered CNV implementations by European genomics resources, in human genetics / rare diseases and cancer genomics.

References: Huang Q et al. (2021). The Progenetix oncogenomic resource in 2021. Database (Oxford), 2021 Jul 17.

Grants: ELIXIR Community-led Implementation Study (2021-2023).

Conflict of Interest: None declared.

P16.032.D Optical genome mapping as a diagnostic tool in cases of unresolved rare diseases

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Background/Objectives: The usefulness of optical genome mapping (OGM) for genetic testing in patients presenting with a rare genetic disease is currently evaluated. For patients for whom all previous genetic testing did not identify an underlying genetic variant, OGM might provide information that allows to end this diagnostic odyssey.

Methods: In this cohort OGM was performed on cases of rare diseases for 28 patient-parents TRIOs. All patients had normal or inconclusive results for chromosomal microarray and diagnostic exome sequencing.

Results: OGM identified putative disease related structural variants in 3 of these cases (10,7 %). The identified variants overlap with known disease genes that correlate to the patient's phenotype. The size of the identified variants was below the resolution of the diagnostic chromosomal microarray and difficult to detect with NGS testing. One patient was found to be composite heterozygous for a structural variant and a pathogenic single nucleotide variant within a recessive disease gene and biparental inheritance.

Conclusion: OGM allowed the identification of putative disease related variants in 10,7 % of our patients with negative chromosomal microarray and NGS testing.

References:.

Grants:.

Conflict of Interest: None declared.

P16.033.A Pilot study demonstrates the feasibility, the diagnostic power and the utility of rapid whole genome sequencing for critically ill pediatric patients in Belgium

***Aimé Lumaka[1]**, Corinne Fasquelle[2], Guillaume Debray[2], Serpil Alkan[3], Adeline Jacquinet[2], Julie Harvengt[2], François Boemer[2], Leonor Palmeira[2], Benoit Charloteaux[2], Anne Brysse[2], Saskia Bulk[2], Vincent Rigo[4], Vincent Bours[1;2]*

[1]University of Liège, Human Genetic Laboratory, GIGA Institute, Liège, Belgium; [2]Centre Hospitalier Universitaire, Center for Human Genetics, Liège, Belgium; [3]CHU de Liège - CHR de la Citadelle, Neuropediatric Division, Liège, Belgium; [4]CHU de Liège - CHR de la Citadelle, Neonatology Division, Liège, Belgium.

Background/Objectives: Genetic diseases are an important causes of admission and death in pediatric intensive care units. Early diagnostic orients the care and prevents irreversible harms. Whole Genome Sequencing in short turnaround time (rWGS) represents a valuable exploration in critically ill pediatrics patients. We evaluated the feasibility, the efficiency and the utility of the rWGS in Belgium.

Methods: We leveraged the collaboration between two pediatric institutions and the center for human genetics of the University of Liège, Cloud-based computing data analysis services and a high-throughput NGS platform to develop a rWGS workflow intended to deliver diagnostics to critically ill pediatric patients before hospital discharge. WGS was performed on NovaSeq PE300cy in trio for 9 and in duo for 1 proband. The study was approved by the Ethical Committee.

Results: Ten unrelated critically ill patients without any clear diagnostic were recruited from the Neonatal Intensive Care Unit (4), the Pediatric Intensive Care Unit (4) and the neuropediatric unit (2). A definite diagnostic was reached in 6 out of 10 patients in 39.29 hours (95% CI 38.3 - 40.3), including six clinically unsuspected diagnosis. rWGS-guided multidisciplinary care was implemented in 5 patients and disease specific care in 1. Time constraints and cost were identified as the main limitations to the broad introduction of rWGS for critically ill pediatric patients.

Conclusion: We successfully implemented the fastest rWGS platform in Europe. Our workflow has one of the highest rWGS yields. This study establishes the path for a nation-wide semi-center rWGS network in Belgium.

References:.

Grants: Government of Wallonia, Belgium: WALGEMED/RWAL1710180.

Conflict of Interest: Aimé Lumaka Sequencing reagents for 5 patients were donated by Illumina Inc., Corinne Fasquelle: None declared, Guillaume Debray: None declared, Serpil Alkan: None declared, Adeline Jacquinet: None declared, Julie Harvengt: None declared, François Boemer: None declared, Leonor Palmeira: None declared, Benoit Charloteaux: None declared, Anne Brysse: None declared, Saskia Bulk: None declared, Vincent Rigo: None declared, Vincent Bours: None declared.

P16.034.B Non-invasive preimplantation genetic testing for optimizing of IVF treatments

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Background/Objectives: The major contributor for unsuccessful IVF cycles has been found to be embryo aneuploidy. The percentage of aneuploidy rate is known to increase with maternal age increasing the problem of unsuccessful IVF treatments. Preimplantation genetic testing for aneuploidies (PGT-A) is a widely used method to screen embryos for chromosomal abnormities. Until now the method has required a TE biopsy making the method invasive and thereby limited to few. The discovery of cell free DNA (cfDNA) in the spent culture media (SCM) has made it possible for the development of a non-invasive PGT-A screening of the blastocysts.

Methods: Amplexa Genetics has implemented and validated the niPGT-A method. The generated WGA is sequenced using the Illumina sequencing platform and processed to identify chromosomal abnormalities.

Results: Amplexa Genetics has demonstrated that we can use cfDNA SCM samples and generate WGA from all SCM samples obtained. Clear calls could be obtained in 93% of all cases as either euploid or aneuploid. We observed that increased maternal age correlated with increased percentage of aneuploidy rate as expected. Moreover, the identified aneuploidy is evenly distributed between losses and gains of all 23 autosomal chromosomes.

Conclusion: The niPGT-A method can be used to screen embryos for chromosomal abnormalities to the same extent as the former and invasive PGT-A method.

References: Scott et al 2012, Hassold T, Hunt P et al 2001, Nature Rev.

Grants: No Grants.

Conflict of Interest: Sabrina Frrederiksen Amplexa Genetics, Christina D. Fenger Amplexa Genetics, Mads Fruensgaard: None declared, Steen B. Laursen Fertilitetsklinik IVF-SYD, Hans Atli Dahl Amplexa Genetics.

P16.035.C A novel enzymatic fragmentation library preparation workflow that prevents sequencing artifacts and increases scalability

***Ross Wadsworth**[1], Zane Jaafar[2], Josh Haimes[2], Thomas Harrison[2], Lindsay Peterkin[2], Kristin Scott[3], Martin Ranik[2], Kristina Giorda[2], Eric van der Walt[1], Brian Kudlow[2]*

[1]Watchmaker Genomics, Cape Town, South Africa; [2]Watchmaker Genomics, Boulder, United States; [3]Colorado State University, Fort Collins, United States.

Background/Objectives: There has been extraordinary progress towards translational research and clinical genomics in the era of NGS — throughput has grown while sequencing costs have gone down. Now, gold standard sonication methods are being reevaluated in light of increased sequencing capacity and the need for highly accurate and scalable workflows. Our goal was to create an automation-friendly workflow and reduce sequencing artifacts to enable highly sensitive clinical and translational applications.

Methods: Here, we utilized sophisticated enzyme engineering and a multidimensional Design of Experiment approach to develop a novel enzymatic library preparation method and ultra-high fidelity amplification module. To assess performance, our solution was compared to conventional enzymatic library preparation workflows using samples ranging from 100 pg to 500 ng of gDNA.

Results: Libraries generated using our optimized workflow reduced chimeric reads and terminal hairpin artifacts 10-fold compared to other enzymatic methods, and reached comparable levels to mechanically sheared DNA controls. Library insert sizes were highly tunable from 150 bp to 550 bp and were consistent across the input titration. To assess the utility of ultra-low input samples, libraries were prepared using a titration from 100 ng to sub-nanogram inputs. Copy number variations (CNVs) were detected across the titration with high sensitivity and specificity using Hidden Markov Model analysis method.

Conclusion: Taken together, this enzymatic fragmentation and library preparation workflow avoids library preparation artifacts that convolute variant calling, is highly scalable, and suitable for ultra-low input samples.

References:.

Grants:.

Conflict of Interest: Ross Wadsworth Watchmaker Genomics, Zane Jaafar Watchmaker Genomics, Josh Haimes Watchmaker Genomics, Thomas Harrison Watchmaker Genomics, Lindsay Peterkin Watchmaker Genomics, Kristin Scott Watchmaker Genomics, Martin Ranik Watchmaker Genomics, Kristina Giorda Watchmaker Genomics, Eric van der Walt Watchmaker Genomics, Brian Kudlow Watchmaker Genomics, Watchmaker Genomics.

P16.036.D Improved transcript detection sensitivity utilizing a novel, rapid whole transcriptome sequencing workflow

*Travis Sanders[1], **Lee French**[2], Julie Walker[1], Jennifer Pavlica[1], Clara Ross[1], Thomas Harrison[1], Ross Wadsworth[2]*

[1]Watchmaker Genomics, BOULDER, United States; [2]Watchmaker Genomics, Cape Town, South Africa.

Background/Objectives: mRNA-seq is a powerful tool for transcriptome profiling but is not applicable to many clinically relevant sample types. Template damage in FFPE samples generates 3'-bias, while overabundant globin mRNAs in blood-derived samples are uninformative. Whole transcriptome sequencing, where overabundant transcripts are depleted, supports these sample types and offers a comprehensive view of the transcriptome - including

biologically relevant non-coding transcripts. However, these workflows are typically long, labor-intensive, and difficult to automate. To address this need, we aimed to develop a highly streamlined and automatable solution tailored for challenging samples.

Methods: To simplify the protocol while minimizing off-target effects, we built algorithms for optimal probe design and streamlined probe hybridization. We specifically engineered enzymes and reformulated buffers in parallel to improve yields. A novel de-crosslinking step was integrated to improve FFPE performance. Combining reactions, reducing incubation times, and eliminating purifications streamlined the overall workflow. With RNA extracted from whole blood and five FFPE blocks, we compared our solution to commercial products using inputs ranging from 10 to 500 ng.

Results: Libraries generated using our solution resulted in improved library yields, unique transcript identification, strand specificity, and rRNA depletion efficiency on a per-FFPE-block basis. Technical replicates showed excellent transcript abundance correlation. Additionally, we observed less than 1% residual rRNA and globin mRNA with no measurable off-target effects using a blood-derived, high-quality sample with as little as 10 ng.

Conclusion: Our novel, simplified workflow delivers robust and reproducible performance while enabling library construction within five hours.

References: N/A.

Grants: N/A.

Conflict of Interest: Travis Sanders Watchmaker Genomics, Lee French Watchmaker Genomics, Julie Walker Watchmaker Genomics, Jennifer Pavlica Watchmaker Genomics, Clara Ross Watchmaker Genomics, Thomas Harrison Watchmaker Genomics, Ross Wadsworth Watchmaker Genomics.

P16.037.A Comprehensive de novo variant discovery with HiFi long read sequencing

Erdi Kucuk[1], *Bart van der Sanden*[1], *Luke O'Gorman*[1], *Michael Kwint*[1], *Aaron Wenger*[2], *William Rowell*[2], *Zev Kronenberg*[2], *christine lambert*[2], *shreyasee chakraborty*[2], *primo baybayan*[2], *Han Brunner*[1], *Alexander Hoischen*[1], *Lisenka Vissers**[1], *Christian Gilissen*[1]

[1]*Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands;* [2]*Pacific Biosciences (PacBio), Menlo Park, United States.*

Background/Objectives: Current technologies fail to accurately explain almost half of neurodevelopmental disorder cases. Long read sequencing (LRS) is promising to comprehensively identify genomic variation in unresolved cases. Pacific Biosciences (PacBio) HiFi sequencing technology provides the highest accuracy among LRS platforms and appears especially suitable for the detection of de novo mutations (DNMs) across single-nucleotide variants (SNVs), indels, and structural variants.

Methods: We sequenced the genomes of 8 trios with unexplained intellectual disability using LRS (PacBio Sequel II System, 30x HiFi) and short read sequencing (SRS; Illumina Novaseq 6000, 40x). Small indels and SNVs were called on LRS using DeepVariant and SRS genomes using xAtlas. We then compared DNMs between the two methods.

Results: We identified an average of 84 DNMs in LRS and 101 DNMs in SRS per trio (85% concordance between platforms) in these 8 trios. 54 variants were uniquely detected by LRS. We were able to design primers for 23 of these, 9 (38%) of which were confirmed as a DNM by Sanger sequencing. We also designed primers for 47 DNMs called only by SRS and confirmed 6/47 (13%). Most false positive SRS DNMs (66%) were missing in the proband samples, whereas most LRS false positive DNMs were inherited (48%). Additionally, we phased on average 95% of LRS DNMs compared to 30% in SRS.

Conclusion: Our results show that current PacBio HiFi sequencing technology achieves single base accuracies that are sufficient to robustly identify genome-wide de novo mutations, including in regions missed by SRS.

References:.

Grants:.

Conflict of Interest: Erdi Kucuk: None declared, Bart van der Sanden: None declared, Luke O'Gorman: None declared, Michael Kwint: None declared, Aaron Wenger Pacific Biosciences, William Rowell Pacific Biosciences, Zev Kronenberg Pacific Biosciences, christine lambert: None declared, shreyasee chakraborty Pacific Biosciences, primo baybayan Pacific Biosciences, Han Brunner: None declared, Alexander Hoischen: None declared, Lisenka Vissers*: None declared, Christian Gilissen: None declared.

P16.038.B Melter: towards achieving efficient semi-automatic reanalysis in rare disease

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Background/Objectives: Through introduction of comprehensive genomic analyses, the proportion of rare disease patients receiving a molecular diagnosis has dramatically increased. However, the majority of cases still remain without molecular diagnosis after a primary analysis[1], despite suspicion of an underlying genetic disease. Reanalyses of manually selected cases have several times led to identification of a disease-causing variant that was missed in the initial analysis. The accumulated pool of unsolved cases constitutes a great opportunity for disease gene discovery.

Methods: We present a concept for systematic, automated reanalysis of unsolved cases using specific triggers to initiate the reanalyses. Such triggers include e.g., updates in clinical features (HPO terms), in phenotype-specific gene panels, or in bioinformatic tools or databases used for annotations. New variants not seen in previous analyses and fulfilling predefined threshold in our variant prioritization scheme are presented for further manual clinical inspection.

Results: Testing an automated approach for reanalysis of previously unsolved cases in a pilot setting will allow measuring the computational resources needed and allows for estimating the effort needed for inspecting variants passed on to manual inspection.

Conclusion: Comparing the change in diagnostic yield with costs will determine if automated reanalysis of unsolved rare diseases cases should be implemented in clinical routine.

References:.

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Grants:.

Conflict of Interest: None declared.

P17 DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

P17.002.D Dried blood spot testing with AmplideX SMA Plus Kit resolves SMN1 and SMN2 exon 7 copy numbers and more

Sarah Edelmon[1], Walairat Laosinchai-Wolf[1], Laura Blasco-Pérez[2], Mar Costa-Roger[2], Ivon Cuscó[2], Gary Latham[1], Eduardo Tizzano[2], ***John Milligan***[1]

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Background/Objectives: Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder commonly caused by homozygous absence of *SMN1*. *SMN2*, an *SMN1* paralog, modulates SMA severity. Breakthrough therapies rely on rapid quantification of *SMN1* and *SMN2* copies, and newborn screening using dried blood spot (DBS) samples has become a public health priority. However, most screening assays only determine presence/absence of *SMN1* exon 7, excluding copy numbers (CN) for *SMN1* and *SMN2* and disease-modifier variants (c.859G<C).

Methods: We tested 42 DBS samples with three DNA isolation methods on three genetic analyzer models (378 measurements total) using the AmplideX® SMA Plus* Kit, which quantifies *SMN1* and *SMN2* exon 7 CN and detects c.859G>C in a single-tube workflow. Genotypes were determined using AmplideX PCR/CE Reporter software. Reference values were determined using matched whole blood (n = 20) or MLPA and sequencing (n = 22).

Results: For each platform, *SMN1* and *SMN2* exon 7 CN and variant status were 95-100% concordant with reference results, including 10 SMA samples with no *SMN1* copies. Confirmed heterozygous or homozygous samples for 859G>C had unique peak profiles, suggesting concordance for zygosity.

Conclusion: These data demonstrate that the AmplideX Kit can accurately resolve *SMN1* and *SMN2* CN and variant status from DBS samples. Further, the ability to generate complete results in under 4 hours may improve the time-to-result over current screening approaches, which rely on follow-up confirmatory diagnostic tests. Given the narrow therapeutic window for maximum treatment efficacy, these benefits may enable earlier decision on treatment and improved patient outcomes.

References: *CE-IVD. For US export only.

Grants: Not applicable.

Conflict of Interest: Sarah Edelmon Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Walairat Laosinchai-Wolf Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Laura Blasco-Pérez: None declared, Mar Costa-Roger: None declared, Ivon Cuscó: None declared, Gary Latham Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Eduardo Tizzano: None declared, John Milligan Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand.

P17.003.A Standardising the variability of variant classification

Zandra Deans[1], *Dave Cregeen[1], Jennifer Fairley[1], Farrah Khawaja[1], Mark Sales[1], Melody Tabiner[2], Rebecca Treacy[1], Ros Hastings[2]*

[1]GenQA, Edinburgh, United Kingdom; [2]GenQA, Oxford, United Kingdom.

Background/Objectives: Expansion of genome-wide testing for disease-causing changes has increased the requirement to classify variant pathogenicity. Multiple classifications are available for different variant types, and clinical settings. GenQA has globally delivered laboratory external quality assessments (EQAs) for variant interpretation to aid standardisation of classification.

Methods: EQAs were provided for classification of single nucleotide variants (SNVs), copy number variants (CNVs) in prenatal and postnatal settings, and somatic variants. Variant details were provided and laboratories were expected to classify them according to the clinical scenarios. Assessment was based on correct classification, evidence provided and, where appropriate, the actionability of the variant.

Results: Nine germline SNV EQAs have been delivered since 2013, during which time the ACMG classification system was introduced. Data from 26 cases will be presented highlighting changes in practice, and the different applications of evidence. A summary of the prenatal and postnatal CNV classification pilots will show consistency in approach, by either using local justification, or applying the ACMG/ClinGen guidelines. They demonstrate how uncoupling evidence-based classification from potential implications for an individual can assist in standardisation of variant classification. Two solid tumours variant classification pilot EQAs have also been delivered. Laboratories used AMP/ACO/CAP tiering guidelines (actionability) and ACMG guidelines1 (pathogenicity). Both showed variability reflecting differences in guideline application.

Conclusion: Accuracy of variant classification is essential to ensure the patient receives the correct result and clinical management. The EQAs demonstrated variability in the use and application of the guidelines, and the continued need for EQAs to educate and promote standardisation.

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Conflict of Interest: Zandra Deans NHS Lothian, Dave Cregeen NHS Lothian, Jennifer Fairley NHS Lothian, Farrah Khawaja NHS Lothian, Mark Sales NHS Lothian, Melody Tabiner Oxford University Hospital NHS Trust, Rebecca Treacy NHS Lothian, Ros Hastings Oxford University Hospital NHS Trust.

P17.004.B Increasing genomics diagnostic success for Indigenous families in Canada with rare diseases

Tatiana Maroilley[1;2;3], *Arezoo Mohajeri[3;4], Jill Mwenifumbo[5], Vladimir Avramovic[1;2], Karen J. Jacob[4], Sarah McIntosh[4], Solenne Correard[4;6], Wyeth W. Wasserman[4;6], Nadine R. Caron[7;8;9], Laura Arbour[4], Anna Lehman[4], Maja Tarailo-Graovac[1;2]*

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Background/Objectives: Genomics has the potential to transform health care by facilitating precision medicine. However, individuals who identify as Indigenous (First Nations, Inuit, and Métis) have limited access to genomics. Background genetic variation for Indigenous Peoples is largely unknown, reducing the chance of accurate interpretation of variants and diagnosis of genetic conditions. The Silent Genomes Project (SGP) aims to increase access

to genomics for Indigenous Peoples in Canada, in a culturally safe way.

Methods: We analysed rare disease (RD) patients' genomes with two independent pipelines. The automated diagnosis pipeline (Level1) implements state-of-the-art methods aligned with clinical standards. The research-based pipeline (Level2) re-analyses genomes with no/uncertain/partial findings. It implements different tools and newly developed approaches to explore complex diagnostic scenarios such as non-coding variations and complex genomic rearrangements (CGR), later experimentally validated (PCR, transcriptomics).

Results: Level1 has analysed 33 probands with suspected undiagnosed RD and reported clinically confirmed pathogenic/likely pathogenic variants for 12 of them (36%). Level1 has also reported 17 variants of uncertain significance (VUS). In total, 20 probands are eligible for Level2. Level2 analysis of seven probands has detected four new findings including novel gene, CGR and translocation.

Conclusion: To facilitate VUS interpretation, the SGP plans to build the first Indigenous Background Variant Library (IBVL) by enrolling Indigenous participants with no RD. With the IBVL and two analytical pipelines, the SGP has the potential to increase diagnostic success in Indigenous patients with RD and reduce disparities in health care. This effort could become a model for other underrepresented populations, addressing gaps in genomic medicine.

References:.

Grants:.

Conflict of Interest: Tatiana Maroilley: None declared, Arezoo Mohajeri: None declared, Jill Mwenifumbo: None declared, Vladimir Avramovic: None declared, Karen J. Jacob: None declared, Sarah McIntosh: None declared, Solenne Correard: None declared, Wyeth W. Wasserman: None declared, Nadine R. Caron Co-Director, UBC Centre for Excellence in Indigenous Health, First Nations Health Authority Chair in Cancer and Wellness at UBC, Special Advisor on Indigenous Health; UBC Health and Faculty of Medicine, Laura Arbour: None declared, Anna Lehman: None declared, Maja Tarailo-Graovac: None declared.

P17.005.C Delivery of a multi-distribution external quality assessment for severe combined immunodeficiency newborn screening

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Background/Objectives: Newborn screening for severe combined immunodeficiency (SCID) by measurement of T-cell receptor excision circles (TRECs) continues to be evaluated and implemented globally. This requires concomitant implementation of external quality assessment (EQA) distributed regularly throughout the year. GenQA has developed and delivered a 2-year pilot EQA consisting of multiple sample distributions.

Methods: Newborn blood spot cards were prepared using blood samples with either normal or absent TRECs and validated to confirm the levels of TRECs using commercially available TREC assays prior to the global distributions. Assessment was carried out by independent expert advisors against peer ratified criteria. Pilot year 1 provided eight samples across two distributions. Pilot year 2 delivered 12 samples across six distributions.

Results: Participants were split evenly between the two commercially available TREC assays with some variation in cut-off levels and the units used to describe results. Over the course of the pilot the quality of result submission improved with ultimately only a few participating laboratories omitting essential information such as assay units, cut-off levels and reference gene results. Older blood spots prepared from normal blood could give results in the affected range despite reference gene results appearing acceptable; therefore, samples for subsequent EQAs were sourced, validated, and distributed within as short a time as possible.

Conclusion: The development of a comprehensive newborn screening EQA is challenging when samples need to be sourced from patients. An EQA format consisting of regular distributions throughout the year of samples suitable for SCID newborn screening analysis has been successfully implemented.

References:.

Grants:.

Conflict of Interest: None declared.

P17.006.D Deep phenotyping – symptom annotation made simple with SAMS

Robin Steinhaus[1;2], *Sebastian Proft*[1;2], *Evelyn Seelow*[3], *Tobias Schalau*[1], *Peter N. Robinson*[4;5], ***Dominik Seelow***[1;2]

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Background/Objectives: Precision medicine needs precise phenotypes. The Human Phenotype Ontology (HPO) structures clinical signs and symptoms and has become the standard annotation for patient phenotypes when describing single gene disorders. However, use of the HPO in other fields than human genetics is not widespread.

Methods: SAMS (Symptom Annotation Made Simple) brings deep phenotyping to routine clinical care, hospital patients, and outpatients. Our web-based application provides three widely used annotation systems: HPO, OMIM, and Orphanet. It also offers a guided differential diagnosis towards a disease when clinical signs (as HPO terms) are entered.

Results: Whilst data can be stored in our database, phenotypes can also be imported and exported as GA4GH Phenopackets without using the database. The web interface can easily be integrated into local databases, e.g., clinical information systems. SAMS offers users the ability to share their data with others, empowering patients to record their own signs and symptoms (or those of their children) and thus provide their doctors with additional information.

Conclusion: We think that our approach will lead to better characterised patients which is not only helpful for finding disease mutations but also to better understand the pathophysiology of diseases and to recruit patients for studies and clinical trials.

SAMS is available at https://www.genecascade.org/SAMS/.

References:.

Grants:.

Conflict of Interest: None declared.

P17.007.A Implementation of genomic medicine in Stockholm healthcare region – update on first 10,000 samples

gmck-RD consortia[1;2;3], ***Anna Lindstrand***[1;3], *Henrik Stranneheim*[2;3], *Kristina Lagerstedt-Robinson*[3], *Nicole Lesko*[3], *Daniel Nilsson*[2;3], *Sofie Vonlanthen*[3], *Jesper Eisfeldt*[2;3], *Malin Kvarnung*[1;3], *Martin Engvall*[3], *Helena Malmgren*[3], *Britt-Marie Anderlid*[3], *Chiara Rasi*[2], *Anders*

Jemt[2;3], Per Marits[3], Maria Johansson Soller[3], Ann Nordgren[1;3], Valtteri Wirta[2;3], Anna Wedell[1;2;3]

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Background/Objectives: In healthcare clinical genetics is transitioning to clinical genomics and especially rare disease diagnostics is increasingly done through panel, exome and genome sequencing. At Karolinska we have formed Genomic Medicine Center Karolinska Rare Diseases (GMCK-RD), a joint unit between healthcare and academia, enabling large-scale genome sequencing of patients. GMCK-RD brings together experts from various medical disciplines with clinical geneticists, bioinformaticians and researchers.

Methods: To facilitate clinical implementation of genome sequencing, we have developed a number of bioinformatic tools and processes covering steps such as variant calling, workflow management, variant prioritization and interpretation, multiomics support, data sharing and quality assurance.

Results: Over 10,000 individuals across a broad spectrum of rare diseases have been analyzed, enabling a diagnosis as well as personalized prediction, prognosis, and treatment in thousands of patients.

Conclusion: Challenges include big data processing, ethical and legal issues as well as rigorous quality control while at the same time enabling continuous development of analysis pipelines and tools. The rapid increase in genomic testing also brings along a high need for scaling up interpretation, including recruitment and training of highly specialized "genomicists" and development of new support solutions. We also need integrated units where highly specialized clinicians work closely together with laboratory experts, enabling patient selection, correct interpretation and rapid translation to individualized treatment. These interactions have also enabled a large number of new disease gene discoveries. By overcoming these issues, we at GMCK-RD have moved healthcare in our region towards precision diagnostics and precision medicine.

References: None.

Grants: None.

Conflict of Interest: None declared.

P17.008.B Generic genome sequencing: one lab flow for all

***Gaby Schobers**[1;2], Ronny Derks[1], Ermanno Bosgoed[1], Debby Hellebrekers[3], Nicole de Leeuw[1], Alexander Stegmann[3], Erik-Jan Kamsteeg[1], Aimee D.C. Paulussen[3], Marjolijn Ligtenberg[1], Arthur van den Wijngaard[3], Christian Gilissen[1;4], Rien Blok[3], Han Brunner[1;2;3], Helger Yntema[1;2], Marcel Nelen[1], Lisenka Vissers*[1;2]*

[1]Radboud university medical center, Human Genetics, Nijmegen, Netherlands; [2]Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands; [3]Maastricht University Medical Center, Clinical Genetics, Maastricht, Netherlands; [4]Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands.

Background/Objectives: Genetic laboratories maintain numerous workflows to diagnose the full spectrum of hereditary and congenital diseases, including traditional approaches and advanced technologies. A single generic workflow would increase efficiency quite dramatically. We therefore assessed whether genome sequencing (GS) can replace all existing workflows supporting germline genetic diagnoses.

Methods: We performed GS (NovaSeq6000TM; 37x mean coverage) on 1,000 cases with 1,271 clinically relevant variants, selected from one year's diagnostic yield in a tertiary referral center, identified through 15 different workflows. Variants were binned by size and type: small variants (SNVs/indels <50 bp), large variants (CNVs and repeat expansions) and other variants (SVs and aneuploidies). VCFs were queried per variant and assessed in Trusight Software Suite (DRAGEN Germline Pipeline, TSS, Illumina).

Results: Overall, 93.9% (1,194/1,271) of variants were detected with GS. Detection rates differed per type, with small variants detected in 95.2% (825/867), large variants in 91.9% (328/357), and other variants in 87.2% (41/47). Importantly, variants were identifiable through routine clinical interpretation strategies, including disease-based clinical filters or gene-specific searches in TSS. Variants that remained undetected were mosaic or located in homologous/repetitive regions.

Conclusion: GS is an efficient generic workflow to capture clinically relevant germline variants in a 'one-test-fits-all-strategy'. Besides those already known for short-read sequencing, no new challenges in variant detection were identified. GS can therefore not only replace exome sequencing, but also >99% of Sanger sequencing, allele specific PCRs, smMIP, MLPA, array, and cytogenetic analyses including karyotyping and FISH. These results provide perspective on how genetic laboratories will evolve in the near future.

References:.

Grants:.

Conflict of Interest: None declared.

P17.009.C How can entire CFTR genotyping contribute in genetically unsolved Cystic Fibrosis cases?

***Simone Ahting**[1], Lutz Nährlich[2], Julia Hentschel[1]*

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Background/Objectives: Genotyping Cystic Fibrosis (CF) patients is crucial for diagnosis confirmation and treatment options. Recent modulator therapies allow for correction of malfunctioning CFTR, but depend on the underlying genotype. Still 5.4% of patients [1] remain undiagnosed after conventional genetic testing and can therefore benefit from entire *CFTR*-genotyping.

Methods: 731 patients with clinically confirmed CF-diagnosis, but ambiguous genotype were assembled using the German CF Registry. Variants were identified and re-classified, if required using ClinVar, HGMD and CFTR1/2 and corrected in the registry database. Genetic testing was offered to all patients whose variants were inconclusive and further patients lacking genetic CF-confirmation were called for testing. Patient samples were analysed using a Next-Generation-Sequencing-custom-design-panel covering all 27 exons including intronic and regulatory regions.

Results: Identification of inconclusive variants led to the discovery of 48 variants not formerly reported in the context of CF. 20 samples with previously unknown or incomplete *CFTR* genotype were sequenced via NGS with an overall success rate of 70%. All results were uploaded to ClinVar and previously unknown variants were reported to CFTR1/2 for database completion.

Conclusion: Entire *CFTR*-genotyping can greatly increase the genetic diagnostic rate of CF-patients and should therefore be considered as a replacement for the current strategies in routine diagnostics. Still unclear cases might further benefit from transcriptome sequencing on nasal epithelial cells to analyse the CFTR-mRNA and better assess intronic variants.

References: [1] German CF Registry annual report 2020 (https://www.muko.info/fileadmin/user_upload/angebote/qualitaetsmanagement/register/berichtsbaende/Berichtsband_2020.pdf).

Grants: Vertex Pharmaceuticals funded this project through the 'Charitable Grant Vertex'.

Conflict of Interest: None declared.

P17.010.D Periodic automated reanalysis and reevaluation of exome data to improve its clinical utility

Cyrielle Kint[1], *Sara Haers*[1], *Linde Proost*[1], *Alexandra Obregon-Tito*[1], *Jeanne Leisk*[1]

[1]*Invitae, Leuven, Belgium.*

Background/Objectives: As more genetic data become available, gene-disease associations and phenotypes are clarified. From June-November 2021 alone, our internal gene-disorder database grew by 184 new rare disorders associated with genetic variation. Additionally, phenotypic associations with genes increased by 28.478 Human Phenotype Ontology terms. Therefore, patients for whom no diagnosis is available today, might be diagnosable next year, next month or even sooner. Similarly, the phenotype of an individual may change over time resulting in the need to adjust analysis to include newly available clinical information. However, leveraging the clinical benefit of this new genetic and phenotypic information is largely contingent on computational tools that can evaluate and integrate it.

Methods: Despite the clinical potential of integrating continuously expanding genetic and clinical information, reanalysis and reevaluation efforts for unsolved whole exome sequencing (WES) tests remain largely manual. This restricts access to the most recent gene-phenotype knowledge, and limits the utility of a WES test to the evaluation of phenotypes present in an individual at the time of testing. To address these deficits, we present an automated reanalysis pipeline utilizing the Moon software that runs every six months, integrating both the most up-to-date gene-phenotype associations and the evolving clinical phenotype of a patient when available.

Results: Automating regular reanalysis of cases has increased the diagnostic utility of our WES tests and provides patients better answers at no additional cost.

Conclusion: This pipeline has been validated and can be used as part of a larger system, as presented here, or as a stand-alone tool (Invitae's Moon).

References:.

Grants:.

Conflict of Interest: Cyrielle Kint Invitae, Invitae, Sara Haers Invitae, Invitae, Linde Proost Invitae, Invitae, Alexandra Obregon-Tito Invitae, Invitae, Jeanne Leisk Invitae, Invitae.

P17.011.A Low-coverage genome sequencing is an efficient approach for the detection of clinically relevant copy-number variants and mtDNA variants

Sander Pajusalu[1;2], *Kaisa Teele Oja*[1;2], *Ustina Šamarina*[2], *Mikk Tooming*[1;2], *Tiina Kahre*[1;2], *Katrin Ounap*[1;2]

[1]*Tartu University, Institute of Clinical Medicine, Department of Clinical Genetics, Tartu, Estonia;* [2]*Tartu University Hospital, United Laboratories, Department of Clinical Genetics, Tartu, Estonia.*

Background/Objectives: Compared to exome sequencing, high-coverage genome sequencing is widely appreciated for its superior ability to detect a wide range of genetic variations including copy-number variants (CNVs) and mitochondrial (mtDNA) variants. However, the high cost is still limiting the use of genome sequencing. We aimed to assess whether low-coverage genome sequencing, a considerably cheaper approach, would detect clinically relevant CNVs and mtDNA variants and would thus be a cost-efficient approach to supplement exome sequencing in rare disease diagnostics.

Methods: To assess the level of sequencing depth needed for variant detection, first, 30x mean coverage genome sequencing data were subsampled to 0.5x, 1x, 2x, and 4x coverage files in silico. CNVs were detected using Control-FREEC and the mtDNA variants were detected using the GATK4 mitochondrial pipeline. Five disease-causing deletions (sized 3.4kb, 4.6kb, 7.2kb, 16kb, and 90kb) and one possibly pathogenic heteroplasmic (14.7%) mtDNA variant was used to assess sensitivity.

Results: For CNV calling, 2x coverage was sufficient to detect all heterozygous CNVs greater than 10kb in size, for smaller CNVs even 4x coverage data did not suffice for CNV detection. Regarding mtDNA variants, 2x coverage resulted in >99% theoretical sensitivity for heteroplasmy levels >10%. The possibly pathogenic heteroplasmic variant was detected without significant change in heteroplasmy estimation in all low-coverage data (0.5x to 4x coverage).

Conclusion: Low-coverage genome sequencing may be used to complement exome sequencing for simultaneous mtDNA variant and CNV detection.

References:.

Grants: Estonian Research Council grants PRG471, MOBTP175, PSG774.

Conflict of Interest: None declared.

P17.012.B MorbidGenes panel: a monthly updated list of diagnostically relevant genes derived from diverse sources

Robin-Tobias Jauss[1], *Bernt Popp*[1], *Konrad Platzer*[1], *Rami Abou Jamra*[1]

[1]*Institute of Human Genetics, University Hospital Leipzig, Leipzig, Germany.*

Background/Objectives: Identifying clinically relevant genetic variants is crucial for a fast and reliable genetic diagnosis. With exome sequencing now standard, diagnostic labs are in need of a, in principle, to-the-day-accurate list of genes associated with rare diseases. Manual curation efforts are slow and often disease specific, while efforts relying on single sources are too inaccurate and may result in false-positive genes.

Methods: We established the MorbidGenes panel based on a list of publicly available databases: OMIM, PanelApp, SysNDD, ClinVar, HGMD and GenCC. A simple logic allows inclusion of genes with sufficient evidence based on a voting algorithm. By providing an API endpoint, users can directly access the list and metadata for all relevant information on their genes of interest.

Results: The panel currently includes 4,677 genes (v.2022-02.1, as of February 2022) with minimally sufficient evidence on disease causality to classify them as diagnostically relevant. Reproducible filtering and versioning allow the integration into diagnostic pipelines. In-house implementation successfully removed false positive genes and reduced time requirements in routine exome diagnostics. The panel is updated monthly, and we will integrate novel sources on a regular basis. The panel is freely available at https://morbidgenes.org/.

Conclusion: The MorbidGenes panel is a comprehensive and open overview of clinically relevant genes based on a growing list of sources. It supports genetic diagnostics labs by providing diagnostically relevant genes in a QM conform format on a monthly basis with more frequent updates planned. Once genomes are standard, diagnostically relevant non-coding regions will also be included.

References:.

Grants:.

Conflict of Interest: None declared.

P17.013.C Who am I? Donor DNA in Patients after Stem Cell Transplantation

Mareike Mertens[1], *Mona Sadlo*[1], *Julia Hentschel*[1]

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Background/Objectives: In Germany, 3,500 persons per year receive a stem cell transplantation resulting in a chimerism. Therefore, germline testing of blood samples is not feasible. The presence of donor DNA in various tissues recipients has previously been documented (Thiede et al. 2000; Imanishi et al. 2007). Yet, the literature lacks a statistical sound assessment of quantitative extend of donor DNA in various tissues.

Methods: We performed a pilot trial with six patients who received an allogeneic stem cell transplant. We analyzed DNA from buccal swabs, nails and reference material (collected before transplantation) with two different approaches: a short tandem repeat (STR) assay containing 28 STR markers (custom-made) to detect donor derived DNA and a NGS-RC-PCR-based SNP-Assay including 34 Loci (Nimagen).

Results: Our study showed that DNA of the stem cell donor could be detected in both buccal swabs and nail samples after transplantation. The detected donor proportion varies between 11 % and 44 % in the buccal swabs and nail samples.

Conclusion: The study confirms the presence of donor DNA in buccal swabs and documents a donor DNA percentage in nail samples of the recipient after allogenic stem cell transplantation. Currently, we extent the study to additional patients, additional tissue types and adress the question how donor DNA proportion varies over time after transplantation to derive more robust conclusions, to provide guidance for germline testing after stem cell transplantation.

References:.

Grants:.

Conflict of Interest: Mareike Mertens Universitätsklinikum Leipzig, Mona Sadlo Universitätsklinikum Leipzig, Julia Hentschel Universitätsklinikum Leipzig.

P17.014.D Challenges and lessons learned for automated variant interpretation pipelines in clinical whole genome sequencing

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Background/Objectives: During the 100,000 Genomes Project, Genomics England developed an automated variant interpretation framework called Tiering. Tiering prioritises variants most likely to be clinically relevant for individual patients based on known gene-disease association, predicted variant consequence type, population frequency and variant segregation with the phenotype in the family. Here, we investigate the performance of tiering.

Methods: We analysed 938 small variants that were classified as pathogenic or likely pathogenic, and considered diagnostic by NHS Genome Medicine Centres, but not prioritised by Tiering at the time of the first interpretation. We identified the reasons these variants were not prioritised.

Results: The most frequent reasons diagnostic variants were not prioritised were:

No gene/disease association data known at time of analysis (43%).
Observed variant segregation with phenotypes in a family did not match mendelian patterns associated with the disease (21%).

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Complexities, such as skewed X inactivation, mosaicism, assumed complete variant penetrance or phenocopies, may account for some segregation errors. Sensitivity can be improved by discarding all information from family members (recovered 63 out of 191 variants) or allowing reduced penetrance, assuming family members may have the diagnostic variant but not be affected (recovered 54 out of 191 variants). Such gains are at the cost of specificity, but given the impact on diagnostic yield this needs to be explored further.

Conclusion: Known gene-disease association data poses the largest challenge for automated variant prioritisation. We show that accommodating complex inheritance patterns with automated variant prioritisation pipelines is particularly challenging.

References:.

Grants:.

Conflict of Interest: Kevin Savage Employed by Genomics England, Susan Walker Employed by Genomics England, Katherine Smith Employed by Genomics England, Antonio Rueda Employed by Genomics England, Javier Lopez Employed by Genomics England, Liam Abrahams Employed by Genomics England, Emma Baple: None declared, Smedley Damien: None declared, Ellen Thomas Employed by Genomics England, Matt Brown Employed by Genomics England, Richard Scott Employed by Genomics England, Augusto Rendon Employed by Genomics England, Rachael Mein: None declared, Zandra Deans: None declared, Sue Hill: None declared, Mark Caulfield Employed by Genomics England, Dalia Kasperaviciute Employed by Genomics England.

P17.015.A Prostate-enriched circulating cells from semen as a source for prostate cancer liquid biopsy: setting and challenges

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Background/Objectives: Prostate cancer (PCa) diagnosis relies on prostate-specific antigen and invasive trans-rectal/perineal biopsy, with a significant rate of false negatives and complications. Hence, there is a growing interest in the development of novel approaches for non-invasive PCa detection. We implemented a protocol to enrich prostate-derived cells from seminal fluid (SF) and urine, to test the hypothesis that liquid biopsy (LB) of SF can be used for PCa diagnosis/prognosis.

Methods: The study cohort included 86 PCa patients scheduled for radical prostatectomy. The day before surgery, we collected a sample of SF, post-ejaculation urine, and peripheral blood from each patient. For 43 patients, PCa specimen was also obtained. After centrifugation, prostate-derived cells were enriched from SF and urine by FACS with the following markers: 7AAD+ (cell viability), Syto-16+ (nuclear staining), CD45- (leucocyte antigen), PSMA+ (prostate-specific antigen), EpCAM+ (epithelial marker).

Results: We obtained on average ≈67,000 and ≈23,000 prostate-enriched cells from semen and urine, respectively. No correlation between the number of collected cells and Gleason Score was observed. Although a critical point of implementation concerns the quantity/quality of nucleic acids (RNA, DNA) obtainable from these cells, we were able to confirm the presence of PCa-specific lesions (TMPRSS2-ERG fusion and a somatic mutation in the SPOP gene) in CTCs from SF and urine, respectively.

Conclusion: The number of prostate-enriched cells in semen/urine alone cannot be used for non-invasive PCa grading. A molecular analysis of the obtained cells could be implemented to identify tumor markers for PCa diagnosis.

References:.

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Conflict of Interest: None declared.

P17.016.B A tailored stepwise strategy for improving diagnostic yield in intellectual disability and developmental disorders

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Background/Objectives: Intellectual disability (ID) / developmental disorders (DD) describe a clinical and genetic heterogeneous group of conditions. Herein, a molecular diagnostic strategy for ID/DD will be illustrated using a singular cohort of patients.

Methods: Exome (ES) and target sequencing were applied in the investigation of a diverse cohort of ID/DD patients with putative autosomal recessive inheritance and a previous normal genetic investigation, namely Fragile-X, conventional karyotype, and chromosomal microarray analysis.

Results: The investigational odyssey of our cohort suggests that (i) targeted sequencing is effective in clinically recognizable forms of ID, e.g. X-linked Optiz G/BBB syndrome; (ii) ES contributes to disclose genetic contribution of complex phenotypes, e.g. in a patient with Usher syndrome and muscle complaints iii) deep phenotyping is important, e.g. in the synergic contribution of heterozygous variants in genes linked to DNA repair, (iv) CNVs and genomic rearrangements can be unnoticed by ES, which we will explain by a genomic rearrangement in SNX14, and (v) interlaboratory collaborations are key for the identification of new ID/DD gene(s) by using MAN2C1 as an example.

Conclusion: According to some authors ES should be used as first-tier test in ID/DD. Despite this trend, the definition of a diagnostic strategy is still difficult even in case with a clear inheritance pattern. To define the best strategy for each patient, communication between medical and laboratory communities is fundamental. In the absence of well-defined guidelines, we propose a stepwise tailored approach for molecular diagnosis, which should be periodically reviewed.

References:.

Grants: UIDB/00215/2020, UIDP/00215/2020) and LA/P/0064/2020.

Conflict of Interest: None declared.

P17.017.C Analytical validation of gene coverages for Newborns Whole Genome Sequencing Pilot programme

Dasha Deen[1], *Susan Walker*[1], *Gabriel Adams*[1], *Alex Stuckey*[1], *Javier Lopez*[1], *Alice Tuff-Lacy*[1], *Matt Brown*[1], *Richard Scott*[1], *Augusto Rendon*[1], *David Bick*[1], *Dalia Kasperavici*[1], *Genomics England Research Consortium*[1]

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Background/Objectives: The Newborn Whole Genome Sequencing (WGS) programme is an NHS-embedded research pilot in the UK that aims (1) to evaluate the feasibility, utility and impact on the NHS of screening for childhood-onset rare actionable genetic conditions; (2) understand how, with consent, genomic and healthcare data could be used to enable research to develop new diagnostics and treatments; and (3) explore the implications of storing an individual's genome for use over their lifetime. Here we present an approach for analytical validation of the coverages of genes for childhood-onset rare actionable conditions in short-read WGS data.

Methods: Statistical modelling was used to define the required coverage. Then the empirical distributions of the coverage for coding regions of 689 genes for rare treatable conditions[1] for SNP and CNV variant calling were assessed in 1,000 test WGS samples sequenced at average depth of 50X.

Results: We defined low coverage positions as the positions that are expected to be covered with less than 16 reads in more than 5% of the samples. We classified the regions of systematic low coverage into three categories: (1) inherently prone to undersequencing; (2) low coverage due to reads with poor base quality and (3) low coverage due to the presence of homology regions.

Conclusion: Our work provides a basis to inform decisions about technology, pipeline and gene selection for Newborn WGS screening. It will also support monitoring of performance and tailoring of approaches to enhance fidelity in error-prone regions by bespoke solutions, reference masking or blacklisting.

References: Bick et al. Am.J.Med.Genet.C.Semin.Med.Genet 187(1):48-54(2021).

Grants:.

Conflict of Interest: Dasha Deen Full time employee of Genomics England, Susan Walker Full time employee of Genomics England, Gabriel Adams Full time employee of Genomics England, Alex Stuckey Full time employee of Genomics England, Javier Lopez Full time employee of Genomics England, Alice Tuff-Lacy Part-time employee of Genomics England, Matt Brown Full time employee of Genomics England, Richard Scott Full time employee of Genomics England, Augusto Rendon Full time employee of Genomics England, David Bick Full time employee of Genomics England, Dalia Kasperavici Part-time employee of Genomics England, Genomics England Research Consortium Full time employees of Genomics England.

P17.018.D Multiplex Ligation-Dependent Probe Amplification (MLPA) is a powerful tool for screening of different CNVs and loci methylation status of various genetic conditions: 7 years' experience

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Background/Objectives: Multiplex ligation-dependent probe amplification is a rapid, reliable, robust and cost-efficient techniques to screen the clinically relevant aberrations in routine diagnostics. It is a multiplex polymerase chain reaction-based method

that can detect changes in the gene copy number status, DNA methylation, and point mutations simultaneously. MLPA is an efficient tool to analyze genomic copy number aberrations at 55-60 different genomic loci. MLPA allows the profiling of prognostic and predictive markers.

Methods: Here, we are going to explore the MLPA results of more than 1500 sample form various genetic conditions such as intellectual disabilities/multiple congenital anomalies (ID/MGA), autism spectrum disorders (ASD), short stature (SS), breast cancer, chronic lymphocytic leukemia (CLL), retinoblastoma (RB), imprinting diseases and prenatal screening for aneuploidies.

Results: MLPA positive results were detected in 3 (22.9%) out of 144 ID/MGA patients, in 7 (17.5%) out of 40 ASD patients, in 11 (22%) out of 50 SS children, in 12 out of 72 RB patients (16.67%). Moreover, MLPA detected 14 foetuses having trisomy out of 48 amniotic fluid samples in addition of their sex determination. Furthermore, MLPA detected 26 genomic abnormalities in 30 CLL patients. There are other interesting results.

Conclusion: Our conclusion about this technology that it has considerable advantages in that it is highly versatile in its applications, malleable in its target loci, highly automated, appropriate for high-throughput testing, competent, and cost effective.

References:.

Grants:.

Conflict of Interest: None declared.

P17.019.A Efficiency and clinical utility of trio-based whole exome sequencing in patients with suspected rare mendelian disorders

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Background/Objectives: Rare genetic diseases are a major cause for severe illnesses in children. Whole exome sequencing (WES) is a powerful tool for identifying the underlying genetic cause. A simultaneous examination of the patient and his/her parents (tWES) has been proven to be a time saving method. To shorten the process of identifying the correct genetic diagnosis is of utmost importance for precise, evidence-based treatment decisions.

Methods: We assessed diagnostic rate and clinical utility of tWES in 224 children with a suspected genetic condition in the German in- and out-patient public healthcare system.

Results: tWES provided a diagnosis in 63 (28%) of all analysed children and in 18 (35%) children treated in intensive care units (ICUs). Due to vigorous improvements in sample processing and data analysis the evaluation time of tWES data has been reduced significantly during the last three years from 41 days in 2019, 34 days in 2020 to 23 days in 2021. Several genes could be identified to be causative, three of them (*GAD1*, *TMEM222* and *ZNFX1*) were previously not associated with a clinical condition. It is worth emphasizing that 15% of children with a genetic diagnosis had larger copy number variations (CNV) that were detected via WES.

Conclusion: tWES has been proven to have a high diagnostic yield in critically ill children. It has a high impact for classification of de novo or compound heterozygous variants and therefore on management and family counselling. We recommend initiating tWES as early as possible, especially or children on ICU.

References: /.

Grants: /.

Conflict of Interest: None declared.

P17.020.B DeepVariant as a variant caller to diagnose rare diseases

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Background/Objectives: GATK best practices workflow is widely used to identify variants to diagnose rare diseases, but studies have shown that DeepVariant (DV) can generate more accurate variant calls. DV also generates biologically relevant genotype quality (GQ) scores, a parameter used to evaluate the quality of the variants. The primary goal of this study was to assess DV's performance against that of GATK in a clinical diagnostics pipeline. Additionally, we have also attempted to understand the relationship between genotype quality (GQ) scores from the two variant callers.

Methods: In this study, we used 2 cases; one is a trio of Ashkenazi Jewish ancestry from NIST reference sample collection that was used to evaluate precision and sensitivity metrics of the pipeline, and one is a synthetic dataset spiked with data from 503 known disease-causative SNVs which we used to assess recall rate on clinical samples and understand the effect of changing the variant caller on GQ scores.

Results: We observed that DV outperformed GATK in terms of both precision (99.92% vs. 98.88%) and sensitivity (99.84% vs. 99.41%) for variants in the high-confidence regions. All 503 SNVs were correctly recalled, and approximately 90% of the SNPs called by GATK had a GQ score of >90, whereas SNVs from DV had a relatively better spread with 90% of the variants between 30 and 60.

Conclusion: Our results show that switching from GATK to DV for variant calling can improve the overall quality of variant calls.

References:.

Grants:.

Conflict of Interest: None declared.

P17.021.C Development of an amplicon-based NGS test for evaluating MLH1 promoter methylation

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Background/Objectives: Detecting loss of expression of DNA mismatch repair (MMR) proteins is highly relevant to identify Lynch syndrome patients. However, *MLH1* inactivation due to promoter hypermethylation occurs in 15% of sporadic colorectal cancers (CRCs), and it is correlated with *BRAF* somatic mutations. Here, we aimed to develop a diagnostic test for assessing *MLH1* promoter methylation based in next generation sequencing (NGS), and to evaluate the concordance of *MLH1* methylation and *BRAF*-V600 mutation status in CRC.

Methods: For *MLH1* methylation analysis DNA from FFPE tumors and saliva was treated with bisulfite, amplified by PCR and evaluated by amplicon sequencing on Ion Proton platform. Sequences were analyzed in CLC Genomics Workbench to evaluate the frequencies of cytosine/thymine bases of four CpGs.

Results: The average percentage of *MLH1* methylation was 2.49% in 20 saliva samples, 2.1% in 6 tumors with MSH2/MSH6 loss and 29.6% (ranging from 1.0% to 91.3%) in 20 tumors with MLH1/PMS2 loss. We confirmed the reproducibility and accuracy of *MLH1* promoter analysis performing a serial dilution experiment with completely methylated and unmethylated control DNAs. For MLH1/PMS2 deficient tumors, the *MLH1* methylation status was concordant with the *BRAF* mutation status in 90% (18/20) of the cases.

Conclusion: Our amplicon based NGS test showed a great sensitivity and specificity for detecting *MLH1* methylation in CRC samples, with a high agreement with the evaluation of *BRAF* mutation. This simple and affordable test could be used as a reflex test to identify patients with sporadic causes of MLH1/PMS2 deficiency in CRC and endometrial cancer.

References:.

Grants:.

Conflict of Interest: None declared.

P17.022.D Automated decision support for the clinical interpretation of copy number variants

Tomáš Sládeček[1;2], *Michaela Gažiová*[1;3], *Marcel Kucharik*[1;2], *Zuzana Pös*[1;4;5], *Ondrej Pos*[1;2;4], *Werner Krampl*[1;2;4], *Rastislav Hekel*[1;4;6], *Ján Radvánszky*[1;4;5], *Jaroslav Budiš*[1;2;6], *Tomas Szemes*[1;2;4]

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Background/Objectives: Clinical interpretation of copy number variants (CNVs) is a complex process that often leads to different decisions between diagnostic centers. In order to alleviate this obstacle, technical standards for the interpretation and reporting of variants have been developed, called ACMG guidelines. Several semiautomatic computational methods have been proposed to recommend appropriate choices. Alternately, the emerging machine learning based tools showed promising ways of even fully automated predictions.

Methods: We evaluated state-of-the-art computational prediction tools on CNV records collected from the ClinVar database. We compared their accuracy and showed the superior accuracy of their combination. We also tweaked the key parameters and underlying data sources to demonstrate their impact on the overall prediction.

Results: We demonstrate that the choice of underlying data sources significantly affects the prediction of CNV pathogenicity. We also show how automated prediction tools, especially their combination, further improve the evaluation of ACMG guidelines and thus lead to more reliable clinical decision support for clinicians.

Conclusion: Modern clinical decision support tools for the clinical interpretation of CNVs are able to provide valuable guidance to clinicians, relieving them of a great share of tedious annotation and interpretation processes.

References.

Gažiová, M., et al. "Automated prediction of the clinical impact of structural copy number variations." Scientific reports 12.1 (2022): 1-14.

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P17.023.A Completing MANE Select, A Joint NCBI and EMBL-EBI Transcript Set for Clinical Genomics and Research

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Background/Objectives: Understanding the impact of clinically relevant variants requires comprehensive gene annotation. However, the historic lack of a standard for clinical reporting complicates the process of consistent interpretation and reporting.

Methods: Ensembl/GENCODE and RefSeq launched the Matched Annotation from NCBI and EMBL-EBI (MANE) collaboration to converge on human gene and transcript annotation and jointly define a high-value set of transcripts and corresponding proteins for use as a universal standard for variant reporting. Each MANE transcript represents an exact match between the exonic sequence of an Ensembl/GENCODE transcript and its counterpart in RefSeq, such that the identifiers can be used synonymously. The MANE Select set identifies a representative transcript for each human protein-coding gene. The MANE Plus Clinical set provides additional transcripts at loci where the Select alone is not sufficient to report all currently known clinical variants.

Results: With MANE release 1.0 we have now released MANE Select transcripts for 99.7% of human protein-coding genes, including all ACMG SF v3.03 genes.

Conclusion: MANE transcripts are accessible from major genome browsers and key resources. Widespread adoption will increase consistency of reporting, facilitate exchange of data regardless of annotation source, and help streamline clinical interpretation.

References:.

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Conflict of Interest: None declared.

P17.024.B Detection of Turner Syndrome in newborn screening: validation of a fragment-analysis based approach

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Background/Objectives: Turner syndrome (TS) affects one in 1,900 newborn girls. Late diagnosis impairs a timely treatment and limits improvement of the outcome. Various experts have been advocating for including TS in newborn screening (NBS) programs (1). Existing methods are not suitable for use in NBS, partly because they fail to work with material from dried blood spots (DBS), fail to detect mosaics or because of elevated costs. Our objective was to overcome these constraints and develop a screening method suitable for public NBS programs.

Methods: We defined 6 STR and 3 SD (segmented duplication) markers, all mapping to the X chromosome, the latter additionally mapping to chromosomes Y, 3 and 16, respectively. The distribution of the STR markers along the X chromosome allows to detect aneuploidies of both chromosomal arms separately. The SD markers allow to compare the copy number of the X chromosome with that of the Y chromosome and the autosomes. Fragments were amplified by multiplex-PCR and separated by capillary electrophoresis. 20 TS patients, confirmed by karyotyping were analyzed and compared against 60 controls. The capability to distinguish 46,XX from discrepant karyotypes was evaluated applying statistics.

Results: All 60 controls and 20 TS cases were correctly identified from DBS, gDNA from whole blood and buccal smears, respectively.

Conclusion: Our SD-STR-QF-Multiplex-PCR system allows to screen for TS, including mosaics and is in line with requirements for NBS programs concerning tissue, ease of use, costs, turn-around-time and scalability.

References: (1) Gravholt, CH, et al: Eur J Endocrinol 2017;177:G1-G70.

Grants:.

Conflict of Interest: Ruth Luschka: None declared, Ivonne Bedei: None declared, Johannes Becker-Follmann Employee of Eluthia, Axel Weber: None declared, Zeynep Agirman Employee of Eluthia, Tarrin Khairi-Taraki Employee of Eluthia, Owns stocks in Eluthia, Ramon Enriquez Schaefer Employee of Eluthia, Owns stock in Eluthia, Roland Axt-Fliedner: None declared.

P17.026.D Sequencing and Commercial Intelligent Ratio based Real-Time PCR Outperform Multiplex Ligation-Dependent Probe Amplification in Prenatal Diagnosis for Spinal Muscular Atrophy

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Background/Objectives: Spinal muscular atrophy (SMA) is a rare, recessively inherited neurodegenerative disorder arising from deterioration of motor neurons in the anterior horn of the spinal cord that in turn results in muscle weakness and atrophy. SMA is mostly caused by deletion or point mutations in the *SMN1* gene. As current therapies are only applicable to patients below two years of age and are expensive, screening tests are of high importance for prevention and early detection of cases. Currently, multiple ligation probe analysis (MLPA) is often considered as the gold standard method for diagnosis, providing an easy and high throughput system for analysing the critical regions in affected patients and carriers.

Methods: Here we report the case of an SMA type I patient with a novel *SMN1* mutation that was misdiagnosed in prenatal screening. She was admitted to the hospital with symptoms of muscle weakness recognised from birth on. The diminished movements were mostly recognised on her hands and arms. Further genetic analysis was performed with next generation sequencing and by SNP Biotechnology SMA Real-Time PCR Detection Kit.

Results: NGS revealed the novel heterozygous *SMN1* c.835_5_835delTCCTTinsTG(IVS7-5_IVS7-9delTCCTTinsTG) mutation in combination with heterozygous *SMN1* exon 7-8 deletion. The novel mutation could also be detected by SNP Biotechnology SMA Real-Time PCR Detection Kit.

Conclusion: In this context we conclude that the use of MLPA analysis may not be the gold standard in SMA screening as less frequent mutations or mutations in the ligand binding regions can be missed.

References:.

Grants:.

Conflict of Interest: None declared.

P18 BIOINFORMATICS, MACHINE LEARNING AND STATISTICAL METHODS

P18.001.A GestaltMatcher 2.0: Open access database and open source software toolkit for facial phenotype descriptors

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Background/Objectives: Many next-generation phenotyping approaches, such as GestaltMatcher and DeepGestalt, have shown that they can assist in finding a diagnosis for individuals with rare genetic disorders. However, most of these tools are based on deep convolutional neural networks that were developed for a broad range of object identification. We aimed to improve the performance by using recent network architectures for facial images, more data augmentation, and ensemble models. Besides, we introduce GestaltMatcher Database (GMDB) to the research community which can be used to train and compare further deep learning models.

Methods: We utilized 4750 frontal face photos of 350 different disorders in GMDB for training and benchmarking. We first replaced the old architecture with a recent one that performs better on face recognition. Afterward, we improved the base model's performance for transfer learning and addressed the data scarcity when training the new GestaltMatcher model. Lastly, we applied test time augmentation and model ensembles to maximize the robustness and accuracy of our model.

Results: We first benchmarked each proposed change and showed that they improve the diagnostic performance on the frequent and rare subsets of GMDB. Moreover, we compared the performance between the old model, the new model, and

an ensemble of models. The modernized models significantly improved the performance on both subsets compared to the old model's performance on GMDB by up to 45%.

Conclusion: In Conclusion GestaltMatcher 2.0 is not only a significant update with respect to performance, but it is the first open source effort for next-generation phenotyping and FAIR data use.

References:.

Grants:.

Conflict of Interest: Alexander Hustinx: None declared, Behnam Javanmardi: None declared, Peter Krawitz Modest; FDNA, Tzung-Chien Hsieh: None declared.

P18.002.B Scalable integration of multiomic single-cell data using generative adversarial networks

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Background/Objectives: The amount of single-cell datasets, their size and the diverse modalities they describe is continuously increasing, prompting the need to develop robust methods to integrate multiomic datasets, whether paired from the same cells or, most challenging, from unpaired separate experiments. Most methods allow the integration of a limited number of omics and make assumptions about the relationships between them. We introduce a deep-learning model to integrate multimodal data supporting high number of modalities and agnostic about their relationships.

Methods: We prototyped our approach on public data for which paired and unpaired experiments exist. Each modality is embedded into feature spaces with same dimensionality across all modalities. The embeddings are used to train a Wasserstein generative adversarial network to understand the couplings between multiple modalities. The output is integrated with the original data and can be used to bridge information across omics.

Results: We introduce "informative training" so that mini-batches include locally similar cells. This paradigm increases the performances in coupling while keeping global structure invariant. Performance of label transfer between unpaired data using learned couplings is comparable to performance achieved when paired data are used. We tested our approach including up to five modalities.

Conclusion: We propose an approach for coupling unpaired multimodal data, ready for the large diversity of single cell technologies available. Our method doesn't make assumptions on crosstalk among modalities nor needs anchors to match them. Moreover, it can be easily extended to an unlimited number of omics.

References:.

Grants:.

Conflict of Interest: Valentina Giansanti: None declared, Marco Antoniotti Università degli Studi di Milano-Bicocca Milan, Italy, CRUK Accelerator Award #22790, Single Cell Cancer Evolution in the Clinic, coPI. Fondo Ateneo Quota Competitiva, Università degli Studi di Milano-Bicocca., Davide Cittaro Ospedale San Raffaele (employer).

P18.003.C Converting single nucleotide variants between genome builds: from cautionary tale to solution

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Background/Objectives: Next-generation sequencing studies are dependent on a high-quality reference genome for single nucleotide variant (SNV) calling. Position information is not directly comparable between builds, so tools such as liftOver and CrossMap are used to convert data from one build to another. However, the positions of converted SNVs do not always match SNVs derived from aligned data, and in some instances, SNVs are known to change chromosome when converted.

Methods: We describe a novel algorithm to identify positions that are unstable when converting between human genome reference builds GRCh37 and GRCh38. We also examine the overlap between these unstable positions and various annotation sets in the genome to explain the source of the instability. Finally, we apply this method to sequencing data to benchmark the accuracy of the converted data with aligned data.

Results: We identified 11.3Mbp of unstable positions on GRCh37 and 20Mbp on GRCh38, all of which are determined by the chain file and independent of the conversion tool. The majority of these positions overlap segmental duplications and/or assembly updates between builds. Pre-excluding SNVs at these positions prior to conversion results in SNVs that are stable to conversion. SNVs at unstable positions had worse accuracy metrics than SNVs at stable positions.

Conclusion: We have identified all positions which are unstable to conversion between GRCh37 and GRCh38 and provided a simple framework to remove these instabilities. This work highlights the care that must be taken when converting SNVs between genome builds.

References: Ormond et al., *Brief Bioinform*, 2021.

Grants: NIH [5U01MH-109499-04] and SFI [16/SPP/3324].

Conflict of Interest: None declared.

P18.004.D Distribution analysis of missense variants in the human genome reveals widespread gene-specific clustering patterns and improves prediction of pathogenicity

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Background/Objectives: Identifying variants that underlie Mendelian phenotypes and cancer represents a major challenge in genetic medicine. However, current criteria still leave many rare missense variants classified as "variants of uncertain significance" (VUS). Many in silico tools have been developed to help with this problem. However, existing tools did not take into account that mutations appear to cluster within specific regions.

Methods: In this work, we address the question of within-gene distribution of pathogenic and benign missense variants, discover significant clustering across the whole coding genome, and use this information to build a positional score. We used a machine learning approach to analyze the within-gene distribution of missense variants observed in hereditary conditions and cancer.

Results: When applied to 840 genes from the ClinVar database, this approach detected a significant non-random distribution of

pathogenic and benign variants in 387 (46%) and 172 (20%) of them, respectively, revealing that variant clustering is widespread. We then developed a pathogenicity predictor, MutScore, that integrates qualitative features of DNA substitutions with the new additional information derived from this positional clustering. Using a random forest approach, MutScore was able to identify pathogenic missense mutations with very high accuracy, outperforming existing predictive tools.

Conclusion: Within-gene clustering of pathogenic and benign DNA changes is an important and previously underappreciated feature of the human exome, which can be harnessed to improve the prediction of pathogenicity and disambiguation of VUS. Website: https://iob-genetic.shinyapps.io/mutscore/.

References:.

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P18.005.A Component-wise L2-boosting for polygenic risk scores based on large cohort data

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Background/Objectives: Polygenic risk scores (PRS) evaluate the individual genetic liability to a certain trait and play an increasingly important role in the field of risk stratification. Most often, PRS models are based on summary statistics of univariate effects derived from genome-wide association studies. To improve the prediction performance of PRS, it is desirable to fit multivariable models directly on the genotypic data. Due to the large and high-dimensional data, efficient algorithms have to be developed to overcome the computational burden.

Methods: We implemented a component-wise L2-boosting algorithm to fit genotyped data from large cohort studies to continuous outcomes using the genotypic variants as linear base-learners. Similar to the snpnet approach for the lasso we iteratively work on smaller batches of variants.

Results: By restricting the set of possible base-learners in a data-driven way in each boosting step, we can increase the computational efficiency without losing prediction accuracy. Furthermore, we show both in a simulation study and via different traits from the UK biobank data, that our method yields competitive results in comparison to other methods such as the lasso.

Conclusion: We introduced a L2-boosting algorithm to effectively derive sparse PRS directly from genotypic data. Due to the modular structure of boosting, the method can be extended to construct PRS for different outcomes such as binary or time-to-event data.

References: Qian J et al. (2020) A fast and scalable framework for large-scale and ultrahigh-dimensional sparse regression with application to the UK Biobank. PLoS 16(10): e1009141. https://doi.org/10.1371/journal.pgen.1009141.

Grants:.

Conflict of Interest: None declared.

P18.007.C Investigating microRNA-associated variation in Multiple Sclerosis

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Background/Objectives: Genome-wide association studies (GWAS) have highlighted over 200 autosomal variants associated with Multiple Sclerosis (MS). However, variants in non-coding regions such as those encoding microRNAs have not been explored adequately, despite strong evidence of microRNA dysregulation in MS patients and model organisms. This work explores microRNA associated variants within MS GWAS studies.

Methods: SNPs within the coordinates of microRNA mature, precursor and flanking sequences and within 3'UTR microRNA-binding sites were collated from six open-access catalogues. These microRNA-associated SNPs were intersected with the 200 susceptibility SNPs and summary statistics from the most recent MS GWAS.

TargetScan7.0, a target-prediction algorithm was used to calculate potential miRNA loss and gain for each candidate SNP.

Results: No MS susceptibility SNPs were identified in microRNA precursor or mature regions directly. However, we identified five susceptibility SNPs among the 34,705 collated 3'UTR binding-site SNPs. Two of the five variants were predicted to change miRNA-binding activity. One variant, rs6742 was associated with the gain of 6 miRNAs and loss of 3 miRNAs. This SNP is an eQTL for SLC2A4RG. Functional validation will be carried out to study the effect of rs6742 on candidate miRNA-interaction, while other MS associated miRNA-SNPs will be assessed by statistical and functional finemapping.

Conclusion: Altogether, we have shown the utility of exploring miRNA variation within MS GWAS data and are generating a novel bioinformatics pipeline for investigating microRNA-associated variants through MS GWAS summary statistics. This pipeline could be useful for interrogating miRNA SNPs in other disease indications.

References: Patsopoulos et al, Science, 365(6460). https://doi.org/10.1126/science.aav7188.

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Conflict of Interest: None declared.

P18.008.D Using GCPBayes to explore pleiotropy at gene-level between breast and ovarian cancers based on GWAS summary statistics data

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Background/Objectives: Cross-phenotype association at gene- or pathway-level can help to detect pleiotropic genes, i.e. the fact that one gene can affect multiple traits, and inform about common mechanisms between diseases. However, the lack of proper pipelines to apply gene-set analysis in this context using GWAS data in a reasonable running time could prevent researchers to apply such methods.

Methods: We designed a user-friendly pipeline to perform cross phenotype association using GCPBayes method [1], a cross-phenotype gene-based method developed by our team. We illustrated the application on publicly available GWAS summary statistics on breast cancer (BC) and ovarian cancer (OC) from BCAC and OCAC consortia and compared the results with previous studies used SNP-level analysis or transcriptome data.

Results: Previous studies suggested 40 pleiotropic genes but only one (RCCD1) was replicated by two studies. Our method retrieved seven of these genes: TERT, BABAM1, CPNE1, RGS19, SMC2, CLIC6, and RCCD1. Besides, we also detected additional 140 new genes with potential pleiotropic signals for BC and OC. However, we are working on a suitable way to narrow our large list of candidate genes for further experimental analyses.

Conclusion: Our method replicated some genes previously found associated to both BC and OC using gene-set rather than SNP-level approach. User-friendly tutorials are available on our group's GitHub page.

References: 1. Baghfalaki et al. 2021, Stat. Med., 40, 1498–1518.

Grants: This work was supported by 'Ligue contre le Cancer', INSERM Cancer, INSERM Cross-Cutting Project GOLD.

Conflict of Interest: None declared.

P18.009.A GenOtoScope: Towards automated ACMG classification of genetic variants associated with congenital hearing loss

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Background/Objectives: Consistent interpretation of a large number of genomic variants is a key challenge of today's clinical genetics. This is particularly relevant for hearing loss (HL). HL is the most common sensory disorder with a vast genetic heterogeneity. Variant assessment can be highly standardized using the "Expert Specification of ACMG/AMP Variant Interpretation Guidelines for Genetic Hearing Loss". However, manual analyses remain time-consuming and prone to inconsistent interpretation and could, therefore, massively benefit from automated variant (pre-)assessment.

Methods: GenOtoScope is an open-source bioinformatics tool written in Python programming language. Two types of interfaces are provided: a freely accessible website to classify single variants, and a command line application capable of processing large variant sets (e.g. full WES data sets).

GenOtoScope automates all 12 ACMG/AMP criteria that can be assessed without further individual patient information or human curator investigations. We benchmarked the performance against two other variant classification tools using two manually curated HL data sets: ClinGen expert clinical validity curation of 164 hearing loss gene-disease pairs (158 variants in 9 genes) and a local data set from Hannover Medical School's Department of Human Genetics (118 variants in 36 genes).

Results: GenOtoScope achieved the best average accuracy and precision for both data sets: Compared to the second best tool, GenOtoScope improved accuracy metrics by 25.75% and 4.57% and precision metrics of 52.11% and 12.13% on the two data sets, respectively.

Conclusion: GenOtoScope has proven capable of standardizing the process and significantly reducing the time of variant assessment and interpretation for HL.

References:.

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Conflict of Interest: Christian Landgraf Volkswagen Foundation (Understanding Cochlear Implant Outcome Variability using Big Data and Machine Learning Approaches), Damianos Melidis Volkswagen Foundation (Understanding Cochlear Implant Outcome Variability using Big Data and Machine Learning Approaches), Gunnar Schmidt: None declared, Anja Schöner-Heinisch: None declared, Sandra Von Hardenberg: None declared, Alisa Förster: None declared, Anna-Lena Katzke: None declared, Anke Lesinski-Schiedat: None declared, Wolfgang Nejdl: None declared, Bernd Auber: None declared.

P18.010.B Using the central dogma to quantify the precision and recall of colocalisation methods

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Background/Objectives: Colocalisation is a fundamental approach for identifying candidate genes that causally mediate complex trait associations. However, benchmarking different colocalisation methods is challenging due to potentially unrealistic assumptions in simulated data and a lack of ground truth about causal associations in real data. We overcame this problem by using cis gene expression and protein quantitative trait locus (QTL) data, assuming that the most likely causal gene for each cis-pQTL is the gene coding for the protein.

Methods: We compared three Bayesian colocalisation methods: coloc v3 and coloc v5 operating at the locus level; and CAVIAR's colocalisation posterior probability (CLPP) defined at the variant level. We used cis-eQTL data from the eQTL Catalogue (103 datasets) and blood plasma cis-pQTLs from the INTERVAL study.

Results: Using the same threshold (PP4 > 0.8), v5 detected 157 additional colocalisations (36% more) relative to v3 and missed only 29 colocalisations (7%) that v3 detected. Of the 851 proteins analysed, v5 found 441 to colocalise with the corresponding coding gene (52% recall), compared to 315 found by v3 (37% recall). However, v5 also detected colocalisations with additional genes, giving it slightly lower precision (32% vs 40%). Both versions of coloc detected more true positive signals than CLPP at its standard threshold (>0.1, 24% recall, 57% precision).

Conclusion: Our results indicate that using fine-mapping-based coloc v5 can significantly improve the yield of colocalisation analysis, but false positives introduced by co-regulation of neighboring genes should be carefully considered.

References:.

Grants: PSG415 "Inferring gene regulatory networks from large-scale gene expression and genotype datasets" (01.01.2020-31.12.2023).

Conflict of Interest: None declared.

P18.011.C A phenotype-gene based graph for symptoms description harmonization and clinically-driven genomic analysis

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Sophie Nambot[3], Cédric Le Maréchal[5], Jean-Francois Taly[6], Wilfrid Carre[7], Gaetan Lesca[4], Claire Bardel[4], Frederic Tran Mau Them[3], Marc Planes[5], Marie-Pierre Audrezet[5], Laure Raymond[6], Charles Coutton[8], Véronique Satre[8], Pauline Le Tanno[8], Mouna Barat-Houari[9], Marjolaine Willems[9], Thomas Guignard[9], Dimitri Larue[2], Denis Bertrand[2], Virginie Bernard[8], Marie De Tayrac[7], Sylvie Odent[7], Laurent Mesnard[10], Damien Sanlaville[4], Laurence Faivre[3], David Geneviève[9], Nicolas Philippe[2], Julien Thevenon[1;8]

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Background/Objectives: Identical symptoms observed in patients may heterogeneously be described by physicians, even though relying on the same Human Phenotype Ontology (HPO). Several tools explore the accuracy of generating diagnostic hypotheses based on HPO terms associations and vicinity in the ontology, although bearing common methodological limitations.

Methods: We build a phenotype-gene graph weighted by consensus of associations identified on both structured and free-text databases extracted by ElasticSearch. To manage the diversity of physicians' descriptions, dimensionality reduction of HPO terms was obtained through Non-Negative Matrix Factorization. Based on this graph, we developed a phenotype-gene matching algorithm called PhenoGenius. We evaluated our approach on a multicentric cohort of 316 patients recruited from a French consortium and 444 patients from literature.

Results: The graph presents more than 2 million phenotype-gene associations, covering 4,974 genes and 9,687 symptoms, whereas the Monarch database contains nearly 640,000 associations. PhenoGenius performances allow a median diagnostic gene rank of 68 (whereas others algorithms range from 144-355). Reducing 9,687 symptoms into 650 groups leads to the reduction of the diagnostic rank dispersion (reducing the standard deviation of 48%) without compromising the ranking performances. Focusing on 650 groups achieve complete coverage of the medical observations and expanded matchings to every medical observation, gaining 24 diagnostics.

Conclusion: This work explored a weighted phenotype-gene association graph, dissociated from the HPO developmental-based hierarchy used to describe patients' phenotypes. PhenoGenius presents an original method that harmonizes and maximizes the usage of clinical symptoms in bioinformatic processes, outperforming currently published approaches.

References:.

Grants:.

Conflict of Interest: Kevin Yauy SeqOne Genomics (part time), Nicolas Duforet-Frebourg SeqOne Genomics (full time), Quentin Testard: None declared, Sacha Beaumeunier SeqOne Genomics (full time), Yannis Duffourd: None declared, Jerome Audoux SeqOne Genomics (full time), Nicolas Chatron: None declared, Sophie Nambot: None declared, Cédric Le Maréchal: None declared, Jean-Francois Taly: None declared, Wilfrid Carre: None declared, Gaetan Lesca: None declared, Claire Bardel: None declared, Frederic Tran Mau Them: None declared, Marc Planes: None declared, Marie-Pierre Audrezet: None declared, Laure Raymond: None declared, Charles Coutton: None declared, Véronique Satre: None declared, Pauline Le Tanno: None declared, Mouna Barat-Houari: None declared, Marjolaine Willems: None declared, Thomas Guignard: None declared, Dimitri Larue SeqOne Genomics (full time), Denis Bertrand SeqOne Genomics (full time), Virginie Bernard: None declared, Marie De Tayrac: None declared, Sylvie Odent: None declared, Laurent Mesnard: None declared, Damien Sanlaville: None declared, Laurence Faivre: None declared, David Geneviève: None declared, Nicolas Philippe SeqOne Genomics (full time), Julien Thevenon: None declared.

P18.012.D Community data-driven approach for generating cross-ethnic population carrier screening panel

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Background/Objectives: The ACMG has recently published tier-based recommendations for carrier screening. While many pan-ethnic conditions are well established, some genes carry pathogenic founder variants (PFVs) prevalent only in specific ethnic groups, and may therefore not be assigned into the correct tier or not represented at all. Using an interconnected, data-driven repository including a cohort of cases from the Israeli population we created a workflow for assistance in generating a cross-ethnic carrier screening panel.

Methods: The dataset included de-identified WES data from a cohort of 2792 Israeli patients. Frequencies of pathogenic/likely pathogenic variants were calculated for each subpopulation and compared them with existing carrier screening panels used in Israel. Manual curation was done for suspected variants using evidence from Franklin community members.

Results: We detected a number of relevant PFVs not included in existing carrier screening panels. These included two variants that should be included in Tier 2 (carrier frequency >1:100) and nine variants for Tier 3 (1:100-1:200). We detected two PFVs of autosomal recessive traits, a PFV in PTPN23, associated with a severe neurodevelopmental disorder (carrier frequency 1:53) as well as a PFV in WFS1 associated with Wolfram syndrome (carrier frequency 1:68).

Conclusion: Community data-driven and sharing approaches may detect PFVs missing from currently available panels. Moreover, with the increasing use of next generation sequencing for carrier screening, it is expected to result in a deluge of variants falsely classified as P/LP. Such data-sharing approaches will facilitate proper classification of frequent variants that have no clinical implications, thereby reducing unnecessary workload and patient anxiety.

Conflict of Interest: Yaron Einhorn Genoox, Moshe Einhorn Genoox, yuval yaron Genoox, dror steinberg: None declared, Noa Henig: None declared, adi mory: None declared, lily bazak: None declared, erez tsur: None declared, Karin Weiss: None declared, Tamar Paperna: None declared, julia grinshpun-cohen: None declared, Amihood Singer: None declared, lina basel-salmon: None declared, Hagit Baris Feldman: None declared.

P18.013.A Benchmarking of long-read structural variant callers using in-house generated Oxford Nanopore data

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Background/Objectives: As long-read sequencing (LRS) technologies mature, several bioinformatics tools designed to identify structural variants (SVs) have been developed. To allow validation of these tools, Zook et al.[1] published a highly curated SV truth set of Genome in a Bottle sample NA24385, consisting of deletions and insertions. We performed a benchmarking analysis with five LRS SV callers against this set utilising in-house generated Oxford Nanopore reads.

Methods: SVs are called with cuteSV, SVIM, sniffles, pbsv, and nanovar. The callers are assessed in terms of resource usage, reproducibility, and calling performance. The latter is evaluated with Truvari giving recall and precision statistics on SV detection. We further investigate the influence of read support, sequencing coverage, SV type and length, and integration of call sets with Jasmine.

Results: CuteSV achieves overall best performance, while nanovar lags behind in both resource usage and calling statistics. A coverage greater than 20x offers no additional advantage for reliable SV detection, while the recommended read support of one third of the coverage proves to be too stringent. Integration of call sets with Jasmine should include three callers to compete with stand-alone call sets.

Conclusion: We propose a minimum coverage of at least 15x for optimal sensitivity and specificity. Read support should be set at one fifth of the coverage to obtain optimised calling performance. CuteSV performs best in both sensitivity and specificity, and resource usage. Further work is however needed to assess results for different SV types and more complex regions.

References: 1. Zook et al. Nat Biotechnol **38**, (2020).

Grants:.

Conflict of Interest: None declared.

P18.014.B DIVAs, a jump forward in the phenotype-driven digenic variants interpretation: a case study of skeletal dysplasia

Federica De Paoli[1], *Giovanna Nicora*[1], *Edoardo Errichiello*[2;3], *Mauro Lecca*[2], *Ivan Limongelli*[1], *Susanna Zucca*[1]

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Background/Objectives: An evolving view of inheritance pattern is rapidly gaining ground in the field of rare genetic diseases, since the paradigm "one gene, one disease" has shown limited diagnostic power. Here, we propose a method (DIVAs) to assess the pathogenicity of digenic variant combinations, considering patient's phenotypes.

Methods: DIVAs is a machine-learning (ML) model exploiting different features to describe each mutated digenic combination capturing gene-gene interaction, single variant impact, a priori genes properties and genes association to HPO-based phenotypes. Family analysis can also be included. The ML model was trained on almost 400 pathogenic digenic combinations and on a benign set of gene pairs, exploiting both public resources and an internal curated database.

Results: On an independent dataset, DIVAs showed promising performances (sensitivity 92%, specificity 99%). Among the large cohort of samples tested with DIVAs, here we focus on two fetuses (from independent pedigrees) with skeletal dysplasia. In both cases DIVAs unveiled a digenic inheritance model. In particular, the algorithm prioritized *DYNC2H1* as first gene, combined with *IFT140* and *TCTN1* in the first fetus, whereas with *IFT172* in the second one. In the first case, familial segregation analysis allowed refining the digenic combination between *DYNC2H1* and *TCTN1*, thus discarding *IFT140*. For *DYNC2H1-IFT172* combination segregation analysis is ongoing.

Conclusion: Our algorithm proved to be reliable to define a likely digenic inheritance in a large cohort of unsolved clinical WES cases with heterogeneous phenotypes, such as skeletal dysplasia, here adopted as paradigm of a typical monogenic condition.

References: PMID:26481352. PMID:26432245.

Grants:.

Conflict of Interest: Federica De Paoli enGenome, Giovanna Nicora enGenome, Edoardo Errichiello: None declared, Mauro Lecca: None declared, Ivan Limongelli enGenome, He has shares of enGenome, an italian bioinformatics company, spin-off of the university of Pavia., Susanna Zucca enGenome, She has shares of enGenome, an italian bioinformatics company, spin-off of the University of Pavia.

P18.015.C Ulisse: beyond the pathway boundaries towards cross-talk and cell-cell communication

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Background/Objectives: Functional analysis is typically based on testing the enrichment of a series of pathways, each defined as a gene set. However, at the molecular level, information flows by means of a complex net of biochemical processes in which pathways do not have clear boundaries, and are highly interconnected, within cells and between cells. We present Ulisse[1], a tool for the analysis of relations between intracellular pathways (pathway cross-talk, PCT) as well as cell-cell communication (CCC).

Methods: Statistical significance is assessed using a permutation-based approach. Molecular interactions: various sources (i.e.: STRING, iReflndex). Gene-pathway associations: MSigDB. Mutational data source: primary tumors from The Cancer Genome Atlas. Ligand-receptor interactions (LRI): Omnipath. Single cell expression data: Single Cell Expression Atlas[2], Single cell Portal[3].

Results: We used Ulisse to study the PCTs affected by the most frequently mutated genes in 10 major cancer types and found cancer-specific and recurring PCTs. We classified the genes involved in these PCTs considering their functional relevance, that is, the number of different PCTs in which a gene is involved in relation to its interactors. We used Ulisse to calculate the CCC using LRI and LR expression between cell types in each tumor sample considered.

Conclusion: Ulisse complements the typical pathway analysis assessing whether the studied genes take part in interactions between pathways. Ulisse can be applied to any gene list emerging from studies in genomics as well as other gene-centered "omics". It can also be applied to quantify cell-cell communication in single cell data.

References: [1] https://doi.org/10.7490/f1000research.1118917.1. [2] https://www.ebi.ac.uk/gxa/sc/home. [3] https://singlecell.broadinstitute.org/single_cell.

Grants:.

Conflict of Interest: None declared.

P18.016.D Improved detection of functionally relevant aberrant splicing with FRASER2

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Background/Objectives: As aberrant splicing is a major cause of rare diseases, detection of aberrantly spliced genes from RNA-seq data is an active research topic. We have recently developed FRASER[1], a denoising autoencoder-based method that outperformed alternative approaches for detection of aberrant splicing on precision-recall analyses. When systematically investigating FRASER results on more than 300 rare disease RNA-seq samples[2] and GTEx v8 samples, we noticed however that many of the detected events captured aberrant weak and cryptic splice site usage that did not result in substantial major isoform usage variation.

Methods: We introduced a new intron excision metric that integrates alternative donor, alternative acceptor and intron retention signal and, therefore, reflects variations in major isoform usage more closely. As with FRASER, we modelled this ratio using a beta-binomial based denoising autoencoder thereby controlling for potential confounding sources of variations.

Results: On a mitochondrial rare disease dataset[2] ($N = 303$), FRASER2 considerably reduced the number of splicing outliers (by 25% to 75%) for a mild loss of sensitivity (only 1 out 26 pathogenic cases not recovered). Moreover, FRASER2 splice outliers were 5 times more enriched for rare splice affecting variants. Also, FRASER2 is less sensitive to sequencing depth and benefits from a reduced multiple testing burden.

Conclusion: By introducing a new splicing metric, FRASER2 is able to maintain the sensitivity of FRASER while providing more functionally relevant outlier calls.

References: [1]Mertes, Scheller et al., Nat Commun (2021).
[2]Yépez, Gusic, et al., Genome Med (2022).

Conflict of Interest: None declared.

P18.017.A Identification of clinically relevant variants in homologous regions in 41,755 exomes

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Background/Objectives: Altogether, homologous regions comprise 3.5% of the exome but are difficult to analyse.

Methods: We devised a new method (Chameleolyser) that identifies single nucleotide variants (SNVs), copy number variants (CNVs) and gene conversion events in homologous regions based on exome sequencing data.

Results: Application to a cohort of 41,755 whole exome sequencing samples yielded 2,191,707 SNVs, 20,432 homozygous deletions and 22,600 homozygous gene conversions with ≤ 10% frequency in the cohort. Validation with PacBio high-fidelity genomes of variants identified in 20 samples confirmed 678/769 SNVs (88.2%), 8/8 gene conversions (100%) and 11/15 homozygous deletions (73%).

We then focused on variation that could potentially be disease-causing. Within the known OMIM disease genes we identified 1,182 homozygous deletions, 2,010 heterozygous loss-of-function (LoF) variants and 54 homozygous LoF variants of which 52 due to gene conversions. We used these variants to conduct a statistical comparison between specific patient groups and control samples. In the 3 top-ranked genes, *STRC*, *OTOA* and *SMN1*, we identified 33 homozygous truncating mutations due to gene conversions as well as 49 homozygous deletions. All of these events were exclusively found in patients with hearing impairment (*STRC* and *OTOA*) and spinal muscular atrophy (*SMN1*) and are causative for disease.

Conclusion: In conclusion, we developed a novel method that can accurately identify clinically relevant copy number and single nucleotide variations in homologous coding regions from diagnostic exome sequencing data.

References:.

Grants: Solve-RD (Horizon 2020 grant agreement No. 779257).

Conflict of Interest: None declared.

P18.018.B Meta-analysis of human retinal transcriptome data: a powerful tool to gain insight into the genomic organization of inherited retinal disease genes

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Background/Objectives: Eye diseases are among the most common inherited human retinal disorders (IRDs). Around 279 genes have been associated with the genetic diversity of IRDs. Over 30% of patients do not harbour mutations in coding regions of IRD genes. We aimed to gain insight into the genomic organization and transcript composition of IRDs genes using human retina RNA-Seq datasets.

Methods: We retrieved 177 bulk RNA-Seq human retina data from non-visually impaired post-mortem donors. RNA-Seq alignments were assembled at a single-sample level and merged to generate a set of assembled transcripts. Transcript expression was quantified by scaling TPM abundance estimates and filtered out transcripts with less than one median TPM. A subset of newly identified candidate transcripts was validated RT-PCR.

Results: We focused our analysis on 219 IRD genes and identified 3367 putative novel transcripts. The latter were the results of a) partial intron retentions, b) exon skipping and extension, c) novel exon and d) connections with other transcriptional units. RT-PCR analysis revealed an overall 50% rate of experimental validation.

Conclusion: To the best of our knowledge, this is the most comprehensive and extended meta-analysis of IRD genes carried out on RNA-Seq data. Our work yielded a reliable expression quantification of IRD transcripts in the human retina, including the identification of novel ones, and paves the way towards a better understanding of the organization of their transcriptional unit and, possibly, of the molecular mechanisms underlying inherited retinal diseases.

References: Pinelli et al., PMID:27235414.
Ratnapriya et al., PMID:30742112.

Grants: European Union, ITN Grant StarT (No. 813490).
Conflict of Interest: None declared.

P18.019.C Alignment-free method for gene copy number estimation from raw next generation sequencing data

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Background/Objectives: Copy variable genes in human genome are often associated with phenotypic traits and susceptibility to diseases. Methods for exact copy number estimation from sequencing data require mapping reads to the reference genome which is time-consuming and often prone to mistakes especially in complex repetitive regions. *K*-mers (small substrings of DNA with length *k*) have been successfully used for efficiently handling huge amounts of genomic data and could be used for fast alignment-free copy number estimation.

Methods: For an accurate copy number estimation, a list of *k*-mers is carefully composed for the gene of interest as well as a flanking single-copy reference region. *K*-mers are selected based on region specificity, uniqueness in the genome and GC content value. The frequencies of these *k*-mers are obtained from raw sequencing data and used for copy number estimation.

Results: The copy numbers have been estimated for amylase genes AMY1, AMY2A and AMY2B for 40 individuals from Estonian Biobank. The results showed high correlation ($R = 0.9932$) with the copy numbers measured experimentally using Droplet Digital PCR and the proportion of concordant results was 93.33%, these numbers were slightly lower when using a read-depth based approach ($R = 0.9925$ and 87.6%). The similar parity of AMY1 and AMY2A copy numbers (95%) corresponds to previous studies.

Conclusion: A *k*-mer based method is not only fast, but accurate approach for gene copy number estimation.

References:.

Grants: EU ERDF grant No. 2014-2020.4.01.15-0012, the cost of the sequencing was partly covered by the Broad Institute (MA, USA).

Conflict of Interest: None declared.

P18.020.D Performance of ACMG/AMP guidelines to tackle digenic variants interpretation: do we need a digenic interpreter?

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Background/Objectives: As monogenic hypothesis is sometimes insufficient to diagnose rare diseases, digenic models are increasingly evaluated. Yet, digenic variant interpretation (DVI) guidelines are still missing. We explore whether the monogenic ACMG/AMP standard guidelines can support interpretation of digenic pairs types (True Digenic (TD), Composite (CO), Dual Molecular Diagnosis (DMD)), highlighting potential utility and drawbacks.

Methods: 121 TDs, 85 COs and 78 DMDs, along with 10.000 benign pairs were collected and interpreted according to the ACMG/AMP guidelines using the eVai software. The eVai score, proportional to the number of triggered ACMG/AMP criteria, was used to develop a digenic classifier (ACMG-based DVI): if both variants' scores exceed a threshold, the pair is predicted pathogenic. We compared ACMG-based DVI against a machine learning (ML) approach tailored for DVI (DIVAs, enGenome).

Results: 58% of DMD are classified as Pathogenic/Likely-Pathogenic according to ACMG/AMP guidelines, 42% of CO are Pathogenic/Likely Pathogenic, 34% of TD are Pathogenic/Likely Pathogenic, and up to 15% of variants (in case of TD) are Benign/Likely Benign. The Area Under the Precision-Recall Curve (PRC) of the ACMG-based DVI is 30% for TD, 46% for CO and 78% for DMD. On the other hand, DIVAs shows 93% mean PRC on TD, 96% on CO and 95% on DMD.

Conclusion: ACMG/AMP guidelines show acceptable performance only for DMD classification. ML-based DVI outperforms the ACMG-based DVI in all categories. New approaches are required to expand variant interpretation towards more complex inheritance patterns, such as digenicity.

References: PMID28911095.
PMID30298955.

Grants:.

Conflict of Interest: Giovanna Nicora enGenome Srl, Federica De Paoli enGenome Srl, Susanna Zucca enGenome Srl, enGenome Srl, Ivan Limongelli enGenome Srl, enGenome Srl, Paolo Magni enGenome Srl, enGenome Srl.

P18.021.A Bioinformatic approach to the detection of genetic variants through next generation sequencing in genes and their pseudogene counterparts

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Background/Objectives: Current short-read NGS methods hinder the precise calling of clinically relevant variants in genes that have pseudogene counterparts. We aim at developing a bioinformatic method that, using Illumina short reads, allows to precisely map variants of interest in the gene CYP21A2 and its pseudogene, which is of great relevance in the diagnostic of congenital adrenal hyperplasia (CAH). We aim at designing a method broadly applicable to other gene/pseudogenes with minimal customization.

Methods: We develop proprietary bioinformatic methods that are capable of phasing long haplotypes from short-read Illumina data. The automated, basic steps of the method are: (i) identify all variant positions that discriminate a gene and its pseudogene/s; (ii) identify all possible haplotypes within the reads in a genomic window; (iii) merge overlapping reads with compatible haplotypes; (iv) compute copy number status for all haplotypes; (v) provide graphical/tabular results for interpretation.

Results: Although our method can identify variants across CYP21A2 here we focus on the clinically relevant, hard to map variant c.C955T, p.Q319X. We have produced results for 49 samples for which we have performed Sanger sequencing. For 10 samples we were not able to reach a clear conclusion of concordance (20%, 10/49). For the rest ($n = 39$), our software was in agreement with Sanger sequencing in 37 samples (94%) and for two samples (5%) we obtained discordant results.

Conclusion: Our haplotype phasing strategy using Illumina short reads has proven capable of precisely identifying when the clinically relevant variant c.C955T, p.Q319X (NM_000500) is present in the CYP21A2 gene or its pseudogene.

References:.

Grants:.

Conflict of Interest: Jairo Rodríguez qGenomics, Nacho Coca qGenomics.

P18.022.B IdeficSV – towards a clinician-friendly tool for the identification and clinical annotation of structural variants in whole-exome sequencing data

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Background/Objectives: Structural variants (SVs) constitute a core cause for genetic variation and may lead to genetic disease or cancer. It is generally assumed that SVs are detectable only by Whole Genome Sequencing (WGS), leading to re-sequencing efforts of 'failed' Whole Exome Sequencing (WES) projects. However, WES can detect SVs at base-pair resolution if a breakpoint lies in the enriched region. Despite its extensive application in clinical practice there is currently no public software with user-friendly graphical interfaces for detection, clinical annotation, and prioritization of SVs in WES data. To make SV analysis accessible for non-bioinformaticians, we have developed IdeficSV, a fast, user-friendly tool for the identification of Structural Variants in WES and optionally WGS data.

Methods: IdeficSV provides an intuitive interface for Windows and Linux for high-throughput screening of BAM files. It detects breakpoints using split-read, paired-end, and read-depth information without requiring reference genome downloads. A 10GB BAM-file is analyzed in ~20 minutes on a typical PC. Results can be exported in popular BED format but we also provide a human-friendly web interface offering candidate SVs prioritization based on clinical phenotypes. IdeficSV also renders IGV screenshots for visual inspection of SVs.

Results: We tested the software on more than 300 NGS datasets and confirmed detected SVs by PCR and Sanger sequencing. We generated a consensus black list of alignment artifacts by annotating frequently present SVs in our cohort. Users can easily provide their own blacklist.

Conclusion: We hope that our tool will foster the recognition of clinically relevant SVs in WES data.

References: -.

Grants: -.

Conflict of Interest: None declared.

P18.023.C Gene-environment interaction effects of early-life stress (ELS) program neurocognitive development in children

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Background/Objectives: Early-life stress (ELS) has well-known associations with low cognitive outcomes and brain functional and structural representations, yet it remains unclear how exactly ELS affects cognitive capacity in humans. Moreover, it is unknown how ELS interacts with genetic influences on development of the brain and cognition in children. We hypothesized that ELS is likely to interact with genetic influences that impact brain development as well as cognitive development in preadolescents.

Methods: We leveraged multimodal data consisting of DNA genotypes, brain imaging (MRI), and neuropsychological assessments (NIH Toolbox) from 4,276 children (ages 9 to 10, European ancestry) from the Adolescent Brain Cognitive Development study. Regression and mediation analyses were used to systematically examine the underlying causal relationships among genome-wide polygenic scores of cognitive capacity, brain structure, and ELS in their influence on child neurodevelopment.

Results: Our analyses revealed significant causal interaction of ELS and genetic factors in modulating child neurodevelopment. In particular, we found genetic influences on cognitive development to be mediated by brain structure development (*partial mediation effect = 0.016,* $P_{FWE}<0.001$), and this gene-to-brain pathway to be significantly moderated by ELS (*abuse*) (*Index of Moderated Mediation = -0.007; 95% CI = -0.012 ~ -0.002;* $P_{FWE}<0.05$).

Conclusion: Our findings indicate ELS to be a negative modulator of genetic influence on brain structural development, thereby leading to disadvantageous neurocognitive development in prepubertal children.

References: Wang, Hee-Hwan, et al. "The Impact of Early Life Stress on the Genetic Influence on Brain and Cognitive Development in Children." medRxiv (2021).

Grants: Funded by the National Research Foundation (NRF) of South Korea (2021R1I1A1A01054995).

Conflict of Interest: None declared.

P18.025.A MANCOR, a powerful test based on correlation to detect predictors of the gut microbiome variability

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Background/Objectives: Multivariate analysis are becoming central in studies investigating features associated with host-gut microbiome relationship. However, statistical methods to assess predictors associated with gut microbiome composition and other meta-parameters are severely lacking. Here we present MANCOR (Multivariate Analysis of Correlation), an innovative and flexible method that addresses this challenge, allowing to test the effect of any predictor on the joint distribution of a multivariate outcome, as measured by its correlation matrix.

Methods: We first compared the performance of MANCOR against existing correlation-based (BoxM, Mantel, etc) methods using simulated data. We then conducted a screening of a hundred predictors (diet, medical history, etc) on 16S-derived gut microbiome data in 1,000 healthy participants from the Milieu Interieur cohort, and compared the results of MANCOR against both standard univariate and multivariate (MANOVA) tests, and diversity-based (alpha) approaches.

Results: In all simulations mimicking microbiome data, existing correlation-based methods display severe type I error rate inflation, and only MANCOR showed both valid calibration and high statistical power. In the Milieu Interieur our test strongly outperformed univariate and multivariate tests, confirming associations with age and sex but with higher power (up to 200% power increase), while detecting additional signals with smoking and diet

variables. Diversity-based test were only able to detect variability with age. Host-genetics screening is ongoing.

Conclusion: Our approach will provide the community a robust and efficient tool to study the role of host-factors (genetics, environment) on a range of microbiome meta-parameters including taxa composition, metagenomic and metatranscriptomic profiles.

References:.

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Conflict of Interest: None declared.

P18.028.D Variant effect prediction based on custom long-read transcriptomes

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Background/Objectives: Our knowledge of transcript annotations is still incomplete and may result in a failure to detect disease-causing variants. For example, in patients with primary immunodeficiencies it could be valuable to annotate transcripts that are only expressed under certain conditions such as host-pathogen interactions. Current variant annotation software uses only precomputed pathogenicity prediction scores based on reference transcripts. The pipeline presented here is designed to annotate variants with custom transcript annotations for downstream prioritization.

Methods: The input of the pipeline is a sample-specific/non-reference long-read transcriptome in fasta format, variant file(s) derived from unsolved exome data of patients with suspected inborn errors of immunity (IEI) in VCF format, and a reference genome build. The Ensembl Variant Effect Predictor is used in conjunction with Polyphen-2 to provide custom variant annotations. Our pipeline is available at https://github.com/cmbi/VEP_custom_annotations.

Results: The input long-read transcriptome contained 37,434 novel transcripts detected through PacBio IsoSeq on peripheral blood cells exposed to various immune stimuli. The re-annotation pipeline was tested on 148 undiagnosed IEI patient's exomes. Out of a total of 802,352 variants, 6.2% had a more severe effect in the novel transcript annotation than in the reference.

Conclusion: Genetic variant annotation may benefit from long-read sequencing approaches that discover novel transcripts. This benefit can be reaped without extensive bioinformatic knowledge using this pipeline. Our pipeline outputs crucial information for further prioritization of potentially disease-causing variants, and will become increasingly useful due to the rising number of long-read RNAseq datasets.

References:.

Grants:.

Conflict of Interest: None declared.

P18.029.A A multi-omics integration framework applied to major depressive disorders

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Background/Objectives: Major depressive disorder (MDD) is a leading cause of disability worldwide that associate with reduced life expectancy. Its clinical heterogeneity and the implication of multiple environmental risk factors contribute to the difficulty in identifying reliable biomarkers for diagnostic and therapeutic purposes. Recently, the emergence of advanced approaches that take into account the biological complexity of multifactorial diseases has broadened treatment strategies in other medical fields, such as cancer. Applied to MDD, this may grant new insight on pathophysiology, and enable patient stratification.

Methods: We recently analyzed transcriptomic (RNA-Sequencing) and epigenomic (microRNA-Sequencing and DNA methylation arrays) processes in blood samples from a well-characterized cohort of individuals with MDD ($n = 65$), and healthy controls ($n = 81$). We then built an integrative framework in 3 steps: first, we applied gene co-expression network and ontology enrichment analyses; second, we used supervised machine learning methods for matrix reduction; third, we prioritized a subset of features (individual protein-coding genes, micro-RNAs, and methylation probes) that maximize the clustering of MDD patient and healthy controls, evaluated with adjusted rand indices (ARI).

Results: We identified gene subnetworks that significantly associate with MDD and also show evidence of enrichment for differentially expressed genes, differential DNA methylation, and regulation by differentially expressed micro-RNAs. Importantly, machine learning identified features that achieve good clustering of cases and controls (best ARI = 0.82).

Conclusion: These results provide support for the hypothesis that, compared to single omics, integration of multi-omic datasets have the potential to significantly improve patient clustering, with implication for the development of MDD biomarkers.

References:.

Grants: French National Research Agency: ANR-18-CE37-0002-02.

Conflict of Interest: None declared.

P18.030.B ILIAD: the ERN-ITHACA federated registry of rare diseases with intellectual disability and anomalies of development

Klea Vyshka[1;2], *Morris Swertz*[3], *Fernanda De Andrade*[3], *Joeri Van Der Velde*[3], *Lennart Johansson*[3], *Dieuwke Prins*[3], *Dorica Dan*[4], *Sofia Douzgou HOUGE*[5], *Andreas Dufke*[6], *Agnies van Eeghen*[7], *Laurence Faivre*[8], *Tiziana Franchin*[9], *Andrew Green*[10], *Raoul Hennekam*[11], *Anne Hugon*[1], *Sylvia Huisman*[11], *Marianne Le Dref*[1], *Tjitske Kleefstra*[12], *David Koolen*[12], *Andrea Manunta*[13], *Jukka Moilanen*[14], *Giovanni Mosiello*[15], *Mahsa Shabani*[16], *Ammi Sundqvist*[17], *Nicholas Szeto*[1], *Marco Tartaglia*[18], *Zeynep TÜMER*[19], *Birute Tumiene*[20], *Lisenka Vissers**[12], *Helger Yntema*[21], *Dagmar Wieczorek*[22], *Giuseppe Zampino*[23], *Alessandra Renieri*[24], *Alain Verloes*[1;25], *Ernithaca consortium*[26]

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Background/Objectives: European Reference Network ITHACA is developing a "meta-registry" called ILIAD, connecting 71 HCPs, databases, and biobanks across the EU for patients with dysmorphic/MCA syndromes and/or intellectual disability. Through the ERN-ITHACA's expert and patient participation network, ILIAD is able to provide an infrastructure for diagnosis, highly specialised multidisciplinary healthcare, evidence-based management, and collection of secure patient data.

Methods: The registry is built on MOLGENIS open-source software, providing flexible rich data structures, user friendly data import and querying, and FAIR interfaces for programmatic data exchange. ILIAD consists of 2 components: a central, web-based registry and a network of linked satellite/client registries forming the ERN-ITHACA registry federation. To date, two client installations have been successful and more are to follow. Data is modelled adhering to international interoperability standards from JRC and EJP-RD.

Results: In addition to the core registry, ILIAD includes thematic sub-registries of patients with biologically proven monogenic or genomic (chromosomal) diagnoses, under the supervision of ERN-based curation teams. ILIAD has adopted a data access policy, for requesting access to the data, Governance of the Registry and ensures compliance with applicable legal and regulatory requirements on the use of Personal Data.

Conclusion: We are well underway to share ERN-ITHACA patient data, yielding high-quality epidemiological insights and expert consensus statements, informing policy decisions that impact rare disease patients in general and care for ERN-ITHACA patients in particular.

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Grants: 3rd EU Health Programme Grant nr. 947617.

Conflict of Interest: None declared.

P18.031.C Predicting chromatin loop status from local DNA sequence alone

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Background/Objectives: The recent flurry of omics technologies and cutting-edge protocols has brought about an increased understanding on the fundamental structure of chromatin. However, specific mechanisms behind the formation of structures of interest such as loops and domains remain elusive and only partially elucidated. While a complex and multifactorial mechanistic nature of loop formation is being unveiled, it is still unclear whether and to which extent the basic DNA sequence contributes to loop formation.

Methods: We use a variety of machine learning models (SVM, neural network) to predict loop status from short 5000 bp fragments. We build these predictions through a new and extensive positioning of loops on the recently-released telomere-to-telomere human genome sequence, combined with a specific embedding inspired from natural language processing models (dna2vec).

Results: Loop status can be correctly predicted with up to 80% accuracy. In contrast with other, highly-performing models (e.g. basenji, akita), our predictions are established independently of the context of the assessed sequence, i.e. locus position or extraneous data like ChiP-seq andRNA-seq.

Conclusion: As recent findings further confirm that chromatin loops are multi-faceted in nature and origin (e.g. cohesin vs non-cohesin mediated), shedding light on the information provided by biological latent variables of interest may prove crucial to investigate regulation mechanisms. Our method paves the way to a generalized use of language-based frameworks to predict DNA structure from its sole sequence.

References:.

Grants:.

Conflict of Interest: None declared.

P18.032.D Enhanced molecular consequence prediction and variant annotation with the Ensembl Variant Effect Predictor

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Background/Objectives: The comprehensive annotation of genomic variation is key to interpreting potential impact on molecular function, thus important in understanding disease aetiology and diagnosis.

Methods: The Ensembl Variant Effect Predictor (VEP) is a powerful toolset for the annotation and prioritisation of genomic variants. We have extended its functionality to incorporate additional protein annotations and variant pathogenicity predictions.

Results: The effect of genetic variation on a protein function depends on the location of the variant in the protein structure. The protein domains a variant falls within are reported alongside matching UniProt protein isoforms. VEP now optionally reports when a variant falls in a molecular interaction site, as described in the IntAct database. To further aid interpretation, the Ensembl VEP

web interface displays variant locations on interactive three-dimensional protein structures.

Further, we have extended the wide range of variant pathogenicity scores available in Ensembl VEP. Recent additions to command line VEP include: ClinPred, an ensemble method for predicting missense variant pathogenicity; CAPICE, a computational method for predicting the pathogenicity of variants of different molecular consequences and allele frequency; PrimateAI, a deep residual neural network for classifying the pathogenicity of missense mutations that incorporates information about protein structure; and EVE, which employs an evolutionary model of variant effect.

Conclusion: These enhancements increase the amount of information available to aid understanding of potential consequences and to predict the impact of sequence variation.

References:.

Grants:.

Conflict of Interest: None declared.

P18.033.A Implementing RNA-fusion detection in routine cancer diagnostics

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Background/Objectives: Healthcare is increasingly relying on massively parallel sequencing in clinical genomics diagnostics. In particular, cancer diagnostics and therapy selection can be assisted by the detection of gene fusions, as an increased number of fusions are observed in common cancer types. However, to operate in a clinical setting, a robust, scalable and portable data analysis pipeline is necessary.

Methods: We have developed the *rnafusion* pipeline designed to examine gene fusions in RNA sequencing. The pipeline is built within nf-core, a community-based framework to build and maintain bioinformatics analysis pipelines. The *rnafusion* pipeline uses multiple callers (e.g. STARfusion, fusioncatcher) to generate a combined comprehensive report of all fusion events, which aids interpretating results.

Results: *rnafusion* has an easily maintainable codebase assembled into a continuous integration environment. Containerisation ensures reproducibility and portability of the analyses. Combining results from different sources grants confidence in fusion events repeatedly identified by different tools and increases the chances of identifying novel fusions.

Conclusion: Maintaining analysis pipelines to the state-of-the-art in terms of software and databases is challenging. As computing capacities and technical achievements advance, a high turnover in data and methods is to be expected, highlighting the need for a fast, reliable and continuous maintenance of analysis pipelines, as well as regular validation procedures. Achieving this gold standard is facilitated within the nf-core framework, enabling a multitude of analyses to be accessible for patient care.

References: Ewels, P.A. et al. The nf-core framework for community-curated bioinformatics pipelines. *Nat Biotechnol* **38**, 276–278 (2020).

Grants:.

Conflict of Interest: None declared.

P18.034.B Evaluating validation approaches for whole exome sequencing based CNV calling in a diagnostic setting

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Background/Objectives: The detection of genomic rearrangements such as copy-number variants (CNV) from Next-generation sequencing (NGS) data is an attractive alternative to the time consuming and costly MLPA and aCGH. Many different tools for CNV detection from NGS data have been developed over the last years. However, the validation of the NGS based CNV calls in a clinical setting is a major challenge, due to the high portion of false-positive calls and the lack of reference material.

Methods: We set up a patient cohort based on 23 samples and used filter settings as well as parameter modification for CNV calls to achieve an accessible number of calls. Here, we aim to evaluate CNV calling performance by comparing NGS based data to various orthogonal methods.

Results: The optimal validation methods differ, depending on the validation parameter of interest. The GIAB dataset is suitable as a truth set for precision calculation while the multitude of different methods used in generating the data makes it difficult to include it in sensitivity calculation based on short-read data. On the other hand, aCGH data is too imprecise to generate viable sensitivity values and MLPA cohorts are usually too small.

Conclusion: We show that the combination of a wide variety of validation methods as a reference system made it possible to compensate for the weaknesses of the individual technologies.

References: Zook, J.M., Hansen, N.F., Olson, N.D. et al. A robust benchmark for detection of germline large deletions and insertions. Nat Biotechnol 38, 1347–1355 (2020). https://doi.org/10.1038/s41587-020-0538-8.

Grants:.

Conflict of Interest: None declared.

P18.036.D Evaluation of in silico pathogenicity predictor tools for the classification of small inframe indels

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Background/Objectives: The use of in silico pathogenicity predictions as evidence when interpreting genetic variants is widely accepted as part of standard reporting guidelines. Although numerous algorithms have been developed and evaluated for classifying missense variants, inframe insertions/deletions (indels) have been much less well studied.

Methods: We created a dataset of 3964 small (<100bp) indels predicted to result in inframe amino acid insertions or deletions using data from gnomAD v3.1 (minor allele frequency of 1-5%), ClinVar and the Deciphering Developmental Disorders (DDD) study. We used this dataset to test ten pathogenicity predictor tools (CADD, CAPICE, EXOMISER, FATHMM-indel, MutPredIndel, MutationTaster2 Provean, SIFT-INDEL, VEST and VVP).

Results: Our dataset consisted of 2224 benign/likely benign and 1740 pathogenic/likely pathogenic variants from gnomAD (n = 809), ClinVar (n = 2882) and, DDD (n = 273). We were able to generate scores across all tools for 95% of the variants, with areas under the ROC curve (AUC) of 0.81-0.96 based on the published thresholds. To avoid biases caused by inclusion of our dataset in

the tools' training data, we also evaluated just DDD variants not present in either gnomAD or ClinVar (70 pathogenic and 81 benign). Using this subset, the AUC of all tools decreased substantially. Overall, VEST performed best, with AUCs of 0.93 (full dataset) and 0.87 (DDD subset).

Conclusion: Diagnostic laboratories and genomic scientists should note that numerous tools now exist for predicting the pathogenicity of inframe indels, which perform on a par with similar tools designed for classifying missense variants, though ease of use varies substantially.

References:.

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Conflict of Interest: Stuart Cannon University of Exeter, Minnie Williams: None declared, Adam Gunning: None declared, Caroline Wright University of Exeter.

P18.038.B Phenotyping Hereditary hemorrhagic telangiectasia using UK primary care Electronic Health Records

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Background/Objectives: Hereditary haemorrhagic telangiectasia (HHT) is a rare multisystemic disease. Underdiagnosis is common due to the disease's complexity and low physician awareness.

Methods: A cohort of HHT patients was identified in the Optimum Patient Care Research Database (OPCRD), a database of 11 million patients' primary care electronic health records (EHR). Cases were identified by the presence of the SNOMED-CT code for HHT.

HHT clinical features were mapped to the appropriate SNOMED-CT codes. Clinical features were examined in advance of their diagnostic date for HHT. Descriptive statistics was performed including patient count and time before diagnosis summarized by median of months.

Results: EHR with HHT diagnostic code: 1119

Clinical features	# EHR	Months before diagnostic code (Median)
Epistaxis	190	-38
Microcytic anaemia	90	-37
Migraine	81	-72
Telangiectasia of the skin	54	-5
Arteriovenous malformation	28	-8
Seizure	23	-74
Spontaneous hematomas	20	-37
Cavernous haemangioma	20	-23
Intestinal polyposis	17	-24
Haematuria	17	-56
Stroke	12	-41
Gastrointestinal haemorrhage	12	-5
Haemoptysis	11	-11
Visceral angiomatosis	8	-33
Pulmonary arterial hypertension	3	-24
Cerebral haemorrhage	1	0

Conclusion: Clinical features of HHT can be identified in patients' primary care EHR in advance of diagnosis. These may be used to develop phenotypical prediction tools to help identify patients at risk of HHT. Further work is needed to validate these results in other data-sets and control comparisons.

References: McDonald, J., & Stevenson, D. A. (2000). Hereditary Hemorrhagic Telangiectasia. In M. P. Adam (Eds.) et. al., *GeneReviews®*. University of Washington, Seattle.

Grants:.

Conflict of Interest: Orlando Buendia Mendelian.

P18.040.D On the way to individualized infection medicine: Metagenomics in Cystic Fibrosis patients as example for the interplay between human genetics and bacterial colonization to predict the course of the disease

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Background/Objectives: The monogenetic disease Cystic Fibrosis leads to mucus obstruction and compromises airway clearance. Dysbiosis in CF lung disease is a result of compromised airway clearance and insufficient host defense. Specific pathogens have been associated with pronounced disease progression. Nowadays scientists can only describe the interplay between CFTR dysfunction and microbial dysbiosis, but are not able to predict the further clinical course.

Methods: We have developed methods for standardized, low-contamination sequencing of airway metagenomes, including published programs and in-house developed in-depth analysis pipelines. This approach allows insight into bacterial growth rate, elimination of false positives and integrates information about viruses and fungi. (1,2,3,4,5).

Results: We have shown that microbial dysbiosis in CF depends on the degree of CFTR dysfunction, is closely related to disease severity and develops after the first four years of life. Airway metagenomes of >100 healthy volunteers (0-60 years) and several hundred patients with various airway diseases were used as a reference database.

Conclusion: The analysis of the CF metagenome is a very suitable example application to show strengths of individualized infection medicine. Both the underlying disease and the respective genetic background of the patient are combined for the prognosis of the course of the disease.

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Grants: VW Stiftung – NWK ZN3432.

Conflict of Interest: None declared.

P18.041.A Synthesized dysmorphic portraits can protect privacy while maintaining the benefits of data sharing

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Background/Objectives: The performance of next-generation phenotyping (NGP) approaches depends highly on the size and diversity of the training dataset. Because collecting a large number of images from individuals with rare disorders at a single institution is not feasible, data sharing between different sites is crucial, and this is usually done through publication. However, since we are re-identifiable by our faces, only about one out of ten patients gives consent. Therefore, we propose synthesizing portraits of rare disorders based on real data that can remain local to overcome this barrier.

Methods: We trained generative adversarial networks on 4,538 frontal images with 139 disorders from GestaltMatcher Database to synthesize faces with rare disorders. We then trained deep convolutional neural networks on the real and synthetic images separately to classify 139 disorders and benchmarked these two models on 360 test images.

Results: We first showed that the model trained on synthetic images achieved comparable performance as that trained on the real images. The saliency map was further utilized to visualize feature importance contributing to the classification, and dysmorphologists confirmed that the key dysmorphic features were preserved in the synthetic images. Lastly, face verification showed that the synthetic images cannot re-identify the underlying individuals.

Conclusion: We conclude that algorithms can benefit from synthetic facial images, representing an alternative to classical means of data sharing. Moreover, facial portraits are still the best teaching material for clinicians in medical genetics. Therefore, beyond the deep learning purpose, it is also of interest to educate the next generation of physicians.

References:.

Grants:.

Conflict of Interest: Tzung-Chien Hsieh: None declared, Alexander Hustinx: None declared, Behnam Javanmardi: None declared, Peter Krawitz Modest.

P18.042.B New RD-Connect GPAP features implemented in collaboration with Solve-RD, EJP-RD and ELIXIR enable the diagnosis of rare disease patients with previously negative WES/WGS

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Background/Objectives: The RD-Connect Genome-Phenome Analysis Platform (GPAP, https://platform.rd-connect.eu/) is an IRDiRC recognised resource for diagnosis and gene discovery in Rare Diseases (RD). The interface allows clinical scientists to collaboratively analyse integrated genome-phenome data under controlled access. The GPAP is a key resource for Solve-RD (http://solve-rd.eu/), EJP-RD (https://www.ejprarediseases.org/) and the ELIXIR RD Community (https://elixir-europe.org/communities/rare-diseases).

Methods: New features have been implemented in the RD-Connect GPAP which processes and indexes pseudonymised genome-phenome data from over 26,000 individuals.

Results: Recent developments facilitate data submission and integration, as well as the analysis and visualisation of the data. These developments include an innovative web interface that improves user experience and analysis capabilities, a module to collate and export phenotypic information using broadly used standards (e.g. HPO, ORDO, OMIM, GA4GH Phenopackets) and a user-friendly tool to create in-silico patient cohorts based on phenotypic and experimental information, a programmatic module to automate genomic analysis and the ability to remotely visualise sequence alignments archived at the European Genome-Phenome Archive (EGA). Some of these developments have contributed to diagnose the first 437 patients through the reanalysis of previously inconclusive WES/WGS data within the Solve-RD project.

Conclusion: New developments in the GPAP have contributed to the identification of hundreds of disease-causing variants in patients with RD and confirm diagnosis hypotheses through patient matchmaking approaches.

References:.

https://pubmed.ncbi.nlm.nih.gov/34075210/, https://pubmed.ncbi.nlm.nih.gov/34075208/, https://pubmed.ncbi.nlm.nih.gov/29487416/.

Grants: EU projects (FP7-305444,H2020-779257,H2020-825575), ISCIII (PT13/0001/0044,PT17/0009/0019), INB and ELIXIR.

Conflict of Interest: None declared.

P18.044.D BAMdelbee: novel software enabling detection of midsize homozygous deletions through unsequenced NGS data analysis

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Background/Objectives: Microdeletions between 70bp and 20,000bp can be disease causing, yet are often too small for detection through chromosomal microarrays (CMA) analysis and too large for standard NGS such as whole-exome-sequencing (WES) and whole-genome-sequencing (WGS) data analyses. Therefore, specialized methods are needed for investigating this troublesome range.

Methods: We created *BAMdelbee*, a novel tool for discovery of homozygous deletions in WES and WGS data, that is effective also in this troublesome range. WES or WGS samples generated by the same method produce BAM files with similar alignment coverage across the genome. *BAMdelbee* locates homozygous deletions by pinpointing regions sequenced in all samples but the ones in question.

Results: We demonstrate how our application can spot homozygous deletions not identified in WES and WGS variant analysis for being too vast, yet undetectable by CMA for being too small.

Conclusion: *BAMdelbee* is unique, being freely available on our website and capable of running on a regular laptop (https://fohs.bgu.ac.il/BirkLab/BAMdelbee). Furthermore, the high specificity of *BAMdelbee* results in scarcity of false positives, a common pitfall in such analyses in existing software. Therefore, it can be easily and effectively integrated into WES and WGS interpretation pipelines for recessive diseases.

References:.

Grants:.

Conflict of Interest: None declared.

P18.045.A AION: an artificial intelligence platform for automated variant interpretation

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Background/Objectives: Variant interpretation is critical to the success of diagnostic tests such as Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS), as it identifies genetic variant(s) causing a patient's clinical features. However, variant interpretation remains time-consuming and costly, requiring ~12 hours per case and causing long turnaround times. Furthermore, manual interpretation is prone to variability in results, potentially impacting diagnostic yield.

Methods: We developed AION, a variant interpretation software platform for diagnostic WES and WGS. AION consists of a pipeline for variant annotation, classification, and prioritization using a white-box machine learning approach. AION performs exhaustive variant interpretation providing a classification and confidence score for each variant in coding regions, and combinations of molecular and clinical diagnoses ranked according to the genetic and clinical data provided per patient.

Results: The performance of AION's classification algorithm was tested in a prospective study of 122,208 variants classified in ClinVar, showing 96% accuracy, 92% sensitivity, and 96% specificity. AION was subsequently tested on 5,062 exomes from simulated patients with different monogenic diseases (including intellectual disability, hereditary cancer, heart disorders…). AION correctly identified the disease-causing variant in 96% of cases (4,845/5,062), outperforming automated ACMG guidelines which identified the pathogenic variant(s) in 61% of cases (3,108/5,062).

Conclusion: AION provides fast, accurate, and interpretable variant interpretation. AION identified pathogenic variants in >95% of cases, increasing diagnostic yield by more than 50% compared to ACMG rule-based classification. Exomes were analysed in 2 minutes, leading to quick turnaround times and the opportunity to increase throughput.

References:.

Grants:.

Conflict of Interest: Kristina Ibáñez Garikano Nostos Genomics, Andrea Bertana Nostos Genomics, Carla Glassl Nostos Genomics, David Alberto Neville Nostos Genomics, Rocio Acuna Hidalgo Nostos Genomics, Nostos Genomics.

P18.046.B Search for complex alleles leading to disruption of mRNA splicing

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Background/Objectives: It is well known that single nucleotide polymorphisms (SNPs) in the human genome are mostly neutral. However, if a mutation appears near to a frequent SNP their cooperative impact on splicing regulatory sequences can be very significant. At the same time, the effect of mutation could be different depending on the allelic states of SNP.

Methods: Bioinformatics analysis was performed using MaxEntScan and SpliceAI. The functional effect of the complex alleles on pre-mRNA splicing using a minigene assay.

Results: We attempted a genome-wide search for such "complex alleles". For this, we created an "alternative" version of the human genome (hg19 assembly) where every frequent SNP contains its alternative allele. We also generated a dataset representing results of an in silico mutagenesis of the human genome sequence within the 10 nucleotides window flanking each SNP. Using the modified SpliceAI algorithm applied on both reference and "alternative" genome versions, we predicted those mutations which could change the splicing pattern in one version of the genome but not in another. As a result, we found about 41 thousand mutations. A detailed analysis of these mutations has shown various mechanisms of disruption of normal splicing patterns. Using the minigene system, we carried out experimental validation of some cases which could be relevant to medical genetics.

Conclusion: This work demonstrates that pathogenic complex alleles are common in the population and can lead to the appearance of hereditary diseases.

References:.

Grants:.

Conflict of Interest: None declared.

P18.047.C Comparison of Polygenic Risk Scores for Coronary Artery Disease in an Italian prospective cohort: the EPICOR study

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and Cancer Prevention, Turin, Italy; [9]AOU Città della Salute e della Scienza, Medical Genetics Unit, Turin, Italy.

Background/Objectives: Coronary artery disease (CAD) is a metabolic disorder that causes the most serious cardiovascular death events in Western countries[1]. The identification of individuals at high risk to develop coronary heart disease is a major clinical need for timely intervention and prevention. We aimed at investigating the predictive potential of Polygenic Risk Scores in an Italian cohort.

Methods: We evaluated the prediction value of PRS in 286 prediagnostic CAD and 290 disease-free individuals belonging to European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (EPICOR study)[2]. Three PRSs were selected from PGS Catalog based on the ancestry of the GWAS study, number variants included in the PRS and developing method to calculate the scores.

Results: Distributions between pre-clinical CAD patients and controls were significantly different for all PRSs evaluated (p-value < 0.0001). Patients whose scores fall above the 90th percentile have a significantly higher risk (OR = 3.24 p-value < 0.0002). In order to test the accuracy of the PRSs, CAD risk factors, age and sex were included in multivariate regression models. Areas under the ROC curve always increases by adding PRS (p-value = 0.016), showing an increase of the prediction potential for CAD.

Conclusion: This data suggest that European CAD PRSs work also in the Italian population and could be used in clinical practice to identify high-risk individuals for CAD development that can benefit from early intervention in terms of life-style changes and as preventive therapeutic approach.

References: 1-Hajar R.2017;18(3):109-114., 2-Palli D et al.Tumori.2003 PMID:14870823.

Grants: Ministero dell'Istruzione, dell'Università e della Ricerca "Dipartimenti di Eccellenza 2018–2022".

Conflict of Interest: None declared.

P18.048.D The reanalysis of large exome sequencing datasets by mobile element insertion detection tools identified causal pathogenic variants

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Background/Objectives: Mobile element insertions (MEIs) compose a small proportion of pathogenic causal variants. Most existing MEI detection tools have been developed for analysis of whole genome sequencing (WGS) data but not specifically for exome sequencing (ES) data.

Methods: Six MEI calling tools (ERVcaller, MELT, Mobster, Scramble, TEMP2, xTea) were evaluated for non-reference MEI calling on ES from 100 trios. We generated a gold standard dataset by visual inspection of 2,942 MEI calls and subsequently evaluated all tools for sensitivity and precision. For comparison all tools were also applied to two WGS samples from the Genome in a bottle consortium (HG0002, NA12878). The best performing tools for ES data were then applied to a further set of 11,157 ES samples (Solve-RD).

Results: We found striking differences in performance between tools when comparing WGS and ES results. MELT and Scramble performed best on ES data with a sensitivity (0.72;0.63) and precision (0.84;0.76), respectively. Application of both methods to the Solve-RD cohort identified a total of 375,082 MEIs, of which 1,647 (0.4%) were found in 2 or less individuals, absent in the dbRIP database and affecting OMIM genes. Patient phenotype matching has so far resulted in the identification of 3 causal pathogenic MEIs (0.03%), but interpretation is ongoing.

Conclusion: MELT and Scramble are the most suitable tools for MEI identification in ES data. Analysis of the Solve-RD dataset has so far yielded three MEIs diagnoses. Analysis of an additional cohort of ~50,000 WES is ongoing.

References:.

Grants: Solve-RD (Horizon 2020 grant agreement No. 779257).

Conflict of Interest: Robin Wijngaard full, German Demidov full, Steven Laurie full, burcu yaldiz full, Wouter Steyaert full, Jordi Corominas Galbany full, Luke O'Gorman full, Christian Gilissen full, Solve-RD (Horizon 2020 grant agreement No. 779257).

P18.049.A Phivea: a novel platform for real-time detection of chromosomal aberrations using long-read sequencing and artificial intelligence

Carmen Garrido Navas[1;2;3], *David Galevski*[4;5], *Anne Kristine Schack*[6;7], *Gjorgji Madjarov*[4], *Aleksandar Nikov*[5], *Lukasz Krych*[6], *Chris Kyriakidis*[7], *Zoran Velkoski*[7]

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Background:.

Genetic diagnosis of chromosomal abnormalities is done through karyotyping/FISH or chromosomal microarrays (CMA) (1). Resolution, although sufficient for large anomalies, is limited for small chromosomal rearrangements and mosaicism. Recently, Optical Genome Mapping (OGM) showed 100% concordance with karyotype and CMA (2)(3) and was able to detect mosaicism (3); however computational analysis is challenging and cost-effectiveness needs to be demonstrated (4). Also, analysis methodology based on CMA, cannot efficiently detect triploidies (5). Oxford Nanopore Technology (ONT) uses long-read fragments facilitating identification of aneuploidies and mosaicism (6)(7) in a rapid and inexpensive way although bioinformatics analysis is under development.

Methods: We developed an end-to-end solution for mass, real-time, and cost effective screening of chromosomal aberrations based on ONT. As proof-of-concept, we evaluated diagnostic ability for Klinefelter syndrome (KS) patients (N = 2), healthy donors (N = 2) and other chromosomal abnormalities (N = 10). DNA libraries were prepared and loaded on the GridION x5. Analysis and diagnosis was performed on Phivea®, a proprietary software solution enabling real-time detection of chromosomal aberrations using state-of-the-art data-driven modelling.

Results: We simultaneously analyzed 48 to 192 replicate samples, reducing costs and increasing throughput. We achieved 98.3% and 97.0% diagnostic specificity and sensitivity respectively for KS, with a LoD below 30%.

Conclusion: Our solution demonstrated a novel approach for detecting chromosomal abnormalities that can reduce costs, increasing throughput and facilitating analysis. Our technology was validated on KS, but results can be directly extrapolated for screening of any other chromosomal aberration.

References: (1) https://doi.org/10.1038/sj.ejhg.5201896. (2) https://doi.org/10.3390/genes12030398. (3) https://doi.org/10.1016/j.ajhg.2021.05.012. (4) https://doi.org/10.1081/E-ECHP-140000148. (5) https://doi.org/10.3390/genes12121958. (6) https://doi.org/10.1534/genetics.115.182311. (7) https://doi.org/10.1016/j.fertnstert.2018.06.014.

Conflict of Interest: Carmen Garrido Navas I am member of the advisory board as Clinical Specialist at gMendel, David Galevski Consultant, Data Scientist, Anne Kristine Schack Employment, Full-time, Gjorgji Madjarov Advisory Board, AI/ML Advisor, Aleksandar Nikov Consultant, AI/ML, Lukasz Krych Advisory Board, Genomics Advisor, Chris Kyriakidis Employment, Full-time, Zoran Velkoski Employment, Full-time.

P18.050.B Structurally informed epistatic features improve variant effect assessment in clinically actionable genes

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Background/Objectives: As genetic testing becomes more relevant to support clinical diagnoses, the interpretation of rare single nucleotide variants (SNVs) remains a major challenge for genetics research. Variant effect predictors (VEPs) are used to inform variant effect assessment. Yet their thresholds to classify a variant as pathogenic are often set for high sensitivity, that results in the misclassification of benign variants. Here, we developed DeMAG (Deciphering Mutations in Actionable Genes), a supervised classifier for interpreting missense variants in actionable genes.

Methods: DeMAG predictor uses conservation-based features and structural features derived from AlphaFold2 3D models. In addition, we designed the "partners feature" that captures epistasis both in sequence and 3D space of the protein. It is a probabilistic score that predicts pathogenicity based on the phenotypic effect of co-evolving residues and spatially close residues of the protein.

Results: DeMAG reached high performance for almost all genes considered and high sensitivity and specificity overall. DeMAG yielded the top performance across clinical and common population validation sets among different VEP tools. The novel partners feature assigns a score to more than 60% of residue positions that lack a clear clinical interpretation. We provide classification of all 1.3 million missense mutations for 59 actionable genes available at demag.org.

Conclusion: DeMAG expands the traditional conservation paradigm to epistatic and structural features to predict variants' effect. DeMAG's high specificity will reduce the number of misdiagnoses due to false positives and it might be better suited to prioritize variants for association studies.

References:.

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Conflict of Interest: None declared.

P18.051.C Hybrid de novo genome assembly of a Kazakh individual

Daniyar Karabayev[1], *Asset Daniyarov*[1], *Askhat Molkenov*[1], *Saule Rakhimova*[1], *Diana Samatkyzy*[1], *Aidana Gabdulkayum*[1], *Ulan Kozhamkulov*[1], *Ainur Akilzhanova*[1], ***Ulykbek Kairov***[1]

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Background/Objectives: State-of-the-art whole-genome sequencing methods cannot read the entire genome at once, therefore genome assembly is required to connect billions of genome fragments. De novo genome assembly with next-generation sequencing data possesses limitations due to the presence of repeated sequences in a genome, which cannot be assembled with short reads. This obstacle can be overcome by using long-read genome sequencing. Here we present a hybrid de novo genome assembly of a Kazakh individual from short and long-read whole-genome sequencing data.

Methods: Long-read genome sequencing of Kazakh male individual was performed on a PromethION nanopore sequencing machine with 76.3 Gb of total reads length. Short-read sequencing was performed on DNBSEQ platform with 99.21 Gb of total reads length. To assemble long reads, Flye and Shasta assembly tools were used independently. Both primary assemblies were polished with short read sequencing data using Racon, and then with Medaka.

Results: Final assembly with Flye and polishing steps resulted in 2,307 contigs with N50 of 21.5 Mb and 2.86 Gb of total length of assemblies. Assembly with Shasta and polished steps resulted in 3,816 contigs with N50 of 19.7 Mb and 2.86 Gb of total length of assemblies.

Conclusion: Hybrid de novo genome assemblies are expected to be used in construction of the first Kazakh reference genome in order to identify and verify structural variants specific for the Kazakh population.

References:.

Grants: This work was supported by grant of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan, #AP09058660, and NU CRP grant 021220CRP2222.

Conflict of Interest: None declared.

P18.052.D Use of different soft-clustering approaches to discover genetic subtypes of chronic kidney disease

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Background/Objectives:.

Chronic Kidney Disease (CKD) is a condition that causes a gradual loss of kidney function and affects approximately 9.1% of the global population. The disease is highly complex, and its genetic disease-causing mechanisms are not entirely understood. In this work, we use different soft-clustering approaches to deconstruct CKD heterogeneity.

Methods: First, we applied two soft-clustering methods to identify overlapping clusters of 493 independent CKD variants and 935 associated traits, identified from published genome-wide association studies (GWAS). Second, we tested whether the novel clusters have any broad clinical consequence by performing a cluster-specific Phenome-Wide Association Study (PheWAS) on 31.701 BioMe biobank participants. We then explored the relationship between these clusters and disease outcomes, calculating Cluster-specific polygenic Risk Scores (CRS) and comparing them to the CKD prevalence (cases = 2871, controls = 2737). Eventually, to further investigate each cluster's mechanistic biology, we

performed a pathway analysis extracting the genes mapped to the top variants.

Results: The two clustering methods identified six comparable clusters whose diversity reflects different aspects of CKD: protective traits, type-2 diabetes, slow eGFR decline, anemia, hypertension, and hemolytic-uremic syndrome. The PheWAS confirmed the top-weighted traits in most of the clusters. Although the clustering results were comparable, Non-negative Matrix Factorization (NMF) performed better in yielding significant PheWAS results. Furthermore, despite having used only CKD risk-increasing alleles, the clusters showed different correlations between an increasing CRS and the prevalence of CKD.

Conclusion: Besides improving the understanding of disease biology, these results support using genetics to reduce CKD complexity by identifying disease subtypes.

Conflict of Interest: None declared.

P18.054.B Venus: Elucidating the impact of amino acid variants on protein function beyond structure destabilisation

Matteo Ferla[1], *Alistair Pagnamenta*[1], *Jenny Taylor*[1], *Brian Marsden*[2]

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Background/Objectives: Exploring functional effects of a missense variant at the protein level requires multiple pieces of information to be interpreted appropriately. This is particularly important when studying a potentially pathogenic variant linked to a rare or monogenic disease. Whereas accurate stability predictions alone are generally informative, other effects, such as disruption of post-translational modifications or weakened ligand binding, may also contribute to the disease phenotype. Furthermore, consideration of nearby variants that are found in the healthy population may strengthen or refute a given mechanistic hypothesis. Whilst there are several bioinformatics tools available that score the deleteriousness of a variant, these do not assemble multiple effects of a variant on the encoded protein, beyond structural stability, and present them on the structure for inspection.

Methods:.

Results: Venus (https://venus.cmd.ox.ac.uk) is a webapp which, given a protein substitution, rapidly estimates the predicted effect on protein stability of the variant, flags if the variant affects a post-translational modification site, a predicted linear motif or known annotation, and determines the effect on protein stability of variants which affect nearby residues and have been identified in healthy populations.

Venus is built onto Michelanglo and the results can be exported to it, allowing them to be annotated and shared with other researchers.

Conclusion: By presenting interactively multiple sources of information on a protein structure (stability, human population variants, post-translational modifications etc.), Venus empowers the investigation into the effects a variant of interest may have on a protein function.

References:.

Grants: NIHR Oxford Biomedical Research Centre Programme. Wellcome Trust Core Award [203141/Z/16/Z].

Conflict of Interest: None declared.

P18.055.C A community developed pipeline for rare disease diagnostics

Anders Jemt[1;2], *Ramprasad Neethiraj*[3], *Mei Wu*[3], *Lauri Mesilaakso*[4], *Annick Renevey*[1], *Gwenna Breton*[5], *Subazini Thankaswamy Kosalai*[6], *Sima Rahimi*[5], *Emil Bertilsson*[2], *Viktor Henmyr*[7], *Valtteri Wirta*[1;2;3], *Henrik Stranneheim*[1;2;8]

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Background/Objectives: Genome sequencing has become a mainstay in rare disease (RD) diagnostics, enabling medical professionals to diagnose challenging cases. However, computational pipelines are getting increasingly complex, resource demanding and are often tightly tied to local computational infrastructure. The community driven nf-core initiative aims to develop and maintain a curated set of open-source best practice bioinformatic pipelines[1]. Herein we describe the development of the nf-core raredisease pipeline.

Methods: The pipeline aligns fastq files, calls and annotates SNV/indels and structural variants. Furthermore, it calls uniparental disomy, runs of homozygosity, repeat expansions and SMN1/SMN2 copy numbers. Variants are ranked according to their predicted pathogenicity using a weighted sum rank model.

Results: The pipeline is an extension of the workflow used in the Stockholm healthcare region to analyze >10,000 samples, with a diagnostic yield of 40% in a first cohort of 3219 patients[2]. The development is a collaborative effort within Genomic Medicine Sweden, aiming to establish this as the national pipeline for RD diagnostics.

Conclusion: Pipelines developed within the nf-core community adhere to strict guidelines ensuring that they will install, run and perform on most computing infrastructure, including cloud. The nf-core raredisease pipeline enables a broad utilization across the diagnostic RD community.

References: [1]Ewels PA et al. The nf-core framework for community-curated bioinformatics pipelines. *Nat Biotechnol*. 2020;38(3):276-278. https://doi.org/10.1038/s41587-020-0439-x. [2]Stranneheim, H et al. Integration of whole genome sequencing into a healthcare setting: high diagnostic rates across multiple clinical entities in 3219 rare disease patients. *Genome Med*. 2021;13(1):40. https://doi.org/10.1186/s13073-021-00855-5.

Grants:.

Conflict of Interest: None declared.

P18.056.D Automated prioritization of copy number variants with ACMG/ClinGen standards

Jiri Ruzicka[1], *Kevin Yauy*[1], *Nicolas Duforet-Frebourg*[1], *Laure Raymond*[2], *Mélanie Broutin*[1], *Jerome Audoux*[1], *Sacha Beaumeunier*[1], *Nicolas Philippe*[1], *Denis Bertrand*[1]

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Background/Objectives: With the rising adoption of long-read sequencing technologies, previously undetected and numerous CNVs (copy-number variants) are accessible and their prioritization becomes necessary for the clinical evaluation. ACMG and ClinGen published guidelines for clinical interpretation of such variations, which allow more consistent prioritization of CNVs.

Methods: We present an original implementation of the recommendations of the ACMG/ClinGen framework, adapted to both small and large CNVs. Classifications were processed using dosage map sensitivity, general population frequency, phenotype matching and disease inheritance patterns. The performance of the model was compared with the published ACMG/ClinGen dataset consisting of 114 CNVs evaluated by two independent experts.

Results: Our classification tool achieved 96.7% specificity for pathogenic variant identification, identifying correctly 15 of 23 CNV assessed as pathogenic by the two evaluators. 2 additional CNV could be classified as pathogenic when phenotypes were available. In 84.2% of CNVs, the prediction was the same as the prediction of at least one evaluator. For the 15.8% of predictions in disagreement, no variants classified as benign were predicted pathogenic and vice-versa.

Conclusion: This implementation of ACMG/ClinGen standards provides an automated and confident classification of CNVs which accelerates the clinical interpretation of structural variants.

References:.

Grants:.

Conflict of Interest: None declared.

P18.057.A dmfind: a network medicine tool for the analysis of genomics data

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Background/Objectives: The network medicine approach helps explain how gene alterations affect the whole system. Network analysis of genomics data is therefore important to explain the genetic architecture of complex diseases. We present dmfind – disease module finder – a network medicine tool for the analysis of genomics data.

Methods: Mutational data: genomic data commons through the package "TCGAbiolinks". Gene-gene interactions: STRING, iRefIndex. dmfind is implemented in R. Graphs are handled by means of the package "igraph". Input gene scores are processed using network diffusion. Gene relevance is quantified by the network smoothing index. Genes are classified by participation coefficient and within-module degree. Computationally intensive analysis are run in parallel using the package "BioCParallel".

Results: We substantially improved the initial release of dmfind[1], introducing a series of novel analyses, and re-implementing the code and its documentation according to the Bioconductor guidelines. The main novelties are: coverage analysis of the interactome over the input ranked gene scores; performance assessment for free parameter tuning; network selection based on network enrichment analysis in top gene scores; gene classification by topological role within and between network communities; network visualization; gene network comparison to find shared and specific regions. We show the functioning of dmfind analyzing the most frequently mutated genes in a few types of cancer and using different interactomes.

Conclusion: The R package dmfind offers a documented pipeline to find gene networks that are significantly enriched in the most altered genes of an "omic" screening. It classifies the genes, suggesting possible key players of the disease under investigation.

References: [1] https://doi.org/10.1038/srep34841.

Grants:.

Conflict of Interest: None declared.

P18.058.B Bone2Gene: deep learning-based diagnosis of rare skeletal disorders

Sebastian Rassmann[1], *Alexander Hustinx*[1], *Kyra Skaf*[2], *Miguel Ibarra-Arellano*[3], *Tzung-Chien Hsieh*[1], *Alexandra Keller*[4], *Ruth Gausche*[5], *Klaus Mohnike*[2], *Peter Krawitz*[1], ***Behnam Javanmardi***[1]

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Background/Objectives: Rare skeletal disorders are an important group of genetic disorders that are highly heterogeneous, making their accurate diagnosis a very challenging process. In this project, we have (so far) collected more than 700 hand X-Ray images from patients diagnosed with 6 different skeletal disorders which we are using to build a diagnostic tool based on Deep Learning (DL).

Methods: DL usually requires massive amounts of training data. However, data for rare genetic disorders is inherently sparse which is further exacerbated by difficulties collecting and digitizing the X-Ray imagery. We address this issue by employing transfer learning from a public bone age dataset. Furthermore, varying data sources e.g. using differing imprinted labels or images showing digitization artifacts potentially induces biases. To eradicate these, we trained DL models to extract only the hands concealing the origin of the X-Ray.

Results: Our bone age DL models trained on the bone age dataset reach a competitive accuracy with a mean age difference (MAD) of ~4.5 months (compared to 5-7 months from human experts). Furthermore, our models achieve a MAD of ~7.5 months (w.r.t. a single human rater) on our skeletal disorder dataset which demonstrates generalizability to (1) unseen datasets and (2) disordered patients. Upon fine-tuning our models on disorder classification, we build a preliminary classifier. However, prediction accuracies widely depend on the class frequency in our dataset (0-95%).

Conclusion: By growing our database we aim to increase the performance and, consequently, deploy Bone2Gene as a clinical assistance tool.

References:.

Grants:.

Conflict of Interest: None declared.

P18.060.D Analyzing clinical RNAseq data with machine learning models greatly improves the genetic diagnosis in pediatric acute lymphoblastic leukemia

Michelle Tang[1;2], *Zeljko Antic*[1], *Stefan Pietzsch*[1], *Jana Lentes*[1], *Winfried Hofmann*[1], *Gunnar Cario*[3], *Gabriele Escherich*[4], *Udo zur Stadt*[4], *Brigitte Schlegelberger*[1], *Martin Horstmann*[4;5], *Martin Stanulla*[6], *Anke Katharina Bergmann*[1]

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Background/Objectives: Since tumour genetics greatly influence the risk stratification and treatment of acute lymphoblastic leukemia (ALL), Next Generation Sequencing (NGS) technologies have been incorporated into routine diagnostics with the cost-

effective targeted RNA sequencing being particularly appealing. However, the analysis and integration of large amounts of NGS data in diagnostic settings remains challenging since a systematic tool is lacking.

Methods: We performed targeted RNAseq on ~1500 pediatric ALL patients from the German pediatric ALL study groups. The megSAP pipeline was applied to analyze the gene expression and fusions. We then combined UMAP (Uniform Manifold Approximation and Projection) and supervised machine learning algorithms to build an interactive tool for visualization and prediction of patients' diagnostic subgroups.

Results: Our tool provides a user-friendly interface for analyzing large cohorts as well as individual cases. New patient' data is uploaded weekly. The UMAP visualization and subgroup prediction are generated immediately (~1 minutes) and accurately (F1~95%). Using this tool, we are able to: (1) identify patients with *DUX4* fusion, when fusion calling and conventional cytogenetic methods failed; (2) stratify patients without aberrant fusion or aneudiploidy, e.g. Ph-like; (3) pinpoint individual outlier cases that need special attention or are usually difficult to distinguish, e.g. masked-hypodiploidy; (4) validate the results from conventional diagnostic methods.

Conclusion: We present a systematic AI tool that easily integrates into the routine diagnostics. Its application helps to improve risk stratification and brings novel insights to research. Our workflow has the power to replace some traditional methods and pave the way for personalized oncology.

References:.

Grants:.

Conflict of Interest: None declared.

P18.061.A Recent updates in the VarFish platform for collaborative variant data analysis

Manuel Holtgrewe[1], *Oliver Stolpe*[1], *Henrike Sczakiel*[2], *Björn Fischer-Zirnsak*[2], *Felix Boschann*[2], *Nadja Ehmke*[2], *Dieter Beule*[1]

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Background/Objectives: VarFish is a web-based for the collaborative analysis of DNA variant data. Varfish is freely available under a permissive open source license.

Methods: We have continuously developed the application further together with our clinical partners and extended it in terms of features and usability.

Results: We present recent advancements in VarFish features including support for easily exporting annotated variants for submission to Clinvar, the analysis of strutural variants, and the support for large cohorts.

Conclusion: VarFish continues to be a versatile and powerful tool for variant analysis in our research and diagnostics applications.

References: Holtgrewe M, Stolpe O, Nieminen M, Mundlos S, Knaus A, Kornak U, Seelow D, Segebrecht L, Spielmann M, Fischer-Zirnsak B, Boschann F, Scholl U, Ehmke N, Beule D. VarFish: comprehensive DNA variant analysis for diagnostics and research. Nucleic Acids Res. 2020 Jul 2;48(W1):W162-W169. https://doi.org/10.1093/nar/gkaa241. PMID: 32338743; PMCID: PMC7319464.

Grants:.

Conflict of Interest: None declared.

P18.062.B Identification of unknown environmental factors that mediate eQTLs using principal interaction component analysis

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Background/Objectives: Expression quantitative trait loci (eQTL) help explain the regulatory mechanisms of trait associated variants. eQTL effect sizes are often dependent on observed and unobserved biological contexts, such as cell type composition and environmental factors. Here, we introduce *PICALO* (Principal Interaction Component Analysis through Likelihood Optimization) which is an unbiased method to identify known and hidden contexts that influence eQTLs.

Methods: *PICALO* uses expectation maximization to identify latent components, referred to as Principal Interaction Components (PICs), which maximally affect eQTL effect-sizes. Here we applied *PICALO* to bulk RNA-seq eQTL datasets in both blood ($n = 2,932$) and brain ($n = 2,439$).

Results: We identified 33 PICs in blood. These PICs interact with 4,533 (35%) unique eQTLs. We identified 21 PICs in brain, which interact with 3,996 (39%) unique eQTLs (FDR<0.05). These PICs capture both RNA quality, cell type composition, and environmental influences.

For instance, a PIC interacting with 375 blood eQTLs is correlated to the presence of prior Cytomegalovirus infection ($r = 0.31$, p-value = 5x10-17). Several of these eQTLs overlap asthma GWAS risk variants, corroborating a previously reported role of Cytomegalovirus in asthma.

We observed that *PICALO* is robust to heterogeneous datasets, yielding biologically highly informative and reproducible interaction components.

Conclusion: *PICALO* is a novel method that allows for the identification of context dependent eQTLs without any prior knowledge, and which outperforms methods that use cell counts or expression-based principal components. *PICALO* therefore has the potential to aid in better understanding the environmental components that play a role in common diseases.

References:.

Grants: ERC Starting Grant 637640.

Conflict of Interest: Martijn Vochteloo: None declared, Patrick Deelen: None declared, Britt Vink: None declared, Bios Consortium: None declared, Sergio Andreu-Sánchez: None declared, Jingyuan Fu: None declared, Alexandra Zhernakova: None declared, Ellen A. Tsai Biogen Inc., Biogen Inc., Heiko Runz Biogen Inc., Biogen Inc., Harm-Jan Westra: None declared, Lude Franke: None declared.

P18.063.C Using CADA – a phenotype-driven gene prioritization tool for custom panel definition in diagnostics of rare disorders using exome sequencing – a retrospective analysis of 380 patients

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Background/Objectives: Phenotype-driven gene target definition using gene panels and known phenotype-gene associations represent an established practice in next-generation sequencing data analysis. In patients with complex phenotypes target selection can be difficult, often resulting in the analysis of multiple

genes not associated with the observed phenotype pattern. We propose using CADA, a phenotype-driven gene prioritization tool for custom panel definition and report on the results of a retrospective analysis in 380 patients.

Methods: Phenotype of 380 patients examined, sequenced and diagnosed at our institution was described in human phenotype ontology (HPO) terms and served as CADA input. We performed a statistical analysis of the results and report on CADA rankings and score distribution for genes with diagnostic findings. We defined custom gene panels using CADA scores at different cut-off values and provide comparison with the standard gene panel approach using Genomics England PanelApp.

Results: Genes with diagnostic findings in our patient cohort had an average CADA rank of 54.6 (SD = 81.8, max = 484) and a median score of 60.5 (SD = 24.6, min = 14.7). Average number of genes in gene panels selected by clinical geneticist was 470. Using CADA score at 5th percentile in our cohort as cut-off value for gene inclusion in custom gene panel would have provided us with smaller target sizes (mean = 324 genes) compared with gene panels selected by a clinical geneticist.

Conclusion: We conclude that using CADA for phenotype-driven gene target definition would lead to smaller gene targets compared to the standard approach using gene panels.

References:.

Grants:.

Conflict of Interest: None declared.

P18.064.D Comprehensive copy number variant analysis of 11,000 previously unsolved rare disease exome sequencing datasets within the Solve-RD project results in diagnoses being reached for 2% of previously unsolved cases

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Background/Objectives: A key objective of the H2020 Solve-RD project is the exhaustive reanalysis of 19,000 exome sequencing (ES) datasets to reach diagnoses that have previously proven elusive. For some of these datasets only limited, if any, copy-number variant (CNV) analyses may have been previously undertaken. The Solve-RD CNV working group is reanalysing all submitted data using a variety of algorithms in order to identify candidate disease-causing CNVs. Here we report on reanalysis of the success of the first 11,000 experiments representing 6,000 families.

Methods: Raw ES reads were realigned to the hs37d5 reference genome and four CNV calling algorithms applied to the new alignments to maximise sensitivity: ClinCNV, Conifer, ExomeDepth, and VarGenius-HZD. Putative CNVs in candidate gene lists provided by four European Reference Networks submitting to Solve-RD, and all CNVs over 500kb in length, were prioritised for further investigation. QC measures were applied to reduce the number of false positive events identified, and IGV track screenshots generated for all candidate CNVs, to facilitate visual inspection and interpretation by clinical researchers.

Results: Analyses to date have already resulted in the identification of CNVs that are fully, or partially explanatory for the disease phenotype in >2% of cases, some of which have remained undiagnosed for more than a decade, and this number is expected to grow further as further events are confirmed.

Conclusion: Disease causing CNVs were identified in an important proportion of patients likely because thorough CNV analysis of exome data remains challenging and thus has not been routinely performed in the past.

References:.

Grants: EU H2020-779257.

Conflict of Interest: None declared.

P18.066.B Fast and powerful statistical method for context-specific QTL mapping in multi-context genomic studies

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Background/Objectives: Recent studies suggest that context-specific eQTLs underlie genetic risk factors for complex diseases. However, methods for identifying them are still nascent, limiting their comprehensive characterization and downstream interpretation of disease-associated variants.

Methods: Here, we introduce FastGxC, a novel statistical method that leverages the correlation structure of multi-context studies to efficiently and powerfully map context-shared and context-specific eQTLs. We prove through analytical derivation and empirical examination that FastGxC shared and context-specific eQTL effect size estimates are a re-parametrization of the standard eQTL effect size estimates in each context and the eQTL-by-context interaction effect size estimates from a linear mixed model with a genotype-by-context effect.

Results: We show via simulations that FastGxC is orders of magnitude more powerful and computationally efficient than previous approaches. We apply FastGxC to bulk multi-tissue RNA-Seq data from the GTEx Consortium and PBMC single-cell RNA-Seq data from CLUES to produce the most comprehensive tissue- and cell-type-specific eQTL maps to date. We validate these maps by establishing that context-specific eQTL variants are enriched in corresponding functional genomic annotations. Finally, we examine the relationship between context-specific eQTLs and human disease and show that FastGxC context-specific eQTLs provide a three-fold increase in precision to identify relevant tissues and cell types for GWAS variants and a two-fold improvement in their rank than standard eQTLs.

Conclusion: In summary, FastGxC enables the construction of context-specific eQTL maps that can be used to understand the

context-specific gene regulatory mechanisms underlying complex human diseases.

References:.

Grants:.

Conflict of Interest: Andrew Lu: None declared, Mike Thompson: None declared, Gracie Gordon: None declared, Andy Dahl: None declared, Chun Jimmie Ye C.J.Y. has received research support from Chan Zuckerberg Initiative,437Chan Zuckerberg Biohub, and Genentech, C.J.Y. is a Scientific Advisory Board member for and hold equity in435Related Sciences and ImmunAI, a consultant for and hold equity in Maze Therapeutics, and a436consultant for TReX Bio., Noah Zaitlen: None declared, Brunilda Balliu: None declared.

P18.067.C DGH-GO: Dissecting the Genetic Heterogeneity of complex diseases using Gene Ontology, an interactive and user-friendly web application

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Background/Objectives: Neurodevelopmental disorders (NDDs) are phenotypically heterogeneous and difficult to diagnose at early-age. The genetic heterogeneity of NDDs matches to their clinical-variability. The different NDDs share biological mechanisms that further complex the patient's stratification, thus, limiting the applications of personalized medicine for NDDs. Existing studies have employed biological networks and machine-learning methods to dissect the genetic heterogeneity. Such methods suffer from many parameter tuning and lack biological interpretations, resulting in the reduced generalizability of the proposed method.

Methods: Here, we presented an interactive and user-friendly application, DGH-GO that allows biologists to dissect the genetic heterogeneity of complex diseases by stratifying the genes, disrupted by any type of genetic variants (SNV, CNVs). The application can also be used to study the shared etiology of complex-diseases.

Results: DGH-GO creates a functional similarity matrix of putative disease-causing genes or known-disease genes for multiple disorders using Gene Ontology (GO). The resultant matrix can be visualized in a 2D space using different dimension reduction methods (T-SNE and Principal-Coordinate-Analysis). Functional similarities from GO and projected space coordinates from dimension reduction methods can be used to identify clusters by employing four different clustering methods (K-means, Hierarchical, Fuzzy and PAM). The user may change the clustering parameters and see their effect on stratification results immediately.

Conclusion: In summary, functional-similarities, dimension-reduction and clustering, coupled with interactive-visualization and control over analysis allows biologists to explore and analyze their datasets, without knowing the execution of complex methods. The proposed application and its application to NDDs is available at https://github.com/Muh-Asif/DGH-GO.

References: Asif et al. 2018, 2019, 2020.

Grants:.

Conflict of Interest: None declared.

P18.068.D Whole-Genome Sequencing: The Long and the Short of It

Sylvan Caspar[1], *Patricia Stoll*[1], *Siro Fritzmann*[1], *Gilles Gut*[1], *Daniel Salerno*[1], ***Janine Meienberg***[1], *Gabor Matyas*[1;2]

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Background/Objectives: Whole-exome sequencing (WES), short-read whole-genome sequencing (SR-WGS) and long-read WGS (LR-WGS) enable the detection of sequence variants at unprecedented scale, leading to new challenges in variant calling. Indeed, the calling of all clinically-relevant ClinVar/HGMD variants is challenging due to the limitations of sequencing and data analysis platforms. Here, we provide new insights into the performance of the most recent sequencing, alignment, and variant-calling pipelines in the detection of ClinVar/HGMD variants.

Methods: We used raw data of SR-WGS (PE150, ~60×) of >50 in-house samples as well as SR-WGS (PE150, ~60×) and LR-WGS (PacBio HiFi, ~30×) of 4 publicly available samples (HG001-HG004). We implemented 12 state-of-the-art analysis pipelines for SR-WGS, LR-WGS, or the combination of both as well as developed a workflow to assess the pipelines' performance in the detection of ClinVar/HGMD variants.

Results: For a ~60× genome, accelerated pipelines decreased the runtime of BWA/GATK from ~2.5 d to ~2-5 h, enabling the analysis of multiple samples. LR-WGS outperformed SR-WGS, particularly in regions with mappability <1, while WES failed to detect variants in non-exonic or GC-rich regions. However, no pipeline alone detected all ClinVar/HGMD variants. By analyzing read depth, strand bias, variant allele fraction, and population-based allele frequency, we identified a substantial number of false-positive calls and ClinVar/HGMD entries.

Conclusion: Sequencing, alignment, and variant-calling pipelines can significantly influence the detection of all ClinVar/HGMD variants, leading to both false-negative and false-positive results. Owing to its inherent advantages in variant detection/calling, LR-WGS should be implemented in clinical practice as soon as it is affordable.

References:.

Grants:.

Conflict of Interest: None declared.

P18.069.A Hybrid semi-automated approach for neonatal screening using whole exome sequencing

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Background/Objectives: The advances in high-throughput sequencing technologies have provided powerful tools for the analysis of genomic variants and allowed for newborn screening that included a huge amount of rare hereditary disorders that would not be detected using conventional screening methods. However the step of variant interpretation remains time and labor consuming and requires a highly qualified specialist. Therefore, a manual analysis of every sample is impossible for routine screening. At the same time, a fully automated approach is not the best decision due to the huge amount of variant data with ambiguous clinical relevance. Therefore, we decided to apply a combined approach that allows for automated analysis of samples with no "suspicious" variants followed by manual analysis of those samples that carry variants of interest.

Methods: The variants were called using GATK best practices pipeline. The automated variant prioritization was performed for

more than 2000 genes associated with severe childhood-onset diseases considering the inheritance type, variant significance information from ClinVar, HGMD, and local databases, variant frequency in gnomAD and local databases. For autosomal recessive disorders we only reported potential homozygotes and compound heterozygotes.

Results: Automated variant prioritization resulted in 45% of cases that carried no variants for manual interpretation. The remaining cases carried "suspicious" variants in 1-3 different genes, that significantly reduced the time needed for analysis.

Conclusion: A combination of automated and manual variant analysis allowed us to speed up data analysis for neonatal screening without sacrificing the sensitivity.

References:.

Grants: The study was carried out within the framework of State Assignment 121092400060-5.

Conflict of Interest: None declared.

P18.070.B Clinically-driven, multi-layered, and interpretable machine learning model for assisted variant interpretation

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[1]SeqOne, Montpellier, France; [2]Biomnis-eurofins, Lyon, France; [3]Assistance Publique – Hôpitaux de Paris, Paris, France; [4]CHU Grenoble, Grenoble, France.

Background/Objectives: With the great expansion of sequencing technologies and artificial intelligence tools, the demand for interpretable classification of variants rises rapidly and highlights the need for a personalized approach based on the clinical context. Unfortunately, the low interpretability of machine learning black-box models limits their adoption in the community.

Methods: We created a multi-layered machine learning model called ClassifyML which scores the pathogenicity of genomic variants and prioritizes their importance for the clinical context. ClassifyML gathers multi-level annotations based on ACMG-AMP evidence criteria, disease heritability patterns, and phenotype matching. The model was trained firstly on the ClinVar variant classification dataset, followed by a second training on a cohort of 316 deep-phenotyped patients recruited from a French consortium.

Results: The model proposes an interpretable output in the form of a continuous importance scale for each criterion, which assists the clinical interpretation of variants. We evaluated our method with a multi-centric cohort consisting of 310 patients. The causing variant was classified as having pathogenic evidence in 291 of 310 cases by the model, with an improvement of the median rank of 39 fold compared to Exomiser (3 against 118).

Conclusion: ClassifyML is an interpretable machine learning model for pathogenicity prediction and variant prioritization. It allows variant classification prediction, patient context integration, and yields human-explainable classifications.

References:.

Grants:.

Conflict of Interest: None declared.

P18.071.C Gene prioritization for rare diseases integrating genotype, RNA-seq and phenotype - lessons from a CAGI 6 challenger team

Vicente Yépez[1], *Christian Mertes[1], Nicholas H. Smith[1], Ines Scheller[1], Julien Gagneur[1]*

[1]Technical University of Munich, Munich, Germany.

Background/Objectives:.

RNA sequencing emerges as a complementary tool to DNA sequencing for rare disease diagnostics. However, gene prioritization methods integrating genotype, RNA-seq and phenotypes have been lacking. To address this need, the SickKids Genome Clinic released a CAGI 6 diagnostics challenge with nearly 80 genomes and RNA-seq samples[1].

Methods: We developed a gene prioritization model integrating variant annotations, mono-allelic expression, gene expression[2] and splicing outliers[3] (through our workflow DROP[4]), together with HPO-encoded phenotypes. The model is a gradient tree boosting machine (XGboost) trained on a cohort of 209 mitochondrial disease patients[5] from which half are diagnosed.

Results: On the mitochondrial disease dataset, our model prioritizes the causal gene first for almost half of the diagnosed cases, and among the top 5 in more than 70% of them. Application to the CAGI6 SickKids cohort revealed a known splice-disrupting pathogenic variant and reported several promising candidates (CAGI6 evaluation pending as of now).

Conclusion: Our approach and publicly available software[6] can help find and prioritize candidates found by DNA and RNA sequencing and can be especially useful to reduce the burden of manual inspection in cohorts of hundreds of samples.

References: 1. http://genomeinterpretation.org/cagi6-sickkids.html.
2. Brechtmann et al, AJHG (2018).
3. Mertes et al, Nat Commun (2021).
4. Yépez et al, Nat Protoc (2021).
5. Yépez, Gusic et al, Genome Med (2021).
6. https://github.com/gagneurlab/cagi6_sickkids.

Grants:.

Conflict of Interest: Vicente Yépez Technical University of Munich, Christian Mertes Technical University of Munich, Nicholas H. Smith Technical University of Munich, Ines Scheller Technical University of Munich, Julien Gagneur Technical University of Munich.

P18.073.A Whole genome sequencing for copy number variation and structural variant analyses

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Background/Objectives: WGS is widely spreading as a first line diagnostic test, both for detecting small variants (SNVs/indels), Copy-Number Variants and Structural Variants (CNVs/SVs). Through the Auragen program, we have validated a routine for CNVs/SVs calling, annotation, and prioritizations devoted to rare mendelian disorders diagnostics. Literature data and reference samples are missing gold standards for such variants, hence we developed an internal analytical validity assessment.

Methods: A reference dataset was constituted through 82 CNVs (52 losses, 30 gains) identified by array-CGH from various

designs (median size of 104kb) and 11 SVs from local projects. GIAB samples were explored for CNVs smaller than 10kb. Multiple softwares were compared to assess precision/recall. Annotation was performed using the VEP software with custom parameters, notably with dbVar. Prioritization was done using in-house scripts.

Results: A combination of two softwares was highlighted, CNVnator and Manta. Dedicated quality control metrics were used for large CNVs calls. Compared to array-CGH, WGS recalled 96% losses (50/52), 87% gains (26/30) and 91% SVs (10/11). Diverging calls were discussed with expert cytogeneticists for pipeline fine-tuning. For 1282 cases involved in Auragen diagnostic routine, in average, 5 losses 2 gains and 4 SVs are delivered for interpretation. Within 517 consecucutive cases, 32 CNVs/SVs were deemed clinically relevant, including two mosaic CNVs.

Conclusion: Our analytical validity assessment developed on Auragen samples suggests the non-inferiority of WGS compared to array-CGH for CNVs discovery in a diagnostic routine.

References: Chaisson et al., 2019 Nat Comm, Delage et al., 2020 BMC Genomics, Zook et al., 2020 Nat Biotechnol.

Grants: PFMG2025 (https://pfmg2025.aviesan.fr/).

Conflict of Interest: None declared.

P18.074.B Increasing the usability of PEDIA approach by integrating open-source GestaltMatcher and CADA into variant prioritization pipeline

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Background/Objectives: The Prioritization of Exome Data by Image Analysis (PEDIA) supports finding the correct diagnosis in patients with rare disorders by including phenotype information into the variant interpretation. However, the current facial analysis approach, DeepGestalt, is not an open-source software and requires data transfer to Face2Gene. This increases the difficulty in integrating PEDIA into the clinics because some patients do not consent to the data transfer to another site. Moreover, the feature-based approaches in PEDIA required updates since advanced algorithms were published in the meantime. Therefore, we propose a version of PEDIA that works with open-source tools only facilitating on-premise usage.

Methods: 679 individuals with 105 monogenic disorders were recruited for benchmarking. We first replaced DeepGestalt with GestaltMatcher for facial image analysis and used CADA and LIRICAL instead of Phenomizer, BOQA, and FeatureMatch for feature analysis. For the exome data, the highest CADD score was used for each gene. We then trained the support vector machine on GestaltMatcher, CADA, LIRICAL, and CADD scores. In the end, the top-10 accuracy was reported.

Results: The new PEDIA approach achieved comparable top-10 accuracy (97%) compared to the previous version. We further deployed the new PEDIA approach together with VarFish to demonstrate how to integrate PEDIA into the existing pipeline in the clinic without transferring data to other sites.

Conclusion: By replacing the non-open-source components, the new PEDIA approach becomes easier to be implemented into the variants prioritization pipeline in the clinic without losing performance.

References:.

Grants:.

Conflict of Interest: Jing-Mei Li: None declared, Meghna Bhasin: None declared, Alexej Knaus: None declared, Peter Krawitz modest; FDNA, Tzung-Chien Hsieh: None declared.

P18.075.C OpenCB: an Open Source BigData clinical genetics platform that scales for large health and research system organizations

*Will Spooner[1], **Pablo Marin**[2], Jacobo Coll[2], Pedro Furio[2], Philip Hamid[1], Junfe Sanahuja[2], Joaquin Tarrega[2], Laura Lopez[1], Marta Bleda[1], Jose Miguel Juanes[2], Rodiel Martinez[2], Ignacio Medina[1;3]*

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Background/Objectives: Current large-scale clinical genomics studies consisting of thousands of whole-genome sequences require a platform with the ability to analyze billions of variants over Terabytes of data. This platform should take into account performance, scalability, flexibility, robustness, accuracy and security under a SAS model based on BIG data. Here we present the latest version of OpenCB, an Open Source project that implements a full-stack solution for genomic data management, analysis and visualization for both population genomics and clinical interpretation of rare diseases and cancer.

Methods: OpenCB is based on three main projects: (1) CellBase, a MongoDB database used for querying genomic annotations, (2) OpenCGA, a variant and clinical data store based on MongoDB and HBASE with Solr indexing and (3) IVA, a web-based analysis client of OpenCGA.

Deployment is based on Docker and Kubernetes under CI/CD.

Results: OpenCB is an open-source suite of interoperable software components that enable genomic data management and analysis at an unprecedented scale. Functionality includes population-scale and clinical interpretation analysis for rare diseases and cancer under a strict data access authorization model, and data visualization modules.

Conclusion: OpenCB provides a scalable and flexible solution for biomedical researchers, clinical scientists, and geneticists. Both genomic and clinical data is stored in one single platform and allow real-time query and analysis. OpenCB is a successful clinical Open Source project that works well from small research genomic centres to big genomic and pharmaceutical companies, and national health and research systems.

Public repository: https://github.com/opencb.

Conflict of Interest: Will Spooner Zetta Genomics, Zetta Genomics, Pablo Marin Zetta Genomics, Jacobo Coll Zetta Genomics, Zetta Genomics, Pedro Furio Zetta Genomics, Philip Hamid Zetta Genomics, Junfe Sanahuja Zetta Genomics, Joaquin Tarrega Zetta Genomics, Laura Lopez Zetta Genomics, Marta Bleda Zetta Genomics, Jose Miguel Juanes Zetta Genomics, Rodiel Martinez Zetta Genomics, Ignacio Medina Zetta Genomics, University of Cambridge, Zetta Genomics.

P18.076.A Identification of a non-canonical transcription factor binding site using deep learning

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Background/Objectives: Most approaches for the detection of transcription factor binding sites are based on position-weight matrices (PWM). These require that one or more common sequence motifs be present in the DNA. Here we show that artificial neural networks (ANN) do not only improve prediction, but also allow the identification of motifs that were hitherto unknown.

Methods: We trained a convolutional-recurrent neural network on SELEX data for GRHL1 binding. The classifier was then applied to 7,857 sequences containing GRHL1 binding sites obtained from ChIP-Seq experiments (length > 197 bp).

Results: The neural network identified 46 potential binding sites for which the PWM-based approach did not suggest any binding at all. Using isothermal titration calorimetry (ITC), we could validate binding between a non-canonical DNA sequence motif predicted by our network and the GRHL1 protein. We could confirm correlation between predicted binding scores and real binding affinity by introducing variants into the binding sites and measuring binding strength by ITC.

Conclusion: Our results show that neural networks do not only outperform PWMs but can also be used to discover unknown TF binding motifs. We will apply our approach to a wider array of transcription factors to identify novel binding sites with a special focus on known disease mutations in promoter regions.

References:.

Grants:.

Conflict of Interest: None declared.

P18.077.A Leveraging functional annotations in genetic discovery for human complex diseases

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Background/Objectives: Although GWAS have identified many genetic variants underlying complex disease, a large fraction of heritability remains unexplained. To investigate this missing heritability, gene-based association tests using GWAS summary data have been proposed, and many disease-associated genes have been identified. However, accuracy in genetic risk prediction remains moderate for most diseases, largely due to challenges in identifying real functionally relevant variants and accurately estimating their effect sizes in the presence of linkage disequilibrium (LD). To identify genes with functionally relevant variants, we propose an optimally weighted score test (OWST).

Methods: OWST uses GWAS summary statistics and models various functional annotations, while allowing for LD estimated from reference genotype data. OWST leverages diverse types of genomic and epigenomic annotations in genetic association study for complex disease. Six traditional tests, including the burden test, the weighted sum of squared score test (SSU), and the weighted sum statistic (WSS) are its special cases.

Results: Simulation results demonstrate that OWST is not only valid but also outperforms comparison methods. We further apply OWST and comparison methods to two schizophrenia (SCZ) datasets from the Psychiatric Genomics Consortium (PGC) (SCZ1 [13,833 cases and 18,310 controls], SCZ2 [36,989 cases and 113,075 controls]) and a UK Biobank Type 2 diabetes dataset [19,119 cases and 423,698 controls]. Results from the three datasets suggest that OWST identified more meaningful genes than comparable methods. Existing literature suggests that these findings are biologically relevant to the etiology of the corresponding diseases.

Conclusion: OWST is a powerful and effective genetic association test.

References:.

Grants:.

Conflict of Interest: None declared.

P19 PERSONALIZED MEDICINE AND PHARMACOGENOMICS

P19.001.D Polygenic risk prediction, how far can it go?

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Background/Objectives: Polygenic risk scores (PRS) based on thousands of genetic variants have become a central tool for genetic prediction in multifactorial diseases, and the prospect of using these PRS in clinical care has received increasing attention. The accuracy of PRS will likely continue to increase with increasing sample size of genome-wide association (GWAS) and the development of new powerful methods. However, questions remain on how much prediction can be achieved in the future and how performances depend on the many parameters involved. Here, we used real data and theoretical models to provide a global perspective on PRS performances.

Methods: We used GWASs from multiple common diseases and published since 2010 to derived PRSs using a harmonized pipeline. The PRSs were then applied to independent datasets to assess performances as a function of sample size, genetic effect size distribution, genomic coverage, and heterogeneity in disease definition and population ancestry. We then use state-of-the-art methodologies to investigate future improvement and expected maximum prediction conditional on multiple parameters.

Results: Among outcomes displaying a clear increase with sample size, some showed a convergence toward the expected maximum, while others suggest important future gain with increasing sample size. For several outcomes, diagnosis heterogeneity (age at onset, self-reported vs doctor diagnosed, etc) induces substantial variability in prediction accuracy. Future improvements appear to be highly dependent on the inclusion of denser variants data with smaller frequencies.

Conclusion: Our harmonized and thorough analyses provide a unique perspective on the potential utility of current and future PRS.

References:.

Grants:.

Conflict of Interest: None declared.

P19.002.A Pharmacogenetic association of diabetes associated genetic risk score with rapid progression of coronary artery calcification following treatment with HMG-CoA-reductase inhibitors — results of the Heinz Nixdorf Recall Study

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Background/Objectives: HMG-CoA-reductase inhibitors (HMGRIs) are currently the most widely used group of drugs in patients with coronary artery disease and are given pre-emptively to patients with high levels of cholesterol, including those with diabetes mellitus (DM). However, HMGRIs also increase the progression of coronary artery calcification (CAC) and the risk of developing DM.

This study aimed to investigate whether HMGRI-intake interacts with the diabetes-associated genetic risk score (GRS) to affect CAC progression using data from the population-based Heinz Nixdorf Recall (HNR) study.

Methods: CAC was measured in 3157 participants using electron-beam computed tomography twice, at baseline (CACb) and five years later (CAC5y). CAC progression was classified as slow, expected or rapid based on predicted values. Weighted DM GRS was constructed using 100 diabetes-mellitus associated single nucleotide polymorphisms (SNPs). We used log-linear regression to evaluate the interaction of HMGRI-intake with diabetes-associated GRS and individual SNPs on CAC progression (rapid vs. expected/slow), adjusting for age, sex and log(-CACb+1).

Results: The prevalence of rapid CAC progression in the HNR study was 19.6%. We did not observe any association of the weighted diabetes-mellitus GRS with the rapid progression of CAC (relative risk [95% confidence interval]: 1.01[0.94;1.10]). Furthermore, no indication of an interaction between GRS and HMGRI-intake was observed (1.08[0.83;1.41]).

Conclusion: Our analyses showed no indication that the impact of HMGRIs on CAC progression is significantly more severe in patients with a high genetic risk of developing DM than in those with a low GRS.

References:.

Grants: This study was supported by the German Heart Foundation/German Foundation of Heart Research.

Conflict of Interest: None declared.

P19.003.B Nagencol project: personalised medicine in familial hypercholesterolemia

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Background/Objectives: Familial hypercholesterolemia (FH) is one of the most common inherited disorders worldwide. Characterised by elevated LDL cholesterol levels since birth, FH confers an increased risk of premature cardiovascular disease (CVD). Therefore, an early diagnosis and adequate treatment is essential. Mutations in LDLR, APOB and PCSK9 are known to cause autosomal dominant FH, but variants in other genes and polygenic inheritance have also been described. The objective of this study is to personalise the management of FH to prevent CVD, by using genomic and clinical data.

Methods: 507 individuals with LDL>190 mg/dl and <65 years old were recruited, and genome sequencing was performed. We analysed genetic variants in 43 genes linked to FH and dyslipidemia, LDL polygenic risk scores (PRS), and pharmacogenetic markers of statins, among others.

Results: A preliminary analysis of 300 genomes revealed: 35% of cases carried pathogenic variants in LDLR, APOB, APOE or ABCG8 (monogenic cause), 24% had high LDL-PRS (polygenic cause) and 6% presented with both monogenic FH and elevated LDL-PRS. Some individuals had VUS in typical genes or pathogenic variants in novel genes, requiring further characterisation. Pharmacogenomic findings showed 27% of individuals carried variants in LpA and 32% in SLCO1B1.

Conclusion: A genetic cause of severe hypercholesterolemia was detected in 65% of cases, as well as variants associated with statin treatment. These data are helping us tailor patient care. The role of non-coding and structural variants, novel FH genes and cardiovascular risk alleles will be evaluated.

References:.

Grants: Navarra Government R&D project call 2019-2021 (0011-1411-2019-000049), "laCaixa" fellowship (LCF/BQ/PI21/11830009) and H2020-MSCA-COFUND-2018 (No847648).

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P19.004.C UGT1A1 mutations may related to furosemide resistance in heart failure patients

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Background/Objectives: Heart failure (HF) can be defined as the heart's inability to pump the amount of blood needed by tissues or to pump some blood only under high filling pressure. Furosemide is used for the treatment of HF however in some patients

furosemide resistance may occur. UGT1A1 enzyme play role in furosemide metabolism therefore in this study it was aimed to investigate the effects of UGT1A1 mutations to furosemide resistance in HF patients.

Methods: Thirty healthy individuals and 50 HF patients who used furosemide during their treatment were enrolled into the study as control and patients groups, respectively. Patient group was also divided into 2 subgroups as responders (n = 25) and non-responders (n = 25) according to the presence of furosemide resistance. After DNA was isolated from peripheral blood, UGT1A1 mutations were investigated by direct sequencing. For understanding the effects of the mutations to three dimentional protein structure, homology modelling and docking studies were applied.

Results: Totally ten mutations were detected at which eight of them are novel and nine of them cause amino acid change. Three mutations were found deleterious and five mutations were detected as probably damaging of protein functions. The binding angles of furosemide with wild type and mutant types were determined. Changes of binding sites and bond structures were detected in five of the mutant types.

Conclusion: UGT1A1 mutations may cause furosemide resistance. Therefore it may possible to prevent the harmful effects of furosemide resistance by using personalized diagnosis and treatment strategies.

References: -.

Grants: -.

Conflict of Interest: None declared.

P19.005.D Polygenic risk score comparator (PRScomp): tested population vs. worldwide populations

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Background/Objectives: We developed a Polygenic risk score comparator (PRScomp), which allows users to evaluate polygenic risk score of a tested population and compare it with worldwide populations.

Methods: A disease/trait database is constructed from GWAS catalog(1) summary statistics data of genetic risk of disease and traits. Genotype data of the tested population is uploaded to the platform and merged with genotype data of reference data set, in order to obtain a merged file including common disease associated SNPs of both datasets. Reference data set comprise genotype data of a total of 138198 disease/trait associated SNPs of 3269 individuals from 1000 Genome Project (1Kg)(2) and Human Genome Diversity Project (HGDP)(3), belonging to 8 worldwide superpopulations and 75 subpopulations.

User can select a desired disease/trait from database, to be assessed on tested population. A curated set of risk markers is obtained and used to calculate summatory polygenic risk score (PRS) by plink software(4). Values obtained are normalized along all populations by z-score.

Results: Distribution of z-scored PRS values of user and 1Kg-HGDP populations are plotted by boxplot, bobble plot on the worldwide map (mean values) and barplot (percentile distribution).

Conclusion: PRScomp offers the opportunity to evaluate population associated risk to disease and traits that could be of great interest in planning and monitoring public health strategies.

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Conflict of Interest: Joan Fibla: None declared, Leandre Palau Employee at Grupo Globalia, Jose Nunes Employee at Grupo Globalia, Oscar Lao: None declared, Ricard López: None declared, Marina Laplana: None declared.

P19.006.A Predictive polygenic score for outcome from first-line oxaliplatin-based chemotherapy in colorectal cancer patients using supervised principal component analysis

Hanla Park[1;2], *Dominic Edelmann*[3], *Federico Canzian*[4], *Tabitha Harrison*[5], *Xinwei Hua*[6;7], *Qian Shi*[8], *Allison Silverman*[9], *Martin Schneider*[10], *Richard Goldberg*[11], *Steven Alberts*[12], *Michael Hoffmeister*[13], *Hermann Brenner*[13;14;15], *Andrew Chan*[16;17;18], *Ulrike Peters*[5;19], *Polly Newcomb*[5;19], *Jenny Chang-Claude*[1;20]

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Background/Objectives: Associations between candidate germline genetic variants and treatment outcome of oxaliplatin, a drug commonly used for colorectal cancer patients, have been reported but not robustly established. This study aimed to construct a polygenic hazard score (PHS) as a predictive marker for oxaliplatin treatment outcome by using a supervised principal component approach.

Methods: Genome-wide association analysis of interaction terms (SNP*type of treatment) was conducted using two phase III trials, 3,098 resected stage III colon cancer patients of NCCTG N0147, and 506 metastatic colorectal cancer (mCRC) patients of NCCTG N9741, separately. SNPs showing interaction with genome-wide significance ($P < 5 \times 10\text{-}8$) were selected for principal component analyses to derive a PHS. We performed replication in an independent, population-based cohort, DACHS.

Results: The PHSs based on the first two principal components of significant NPs (15 SNPs in resected stage III colon cancer and 13 SNPs in mCRC) in the discovery cohorts showed significant interaction with treatment type in models adjusted for clinical covariables. However, clinical models including PHS interaction terms were not replicated in DACHS. The prediction error of the 3-year survival remained unchanged when comparing clinical models to clinical models including interaction terms as 0.04 in resected stage III colon patients and 0.18 in mCRC patients.

Conclusion: Integration of the PHS with clinical factors did not provide a statistically significant improvement in the prediction of patient benefit from the oxaliplatin-containing regime. Our negative results highlight the challenges in providing evidence for a potential polygenic score for oxaliplatin efficacy.

References:.

Grants:.

Conflict of Interest: Hanla Park: None declared, Dominic Edelmann: None declared, Federico Canzian: None declared, Tabitha Harrison: None declared, Xinwei Hua: None declared, Qian Shi Celgene/BMS, Roche/Genentech, Janssen, Novartis., Chugai Pharmaceutical Co., Ltd, Johnson & Johnson, Amgen, and Merck & CO., Yiviva Inc, Boehringer Ingelheim Pharmaceuticals, Inc, Regeneron Pharmaceuticals, Inc., Hoosier Cancer Research Network., Allison Silverman: None declared, Martin Schneider: None declared, Richard Goldberg: None declared, Steven Alberts: None declared, Michael Hoffmeister: None declared, Hermann Brenner: None declared, Andrew Chan: None declared, Ulrike Peters: None declared, Polly Newcomb: None declared, Jenny Chang-Claude: None declared.

P19.007.B Whole genome germline genetic testing: a pilot of its role as an additional tool to general health screening in General Practice, the first in the UK

Ann-Britt Jones[1;2], *Michael Sandberg*[3], *Gabriella Pichert*[4], *Lucy Side*[4], *Tessa Homfray*[4], *Sophie Hicks*[4], *Matthew Dixon*[5], *Vincenzo Cirigliano*[5], *Bibiana Palao*[5], *Luis Izquierdo*[5], *Elizabeth Bancroft*[2], *rosalind eeles*[1;2;4]

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Background/Objectives: Whole genome sequencing (WGS) is now possible due to improvements in next generation sequencing technology. This allows opportunity to incorporate genetic data into primary care health screening. This study aimed to set up a model pathway to undertake WGS for actionable findings and assess its role in enhancing health screening.

Methods: We have undertaken WGS in 100 individuals as part of a medical screen. To date, 20 have been analysed. Individuals were recruited from a private general practice. We reviewed past medical and family history from electronic health records (EHR). Germline genetic testing consisted of 84 cancer and 77 cardiac genes and WGS, including higher penetrance monogenic mutations, recessive carrier alterations and pharmacogenomics. A multidisciplinary clinical team reviewed integrated results through an iterative process.

Results: Twenty-five percent (5 out of 20) individuals had an actionable genetic variant in either cancer or thromboembolic genes. No cardiovascular risk associated variants were found. Eighty percent of the participants had an autosomal recessive carrier variant. Pharmacogenomics results yielded significant variants in seven individuals (*DYPD*; *CYP2C19*). Forty percent of patients had a significant change in management.

Conclusion: WGS in this pilot study altered risk-reducing measures in 25% of individuals and altered the screening programme in 40% of individuals. We identified clinically significant actionable variants in unaffected participants and showed integrating genetic screening into primary care is clinically valuable.

References:.

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Conflict of Interest: None declared.

P19.008.C Selection of biomarkers for determining the degree of aggression of bladder tumors - strategy and a flexible workflow

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Background/Objectives: Bladder cancer is a heterogeneous group with several subgroups with different clinicopathological features: Low-grade non-infiltrating progressive and non-progressive cancers, High-grade muscle-invasive cancer with relatively good prognosis and those with poor prognosis.

This required improving the current knowledge that underlies this variation in tumour behavior. The aim of our study was to select potential bladder cancer biomarkers and to test the methodology for biomarkers development.

Methods: A total of 87 bladder cancer samples staged pTa to pT4 and 4 negative controls were collected. Pool gene expression analysis of 168 genes involved in pathways for Cancer drug resistance and metabolism (PAHS-004) as well as for Cancer drug targets (PAHS-507), Qiagene was performed on 40 samples staged pTa, pT1 and pT2. Conformational analyses were performed on 12 individual samples (4 per stage). The subsequent analyzes selected genes for tumor invasion. Further validation on the selected genes was performed in a cohort of additional 40 bladder cancer samples from all tumor stages pTa to pT4.

Results: The four genes: AP1S1, FIGF, HDAC11 and CDK9 were selected for gene-expression analysis based on differences in the expression levels between invasive and non-invasive bladder carcinoma. The results revealed 4-12 fold change difference in the expression level between pT2 and pTa/pT1 non-invasive tumors for FIGF, HDAC11 and CDK9 genes. AP1S1 failed to show significant difference in expression levels between tumor stages.

Conclusion: FIGF, CDK9 and HDAC11 can be considered as potential biomarkers for the characterization of invasive tumor phenotype.

References: No.

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Conflict of Interest: Olga Antonova BG NSF No KP-06-OPR01/3-2018, Zora Hammoudeh BG NSF No KP-06-OPR01/3-2018, Boris Mladenov BG NSF No KP-06-OPR01/3-2018, Zornitsa Yordanova BG NSF No KP-06-OPR01/3-2018.

P19.009.D Utilising drug switches as a proxy for angiotensin-converting enzyme inhibitor-induced cough discovers novel genetic signals

Kayesha Coley[1], *David Shepherd*[1], *Catherine John*[1], *Richard Packer*[1], *Robert Free*[2;3], *Edward Hollox*[4], *Louise Wain*[1;2], *Martin D Tobin*[1;2], *Chiara Batini*[1]

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Background/Objectives: Switching drugs from one class to another is often indicated in response to adverse reactions. Approximately 15% of patients taking angiotensin-converting enzyme inhibitors (ACEIs) experience persistent dry cough, resulting in switches from ACEIs to angiotensin-II receptor blockers (ARBs). Drawing on previously validated electronic health record-based phenotypes, we used such drug switches as a proxy to study genetic determinants of ACEI-induced cough.

Methods: Using EXCEED Study and UK Biobank primary care data, cases switched from ACEIs to ARBs within 12 months of initiating ACEIs, and controls were continuous users of ACEIs. In a two-stage meta-analysis, stage 1 included all cohort and ancestry-specific genome-wide association analyses. Variants reaching suggestive significance (p-value $<5 \times 10^{-6}$) were meta-analysed with publicly-available summary statistics of ACEI-induced cough in stage 2.

Results: A total of 39,871 individuals (5,435 cases; 34,436 controls) from 4 ancestral groups (African, Asian, Chinese and European) were included in the stage 1 meta-analysis of ~17M variants. In stage 2, we identified nine genome-wide significant (p-value $<5 \times 10^{-8}$) risk loci for which positional and gene expression analyses implicated 35 protein-coding genes, one of which (*KCNIP4*) has been previously associated with ACEI-induced cough. Novel genes included *KCNA2*, supporting the importance of potassium ion channels in cough reflex modulation, and *NTSR1*, highlighting the role of neuropeptides in neurogenic inflammation and cough hypersensitivity.

Conclusion: In this muti-ancestry meta-analysis we have identified associated genes for which functional follow-up has given insight to the currently unclear mechanism of ACEI-induced cough, and will help identify candidates for predictive pharmacogenetic biomarkers.

References:.

Grants:.

Conflict of Interest: Kayesha Coley: None declared, David Shepherd: None declared, Catherine John: None declared, Richard Packer: None declared, Robert Free: None declared, Edward Hollox: None declared, Louise Wain Research grants from GlaxoSmithKline (as principal investigator) and Orion Pharma (as co-principal investigator), Consultancy/advisory board for Galapagos, Martin D Tobin Research collaborations with Orion Pharma and GlaxoSmithKline unrelated to the current work., Chiara Batini: None declared.

P19.010.A Results of a two-year program of whole genome sequencing at a medical check-up unit

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Background/Objectives: Whole genome sequencing (WGS) of individuals without a medical indication is known as elective WGS. Elective genome is feasible to identify both primary (related to the patients or family history) and secondary (clinically relevant still not yet evident) findings.

We hypothesize that the unbiased selection of these individuals in an experienced medical check-up unit would add value to standard check-up findings.

Methods: 361 patients attended to the Medical Check Up Unit of the Clínica Universidad de Navarra and underwent a "genomic check-up" consisting in a complete medical interview, biochemistry, low-intensity whole body scan and selected genome analysis, curated with a proprietary platform (myGenome, Veritas Intercontinental).

Results: seventy-one clinically relevant genetic variants were identified in 64 of 361 patients (17.7%), including 7 patients with 2 variants. The detected variants were related to hemochromatosis (8.3%), hereditary cancer predisposition (6.9%), hereditary cardiovascular disease (2.2%), and other diseases (2.2%). Clinical phenotype-genotype associations were made based on the available clinical data. In addition, 66.2% patients were carriers of one or more recessive conditions, 88.6% presented risk alleles for multifactorial diseases and relevant pharmacogenomics associations were detected in all patients.

Conclusion: the inclusion of elective genomic sequencing in a structured medical consultation optimizes the identification of primary and secondary genetic findings and enables a better correlation of the genetic findings with the real clinical scenario of patients and their follow up.

References: Senol-Cosar O, et al. Genet Med 21:2765-2773 (2019); Hou YC, et al. Proc. Natl Acad. Sci. 117(6):3053-3062 (2020).

Grants: None significant to the purpose of this research.

Conflict of Interest: None declared.

P19.011.B Homozygous CYP2C9*14 allele resulting in profound warfarin sensitivity

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Background/Objectives: Rapid genomic testing facilitates early genetic diagnosis, while the data generated also provides opportunity for additional analyses in future, as clinical features evolve. We describe a neonate who initially presented with cleft lip/palate and congenital heart disease and underwent exome sequencing (ES) in 2018 without a diagnosis being made. Re-analysis of the ES data was performed after the clinical presentation evolved to severe dilated cardiomyopathy. Clinical management was complicated by profound sensitivity to warfarin and significant bleeding.

Methods: Clinical rapid trio ES was performed in 2018 on DNA extracted from peripheral blood using Agilent Sureselect QXT CREv1 kit, following by sequencing on Illumina NextSeq500. Re-analysis using updated clinical information and updated phenotype-driven virtual gene panels was performed in 2021.

Results: A homozygous pathogenic variant in *PPP1R13L* (c.1068dupC; p.(Ser357Leufs*49)) was identified as causing the primary clinical features (dilated cardiomyopathy and cleft lip/palate), this gene-disease association having been described in 2020 in five unrelated families. In addition, homozygosity for the well-established *CYP2C9**14 (p.Arg125His) warfarin sensitivity allele was identified, explaining the extreme warfarin hypersensitivity. Alternate anticoagulation with intravenous bivalirudin and low molecular weight heparin successfully preventing thromboembolic complications.

Conclusion: Heterozygosity for the *CYP2C9**14 allele has been described in individuals with increased sensitivity to the anticoagulant effects of warfarin. This is the first case report of an individual homozygous for the *CYP2C9**14 allele with profound warfarin sensitivity. In addition to expanding the phenotypic spectrum associated with the *CYP2C9**14 allele, this case report highlights the value of genomic data as a healthcare resource.

References:.

Grants: Australian Government GHFM76747.

Conflict of Interest: None declared.

P19.012.C Genetic and environmental determinants of drug adherence

Mattia Cordioli[1], *Andrea Corbetta*[1], *Sakari Jukarainen*[1], *Tuomo Kiiskinen*[1], *Samuli Ripatti*[1;2;3], *Andrea Ganna*[1;4;5]

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Background/Objectives: One of the major factors behind the efficacy of pharmacological treatments is patients adherence to the prescribed therapy regimen. While demographic and socioeconomic factors play a role in determining adherence, there have been few investigations on the potential effects of genetic variation on drug adherence. By using genetic data from the FinnGen study (N = 356,077), data from Finnish nation-wide health registries and the drug purchase registry (68,826,654 total purchases), we provide a systematic investigation of adherence determinants across multiple medications.

Methods: We looked at adherence (ratio between the total purchased quantity and total days of purchasing) and early discontinuation (stopping after one purchase) for six different class of medications: statins, blood pressure (BP) medications, breast cancer medications, antiplatelets, anticoagulants and glaucoma medications.

For each, we run a GWAS of adherence and early stopping *vs* good adherence. We estimated genetic correlation (r_g) between these and 29 publicly available traits.

Results: Adherence to BP medications or statins positively associated with psychological or behavioural traits such as educational attainment, participation to follow-up questionnaires, subjective well-being and with predisposition to type2 diabetes, higher BMI, higher systolic BP. Negative associations were observed between adherence and risk tolerance, loneliness and schizophrenia. Similar association patterns were observed for early discontinuation of BP medications.

Conclusion: Overall, results suggest adherence is related to behavioural aspects and perception of underlying risk factors or disease severity rather than to biologic determinants, allowing for a better identification of patients at high risk of non-adherence in drug taking.

References: -.

Grants: -.

Conflict of Interest: None declared.

P19.013.D Polygenic scores for cardiovascular disease - how could they be implemented?

Tanya Brigden[1], *Saskia Sanderson*[2], *Joanna Janus*[1], *Chantal Babb de Villiers*[1], *Sowmiya Moorthie*[1], *Mark Kroese*[1], *Alison Hall*[1]

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Background/Objectives: With the increased interest in the possible use of polygenic score analysis for clinical care, we explored the implications arising from the possible implementation and delivery of polygenic score analysis for cardiovascular disease in practice, specifically in the context of the NHS Health Check programme. Underpinning our analysis is the assumption that a robust polygenic score model has been developed, suitable for implementation.

Methods: We adopted a mixed methods research approach, including a literature review of peer-reviewed publications and grey literature and semi-structured interviews with domain specific experts.

Results: Our analysis suggests that polygenic score analysis could be incorporated into NHS Health Checks with only modest changes relating to the interface between healthcare professionals and patients. However, such implementation will be contingent on a robust testing infrastructure being in place.

Looking further forward, incorporating polygenic scores may generate opportunities for developing personalised risk assessments targeted at individuals or sub-groups.

Conclusion: More evidence is needed on the impact of polygenic score analysis on healthcare provider behaviour, particularly the impact of a high polygenic score on the likelihood of a health professional offering a prescription or recommending lifestyle change. If health systems choose to adopt personalised risk assessment incorporating polygenic score analysis, a key priority will be to address any inequalities and inequities that might arise.

References: T Brigden, S Sanderson, J Janus, C Babb de Villiers, S Moorthie, M Kroese, A Hall, Implementing polygenic scores for cardiovascular disease into NHS Health Checks, PHGF 2021.

Grants:.

Conflict of Interest: None declared.

P19.014.A High throughput genetic characterization of Italian patients affected by multi-drug resistant rheumatoid and psoriatic arthritis

Paola Tesolin[1], *Francesca Bertinetto*[2;3], *Arianna Sonaglia*[4], *Maria Pina Concas*[5], *Anna Morgan*[5], *Giuseppe Giovanni Nardone*[5], *Alen Zabotti*[4], *Paolo Gasparini*[1;5], *Antonio Amoroso*[2;3], *Luca Quartuccio*[4], *Giorgia Girotto*[1;5]

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Background/Objectives: Rheumatoid and Psoriatic arthritis (RA and PsA) are multifactorial autoimmune diseases for which very few susceptibility genes have been described so far. We genetically characterised a highly selected cohort of non-responder patients.

Methods: The accurate clinical examination of eleven patients affected by refractory RA/PsA was followed by several high-throughput genetic testing:

1. Targeted resequencing focused on HLA genes.
2. WES to detect rare and pathogenic variants. T-test was employed to compare variants burden of RA/PsA group with a WGS control cohort.
3. SNP-arrays to identify CNV.

Results: As regards 1) the 11 patients carry at least one HLA allele that might predispose to those diseases (i.e. HLA-DRB1*04; *DRB1*10:01 and DRB1*01). Two subjects also carry

a particular ancestral haplotype (i.e. HLA-A*33:01;B*14:02;C*08:02;DRB1*01:02;DQB1*05:01). For analysis 2), 27 rare variants (MAF<0.01) within 22 candidate RA/PsA genes have been detected and checked in the WGS control cohort. For example, two patients carry different pathogenic variants (i.e. heterozygous missense and homozygous truncating) within *UGT2B17*, a gene involved in hormones and drug metabolisms. Other two carry pathogenic heterozygous missense variants within *SERPING1* and *VPS13D*, having a role in complement-cascade and IL6-signaling, respectively. Further, we identified four heterozygous missense variants within *HACE1*, *IRF2BP2* and *DVL1*, which might modulate TNFα signalling. In analysis 3) we identified CNVs within genes involved in crucial mechanisms of RA/PsA (e.g. *MSR1)*.

Conclusion: This multistep approach allowed the discovery of novel RA/PsA candidate genes and the definition of genotype-phenotype correlations. Thus, we highlighted new molecular targets to help clinicians define the best therapeutic approach.

References:.

Grants:.

Conflict of Interest: None declared.

P19.015.B Transferability of genetic loci and polygenic scores for cardiometabolic traits in British Pakistanis and Bangladeshis from a real-world healthcare cohort

Qinqin Huang[1], *Neneh Sallah*[2], *Diana Dunca*[2], *Bhavi Trivedi*[3], *Karen Hunt*[3], *Sam Hodgson*[4], *Samuel Lambert*[5], *Elena Arciero*[1], *John Wright*[6], *Chris Griffiths*[3], *Richard Trembath*[7], *Harry Hemingway*[2], *Michael Inouye*[5], *Sarah Finer*[3], *David van Heel*[3], *Thomas Lumbers*[2], *Hilary Martin*[1], *Karoline Kuchenbäcker*[2]

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Background/Objectives: Individuals with South Asian ancestry have a higher risk of heart disease than other groups in Western countries. However, most genetic research has focused on European-ancestry individuals and it is largely unknown whether the findings are applicable to other groups.

Methods: Using data from 22,000 British Pakistanis and Bangladeshis with linked electronic health records from the Genes & Health cohort (G&H), we conducted genome-wide association studies (GWAS) of coronary artery disease (CAD) and its key risk factors, body mass index (BMI), lipid biomarkers and blood pressure. We estimated power-adjusted transferability (PAT) ratios, a new technique to assess the extent to which loci are transferable. We tested how well polygenic scores (PGS) developed in European ancestry studies performed in G&H.

Results: We observed high transferability for cardiometabolic loci from European GWAS that were sufficiently powered to replicate in G&H: the PAT ratios were all ≥1 for the risk factor traits, but only 0.62 for CAD (binomial $p = 0.05$). The relative accuracy of PGS compared to European ancestry was ≥0.95 for HDL-C, triglycerides, and blood pressure, but lower for BMI (0.78) and CAD (0.42). We observed additional predictive power of PGS beyond clinical risk factors (QRISK3) for CAD, with a significant net reclassification index (NRI) =3.9% (95% CI 0.9 – 7.0).

Conclusion: Transferability of GWAS loci and PGS was high for lipid and blood pressure traits but lower for BMI and CAD. Our analyses indicate clinical validity for adding PGS to existing clinical risk prediction tools in primary prevention settings for this population.

References:.

Grants:.

Conflict of Interest: Qinqin Huang: None declared, Neneh Sallah NS is now employed by GlaxoSmithKline., Diana Dunca: None declared, Bhavi Trivedi: None declared, Karen Hunt: None declared, Sam Hodgson: None declared, Samuel Lambert: None declared, Elena Arciero: None declared, John Wright: None declared, Chris Griffiths: None declared, Richard Trembath: None declared, Harry Hemingway: None declared, Michael Inouye: None declared, Sarah Finer: None declared, David van Heel: None declared, Thomas Lumbers: None declared, Hilary Martin: None declared, Karoline Kuchenbäcker: None declared.

P19.016.C "Actionable" host genetic markers for repurposing "add-on therapy" in COVID-19

Chiara Fallerini[1;2], *Francesca Fava*[1;2;3], *Margherita Baldassarri*[2;3], *Sabino Scolletta*[4], *Federico Franchi*[4], *Serafina Valente*[5], *Bruno Frediani*[6], *Elena Bargagli*[7], *Andrea Antinori*[8], *Simone Lanini*[9], *Andrea Isidori*[10], *Mario Umberto Mondelli*[11;12], *STEFANIA MANTOVANI*[11], *Massimo Vaghi*[13], *Gülsah Gabriel*[14;15;16], *Francesca Mari*[2;3;17], *GenCovid Multicenter Study*[17], *Alessandra Renieri*[2;3;17]

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Background/Objectives: COVID-19 still represents a life-threatening disease in individuals with a specific genetic background. We successfully applied a new Machine Learning method on WES data to extract a set of coding variants relevant for COVID-19 severity. We aim to identify personalized add-on therapy.

Methods: A subset of identified variants, "actionable" by repurposed drugs, were functionally tested by in vitro and in vivo experiments.

Results: Males with either rare loss of function variants in the *TLR7* gene or L412F polymorphism in the *TLR3* gene benefit from IFN-γ, which is specifically defective in activated PBMCs, restoring innate immunity. Females heterozygous for rare variants in the *ADAMTS13* gene and males with D603N homozygous polymorphism in the *SELP* gene benefit from Caplacizumab, which reduces vWF aggregation and thrombus formation. Males with either the low-frequency gain of function variant T201M in *CYP19A1* gene or with poly-Q repeats ≥23 in the *AR* gene benefit

from Letrozole, an aromatase inhibitor, which restores normal testosterone levels, reducing inflammation and which rescues male golden hamsters from severe COVID-19.

Conclusion: By adding these commonly used drugs to standard of care of selected patients, the rate of intubation is expected to decrease consistently, especially in patients with high penetrance rare genetic markers, mitigating the effect of the pandemic with a significant impact on the healthcare system.

References: Fallerini C et al. Common, low-frequency, rare, and ultra-rare coding variants contribute to COVID-19 severity. Hum Genet. 141(1):147-173 (2022).

Grants: FISR 2020 / Tuscany Region COVID-19 / INTERVENE - GA No. 101016775 / Soka Gakkai PAT-COVID.

Conflict of Interest: None declared.

P19.017.D Input of PRS313 for breast cancer risk stratification in patients with atypical breast lesion

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Background/Objectives: The genetic architecture of breast cancer (BC) susceptibility implies Mendelian inheritance related to "high-risk" genes like *BRCA1/2*, and polygenic inheritance estimated through GWAS. Polygenic risk scores (PRS) derived from GWAS help assessing such polygenic inheritance. PRS contribution to BC risk estimation was demonstrated in several Caucasian female populations, including women with high/moderate risk due to BRCA and CHEK2 mutations[1-3]. Atypical breast Lesions (AL) confer moderate to high risk of BC to carriers, but no routine prognostic markers are defined.

In this single-center study, we retrospectively evaluated the performances of PRS_{313} to refine BC risk within a cohort of patients with AL.

Methods: PRS_{313} was determined in non-tumor breast samples in 150 cases and 150 controls, defined respectively by history of AL and BC within 5 years, and history of AL without BC. Fluidigm© Microfluidic genotyping was performed. We assessed (1) the reproducibility of genotyping on FFPE compared to frozen tissues (2) the performances of PRS to detect high-risk women within this population, through sensitivity, specificity and area under the curve (AUC), and (3) evaluated odds-ratios between first and last decile of patients.

Results: Performance's parameters are currently under evaluation; results in our population will be discussed compared to patients with BC risk factors from literature.

Conclusion: PRS_{313} is a promising tool to better refine BC risk in specific female populations with moderate to high risk and may help clinical decisions.

References: 1- Mavaddat &al, Am J Hum Genet 2019.
2- Lakeman &al, Genet Med 2021.
3- Muranen &al, Genet Med 2017.

Grants: AOR-BFC-Plateforme-2020. AOI-Biocollection-2020.

Conflict of Interest: None declared.

P19.018.A A simple tool to enhance polygenic risk prediction in coronary heart disease and type 2 diabetes

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Background/Objectives: Large-scale biobank initiatives and commercial repositories store genomic data collected from millions of individuals, and tools to augment easily derived polygenic risk scores with simple questionnaire-based clinical risk factors are needed for disease prevention.

Methods: Here, we describe the derivation and validation of simple genomics-enhanced risk tools for two common cardiometabolic diseases, coronary heart disease (CHD) and type 2 diabetes (T2D). Data used for our analyses include the FinnGen ($N = 309{,}154$) and the UK Biobank ($N = 343{,}672$) studies. The risk tools integrate contemporary genome-wide polygenic risk scores with simple questionnaire-based risk factors, including demographic, lifestyle, medication, and comorbidity data, enabling risk calculation across resources where genome data is available.

Results: Compared to routinely used clinical risk scores for prediction of incident CHD and T2D (PCE/QRISK3 for CHD and QDiabetes/FINDRISC for T2D), the new risk tools show at least equivalent risk discrimination, improved risk reclassification (net reclassification improvements ranging from 3.7 to 6.2), and effective 10-year risk stratification. The predictive performance was best in women, younger (age < 55), and non-obese (BMI < 30) individuals, groups in which clinical risk scores often show limited utility. The performance can be further improved with routine lipid and blood pressure measurements.

Conclusion: Without the need for blood tests or evaluation by a health professional, the risk tools provide a powerful yet simple method for preliminary cardiometabolic risk assessment for individuals with genome data available.

References:.

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Conflict of Interest: None declared.

P19.020.C Automated identification of a cancer patient treatment: from sequencing to treatment prioritisation

***Nicolas Soirat**[1;2;3], Denis Bertrand[1], Sacha Beaumeunier[1], Nicolas Philippe[1], Dominique Vaur[3], Sophie krieger[2;3], Anne-Laure Bougé[1], Laurent Castera[3]*

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Background/Objectives: The emergence of sequencing allowed the scientific community to gather a tremendous amount of cancer genomic data, characterising biomarkers responsible for tumorigenesis that might indicate potential treatments. The use of short-read sequencing to identify cancer patient treatment is becoming a more common practice in hospitals. To standardise the treatment identification some prediction frameworks have been developed, but they mostly focus on a single alteration type and very few have been implemented.

Methods: We design a targeted DNA and RNA panel covering 639 cancer genes and 57 fusion genes to obtain a comprehensive patient genomic landscape. We developed a decisional algorithm which prioritises all known variant-therapy associations. Several rules give a score for each association based on more than 20 variant features indicating the variant impact in cancer, the patient indication and similarity of patient variant with variant in therapeutic databases.

Results: We generated a thousand simulated tumours, each containing passenger mutations and a targetable mutation from the Civic database. Our method correctly classifies the targetable mutation in its top predictions (average rank 2.19). Furthermore, on a cohort of 12 patients, we obtain similar results as 2 clinical routine approaches using our fully automated protocol. Currently, we are expanding our validation to a pan-cancer cohort of 500 patients.

Conclusion: We design a complete framework for multiple variant drug association identification in order to make easier therapeutic choices for a clinician. We succeed to integrate it into our variant calling workflow and show good performance of our method to prioritise targetable variants.

References:.

Grants:.

Conflict of Interest: None declared.

P19.021.D Quantifying the causal impact of modifiable risk factors and biomarkers on total healthcare burden via Mendelian randomization

Jiwoo Lee[1], *Sakari Jukarainen*[2], *Pradeep Natarajan*[1], *Andrea Ganna*[2]

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Background/Objectives: Public health aims to reduce the burden of modifiable risk factors to improve population health and decrease healthcare burden. Previous studies have attempted to quantify the total healthcare burden (e.g. total healthcare expenditure) associated with certain modifiable risk factors such as body mass index (BMI), systolic blood pressure (SBP), or cholesterol. However, such studies are based on observational data or single disease clinical trials. Thus, they are likely to suffer from confounding or ignore the impact of modifiable risk factors on broader healthcare burden, which is mediated by multiple diseases.

Methods: Here, we address these limitations by using genetic instruments to assess the causal impact of modifiable risk factors on healthcare burden and expenditure. We leveraged primary care, hospital visit, and medication costs for 236,101 FinnGen participants to quantify the total healthcare burden of each participant since 2011. We performed Mendelian randomization using genetic instruments for 15 modifiable risk factors and biomarkers.

Results: We found that a SD increase in SBP, body mass index BMI, and low-density lipoprotein cholesterol (LDL-C) was causally associated with an increase in observed healthcare costs of 27.6% [95% CI: 20.5%–35.2%], 17.1% [11.7%–22.8%], and 5.2% [2.2%–8.2%], respectively, while high-density lipoprotein cholesterol (HDL-C) was associated with a decrease in observed healthcare costs by 5.2% [1.8%–8.5%].

Conclusion: Taken together, we quantified the causal impact of modifiable risk factors and biomarkers on total healthcare burden.

References: None.

Grants: None.

Conflict of Interest: None declared.

P19.022.A Genome-wide association studies of advanced cancer patients identified loci associated with opioid-induced nausea-vomiting

Francesca Minnai[1], *Morena Shkodra*[2;3], *Sara Noci*[4], *Cinzia Brunelli*[2], *Alessandra Pigni*[2], *Frank Skorpen*[5;6], *Pål Klepstad*[5;7], *Stein Kaasa*[3;7], *Oscar Corli*[8], *Maria Caterina Pallotti*[9], *Marco Cesare Maltoni*[9], *Augusto Caraceni*[2;10], *Francesca Colombo*[1]

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Background/Objectives: Advanced cancer patients usually receive opioids to treat cancer pain; unfortunately, 10-20% do not benefit from treatment and even higher percentages experience side effects such as nausea and vomiting. Previous studies suggested a possible role of genetics in determining individual variability in the response to opioids. The aim of this genome-wide association study is to identify new genetic markers of opioid toxicity.

Methods: The study included 1,038 European cancer patients receiving morphine, oxycodone, buprenorphine or fentanyl. The patients were included in one cross-sectional and two longitudinal studies. Nausea and vomiting was measured by the European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC-QLQ-C30). A composite nausea-vomiting score (NVS), ranging from 0 to 100, was calculated. Patients were genotyped using Axiom PMRA arrays. Genome-wide linear regressions between NVS and the genotypes of 432,087 variants were performed using PLINK software.

Results: NVS significantly associated ($P < 5.0x10^{-8}$) with the genotype of 12 variants (six, five, and one on chromosome 2, 6 and 15, respectively). The top-significant variants at each locus were rs6723108 (beta = 7.26, $P = 7.47x10^{-11}$), rs2596503 (beta = 10.0, $P = 2.96\ x10^{-11}$), and rs1129138 (beta = -5.81, $P = 1.65\ x10^{-9}$), respectively. The gene nearest to rs6723108 is *TMEM163*, encoding a transmembrane protein transporting zinc ions, whereas variants on chromosomes 6 mapped in the HLA locus.

Conclusion: These results indicate that opioid induced nausea and vomiting are regulated by germline variants. However, further studies are needed to validate these results and to identify the molecular mechanisms underlying the observed associations.

References: https://doi.org/10.1038/s41598-019-57358-y.

Grants: AIRC MFAG 2019 - ID. 22950 project.

Conflict of Interest: None declared.

P19.024.C Typing CYP2D6 star alleles from fully phased variants using PacBio HiFi reads

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Background/Objectives: The *CYP2D6* locus is well known for its importance to pharmacogenetics as well as for its high diversity and complex genomic setting. Resolving individual alleles at this locus using short-read sequencing technologies requires inference-based methods due to ambiguous mapping in the presence of highly homologous pseudogenes. In contrast, long-range sequencing with

PacBio HiFi reads directly resolves and phases a wide range of complicated and difficult genetic loci without inference. We present a novel bioinformatics workflow using PacBio HiFi reads which enables rapid and precise diplotyping and star(*)-allele classification of *CYP2D6*.

Methods: In this work we designed a set of primers to amplify full *CYP2D6* genes and flanking sequence. A multi-primer approach was used to separately amplify primary *CYP2D6* genes, duplicate genes, hybrid genes, and fully deleted *5 alleles. We applied this targeted strategy to 22 samples from Coriell and sequenced the amplicons on a PacBio Sequel II System. To generate resolved CYP2D6 *-allele diplotypes we describe a two-step process: 1) Cluster and consensus of PacBio HiFi reads, 2) Direct comparison of phased variant sets from consensus sequences to star-alleles described in PharmVar. Additional information regarding fusion alleles is also provided to further identify hybrid categories.

Results: Direct CYP2D6 *-allele typing generated by this workflow resulted in concordant results compared to orthogonal technologies. Differences between previous technologies' results and PacBio HiFi sequencing were due to higher resolution and improved calls via our method, including better CNV calls, *5 deletion calling, and high resolution subtyping for all alleles.

Conclusion:.

References:.

Grants:.

Conflict of Interest: John Harting Pacific Biosciences, Stock/options, Zev Kronenberg Pacific Biosciences, stock/options, Nina Gonzaludo Pacific Biosciences, stock/options, Jenny Ekholm Pacific Biosciences, stock/options, Geoff Henno Pacific Biosciences, stock/options, Edd Lee Pacific Biosciences, stock/options.

P19.025.D Clinicians' perceptions towards precision medicine tools for cardiovascular disease risk stratification in South Africa

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Background/Objectives: Cardiovascular disease (CVD) risk stratification in African populations could improve with the inclusion of genetic risk to develop a clinically relevant precision medicine (PM) approach. African genetic data are scarce and current stratification approaches remain unvalidated. Successful PM implementation requires medical fraternity support. This study assessed clinicians' knowledge, perceptions, and confidence towards implementing a PM tool for CVD risk stratification in the South African public health setting.

Methods: Electronic self-administered questionnaire was provided to clinicians in public hospitals in Johannesburg. Knowledge, perception, and confidence towards PM-based CVD risk stratification and perceived barriers were evaluated. Bivariate analysis assessed the effect of clinical speciality, practice level, research involvement, postgraduate study, clinical experience, exposure to genetics training and traditional CVD risk stratification on mean scores.

Results: Of the 94 respondents, 23.4% currently use clinical genetic testing, and 14.1% have formal genetics education and training. 79.5% had a low mean knowledge score, with higher scores associated with genetic training ($p < 0.005$) and research involvement ($p < 0.05$). 56.3% felt confident in applying the PM-based CVD risk stratification, with significantly higher scores amongst those involved in research ($p < 0.005$) or already undertaking CVD risk stratification ($p < 0.005$). Despite limited knowledge, 83.3% of respondents perceive PM approaches positively, with screening approaches tailored to African populations being the most valued benefit. Cost and limited access to genetic services were considered the greatest barriers to implementation.

Conclusion: Addressing the gap in genetic training is necessary to increase confidence in utilising and effectively implementing PM in South Africa.

References:.

Grants: National Human Genome Research Institute (NHGRI) grant U54HG006938.

Conflict of Interest: Michelle Kamp: None declared, Oliver Pain: None declared, Cathryn Lewis Advisory board member, Myriad Neuroscience, Michele Ramsay: None declared.

P19.026.A Modelling the patient journey of osteoarthritis in the context of obesity, using longitudinal health care data and genetics

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Background/Objectives: Osteoarthritis (OA) is a chronic degenerative joint disease, causing decreased quality of life and a high economic health-care burden. The utility of known OA risk factors for predicting OA progression is insufficiently understood [1,2]. We developed predictive risk models of OA progression, across patient subgroups, to improve understanding of the heterogenous OA aetiology and contribute to early diagnosis and treatment.

Methods: UK biobank data was explored using machine learning (ML) models to predict onset and progression of OA (N~100,000). The ML models integrated longitudinal information across primary and secondary health care data, as well as basic anthropometrics, lifestyle and nutrition, socioeconomic status, and genetic data. Several approaches were explored for incorporating genetic features. These included focusing on known common risk variants either associated with OA [3] or located within functionally relevant OA genes, and genetic risk scores.

Results: A subgroup of people with obesity had increased risk of OA compared to a normal weight population (OR: 2.95). In this subgroup, XGBoost predicted people with a risk of rapid progression to OA following obesity (1-6yrs) with ROC-AUC:0.66 with age, walking pace, BMI and health rating as the most predictive features. People with slower progression to OA following obesity (6-10yrs) were predicted with similar performance (ROC-AUC:0.65). Genetic risk scores of selected OA-associated genes were important for prediction of slow progression.

Conclusion: Predictive models of OA progression identify people who would most benefit from early intervention and enable the discovery of drug target candidates.

References: [1] PMID:26904959.
[2] PMID:30523334.
[3] PMID:34450027.

Grants: Danish Diabetes Academy NNF17SA0031406 and the Innovation Fund Denmark.

Conflict of Interest: Rikke Linnemann Nielsen Novo Nordisk, full-time employment, Thomas Monfeuga Novo Nordisk, full-time employment, Zahra McVey Novo Nordisk, full-time employment, Luis G. Leal Novo Nordisk, full-time employment, Line E. Lund Novo Nordisk, full-time employment, August Thomas Hjortshøj Schreyer: None declared, Carol Sun Novo Nordisk, full-time employment, Marianne Helenius: None declared, Robert Kitchen

Novo Nordisk, full-time employment, Ramneek Gupta Novo Nordisk, full-time employment.

P19.027.B Pharmacogenetic analysis of a protein-protein interaction network in cyclosporine pathway in Greek patients with psoriasis under cyclosporine treatment

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Background/Objectives: Psoriasis is a chronic, inflammatory skin disease that affects 2-3% of the population worldwide. Although cyclosporine is a well-established systemic therapy for moderate to severe psoriasis, patients show important heterogeneity (~70%) in their response to treatment. The aim of our study was the pharmacogenetic analysis of Greek patients with psoriasis based on the construction of the cyclosporine-pathway Protein-Protein Interaction (PPI) network.

Methods: The cyclosporine related protein interactome was constructed through the PICKLE meta-database (www.pickle.gr). Based on this, we selected 30 single nucleotide polymorphisms mapped on 22 of the key molecular nodes in the cyclosporine signaling cascade, filtered through their functional significance and/or MAF≥5%. Forward, reverse and the appropriate iPLEX extension PCR primers were designed using the ADS software. SNP genotyping of the 200 patients included in our study was performed through the iPLEX®GOLD panel of the MassARRAY®System (Agena Biosciences). In addition to single-SNP analysis carried out in Stata 13.1, haplotypes were constructed with the Hapstat 3.0 software.

Results: Preliminary single-SNP analyses showed statistically significant associations between *PPP3R1* rs1868402 ($P = 0.02$) and *MALT1* rs2874116 ($P = 0.03$) polymorphisms with positive response to cyclosporine therapy, but also a trend for association for 9 more genetic biomarkers. Haplotype analysis further enhanced the predictive value of rs1868402 as a pharmacogenetic biomarker for cyclosporine therapy ($P = 0.0197$).

Conclusion: Our findings have the potential not only to improve our prognosis of cyclosporine therapy in psoriasis patients but more importantly to be applied as a methodological approach in the pharmacogenetics of biological therapies in complex diseases.

References:.

Grants:.

Conflict of Interest: None declared.

P19.029.D Whole-exome sequencing re-analysis of families with suspected Gastric Cancer tumour risk syndromes strengthens the role of PALB2 in diffuse gastric cancer and lobular breast cancer predisposition

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Background/Objectives: Hereditary diffuse gastric cancer (HDGC), is the major gastric cancer tumour risk syndrome (GC-TRS) and is caused by loss-of-function germline variants in *CDH1* or *CTNNA1*, which predisposes for diffuse gastric cancer (DGC) and/or lobular breast cancer (LBC). Overall, >50% of all clinically-diagnosed HDGC remain unsolved. We hypothesise that other tumour risk syndrome (TRS) genes explain unsolved GC-TRS cases.

Methods: We systematically searched the literature for individuals/families with cancer-history fitting clinical criteria for GC-TRS and bearing Pathogenic/Likely pathogenic variants (PVs) in TRS genes. Within the SOLVE-RD project, we re-analysed germline WES from 61 unsolved GC-TRS suspected families from the Netherlands, the United Kingdom and Canada. A cohort of 23 unsolved Portuguese GC-TRS cases was used to validate actionable candidate single nucleotide variants (SNVs) or copy number variants (CNVs).

Results: The literature search returned 28 PVs affecting 16 TRS-genes in 27 GC-TRS families, with *PALB2* pathogenic SNVs occurring in 7/27 (26%) families dominated by DGC and LBC. SOLVE-RD re-analysis identified 1/61 (1.6%) GC-TRS family bearing a *PALB2* pathogenic CNV, and a further case with LBC (1/23;4.3%) was identified in the validation cohort, bearing a *PALB2*-PV, and no other candidate variants.

Conclusion: GC-TRS families may be explained by non-classical genes, with *PALB2* as the most frequently mutated gene found here (2.4%) in families fulfilling HDGC criteria and unsolved by *CDH1* or *CTNNA1*. This study supports the role of *PALB2* SNVs and CNVs in the predisposition for DGC and/or LBC, and widening of genetic testing for GC-TRS suspected-families.

References:.

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Conflict of Interest: None declared.

P19.030.A Functional characterization of RUNX1 germline variants enhances translation of genetic data into personalized care

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Background/Objectives: Familial platelet disorder with predisposition to hematologic malignancies (RUNX1-FPD) is caused by heterozygous pathogenic *RUNX1* germline variants. Following current variant classification guidelines, *RUNX1* missense variants must frequently be classified as variants of uncertain significance (VUS) allowing no clinical conclusions. To support variant classification, we have established, validated and clinically applied a set of functional assays addressing various aspects of RUNX1.

Methods: We analyzed RUNX1's heterodimerization with CBFB using FRET, its phosphorylation by western blotting, and its ability to activate transcription in transactivation assays in HEK293T and HEL cells. Individual assay results were combined in a functional score and integrated into variant classification.

Results: To date, we investigated 11 (likely) pathogenic controls, one benign control, and 19 *RUNX1* variants of interest. We showed that the majority of *RUNX1* variants detected in RUNX1-FPD can be evaluated by our functional assays and demonstrated that transactivation assays are suitable first-line screening tools. Integrating functional data led to reclassification of five *RUNX1* VUS to likely pathogenic, two *RUNX1* VUS to likely benign and supported the (likely) pathogenic classification of four additional variants.

Conclusion: Functional assays support *RUNX1* variant classification and can be key to obtain a genetic diagnosis. These assays facilitate translation of genetic data into personalized medical care for patients and relatives at risk. Currently, we analyze additional missense VUS (n = 16) and aim to establish functional high-throughput assays as well as test tools for N-terminal RUNX1 variants to further enhance *RUNX1* variant classification.

References:.

Grants: EHA John Goldman Clinical Research Grant 2017; BMBF MyPred (01GM1911B).

Conflict of Interest: None declared.

P19.031.B Costs and diagnostic yield of whole genome sequencing in neurodevelopmental disorders

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Background/Objectives: Whole genome sequencing (WGS) has the potential to be a comprehensive genetic test, especially relevant for individuals with neurodevelopmental disorders, syndromes and congenital malformations. However, the cost consequences of using WGS as a first-line genetic test for these individuals are not well understood. The study objective was to compare the healthcare costs and diagnostic yield when WGS is performed as the first-line test instead of chromosomal microarray analysis (CMA).

Methods: Two cohorts were analyzed retrospectively using register data, cohort CMA (418 patients referred for CMA at the department of Clinical Genetics, Karolinska University Hospital, during 2015) and cohort WGS (89 patients included in a WGS-first prospective study in 2017). The analysis compared healthcare consumption over a two-year period after referral for genetic testing and diagnostic yield over a two- and three-year period after referral.

Results: The mean healthcare cost per patient in cohort WGS was $2,339 lower compared to cohort CMA ($-2,339, 95%CI, -12,238-7,561; P = 0.64) including higher costs for genetic investigations ($1,065, 95%CI, 834-1,295; P < 0.001) and lower costs for outpatient care ($-2,330, 95%CI, -3,992-(-669); P = 0.006). The diagnostic yield was 23% higher for cohort WGS (cohort CMA 20.1%, cohort WGS 24.7%) (0.046, 95%CI, -0.053-0.145; P = 0.36).

Conclusion: WGS as a first-line diagnostic test for individuals with neurodevelopmental disorders is associated with statistically non-significant lower costs and higher diagnostic yield compared with CMA. This indicates that prioritizing WGS over CMA in health care decision making will yield positive expected outcomes as well as showing a need for further research.

References:.

Grants:.

Conflict of Interest: None declared.

P19.032.C Polygenic risk scores for disease progression in clinically related events

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Background/Objectives: Predicting who is at elevated risk of developing a severe disease remains an important task in many clinical disciplines to optimize screening and prevention.

Methods: Here, we test the utility of polygenic risk scores (PRSs) in a potentially actionable clinical setting where a patient presents with an unspecified symptom using electronic health records of 356,077 Finns. Examples for such scenarios are individuals with an unclear possible seizure at risk to develop epilepsy or individuals with unclear stomach complaints (irritable bowel syndrome) at risk to develop an autoimmune inflammatory bowel disease (IBD).

Results: In 7,083 individuals with irritable bowel syndrome, we found that individuals with the top 10% of IBD-PRS showed an increased risk (HR 2.24, 95% CI 1.60-3.15) of developing IBD after 10 years compared to individuals with lower IBD-PRS. In young adults (age < 40) the effect of IBD-PRS was larger: the top 10% of IBD-PRS had a risk of developing IBD of ca. 14%. Individuals in the bottom 25% of IBD-PGS were consistently at low risk (<5%) to develop IBD. Disease-specific PRS had a more modest benefit in scenarios of high genetic correlation between two endpoints such as predicting myocardial infarction in individuals with angina pectoris or onset of type 2 diabetes in women with gestational diabetes. Analyses are replicated in a biobank network (n = 1.2 Million).

Conclusion: We are thus presenting scenarios and methods to improve the utility of PRSs for predicting disease progression.

References:.

Grants: Horizon 2020(101016775).
Conflict of Interest: None declared.

P19.033.D SwissGenVar: A genetic data-sharing platform and knowledge-database for harmonization and up-scaling of clinical grade interpretation of genetic variants to foster personalized health care in Switzerland

Dennis Kraemer[1], *Dilenn Terumalai*[2], *Livia Famiglietti*[3], *Stephanie Meier*[4], *Javier Sanz*[5], *Samuel Koller*[6], *Pascal Joset*[4], *Fabienne Maurer*[7], *Thierry Nouspikel*[8], *André Schaller*[5], *Isabel Filges*[4], *Andrea Superti-Furga*[7], *Marc Abramowicz*[8], *Wolfgang Berger*[6], *Sven Cichon*[4], *Valérie Barbié*[2], *Anita Rauch*[1]

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Background/Objectives: Representing an effort of all Swiss academic centers for Medical Genetics under the umbrella of the Swiss Personalized Health Network (SPHN), SwissGenVar aims to create the infrastructure for a scalable and user-friendly Swiss-wide depository and sharing platform of genetic variant data generated during routine diagnostic procedures and research projects. SwissGenVar intends to provide an environment for expert knowledge-sharing to harmonize and up-scale the significance interpretation of genetic variants at clinical-grade. Broader visibility will be gained by interfacing with international databases, thus supporting global initiatives in personalized health care.

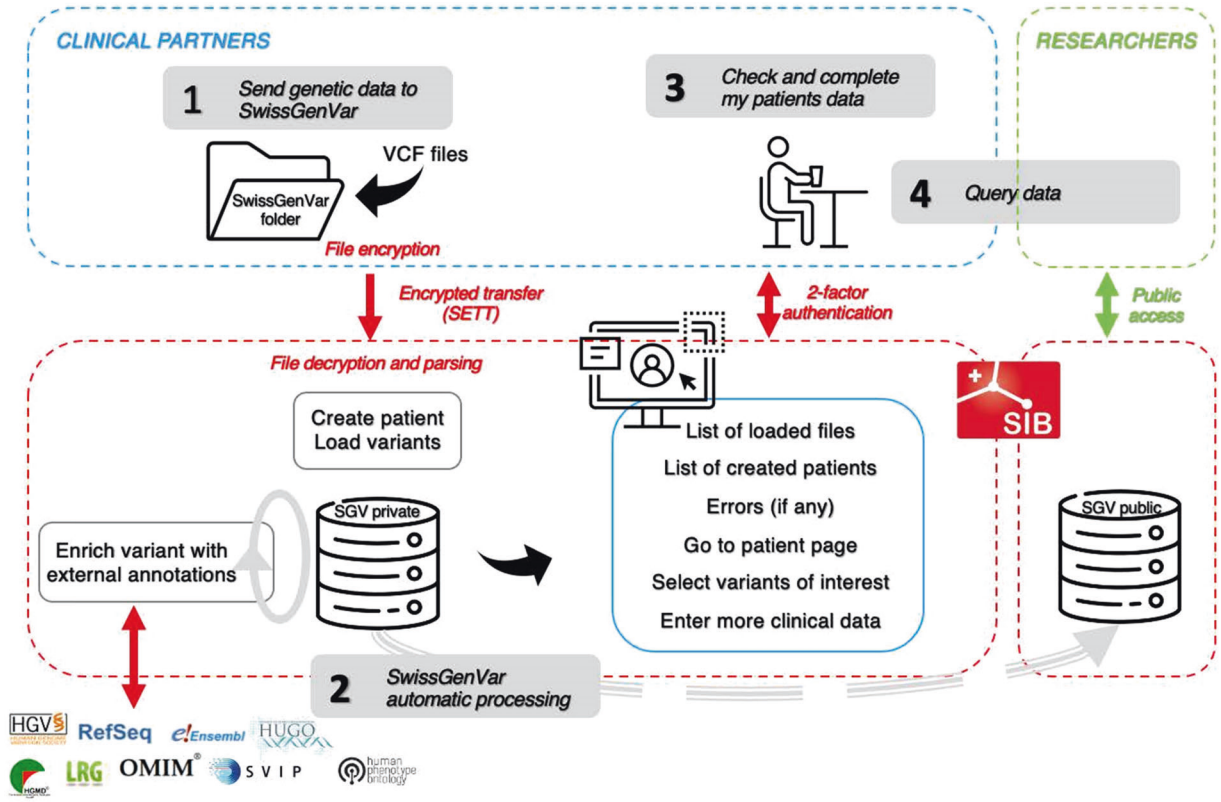
Methods: For the operationalisation of the genomic and related phenomic data, SwissGenVar cross-expert working groups defined a set of variant (type)-related genetic and clinical data by utilizing an interchangeable data format for direct platform implementability. Furthermore, the database structures and functionalities required for fulfilling the objectives of SwissGenVar were elaborated by dedicated operational teams.

Results: Based on this, SwissGenVar database covering variant- and patient-centered query interfaces was designed. Its ontology refers to established external and custom data catalogs reflecting the different variant annotations as well as the demographical and phenotypical features consented by the individual variant carriers. After a preliminary testing phase, the database was fed by research vcf-files using an established encrypted data transfer tool and secure access management system. Further exploration of the platform functionality and required adjustments are ongoing.

Conclusion: SwissGenVar will be a necessary first step to scale-up clinical-grade genetic testing in Switzerland and will foster personalized health research involving genetic risk stratification and disease classifications.

References: https://pages.sib.swiss/project/swissgenvar/Project.

Grants: SPHN Infrastructure development project.



Conflict of Interest: None declared.

P19.034.A MicroRNAs as biomarkers for radiotherapy toxicity in breast and prostate cancer patients

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Lambrecht[11], Sarah Libert[12], Susana Magriña[5], Giovanna Muggiolu[12], Tiziana Rancati[13], Tim Rattay[14], Victoria Reyes[5], Sylvie Sauvaigo[12], Paloma Sosa-Fajardo[4;10], Christopher Talbot[14], Ana Vega[3;4;15], Liv Veldeman[16], Paolo Zunino[17], Jordi Giralt[5;18], Jenny Chang-Claude[2;19], Sara Gutiérrez-Enríquez[1]

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Background/Objectives: Late adverse effects caused by radiotherapy (RT) are a common cause of morbidity amongst cancer survivors and are determined in part by genetic susceptibility. The international RADprecise project aims to personalize RT for cancer patients. One of its objectives is to identify differentially expressed microRNAs as novel biomarkers for radiosensitivity in whole blood of prostate (PC) and breast cancer (BC) patients.

Methods: We used data and RNA samples from a large established cohort of BC and PC patients from the REQUITE European project (www.requite.eu). Patients with moderate/severe toxicity were matched to patients without, two years after RT: 62 and 61 patients for BC, and 76 and 52 for PC, respectively. MicroRNA libraries were prepared with whole blood RNA using NEBNext® Small RNA Library for Illumina sequencing in a HiSeq. sRNAtoolbox (https://arn.ugr.es/srnatoolbox/) was used to perform the bioinformatics analysis.

Results: The first results show that in BC patients there are 75 microRNAs under- and 68 over-differentially expressed (FDR <= 0.05) between toxicity and non-toxicity groups. For PC patients no differentially expressed microRNAs were found. However, significant differences were observed between the two centers where the PC patients were treated. A multivariate analysis including clinical data, treatment and location will be performed.

Conclusion: MicroRNAs may be a good indicator of RT-induced late toxicity in BC patients. The most differentially expressed microRNAs according to RT toxicity in this discovery analysis will be validated in another 400 RADprecise patients using NanoString technology.

References:.

Grants: ERAPERMED2018-244 (ERAPerMed JTC2018), SLT011/18/00005 and FP7 #601826.

Conflict of Interest: None declared.

P19.035.B Genetic Longevity Scores for the Croatian population aged 85+

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Background/Objectives: Ages of 90.0 and 95.0 are widely considered as thresholds for longevity and extreme longevity, respectively. The goal of this study was to construct unweighted and weighted Genetic Longevity Scores (uGLS, wGLS) and to test their ability to predict a chance of survival beyond these ages.

Methods: DNA samples of 314 unrelated elderly individuals (85.0+ years) were genotyped for 42 putative longevity SNPs in 27 genes, and all SNPs that had a $p < 0.20$ in univariate analyses were selected for testing in the multivariate logistic regression models. SNPs from the best model for each cut-off age at death were chosen for GLS calculation, with unweighted score representing a sum of longevity-related alleles, and weighted score a sum of each SNP's value multiplied by its respective beta coefficient from the multivariate model.

Results: GLS included nine SNPs (rs7412, rs50871, rs12206094, rs2267723, rs9536314, rs16847897, rs1800629, rs1042522, rs17202060) for the survival age of 90.0, and five SNPs (rs429358, rs12203592, rs4837525, rs6067484, rs1042522) for the age of 95.0. There was no significant sex difference in mean values of any GLS. All four GLSs (uGLS90.0, wGLS90.0, uGLS95.0, wGLS95.0) were positively correlated with age at death ($p < 0.01$). ROC curve analysis showed all four scores are predictive for reaching the longevity milestones. With area-under-curve of 0.690, weighted GLS90 was shown to be the most predictive.

Conclusion: GLS based on nine longevity SNPs is predictive of survival to 90.0 years, and five SNP-based GLS is predictive of reaching 95.0 years in the elderly Croatian population.

References: /.

Grants: CSF IP-01-2018-2497 (HECUBA).

Conflict of Interest: None declared.

P19.036.C A multi-omics approach to visualize early neuronal differentiation in 4D

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Background/Objectives: Neuronal differentiation of pluripotent stem cells is an established method to study physiology, disease and medication safety. However, the sequence of events in human neuronal differentiation and the ability of in vitro models to recapitulate early brain development are poorly understood. Moreover, the role of epigenetic regulation on the establishment and maintenance of cellular identity during early neuronal differentiation processes is not delineated.

Methods: We developed a protocol optimized for the study of early human brain development and neuropharmacological applications. We performed RNA-seq, global DNA methylation, single-cell RNA-seq and ATAC-seq data integration across timepoints (4D analysis), to correlate the expression of transcription factors with time- and population-specific chromatin states in hESCs, and during differentiation.

Results: Gene expression and epigenetic profiles were comprehensively characterized at four timepoints, as the cells differentiate from embryonic stem cells towards a heterogenous population of progenitors, immature and mature neurons bearing telencephalic signatures. A multi-omics roadmap of neuronal differentiation, combined with searchable interactive gene analysis tools, allows for extensive exploration of early neuronal development and the effect of medications.

Conclusion: In this study, we describe the generation of a novel neuronal differentiation protocol where we used the unparalleled power of multi-omics to understand early events of anterior neuroectodermal fate specification. We assessed the functional regulation of transcription factors and developmentally regulated genes, from loss of pluripotency towards neuronal differentiation.

References:.

Grants:.

Conflict of Interest: None declared.

P19.037.D Genetic variants of serotonergic pathway affect the development of visual hallucinations due to dopaminergic treatment in Parkinson's disease

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Background/Objectives: Serotonergic system plays an important role in the pathogenesis of Parkinson's disease (PD) as it takes over dopamine production after dopaminergic neurons are degenerated to a great extent. The aim of this study was to evaluate the effect of genetic variability of key serotonergic pathway genes on the occurrence of visual hallucinations (VH) as adverse events (AE) of dopaminergic treatment in PD.

Methods: We recruited 231 PD patients and collected their demographic and clinical data. We evaluated the presence or absence of VH due to dopaminergic treatment. We genotyped patients for the following SNPs: *HTR1A* rs6295, *HTR1B* rs13212041, *TPH2* rs1843809, rs7305115, rs4290270, rs4570625, *SLC6A4 5-HTTLPR*, and *SLC6A4* rs25531. Logistic regression was used for analysis. Results were adjusted for age at diagnosis.

Results: Carriers of the *HTR1A* rs6295 GC genotype (OR = 2.58; 95%CI = 1.15−5.78; p = 0.021), *TPH2* rs4290270 AA genotype (OR = 2.78; 95%CI = 1.08−7.03; p = 0.034), and at least one *TPH2* rs4570625 T allele (OR = 1.86; 95%CI = 1.00−3.44; p = 0.047) had increased risk for VH. Additionally, carriers of at least one *SLC6A4 5-HTTPLR* rs25531 S (OR = 0.52; 95%CI = 0.28-0.96; p = 0.037) or at least one L_G allele (OR = 0.37; 95%CI = 0.14−0.97; p = 0.044) had decreased odds for VH. Carriers of the *TPH2* TAAT haplotype had increased risk for VH (OR = 1.94; 95%CI = 1.06-3.55; p = 0.032) compared to the *TPH2* TGTG carriers. Gene-gene interactions did not show any associations with VH.

Conclusion: The genetic variants in serotonergic system should be further investigated as predictive biomarkers of VH as they could contribute to better and more personalized care of PD patients.

References:.

Grants: ARRS, grants P1-0170 and J7-2600.

Conflict of Interest: None declared.

P19.038.A Genetic variability in vitamin D receptor gene may influence the kidney graft function

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Background/Objectives: Vitamin D is crucial for the functioning of several organ systems. Vitamin D deficiency has also been associated with an increased risk of chronic kidney disease and worse outcomes in kidney transplantation. Since vitamin D elicits its actions by binding to vitamin D receptor (VDR), polymorphisms within *VDR* gene may also result in impaired regulation of genes involved in immune response and kidney graft function. Our aim was to evaluate the association of *VDR* polymorphisms with kidney graft function in the first year after transplantation.

Methods: We performed a retrospective study in 143 Slovenian patients with functional kidney graft. All patients were genotyped for common *VDR* polymorphisms: rs11568820 (Cdx2), rs4516035 (GATA), rs2228570 (FokI), rs1544410 (BsmI), rs731236 (TaqI) and rs739837 (BglI). Nonparametric tests and logistic regression were used for statistical analysis.

Results: *VDR* rs4516035 polymorphism was associated with increased serum creatinine and decreased eGFR at the end of the first year after transplantation in the additive (P = 0.001 and P = 0.008, respectively) and dominant (P < 0.001 and P = 0.003, respectively) genetic models. *VDR* rs2228570 was associated with occurrence of BK polyomavirus and cytomegalovirus infections in the first year after transplantation, with carriers of two polymorphic rs2228570 alleles having a lower infection rate (OR = 0.31, 95% CI = 0.10-0.99, P = 0.047) than carriers of two normal alleles. None of the investigated *VDR* polymorphisms was associated with overall rejection in the first year after transplantation.

Conclusion: *VDR* polymorphisms may be associated with worse kidney graft function and lower risk of graft viral infection.

References:.

Grants: ARRS, grants P1-0170 and P3-0323.

Conflict of Interest: None declared.

P19.039.B Association of genetic polymorphisms in glucocorticoid pathway with dexamethasone treatment in COVID-19 patients

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Background/Objectives: Corticosteroids are widely used for the treatment of coronavirus disease (COVID)-19 caused by SARS-CoV-2 as they attenuate the immune response with their anti-inflammatory properties. Genetic polymorphisms of glucocorticoid receptor, metabolizing enzymes or transporters may affect treatment response to dexamethasone. The aim of this study was to evaluate the association of polymorphisms in glucocorticoid pathway with disease severity and duration of dexamethasone treatment in COVID-19 patients.

Methods: Our study included 107 hospitalized COVID-19 patients treated with dexamethasone. We isolated DNA from peripheral blood and genotyped all samples for polymorphisms in NR3C1 (rs6198, rs33388), CYP3A4 (rs35599367), CYP3A5 (rs776746), GSTP1 (rs1695, rs1138272), GSTM1/GSTT1 deletions and ABCB1 (1045642, rs1128503, rs2032582 Fisher's and Mann-Whitney tests were used in statistical analysis.

Results: The median (min-max) age of the included patients was 62 (26-85) years, 69.2 % were male and 30.8 % female and they had moderate (1.9 %), severe (83 %) or critical (15.1 %) disease. NR3C1 rs6198 polymorphism was associated with more severe disease in additive genetic model ($P = 0.022$). NR3C1 rs6198, ABCB1 rs1045642 and ABCB1 rs1128503 polymorphisms were associated with a shorter duration of dexamethasone treatment in additive ($P = 0.048$, $P = 0.047$ and $P = 0.024$, respectively) and dominant genetic models ($P = 0.015$, $P = 0.048$ and $P = 0.020$, respectively), while carriers of the polymorphic CYP3A4 rs35599367 allele required longer treatment with dexamethasone ($P = 0.033$). Other polymorphisms were not associated with disease severity or dexamethasone treatment duration.

Conclusion: Genetic variability of glucocorticoid pathway genes was associated with the duration of dexamethasone treatment of COVID-19 patients.

References:.

Grants: ARRS P1-0170 and P3-0296.

Conflict of Interest: None declared.

P19.040.C The NUDT15 and TPMT genes variants in Polish IBD patients treated with thiopurines

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Background/Objectives: For years, thiopurines have been essential immunomodulatory agents in maintaining inflammatory bowel disease (IBD) remission. However, nearly one-third of patients had modified or discontinued medication due to numerous side effects. Genetic polymorphism of the *TPMT* and *NUDT15* genes is related to enzyme activity, patients' reaction to thiopurines therapy, and drug dosing. We aimed to determine the *NUDT15* and *TMPT* gene variants in a Polish cohort in the context of personalized medicine.

Methods: A group of 88 Polish IBD patients treated with thiopurine agents was enrolled. The coding region of the *NUDT15* gene was analyzed using Sanger sequencing, and four common loci c.238G>C, c.292G>T, c.460G>A, and c.719A>G of the *TPMT* gene using pyrosequencing were genotyped.

Results: Obtained results found four variants of the NUDT15 gene; two in the first exon: c.36A>G (p.Pro12Pro, rs61746486) and c.123A>C (p.Gly41Gly, rs138959770) with minor allele frequency (MAF) of 0.57%, one in the first intron: c.IVS1-91G>A (rs41284205) with MAF of 1.7% and one in the 3′ untranslated region: c.*7G>A (rs61973267) with MAF of 2.84%. For the TPMT gene, two polymorphisms: c.460G>A (p.Ala154Thr) in exon 6 and c.719A>G (p.Tyr240Cys) in exon 9, as compound heterozygous were detected, which corresponds to the presence of the TPMT*3A allele with a frequency of 3.41%.

Conclusion: This study confirmed the importance of the *TMPT* gene in the pharmacogenetics of Polish IBD patients treated with thiopurines. However, it did not demonstrate thiopurine-related toxicity variants in the *NUDT15* gene.

References:.

Grants: Polish National Science Centre, grant no. 2016/23/D/NZ2/01620.

Conflict of Interest: Joanna Żuraszek Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, Maryam Dangana Department of Biochemistry and Biotechnology, Poznań University of Life Sciences, Poland, Oliwia Zakerska-Banaszak Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, Aleksandra Zielińska Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, Michał Walczak Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, Marlena Szalata Department of Biochemistry and Biotechnology, Poznań University of Life Sciences, Poland, Ryszard Słomski Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, Marzena Skrzypczak-Zielinska Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, Polish National Science Centre, grant no. 2016/23/D/NZ2/01620.

P19.041.D INTERVENE: exploring the generalizability of polygenic risk scores on disease

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Background/Objectives: The INTERVENE project is a collaboration between 7 biobank projects, harmonizing data on 1.3 million

participants. We aimed to estimate absolute risk by disease specific PRS strata and to understand the incremental benefit in prediction by phenotypic risk factors.

Methods: 38 diseases were selected to have high heritability, powerful GWAS data and contribution to global burden of disease. This includes diseases such as type 1 diabetes (T1D), rheumatoid arthritis (RA), and diseases less explored in the PRS literature such as melanoma and glaucoma. Analytical consistency is reached through comprehensive endpoint harmonization and systematic PRS deployment through Docker software.

Results: 32 disease specific PRS were tested for association in at least one of either FinnGen, Estonian Biobank or UK Biobank. Association strength varied by disease, however, two notably strong associations were prostate cancer (meta-analysis: OR per SD = 1.95, 95%CI = 1.92-1.98) and T1D (meta-analysis: OR per SD = 2.25, 95%CI = 2.17-2.32).

While estimates for most diseases showed agreement across the biobanks, some showed high variability, such as gout, inflammatory bowel disease and RA. Indeed, RA differed in association across all three biobank projects (Estonian Biobank: OR = 1.41, 95%CI = 1.33-1.49; FinnGen: OR = 1.64, 95%CI = 1.60-1.68; UK Biobank: OR = 1.98, 95%CI = 1.82-2.16). These analyses are now being replicated in other ancestries and across more biobanks.

Conclusion: Through our multi-biobank collaboration, we have developed an efficient replication machinery for studies on polygenic risk. Through this, we show variation in PRS association exists for specific diseases. Understanding such variation will be a vital step towards PRS's clinical implementation.

References:.

Grants: Horizon 2020 (101016775).

Conflict of Interest: None declared.

P19.042.A Rethink of EGFR signaling in triple negative breast cancer, with its interconnection with MAPK and PI3K/AKT signaling using bioinformatic approaches

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Background/Objectives: Triple-negative breast cancer (TNBC) has the worse survival rate reported to other breast cancer subtypes. Resistance mechanisms are based on the interplay between numerous factors and signaling pathways, which crosstalk and feedback.

Methods: To understand the interplay between the signaling pathways was downloaded the NCBI gene list related to pathway in cancer, mitogen-activated protein kinase(MAPK) and phosphatidylinositol 3-kinase(*PI3K*)/protein kinase B(*AKT*) signaling. The next step was to overlap the genes list using Venn diagram, then was evaluated the overall survival using online tools, based on a patient cohort of 176 TNBC patients from TCGA.

Results: The Venn diagram among the selected gene list emphasis a common signature of 52 genes; among this, 23 were correlated with overall survival rate, these genes being related to the EGFR tyrosine kinase inhibitor resistance, as KEEG gene ontology classification shows.

Although epidermal growth factor receptor (EGFR) is overexpressed and correlated with overall survival rate in TNBC, most of the clinical trials with EGFR inhibitors in TNBC have heretofore been unsuccessful. To develop effective EGFR-targeted therapy for TNBC, the specific mechanisms of EGFR signaling and the interconnection with MAPK and PI3K/AKT signaling mediated immune response in TNBC need to be elucidated. Additional key immune response regulators (IL1A,IL4,IL6,IGF2,VEGFA/B/C,EGFR,FGR2) were proved to be connected with overall survival rate in TNBC.

Conclusion: Our work provides the important role of the crosstalk among the MAPK and PI3K/AKT signaling; this signature paving the way to precision TNBC care.

References:.

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Conflict of Interest: None declared.

P19.043.B Towards global contribution to precision medicine: high-quality genome assemblies from Central African trios

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Background/Objectives: Precision medicine cannot be achieved without global participation. While the cost of generating a telomere-to-telomere genome assembly is out of reach for most laboratories, it is feasible to generate human assemblies which approach reference quality. To this end, our team has collaborated to recruit and consent participants from genetically diverse trios from the Democratic Republic of the Congo for high quality genome sequencing.

Methods: Our ethics proposal was based on the 1000 Genomes protocol[1]. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood from participants. Each member of the trio was sequenced with Illumina to a depth of 30x. DNA was extracted from PBMCs and was sequenced on four PacBio HiFi flowcells for each proband, representing approximately 300x coverage. Assemblies were trio binned with yak, assembled with HiFiASM[2], and scaffolded with Bionano optical maps.

Results: Trios were recruited in Kinshasa through our network of physician scientists who prioritized geographic and ethnolinguistic diversity. We chose four trios for sequencing by selecting those thought to be historically distant from one another. With these assemblies, we show that unmapped reads from experiments on novel genomes from Congo, aligned to GRCh38p13, can indeed be placed. We further show that African Pangenomic Contigs[3] can be placed on these genomes, resolving outstanding issues in missing data.

Conclusion: HiFiASM assemblies, scaffolded with Bionano, represent a strong advancement for placement of known, unmapped genomic data, thus representing a step forward in precision medicine.

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Conflict of Interest: Jonathan LoTempio: None declared, Kizito Mosema: None declared, D'Andre Spencer: None declared, Kevin

Karume: None declared, Adam Wang: None declared, Miguel Almalvez: None declared, Yulong Fu: None declared, Celeste Musasa: None declared, Johanna Nsibu: None declared, Matthew Bramble: None declared, Erick Kamangu: None declared, Désiré Tshala Katumbay: None declared, Dieudonné Mumba: None declared, Eric Vilain Bionano Genomics Inc, stock options, Bionano Genomics Inc Scientific Consulting.

P19.044.C Gene editing in human iPSC models derived from patients affected by inherited retinal dystrophies

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Background/Objectives: Inherited retinal dystrophies (IRD) are caused by mutations in more than 300 genes. Previous work in our lab reported the generation of several human induced pluripotent stem cell (iPSC) lines from patients carrying mutations in different IRD-associated genes. These genes comprise *ABCA4*, *BEST1*, *PDE6A*, *PDE6C*, *RHO* and *USH2A*. The aim of this study is to correct the identified mutations through gene editing and to revert the phenotype observed in the unedited cells as a source of cells for potential iPSC-mediated therapy.

Methods: We used CRISPR/Cas9 and transcription activator-like effector nuclease (TALEN) in patient derived human iPSCs to correct the variants from each subject. For that we designed specific sgRNAs and TAL mRNA pairs to target the mutation.

Results: We have found differences in DNA cleavage efficiency in the different iPSC lines used depending on the type of the mutation and the gene. We also observed differences in editing efficiency between CRISPR and TALEN approaches among iPSC lines.

Conclusion: There is an unmet need to develop therapies to treat IRD. Genome editing allows correcting the disease mutations directly in patient's DNA. By using CRISPR/Cas9 or TALEN-mediated genome editing we are able to specifically cut the DNA at the desired location and to revert the mutation with a corrected DNA template.

References:.

Grants:.

Conflict of Interest: None declared.

P19.045.D Early-onset prostate cancer: Association of polygenic background in combination with family history and rare pathogenic variants

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Background/Objectives: We aimed to comprehensively assess the role of polygenic risk score (PRS) on the early-onset prostate cancer (PC) vs late-onset, in the absence or presence of a family history of PC (FH) or rare pathogenic variants (PV, across 5 PC susceptibility genes - HOXB13, *BRCA2, ATM, CHEK2, BRCA1*).

Methods: We identified 9,824 PC cases from 192,779 men (data from UK Biobank). A PRS for PC was calculated and used to stratify individuals into low, intermediate, and high-risk groups. We then assess the impact of PRS stratification on the age of onset (i.e., early-onset considered before 55 years old vs late-onset) in the absence or presence of a FH or PV (i.e., non-carriers vs carriers).

Results: The odds ratio (OR) of PC per standard deviation of PRS were higher for early-onset cancer (OR = 2.1) than late-onset cancer (OR = 1.5).

Depending on the PRS, OR for individuals without FH ranges between 0.4-2.4 for early-onset, compared to 0.5-1.8 for late-onset; while OR for individuals with FH ranged between 1.5-8.3, and 1.0-3.2, respectively. OR for non-carriers ranges between 0.4-2.5 for early-onset, compared to 0.5-1.9 for late-onset; while OR for carriers ranged between 0.9-7.8 and 1.5-5.3, respectively.

Conclusion: These findings show that the modifying role of PRS on prostate cancer risk is stronger for early-onset than for late-onset. This tendency is pronounced in combination with family history and carrier of rare pathogenic variants.

References:.

Grants:.

Conflict of Interest: None declared.

P19.046.A Genotype-first breast cancer prevention: transferring monogenic findings from a population biobank to clinical setting

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Background/Objectives: Although hereditary breast cancer (BC) screening and management is well established in clinical settings, it only captures a fraction of genetic predisposition at the population level. We have addressed this gap by returning data on BC predisposing monogenic variants to female participants of the Estonian Biobank (EstBB).

Methods: We describe our experience from a national pilot study (2018–2021) in collaboration with two Estonian regional hospitals. We screened the EstBB cohort (n = 154,201 of whom 66.1% were female) to identify carriers (22-79y) with monogenic variants conferring high or moderate BC risk in any of 11 genes listed in clinical guidelines (*BRCA1*, *BRCA2*, *TP53*, *STK11*, *PTEN*, *CDH1*, *ATM*, *PALB2*, *CHEK2*, *NBN*, *NF1*). 180 female carriers were re-contacted and 109 (61%) consented for further clinical management by clinical geneticists and oncologist. That included confirmation of genetic variant, cascade screening, imaging studies, laboratory testing, preventive surgery etc.

Results: Our results show that only one-third of participants were eligible for BC screening according to the current clinical criteria. 16 participants had previous cancer history. Only 10% were aware of their genetic finding. BC was diagnosed in six participants. Majority of the participants considered receiving the genetic risk information as valuable.

Conclusion: Our results support population-based genomic screening for hereditary BC prevention.

References:.

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Conflict of Interest: None declared.

P19.047.B Pharmacogenetic markers of treatment response to latanoprost in patients with primary open-angle glaucoma and ocular hypertension

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Background/Objectives: The only proven effective treatment of primary open-angle glaucoma (POAG) is to reduce the intraocular pressure (IOP). Treatment with prostaglandin analogues (PGAs) such as latanoprost eye drops leads to highest IOP reduction. However, in some patients sufficient IOP reduction may not be achieved or the disease may progress despite low IOP. The aim of our study was to search for pharmacogenetic markers of treatment response to latanoprost in patients with POAG and ocular hypertension.

Methods: Our pilot study included 52 treatment naive patients with POAG or ocular hypertension. Response to latanoprost was evaluated as IOP reduction in the most affected eye after 6 weeks of treatment. Patients were genotyped for polymorphisms in genes of latanoprost activation (*CES1P1* rs3785161), transport (*SLCO2A1* rs34550074 and rs4241366), and receptor (*PTGFR* rs3753380) and in antioxidative (*GSTM1**0, *GSTT1**0, *GSTP1* rs1695 and rs1138272, *SOD2* rs4880, *CAT* rs1001179, *GPX1* rs1050450) and inflammatory (*TNF* rs1800629, IL1B rs16944 and rs1143623, *IL6* rs1800795) pathways.

Results: *SOD2* rs4880 CT (Padd = 0.036), *CAT* rs1001179 TT (Padd = 0.012) and *TNF* rs1800629 GG (Pdom = 0.012) genotypes were significantly associated with a decrease in absolute IOP after latanoprost treatment in the additive model, while carriers of at least one *GSTT1* gene copy achieved higher percentage of IOP reduction in the dominant model (P = 0.024). The other investigated polymorphisms were not statistically significantly associated with response to latanoprost treatment.

Conclusion: Pharmacogenetic biomarkers associated with treatment response to latanoprost may contribute to more effective and personalized treatment of POAG and ocular hypertension.

References:.

Grants: ARRS grants P1-0170 and P3-0333.

Conflict of Interest: Makedonka Atanasovska Velkovska: None declared, Tanja Blagus: None declared, Katja Goricar: None declared, Barbara Cvenkel: None declared, Vita Dolzan ARRS grant P1-0170.

P19.048.B NAGENpediatrics: Rapid Whole Genome Sequencing in Neonatal/Pediatric Intensive Care in Navarra, Spain

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Background/Objectives: Whole-genome sequencing (WGS) is a rapid and cost-effective technique that significantly impacts the clinical setting, particularly in the paediatric context. Approximately 2-3% of newborns have a congenital anomaly, and at least 50% of these have a genetic cause. According to the literature, rapid genomic testing (2-3 weeks) allows the diagnosis of around 21-26% of critically ill children, influencing clinical outcomes. This pioneering study at regional level aims to evaluate the diagnostic and therapeutic utility of rapid WGS in acutely unwell children.

Methods: This ongoing study involved the participation of a multidisciplinary team and the recruitment of 34 trios over one year. A specialized paediatric clinical team initially assessed their eligibility and each family received a detailed genetic counselling consultation, provided by a registered genetic counsellor. WGS was performed on germline DNA, and variants were filtered and reported by extensive bioinformatic analysis.

Results: We identified pathogenic variants in genes in line with the clinical manifestations in 14 families, reaching a diagnostic yield of 41%. Genetic results, delivered in an average time of 2-3 weeks, significantly reduced the diagnostic odyssey and influenced clinical decisions.

Conclusion: We demonstrate the implementation of precision medicine through rapid WGS in the paediatric context in the region of Navarra, Spain, with potential extensive benefits for patients, clinicians and the Regional Health System. We describe new genetic variants associated with rare diseases, not identifiable by other diagnostic methods in rapid turnaround times.

References: Stark, Z et al., 2022.

Grants: Proyecto estratégico I+DS3 2020-2022. GEMAIV Gobierno de Navarra.

Conflict of Interest: None declared.

P20 POPULATION GENETICS AND EVOLUTIONARY GENETICS

P20.001.C Genome-wide polygenic risk score and lifestyle risk factors for hypertension among 11,252 Japanese individuals

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Background/Objectives: Among non-European populations, it is an urgent task to construct a PRS (polygenic risk score) and to assess its utility with a combination of traditional risk factors. We aimed to examine the associations between genome-wide PRS and hypertension prevalence and interaction with conventional risk factors in a general Japanese population.

Methods: This cross-sectional study included 11,252 Japanese individuals who participated in the J-MICC Study (ten nationwide study sites). By leveraging publicly available GWAS results from the BioBank Japan, we developed PRS in the Target dataset using PRSice-2 and evaluated its performance in the Test dataset. Hypertension was defined as systolic blood pressure (SBP) > 130 mmHg, diastolic blood pressure (DBP) > 85 mmHg, or taking an antihypertensive drug.

Results: Compared with the middle PRS quintile, the OR (95% CI) of hypertension at the bottom and top PRS quintiles were 0.74 (0.57–0.97) and 1.73 (1.32–2.27), respectively. For continuous outcomes, differences (95% CI) between the middle and the top quintile were 4.55 (2.26–6.85) mmHg for SBP and 2.53 (1.80–3.26) mmHg for DBP. The OR (95% CI) of non-drinkers in the top PRS quintile (1.70, 1.11–2.61) were lower than drinkers in the top quintile (2.53, 1.70–3.79), although interaction term was not significant.

Conclusion: Our findings highlighted that a combination of PRS and traditional risk factors could be useful for risk stratification of hypertension in a Japanese population.

References:.

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Conflict of Interest: None declared.

P20.002.D Inherited and de novo variation in Lithuanian genomes: introduction to the analysis of the generational shift

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Background/Objectives: Most genetic variants are rare and specific to the population, highlighting the importance of characterizing local population genetic diversity. Many countries have initiated population based whole-genome sequencing (WGS) studies. Lithuanian genomes are underrepresented in the public databases.

Methods: Here we report initial findings of a high-coverage (an average of 36.27x) whole genome sequencing study for 25 trios of the Lithuanian population to infer the distribution of single nucleotide variations (SNVs) throughout the genome and biological aspects of novel variations from one generation to another.

Results: Each genome on average carried approximately 4,701,473 (±28,255) variants, where 80.6% (3,787,626) were SNPs, and the rest 19.4% were indels. An average of 12.45% were novel according to dbSNP (build 150). The WGS structural variation (SV) analysis identified on average 9,133 (±85.10) SVs, of which 95.85% were novel. De novo single nucleotide variation (SNV) analysis identified 4,417 variants, where 1.1% de novo SNVs were exonic, 43.9% intronic, 51.9% intergenic, and the rest 3.13% in other sites of genome like UTR or downstream sequence. Three possible pathogenic de novo variants in the *ZSWIM8*, *CDC42EP1* and *RELA* genes were identified.

Conclusion: Our findings provide useful information of local human population genomic variation, especially for de novo variants and will be a valuable resource for further genetic studies.

References:.

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Conflict of Interest: None declared.

P20.003.A Pursuing public health benefit within national genomic initiatives: learning from different policies

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Background/Objectives: Substantial public health benefits are expected of population-based genomic endeavours, such as national genomics initiatives. To date, little is known about the actual public health benefit that is yielded. We explore to what extent and how public health benefit is pursued in national genomics initiatives.

Methods: A mixed-method study was conducted, comprising a literature-based comparison of 11 purposively sampled national genomics initiatives (Belgium, Denmark, Estonia, Finland, Germany, Iceland, Qatar, Saudi Arabia, Taiwan, United Kingdom (UK), and United States of America (USA), and five semi-structured interviews with experts (Denmark, Estonia, Finland, UK, USA). The results were analysed using an adapted public health policy cycle: agenda setting, governance, (research) strategy towards health benefit, implementation, evaluation.

Results: Public health benefit within national genomics initiatives was pursued in all initiatives and in all phases of the public health policy cycle. The operationalization of public health benefit seemed dependent on the outcomes of agenda setting, as well as design of governance. Some initiatives focus on a research-based strategy to contribute to public health, while others focus on research translation into healthcare, or a combination of both. Both quantitative and qualitative methods were reported to evaluate public health benefits. However, the created health benefit for the general public, both short- and long-term, appears difficult to determine.

Conclusion: Genomics initiatives hold the potential to deliver health promises of population-based genomics. To ensure effective research translation, implementation, and ultimately improve public health, development of evaluation tools and clarity in roles and responsibilities of stakeholders is crucial.

References:.

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Conflict of Interest: Suzanne Onstwedder Part-time employed at National Institute for Public Health and the Environment (the Netherlands), part-time employed at Amsterdam UMC, Marleen Jansen Part-time employed at National Institute for Public Health and the Environment (the Netherlands), part-time employed at Amsterdam UMC, Teresa Leonardo Alves Fulltime employed at National Institute for Public Health and the Environment (the Netherlands), Fulfills a position in the Advisory Board of a not-for-profit organization called Mieux Prescrire, but receives no financial payment., Martina Cornel Fulltime employed at Amsterdam UMC, the Netherlands, Tessel Rigter Part-time employed at National Institute for Public Health and the Environment (the Netherlands), part-time employed at Amsterdam UMC.

P20.004.B The contribution of rare whole genome sequencing variants to plasma protein levels and to the missing heritability

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Background/Objectives: Despite the success of genome-wide association studies, much of the genetic contribution to complex traits remains unexplained. Here, we analysed high coverage whole-genome sequencing data, to evaluate the contribution of rare genetic variants plasma protein concentrations and to the heritability.

Methods: Whole-genome sequencing was performed using Illumina short read technology (X-ten) and protein levels for 460 putative biomarkers was measured using the Olink Proseek Multiplex panels (CVD II, CVD III, INF I, ONC II and NEU I) and the protein extension assay. Association analyses were performed using SKAT with different weights and minor allele frequency cut-offs.

Results: The frequency distribution of genetic variants was skewed towards the rare spectrum, and damaging variants were more often rare. We estimated that less than 4.3% of the narrow-sense heritability is expected to be explained by rare variants in our cohort. Using a gene-based approach and SKAT, we identified *Cis*-associations for 237 of the proteins, which is slightly more compared to a GWAS (N = 213), and we identified 34 loci in *Trans*. Several associations were driven by rare variants, and rare variants had on average larger phenotypic effects.

Conclusion: We conclude therefore that rare variants could be of importance for precision medicine applications, but have a more limited contribution to the missing heritability of complex diseases.

References:.

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Conflict of Interest: None declared.

P20.005.C The effect of LPA p.Thr1399Pro on lipoprotein(a) concentrations and coronary artery disease is modified by an interaction with the LPA splice site variant KIV-2 4925G>A

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Background/Objectives: High Lipoprotein(a) [Lp(a)] concentrations are a major genetic risk factor for coronary artery disease (CAD) in the general population[1]. Lp(a) variance is determined by the complex LPA gene, with a coding CNV ("KIV-2 repeat") encompassing up to 70% of the coding region and determining 30-70% of Lp(a) variance[1]. Additionally, many LPA variants further contribute to Lp(a) variance[1].

Methods: The effect of the interaction between the largely investigated but ambiguous coding SNP rs41272110 (p.Thr1399Pro) and the splice variant 4925G>A located in the KIV-2 repeat was investigated by quantile regression in 10,405 individuals from three German studies (GCKD, KORA F3 and KORA F4). Survival analysis was used to assess the impact of both variants on CAD risk in UKBiobank (n = 186,088 exomes).

Results: We observed a highly significant SNP interaction (p < 3.03e-04). Rs41272110 alone showed no impact on Lp(a) (β = -0.06 [-0.79;0.68], p = 0.879) and CAD risk (HR = 1.01 [0.97;1.04], p = 0.731), but in a model containing both variants, rs41272110 was associated with markedly increased Lp(a) (β = +9.40 mg/dL, [6.45;12.34], p = 4.07E-10) and higher CAD risk (HR = 1.10, [1.04;1.16], p = 6.9e-04). The effect of rs41272110 is seen only in carriers of rs41272110 not carrying 4925G>A (i.e. sizeable 4% of the population), but at population level it is masked by partial association (R2 = 0.836-0.872, D′ = 0.984-0.985) and interaction with KIV-2 4925G>A.

Conclusion: We identify a SNP interaction and a novel CAD risk variant in the LPA gene. Our work emphasizes that missed SNP interactions in the genome may lead to inaccurate determination of phenotypic effects.

References:.

[1]Coassin S et al, 2017, https://doi.org/10.1093/eurheartj/ehx174.

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Conflict of Interest: Rebecca Grüneis: None declared, Claudia Lamina: None declared, Silvia Di Maio: None declared, Sebastian Schoenherr: None declared, Peter Zöscher: None declared, Lukas Forer: None declared, Annette Peters: None declared, Christian Gieger: None declared, Anna Köttgen: None declared, Florian Kronenberg served on the advisory boards and has received lecture fees from Novartis and Amgen., Stefan Coassin: None declared.

P20.006.D Functional differences between the modern human and Neandertal aryl hydrocarbon receptor

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Background/Objectives: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor important in the response to toxicants. AHR in modern humans differs from the AHR of Neandertals, Denisovans or other primates by having an alanine to valine substitution at position 381. Previous studies where these variants were overexpressed have yielded contradictory results. Here, we investigate the activity of the two versions of AHR by genome editing.

Methods: We introduced the codon for the Neandertal-like amino acid in human cells using CRISPR/Cas genome editing, exposed the edited and non-edited cells to AHR ligands and measured the expression of the AHR target genes cytochromes P450 1A1 and P450 1B1 with real-time quantitative PCR and additional target genes by RNA-Sequencing.

Results: We show that the induction of the transcription of the AHR target genes does not differ in cells expressing the ancestralized and modern versions of AHR when they are exposed to the microbiome-derived metabolite indirubin. In contrast, the ancestral AHR variant induces a transcriptional response at >1000-fold lower concentrations than the modern human AHR variant when cells are exposed to the environmental pollutant benzo(a)pyrene and the endogenous tryptophan metabolite kynurenic acid.

Furthermore, the ancestralized cells are similar to comparable chimpanzee cells in that they express higher levels of AHR target genes when no ligands are added.

Conclusion: Compared to the ancestral variant seen in archaic hominins, modern human AHR activates its target genes to a lesser extent when exposed to kynurenic acid and benzo(a)pyrene. This may be advantageous in that less DNA adducts might be formed.

References:.

Grants:.

Conflict of Interest: None declared.

P20.007.A Phenome-wide association studies of genetic variants in microRNAs

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Background/Objectives: Genetic variants in microRNAs (miRNAs) can affect their biogenesis or functionality. Previous research showed these variants could be associated with disease, but their implication on a wide range of phenotypes remains unknown. Here we aim to study the effects of those variants on human phenome.

Methods: We utilized genotype and hospital episode statistics data to investigate associations between genetic variants in miRNAs and clinical diagnoses in 423,419 participants in the UK Biobank. We extracted 346 genetic variants in seed, mature, or precursor genes of miRNAs. We included 905 diagnoses with at least 200 cases. Phenome-wide association studies were conducted for each genetic variant separately, adjusting for age, sex, genotyping array, and the first five principal components.

Results: At FDR<0.05, we identified 122 associations between 42 genetic variants (6 in seed, 9 in mature, and 27 in precursor genes of miRNAs, respectively) and 63 diagnoses across 14 disease groups. Of those, 47 associations (38.5%) were genome-wide significant. The strongest associations were reported between genetic variants in the major histocompatibility complex (MHC) region, including rs4285314 in the precursor gene of miR-3135b and celiac disease (P = 1.80e-162) and other immune-related disorders. Excluding MHC region, rs368791729 and rs370955537 in mature miR-3939 remain the most significant findings associated with hypothyroidism (P = 8.06e-11 and P = 4.17e-10, respectively).

Conclusion: Our study shows that genetic variants in miRNAs could have clinical implications and offers an opportunity to investigate their clinical importance. Further studies are needed to validate the findings, assess their causality, and explore the underlying mechanism.

References:.

Grants:.

Conflict of Interest: None declared.

P20.008.B Genetics and material culture support repeated expansions into paleolithic Eurasia from a population hub out of Africa

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Background/Objectives: The population dynamics that followed the Out of Africa expansion of *Homo sapiens* and the whereabouts of the early migrants before the differentiation that ultimately led to the formation of Oceanian, West and East Eurasian macro populations have long been debated. Shedding light on these events may, in turn, provide clues to better understand cultural evolution in Eurasia 50.000-35.000 years ago.

Methods: Here we analyze the available Eurasian Paleolithic aDNA to provide a comprehensive population model (primarily using admixturegraphs) and validate it in light of available material culture.

Results: We show that the genetic modeling of Paleolithic genomes largely matches the material culture evidence and suggests multiple waves of colonization of Eurasia.

The only representative of the first expansion has been recovered in Europe and might be linked with the so called "transitional cultures" documented there 48.000-43.000 years ago. A second, major, population wave is broadly associated with Initial Upper Paleolithic lithics and populated West and East Eurasia around 45.000 years ago, before getting largely extinct in Europe. Another expansion, started before 38.000 years ago, is broadly associated with Upper Paleolithic industries and repopulated Europe with sporadic admixtures with the previous wave and more systematic ones while moving through Siberia.

Conclusion: Leveraging on our integrated approach we propose that, starting from a Eurasian population Hub, the broader colonization of the continent occurred through multiple events of expansion and local extinctions; with each expansion characterized by distinct chronologies, genetics and lithic technologies.

References:.

Grants: UniPd PRID 2019, EU Horizon 2020 (N.724046–SUCCESS).

Conflict of Interest: None declared.

P20.009.C Heterogeneity of lung function signals and genetic risk score in predicting Chronic Obstructive Pulmonary Disease in a multi-ancestry study of 580,869 individuals

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States; [9]Centre for Genetics and Genomics Versus Arthritis, Centre for Musculoskeletal Research, The university of Manchester, Manchester, United Kingdom; [10]National Institute for Health Research Biomedical Research Centre – Respiratory theme, Glenfield Hospital, Leicester, United Kingdom; [11]Division of Respiratory Medicine, University of Nottingham, Nottingham, United Kingdom.

Background/Objectives: Eurocentric biases in genome-wide association studies have risked exacerbating health disparities. We aimed to: (i) investigate genetic ancestry-related heterogeneity in effect sizes of lung function associated loci; (ii) develop and assess a multi-ancestry genetic risk score (GRS) for lung function (the ratio of forced expiratory volume in 1 second to forced vital capacity, FEV_1/FVC); and (iii) evaluate the performance of the multi-ancestry GRS in prediction of chronic obstructive pulmonary disease (COPD).

Methods: We assessed the heterogeneity of lung function genetic signals using MR-MEGA. We constructed two GRS both including 442 SNPs associated with lung function in 580,869 individuals across five ancestry groups: (i) using ancestry-specific weights estimated from a fixed-effects meta-analyses across cohorts (discovery dataset) within the same ancestry group; (ii) using weights estimated from a multi-ancestry meta-regression (MR-MEGA) across all cohorts. We evaluated GRS performance over ancestry-matched groups in UK Biobank (independent testing datasets).

Results: We observed 109 (11.4%) signals with nominal evidence of ancestry-correlated heterogeneity (binominal test $p = 5.17x10^{-15}$). In UK Biobank, the multi-ancestry GRS consistently outperformed ancestry-specific GRS in association with FEV_1/FVC and COPD. The variance of FEV_1/FVC explained in European ancestry individuals by multi-ancestry GRS has increased from 5.02% to 7.77% compared to our previously published GRS derived in European samples; from 0.74% to 2.35% for African ancestry and from 2.02% to 3.84% for East Asian ancestry.

Conclusion: 11% of lung function loci exhibit ancestry-related heterogeneous effects. Multi-ancestry GRS for FEV_1/FVC better predicts COPD compared to a GRS based on same-ancestry GRS.

References:.

Grants:.

Conflict of Interest: Jing Chen full-time, Nick Shrine full time, Abril Izquierdo full, Anna Guyatt full, Richard Packer full, Chiara Batini full, Xiaowei Hu full, Ain W Manichaikul full, Brian Hobbs full, Michael Cho full, Tamar Sofer full, Karsten Suhre full, Alfred Pozarickij full, Robin G Walters full, Stephanie London full, Andrew Morris full, Louise Wain full, Ian P. Hall full, Martin D Tobin full.

P20.010.D Estimating diagnostic noise in panel-based genomic analysis

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Background/Objectives: Gene panels with strict variant filtering rules are often used for clinical analysis of exomes and genomes. Panels sizes vary, depending upon the genetic heterogeneity of the clinical presentation, affecting the sensitivity and specificity of the test. We investigated diagnostic variant filtering in a population setting using gene panels developed to diagnose a range of heterogeneous monogenic diseases.

Methods: We used the Genotype-2-Phenotype database with the Variant Effect Predictor plugin (VEP-G2P) to identify rare non-synonymous variants in exome sequence data from 200,643 individuals in UK Biobank. We evaluated five clinically curated gene panels: developmental disorders (DD; 1716 genes), heritable eye disease (536 genes), skin disorders (293 genes), cancer syndromes (91 genes) and cardiac conditions (49 genes). We used phenotypic data to evaluate potential clinical overlap between individuals in UK Biobank and gene-disease panels.

Results: As expected, bigger gene panels resulted in more prioritised variants, from ~0.3 per person in the smallest panel (cardiac) to ~3.5 using the largest panel (DD) with more in individuals of non-European ancestry. The number of prioritized variants increased with gene length, with some notable outliers. Most prioritized variants were missense, but the number can be reduced by applying REVEL score thresholds or ClinVar pathogenicity assertions, or phasing heterozygotes in biallelic genes.

Conclusion: Although large gene panels may be the best strategy to maximize diagnostic yield in genetically heterogeneous diseases, they will frequently generate false positives. Extreme caution should therefore be applied when interpreting candidate diagnostic variants found incidentally.

References:.

Grants: MR/T00200X/1.

Conflict of Interest: None declared.

P20.011.A Reidentifying canonical metabolic networks using observational data

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Background/Objectives: Molecular traits are interesting candidates for causal inference as they can be biomarkers for disease. Mendelian randomization (MR) identifies causal relationships between complex traits. Unfortunately, application of MR to molecular traits is difficult due to lack of instruments and the fact that these networks are only partially observed leading to confounding or pleiotropy.

Methods: We assessed multivariable MR (MVMR) performance in complex network scenarios through simulations of the caffeine metabolism network (KEGG ID hsa000232). In order to test how well KEGG metabolite networks can be recovered in general, we applied MVMR to metabolite QTLs (mQTL) obtained from two studies comprising up to 30,724 individuals. For this, we first identified 76 metabolites (with 190 reactions between them) in KEGG for which we have mQTLs and then tested how well MVMR can distinguish confirmed reactions from unsupported links.

Results: In various simulation settings, MVMR with Steiger-filter re-identified the caffeine metabolic network edges with well-controled false positive rates (mean: 0.050, min: 0.030, max: 0.080) and high power (mean: 0.695, min: 0.652). MVMR based on mQTLs successfully reidentified the caffeine network (AUC 0.77), even when mQTLs for many (9/19) metabolites are unavailable. When extending the network to all 76 matching KEGG metabolites, we found slightly attenuated discriminative ability (AUC: 0.69).

Conclusion: We show that metabolic networks can serve as ground truth for causal inference. MVMR demonstrated discriminative ability for these networks. Our results can also be used to understand why often MVMR fails when applied to molecular traits.

References:.

Grants:.

Conflict of Interest: None declared.

P20.012.B Characterization of Danube Swabian samples on a high-resolution genome-wide basis

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Background/Objectives: German-derived ethnicities are one of the largest ethnic groups in Hungary, which date back to the 11th century. Germans came to Hungary in multiple waves throughout history. The biggest immigration wave took place following the collapse of the Ottoman Empire in East-Central Europe. To date, there are no studies available regarding the investigation of their genetic makeup. Here we intended to assess Danube Swabian samples using standard methods in order to determine their suitability for more comprehensive investigations.

Methods: We analysed 47 Danube Swabian samples collected from elderly individuals living in Southwest Hungary. Based on self-declaration, they did not admix with other ethnicities for 3-6 succeeding generations. Using Illumina 720K genotype data, we conducted allele frequency- and haplotype-based genome-wide marker data analyses like maximum likelihood ancestry estimation methods, formal tests of admixture, identical by descent and homozygous by descent DNA segment analyses.

Results: Haplotype-based analyses like identity by descent and homozygous by descent segment analyses show that the investigated Danube Swabians remained isolated from other ethnic groups. This was also supported by D-statistics results. The investigated Danube Swabians are unadmixed, and our results suggest that their main source of ancestry can be traced back to Germany.

Conclusion: This is the first genome-wide analysis of Danube Swabians and according to the results, our analysed Swabian samples remained remarkably isolated. This makes our data exceptional and give us a great advantage in the reconstruction of their origin and the identification of their major archaic genomic patterns.

References:.

Grants: Supported by NKFIH 138669 and AHT 386706 grants.

Conflict of Interest: None declared.

P20.013.C Population genetic profiling of hereditary transthyretin amyloidosis in Bulgaria and possible non-coding genetic modifiers

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Background/Objectives: In Bulgaria there is high prevalence of vATTR and founder effect for Glu89Gln TTR variant. Haplotype analysis of the other known TTR mutations, located in exons 2 and 3 and screening of exons 1 and 4 in suspected patients is important for exploration of the vATTR diversity. The search of genetic modifiers with possible effect on the manifestation of vATTR could have impact on the disease heterogeneity research.

Methods: Haplotype analysis was performed with microsatellite markers on chromosome 18. DNA samples from vATTR patients and their healthy relatives were analysed. TTR screening in exons 1 and 4 of other suspected patients, DBS screening of Roma newborns for Gly47Glu and regulatory SNP detection in Glu89Gln cohort were performed via Sanger sequencing. All samples were selected from our DNA bank.

Results: Founder effect was proved for the variants Val30Met and Ser77Phe. The results from the DBS screening of newborns for Gly47Glu were negative. We didn't find any TTR pathogenic variants in exons 1 and 4 in the tested patients. Two of the selected regulatory SNPs in the Glu89Gln cohort are linked in haplotype and hypothetically could correlate to the late disease onset with cardiac manifestation.

Conclusion: Bulgaria has several endemic loci with founder effect for vATTR with distinct genetic background. The presence of Gly47Glu is sporadic event. TTR exons 2 and 3 are proven hot-spot for vATTR in Bulgaria. More studies should be done in order to reach statistical significance for the hypothesis of the effect of regulatory SNPs.

References:.

Grants: WI241542 2018 GLOBAL ASPIRE TTR Amyloidosis.

Conflict of Interest: None declared.

P20.014.D Validation of low-pass sequencing approach for HGDP populations

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Background/Objectives: Low-pass sequencing is whole genome sequencing to an average depth 1× combined with genotype imputation. It has been proposed as a cost-effective alternative to genotyping arrays to identify genetic variants [1]. Most genotyping arrays best capture variation common in populations of European ancestry, while low-pass sequencing mitigates this ascertainment bias and can therefore be applied in underrepresented populations. Furthermore, unlike genotyping arrays, it allows effective identification of novel variation. In this study, we applied low-pass sequencing to over 1000 individuals from 52 world populations from the HGDP panel [2], to evaluate the performance compared to standard 30× whole genome sequencing.

Methods: Libraries from 1062 HGDP individuals (CEPH, Fondation-Jean-Dausset) have been prepared with a miniaturized assay (adapted from Illumina), optimized and fully automated in our laboratory. Libraries (N = 720) were pooled to obtain low coverage (target 1×) and sequenced on a NovaSeq S4 flowcell (Illumina). Coverage metrics were assessed using our custom pipeline. Haplotype imputation was performed on a subset of libraries with a dedicated pipeline (Gencove).

Results: We obtained homogeneous results for all libraries, with mean depth of coverage 1.0× (interquartile range: IQR = 0.2) and mean breadth of coverage 55.4% (IQR = 7.0%). Coverage was relatively homogeneous across all chromosomes.

Conclusion: This is a proof-of-principle study to evaluate low-pass sequencing approach and test its benefits and limits by analyzing genomes of individuals from populations underrepresented in genome reference panels.

References: [1] Li et al. 2021. Genome Res 31(4):529-537.
[2] Bergström et al. 2020. Science 370(6516):557-564.

Grants: LabEx GenMed (grant number ANR-10-LABX-0013).

Conflict of Interest: None declared.

P20.017.C Using non-linear and stratified Mendelian randomisation to explore relationships between body mass index and the plasma proteome

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Background/Objectives: Elevated body mass index (BMI) raises the risk of diseases such as Type 2 Diabetes (T2D). The circulating proteome has been explored as an intermediate between adiposity and disease, where alterations in growth hormone receptor (GHR) and insulin-like growth factor binding proteins (IGFBP1/2) likely mediate some risk. These associations are often modelled using linear methods but recent studies have indicated that such relationships may be non-linear. This is important when measuring the effect of BMI on protein levels as it may have implications for understanding how weight loss interventions influence disease risk reduction.

Methods: We estimated linear and non-linear associations between BMI and protein (SomaLogic, N>3600) levels using observational and Mendelian randomisation (MR) frameworks in INTERVAL, a UK blood donor cohort (N = 2737). To estimate non-linear associations, an R package was developed ("glsmr" - generalized additive model (GAM) and linear stratified MR). Non-linear effects were identified by comparing the observational linear model and non-linear GAM models using an F-test. In addition, BMI stratified (BMI = 18.5-25kg/m^2, 25-30kg/m^2 and 30-40kg/m^2) linear observational and MR analyses were performed to estimate BMI-protein associations in each stratum.

Results: We observed attenuated estimates for BMI on levels of proteins including leptin, GHR and IGFBPs when comparing the obesity stratum with normal-weight and overweight strata.

Conclusion: These analyses suggest weight loss interventions may be less effective at higher BMIs where plateauing associations between BMI and protein level is observed. This could have implications for weight loss interventions and disease risk reduction.

References:.

Grants: University of Bristol alumni, BHF, EPSRC Prostanoid programme, Wellcome Trust.

Conflict of Interest: None declared.

P20.018.D Simulated European Genome-phenome Dataset of 1,000,000 Individuals for 1+ Million Genomes Initiative

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Background/Objectives: We are in the process of simulating 1,000,000 individual genomes accompanied with simulated quantitative and qualitative phenotypes for the 1+MG initiative. We will use a mosaic of publicly available sequence data sets from European populations. This data is needed to set a founding population (original chromosomes) and parameters for the simulation of million genomes.

Methods: Our method of choice is the chromosome-based simulation method (Terwilliger, 1993) which allows rapid simulation of populations. In this simulation we drop chromosomes through a population for a few hundred generations of drift, while keeping track of recombination events in every meiosis, so that at the end of the simulation we will have a set of families whose genomes will be highly recombined mosaics of the original chromosomes. The dataset will have all the characteristics and data issues that any "true" genome set would.

Phenotypes will be simulated based on epidemiological models, comprised of parameters such as heritability, contribution of environmental factors, prevalence, and others which must be hypothesized (number of genes, allelic complexity, relative effect size). Based on such parameters, a genotype → phenotype relationship is simulated.

Results: Simulated data can be used to benchmark the national service implementations, technical standard compatibility and best practices in sensitive human data management. Purpose is to create a truly anonymised dataset of million genomes in different formats that can be used and shared without fear for data security issues.

Conclusion: We are already simulating hundreds of thousands individuals and we utilize supercomputer resources of Finnish CSC IT Center for Science.

References:.

Grants:.

Conflict of Interest: None declared.

P20.020.B Variation at ADH1B-ADH1C is associated with alcohol and drug dependence in Ukrainians and has pleiotropic effects on metabolic traits

Vitalina Bashynska[1;2], *Oksana Zahorodnia*[3;4], *Yuliia Borysovych*[3;5], *Yaroslav Zaplatnikov*[6;7], *Valeriia Vasylieva*[6], *Ihor Arefiev*[6], *Darina Osichanskaya*[8], *Anna Pastyria*[9], *Dmytro Krasnienkov*[10], *Oksana Zabuga*[10], *Kateryna Murlanova*[11], *Alexander Koliada*[11], *Artur Karapetov*[8], *Oleksandra Melnychuk*[12], *Olena Boiko*[12], *Gennady Zilberblat*[12], *Nataliia Slobodianyuk*[1], *Larysa Bal-Prylypko*[1], *Inga Prokopenko*[13], *Marika Kaakinen*[13;14]

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Background/Objectives: Substance use disorders (SUDs), including drug (DD), alcohol (AD) and nicotine (ND) dependence, are global public health threats. SUDs are highly co-morbid with each other and there is evidence for co-morbidity with other health-related traits, including psychiatric and metabolic traits. This suggests underlying shared genetic effects. We investigated variation at the mainly alcohol-associated region *ADH1B-ADH1C* for a range of SUDs in Ukrainians and the potential pleiotropic effects on other traits.

Methods: We genotyped rs1789891 at *ADH1B-ADH1C* in 507 individuals with and without SUDs from Ukraine (mean age 32.6±9.6 years). The individuals' case/control statuses were defined for each phenotype depending on their ICD-10 diagnosis and the standard questionnaire measures. We performed logistic regression for DD (in general and for opiate (OD)/stimulators (AmphD) dependence), AD, and ND, adjusting for sex and other SUDs phenotypes. We further conducted a phenome-wide association study of rs1789891 in UK Biobank (UKBB) data using GeneATLAS.

Results: rs1789891 in Ukrainians was associated with AD ($P = 0.0087$) and DD ($P = 0.0321$), specifically with AmphD ($P = 0.0261$), with the direction of effect corresponding to the established results, but not with OD, ND, or alcohol consumption. rs1789891 showed an association in the UKBB with alcohol intake frequency ($P = 2.45x10^{-24}$) and suggestive evidence for associations with F10 AD ($P = 3.27x10^{-8}$), Waist/Hip circumference ratio ($P = 3.02x10^{-6}$), and Non-insulin-dependent diabetes mellitus ($P = 3.03x10^{-6}$).

Conclusion: Our results suggest variation at *ADH1B-ADH1C* may have pleiotropic effects on multiple SUDs as well as metabolic phenotypes.

References:.

Grants: University of Surrey Faculty Research Support Fund, US-Ukraine Biotech Initiative Small Research Grant, Crowd.Science, Diagen genetic laboratory.

Conflict of Interest: Vitalina Bashynska Lightgene LLC, part-time employment (modest contribution) - scientific consultant in molecular biology, PI and co-I in several research grants (those relevant to the research are declared in the 'Grants' section, Have received in-kind support (availability of equipment) from Diagen genetic laboratory (2018-2020) and Institute of Gerontology NAMS Ukraine (2021-2022), Oksana Zahorodnia: None declared, Yuliia Borysovych: None declared, Yaroslav Zaplatnikov: None declared, Valeriia Vasylieva: None declared, Ihor Arefiev: None declared, Darina Osichanskaya: None declared, Anna Pastyria: None declared, Dmytro Krasnienkov: None declared, Oksana Zabuga: None declared, Kateryna Murlanova: None declared, Alexander Koliada: None declared, Artur Karapetov: None declared, Oleksandra Melnychuk: None declared, Olena Boiko: None declared, Gennady Zilberblat: None declared, Nataliia Slobodianyuk: None declared, Larysa Bal-Prylypko: None declared, Inga Prokopenko PI and co-I in several research grants (those relevant to the research are declared in the 'Grants' section, Marika Kaakinen PI and co-I in several research grants (those relevant to the research are declared in the 'Grants' section.

P20.021.C Haplotype graph assembly using Swedish individuals

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Background/Objectives: Next generation sequencing (NGS) methods such as short-read whole genome sequencing (SR-WGS) has transformed clinical analysis, solving complex genomic rearrangements. Regardless, capturing the complexity of the genome and relating phenotype-genotype is challening using SR-WGS, resulting in patients remaining without a molecular diagnosis. Using long-read data to create a Swedish haplotype graph will allow association of single-nucleotide polymorphisms (SNPs) with haplotypes, easing prediction of disease susceptibility and phenotype-genotype interactions in Swedish patients.

Methods: To enable phasing of SNPs we built a haplotype graph using 62 Swedish individuals sequenced with linked-read sequencing. A custom de Bruijn graph-algorithm was applied to assemble the graph. Using 1000 additional Swedish genomes from the SweGen[1] dataset sequenced with mostly short-, but also long-read WGS, we identified common haplotypes in the Swedish population. The haplotypes were compared to phased genomes from the 1000 Genomes Project (1KGP)[2] and haplotype reference consortium (HRC)[3].

Results: The de Bruijn graph with a kmer length of 2 SNPs is represented by nodes and edges, where traversal will result in a de Bruijn sequence representing a haplotype. We identified >1000 haplotypes, covering 90% of the genome. Using haplotype panels from the 1KGP and HRC, we distinguished population-specific haplotypes and inheritance patterns.

Conclusion: We built a population-specific haplotype graph of Swedish individuals, capturing local genetic diversity. The graph enables accurate phasing and genomic imputation in clinical analysis, as well as GWAS and linkage analysis studies of complex SNPs.

References: 1) Ameur, A., et al., https://doi.org/10.1038/ejhg.2017.130.

2) 1000 Genomes Consortium, https://doi.org/10.1038/nature15393.

3) Haplotype Reference Consortium, https://doi.org/10.1038/ng.3643.

Grants:.

Conflict of Interest: None declared.

P20.022.D Lessons learned from the first national population-based carrier-screening program for Duchenne Muscular Dystrophy

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[1]*Carmel Medical Center, Genetics Institute, Haifa, Israel;* [2]*Israel Ministry of Health, Jerusalem, Israel.*

Background/Objectives: The Israeli Ministry of Health genetic screening program for reproductive purposes is mainly aimed at severe incurable diseases with high rates of infant and childhood morbidity and/or mortality, a carrier frequency of at least 1:60 and/or a disease frequency of 1 in 15 000 live births. Recently, Duchenne Muscular Dystrophy (DMD) testing was included in panethnic screening tests, and our objective was to summarize the results of first year implementation of this test.

Methods: Data acquisition for this study was performed by retrospective search of Ministry of Health database, encompassing the reports of all national laboratories performing genetic screening tests. Deletion and duplication testing of the 79 DMD exons was performed by multiplex ligation-dependent probe amplification technology. In case of single exon deletion, sequencing of the specific exon was performed to rule out underlying single nucleotide polymorphism (SNP).

Results: Of overall 35,830 DMD tests, 49 female carriers were noted (0.14%, 1:731). Of these, 41 constituted in-frame deletions and duplications, and only 8 (0.02%, 1:4479) were out-of-frame variants. Additional 201 single exon deletions were subsequently defined as false-positives due to underlying SNP (0.56%, 1:178), the majority of these are exon 8 deletion in North African Jewish population, and exon 48 deletion in Arab Muslim population.

Conclusion: Interpretation of population-based DMD carrier-screening might be highly complex, occasionally requiring

additional genetic testing methods and ethical considerations. Multicenter data registry, including ethnic origin and familial segregation in selected cases, is crucial for optimal definition of the results and informed decisions regarding prenatal testing.

References:.

Grants:.

Conflict of Interest: None declared.

P20.024.B a genome-wide screening of plasma circulating microRNAs associated with obesity, body fat distribution and fat mass: the Rotterdam Study

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Background/Objectives: MicroRNAs represent a class of small non-coding RNAs that regulate gene expression post-transcriptionally and are implicated in the pathogenesis of different diseases. However, limited studies have investigated the relationship between circulating miRNAs and obesity-related traits in large-scale using population-based data.

Methods: We conducted a genome-wide profile of circulating miRNAs in plasma, collected between 2002 and 2005, in 1208 participants from the Rotterdam Study. Obesity was measured by body mass index (BMI) and waist-to-hip ratio (WHR) and body composition including fat mass index (FMI) and android-fat to gynoid-fat ratio (AGR) were measured using Dual X-ray Absorptiometry. Multivariable linear regression models were used to assess the association of 591 miRNAs well-expressed in plasma with BMI, WHR, FMI and AGR adjusted for potential covariates. We further sought for the association of identified miRNAs with cardiovascular and metabolic diseases in previous studies.

Results: Plasma levels of 12 miRNAs were associated and overlapped between all four traits (at Bonferroni-corrected $P < 8.46 \times 10^{-5}$). The most significant association was with miR-193a-5p, which was also associated with type 2 diabetes and hepatic steatosis in the Rotterdam Study. Besides, 4 miRNAs were particularly associated with fat mass and body fat distribution; among them, three miRNAs were associated with FMI, and miR-378i only with AGR. Five of the obesity-related miRNAs and two of the fat distribution-associated miRNAs have been linked previously to cardiovascular disease.

Conclusion: This study indicates that plasma levels of several miRNAs are associated with obesity traits and body fat distribution that may serve as potential biomarkers for obesity-related diseases.

References:.

Grants:.

Conflict of Interest: None declared.

P20.026.D Multiple occurring copy number variants that matter

Michaela Hyblova[1;2], *Marcel Kucharik*[3], *Martina Sekelska*[1;2], *Gabriel Minarik*[1;2]

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Background/Objectives: NIPT has proven its efficacy, reliability, and high positive predictive value (PPV) in common aneuploidy screening, sex chromosomal aneuploidy, or even copy number variants. Simultaneously, they may be a precious source of information on maternal genome variation. Large CNVs are usually not an issue to interpret, however, many small and frequent CNV remain ambiguous. We reviewed our NIPT data on such CNV and determine which are the most common, compare them with frequencies in Gnomad. We defined them as multiple occurring variants (movs).

Methods: Paired end whole genome sequencing with low coverage on NextSeq was performed on pregnant plasma samples with at least 5 million reads. CNV detection algorithm was applied to more than 6000 unique analyses.

Results: We identified 20 movs not surprisingly most of them are duplications (16) and 4 deletions. Few of them have conflicting interpretations, and almost none of them is a clear benign variant. The frequencies varied from 0.2-1.9%. In 4 cases there was no match, in 3 cases partial overlay and 13 cases with almost complete different or comparable frequency compared to Gnomad, respectively.

Conclusion: We attempted to assess CNVs marked as movs in the context of genomic content and allele frequency.

References:.

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Conflict of Interest: Michaela Hyblova a part-time employee of Medirex Group Academy n.p.o. and Trisomy test Ltd., collaborator on several running grants, Marcel Kucharik full time job in Geneton Ltd., Collaborator on several running grants., Martina Sekelska a part-time employee in Medirex Group Academy n.p.o. and Trisomy test Ltd., Collaborator on several running grants., Gabriel Minarik a part-time employee in Medirex Group Academy n.p.o. and Trisomy test Ltd., principal investigator on running grants.

P20.027.A Genetic ancestry supports cultural history of northwestern Himalayan Muslims

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Background/Objectives: The Himalayas, a linguistic and cultural metacenter inhabiting diverse human populations has also been a focal point of invasions from both the west and south-eastern regions. With the varied and complex pattern of human settlements, the northwestern Himalaya has majorly evolved under the influence of Afghan-Iranian cultures from the west blending with the Tibetan culture from the north and Indic culture from the south. Despite the social amalgamation, the Muslim populations are believed to retain their distinct genetic signature due to restricted gene flow.

Methods: To establish the influence of social amalgamation on the genetic makeup, we investigated population structure based on 20 highly polymorphic autosomal STR markers in 187 individuals comprising three Muslim populations (Kashmir, Ladakh, and Himachal Pradesh) inhabiting northwestern Himalaya, India. Further, we evaluated the previous theories about the social structure and subsistence of cultures and identified the genetic affinities with 16 different Muslim populations comprising 3610 individuals.

Results: Overall, the phylogenetic analysis demonstrated Kashmiri Muslims in close affinity with other Muslim populations of the neighbouring countries, while the Muslims of Ladakh and Himachal Pradesh showed two different lineages. The Bayesian and Non-Bayesian clustering analysis revealed diverse population genetic structures between the three Muslim populations of western Himalaya, India.

Conclusion: These genetic patterns suggest that the genetic signature of all three populations corroborate with the historic patterns of human settlements and the Muslim populations still retain their genetic signature despite social amalgamation over the centuries.

References:.

Grants:.

Conflict of Interest: None declared.

P20.028.B Homozygosity predominantly affects hypertrophic cardiomyopathy minor genes in an Egyptian clinical cohort

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Background/Objectives: Consanguinity is prevalent in Egypt (35%) resulting in a high incidence of homozygosity. The influence of homozygosity on the genetics of hypertrophic cardiomyopathy (HCM) has not been adequately studied. The aim of this study is to define the genetic architecture of HCM in Egypt using ethnically-matched case and control cohorts.

Methods: Prospective Egyptian patients (n = 514) and controls (n = 400) were recruited to Aswan Heart Centre for clinical phenotyping and genetic testing for 174 genes implicated in inherited cardiac conditions (Illumina). Rare variation (gnomAD filtering allele frequency <=4x10-5) in 13 validated HCM genes were classified according to the American College of Medical Genetics (ACMG) guidelines and compared with a prospective HCM cohort of predominantly European ancestry (n = 684).

Results: Significantly fewer rare variants detected in Egyptian patients could be classified as (likely) pathogenic compared to Europeans (40.8% vs. 61.6%, p-value = 1.6x10-5). Incorporating analysis from these Egyptian case-control cohorts into the ACMG guidelines increased this yield to 53.8%. Homozygous variants were more frequently observed in Egyptian patients (4.1% vs 0.1%, p-value = 2x10-7), with variants in the minor HCM genes MYL2, MYL3 and CSRP3 more likely to present in homozygosity than the major genes (MYH7, MYBPC3 and troponins), suggesting such variants are less penetrant in the heterozygous state.

Conclusion: The integration of Egyptian-specific genetic and phenotypic data significantly improves variant interpretation in HCM and consequently the precision of genetic testing. The observed prevalence of homozygosity and rare variation in minor HCM genes in Egyptian patients provides important insights into its disease-mechanisms and genetics.

References:.

Grants:.

Conflict of Interest: None declared.

P20.030.D Deamination of adenine to guanine is a signature of oxidative damage in mitochondrial DNA

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Background/Objectives: Since mitochondria maintain the oxidative metabolism, it has been traditionally assumed the mitochondrial DNA is affected by the oxidative damage. However, the classical signature of the oxidative damage, G>T, is extremely rare in mtDNA, suggesting that either there is no oxidative damage in mitochondria or it has another - mitochondria specific and yet unknown signature.

Methods: Since oxidative damage is a normal byproduct of oxidative metabolism, which in turn is associated with different life-history traits, we performed a large-scale comparative-species study associating mtDNA mutational spectrum with life-history traits. We reconstructed mtDNA mutational spectra for thousands of vertebrate species analyzing all available intraspecies mitochondrial polymorphisms at fourfold degenerate synonymous sites.

Results: First, within mammals, we correlated mtDNA mutational spectrum of hundreds species with their life-history traits and observed that the fraction of Ah>Gh transitions (heavy chain notation) is higher in species with longer generation length (https://doi.org/10.1101/2021.12.03.460832). Second, focusing on cold-blooded fishes (Actinopterygii) we observed that the frequency of the same transition Ah>Gh positively correlates with the ambient temperature (https://doi.org/10.1101/2020.07.25.221184). Finally, analyzing somatic mtDNA mutations in different human tissues we observed that Ah>Gh is more common in normoxic tissues, enriched in molecular oxygen. (https://doi.org/10.1101/589168).

Conclusion: Aging in mammals, high ambient temperature in fishes and normoxia in human tissues can be similar in terms of the increased oxidative damage. Thus we propose that Ah>Gh is a mitochondria specific mutational signature of the oxidative damage.

References:.

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Conflict of Interest: None declared.

P21 FUNCTIONAL GENOMICS AND EPIGENOMICS

P21.001.A Gene regulation in T-cells from PsA patients differs between peripheral blood and the inflamed joints: implications for the interpretation of GWAS signals

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Background/Objectives: GWAS studies have identified the variants associated with complex diseases. Most of these affect regulatory elements which are cell type and state specific, and can affect distally located genes via chromatin interaction mechanisms.

We and others have used functional genomics techniques in cell lines to provide putative mechanisms for many loci with previously unknown function. It is known that significant differences exist between cell lines and primary cells. However, differences between between samples from healthy volunteers and patients, in particular from the affected tissue, have not been exhaustively investigated. Here we assess the impact of using primary cells derived from PsA patients in functional genomics studies.

Methods: RNA-seq and ATAC-seq were performed on CD4+ and CD8+ T cells isolated from blood from 10 controls and 48 PsA patients and from 6 synovial fluid samples.

Results: We find subtle differences between PsA patients and healthy controls in cells isolated from blood. In contrast, T cells isolated from synovial fluid showed substantially more differences compared to those isolated from patient's blood. Interestingly, we find that CD4+ T cells display more DE genes compared to CD8+ T cells. These genes also enriched more strongly for immune pathways compared to synovial CD8+ T cells.

Conclusion: This study indicates the importance of not only studying GWAS loci in relevant primary cells from patients, but also that attention needs to be given to cells isolated from the affected site.

References:.

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Conflict of Interest: None declared.

P21.002.B Knockout of AGBL5 in human retinal pigment epithelium cells disrupts ciliogenesis and provides insight into transcriptomic changes

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Background/Objectives: *AGBL5* encodes a deglutamylase that regulates functional tubulin glutamylation levels in cilia. Mutations are associated with Retinitis Pigmentosa (RP). However, disease mechanisms associated with *AGBL5* variants require further investigation. This study investigated the effect of patient *AGBL5* mutations in *AGBL5* knockout ARPE19 cells to identify the potential molecular function of AGBL5 and mechanisms causing retinal dysfunction.

Methods: Immunofluorescence confocal imaging, western blotting, transfection with an AGBL5-eGFP expression construct and site-directed mutagenesis were used to investigate cilium structure modifications and protein expression in wild-type (WT) ARPE19 cells and CRISPR knockout cells *AGBL5*$^{-/-}$, and to characterise the effect of variants in patients with RP. RNA-seq data was used to investigate transcriptomic changes.

Results: *AGBL5*$^{-/-}$ cells have reduced levels of AGBL5 expression, hyperglutamylation, significantly shorter cilia, and lower percentage of ciliated cells than WT cells ($p < 0.001$). WT AGBL5-eGFP expression in mutant cells rescues the ciliary phenotype, whilst mutated versions affect ciliogenesis at variable levels, ranging from increased ciliogenesis to loss of cilia. Gene ontology (GO) analysis of genes overexpressed in *AGBL5*$^{-/-}$ cells shows enrichment of genes with GO terms glutamate receptor signalling, nervous system development and negative regulation of neuron apoptotic process.

Conclusion: This work reports a clear phenotype of AGBL5 deficient human cells consisting of hyperglutamylation, short cilia and reduced ciliogenesis, provides functional and transcriptomic data that could be used as a basis for investigating targets for treatment of retinal degeneration and supports the use of mutant cells models to obtain functional data of patient mutations.

References:.

Grants:.

Conflict of Interest: None declared.

P21.003.C DNA methylation signature for JARID2-neurodevelopmental syndrome

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Background/Objectives: JARID2 pathogenic variants cause a neurodevelopmental syndrome, that is characterized by developmental delay, cognitive impairment, hypotonia, autistic features, behavior abnormalities and dysmorphic facial features. JARID2 encodes a transcriptional repressor protein that regulates the activity of various histone methyltransferase complexes (1).

Methods: DNA methylation (DNAm) profiles were generated. Raw data was qc processed and analyzed in R. A Linear model (limma) was applied to obtain differential methylated positions between patients and controls. An unsupervised model was applied in order to assess the robustness of the JND classifying probes, wherein we followed an eight round cross-validation. Subsequently we applied a support vector machine to construct the final JND DNAm signature.

Results: Genome-wide DNAm analysis indicated a clear and robust separation between patients with pathogenic variants and controls. Cross-validation analysis confirmed the findings of a distinct and reproducible episignature. Patients carrying a Variation of Uncertain Signification (VUS) clustered with the control group.

Conclusion: We identified a highly specific genome-wide DNAm signature for patients with JARID2-neurodevelopmental syndrome. The signature can be used to assess and reclassify JARID2 genomic variants. In addition, the JARID2 signature can be added to the growing list of syndromes that can be confirmed by EpiSign analysis (LHSC Epigenetics), thus further conforming the value of EpiSign as a diagnostic tool in patients with suspected genetic disorders.

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P21.004.D The effect of polymorphic inversions on DNA methylation and its modulation by the early-life exposome

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Background/Objectives: Polymorphic genomic inversions are chromosomal variants that play important roles in chromosome evolution, environmental adaptation, and complex traits. Although their effect on local gene expression is demonstrated, it is less known the extent to which inversions influence DNA methylation and how this influence depends on environmental exposures.

Methods: We analysed data of 1,009 children from the Human Early Life Exposome (HELIX) project and 39 prenatal heart tissue samples. We investigated the DNA methylation patterns in blood of three common human inversions, at 8p23.1, 16p11.2, and 17q21.3, and the modulation of these patterns by 64 early-life exposures.

Results: We found inversion-state specific methylation patterns that extended across the three inversion regions in both datasets. Additionally, at CpG level, we identified several inversion-exposure interactions on methylation levels for the early-life exposome. Within those interactions, we observed, for instance, that children homozygous for inv-8p23.1 and exposed to higher meat intake hypermethylated the TDH gene; while those exposed to higher manganese hypomethylated GATA4; and those exposed to parental smoking hypomethylated TRMT9B. These genes have been associated with obesity, heart development, and respiratory disease, respectively.

Conclusion: Although numerous significant inversion-exposure interactions in important genes deserve further scrutiny, our data suggest that the effect of numerous environmental exposures in childhood depends on the individual genetic background given by the inversions via allele-specific methylation patterns.

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Conflict of Interest: None declared.

P21.005.A Ago-RIP sequencing identifies new microRNA-449a-5p target genes increasing sorafenib efficacy in hepatocellular carcinoma

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Background/Objectives: Multi-tyrosine kinase inhibitor sorafenib has been used as standard treatment in advanced hepatocellular carcinoma (HCC) for the last fourteen years. Unfortunately, drug resistance develops in many cases. Therefore, we aimed to

mitigate drug resistance and to improve sorafenib efficacy in HCC. MicroRNAs play a significant role in targeting genes involved in tumor control suggesting microRNA/sorafenib combination therapy as a promising treatment option in advanced HCC.

Methods: MiR-449a-5p target genes were identified by Ago-RIP-sequencing and validated by luciferase reporter assays and expression analyses. Target gene expression and survival data were analyzed in public HCC datasets. Tumor-relevant functional effects of miR-449a-5p and its target genes as well as their impact on the effects of sorafenib were analyzed using in vitro assays. A transwell co-culture system was used to survey anti-angiogenic effects of miR-449a-5p.

Results: PEA15, PPP1CA and TUFT1 were identified as direct target genes of miR-449a-5p. Overexpression of these genes correlated with a poor outcome of HCC patients. Transfection with miR-449a-5p and repression of miR-449a-5p target genes inhibited cell proliferation and angiogenesis, induced apoptosis and reduced AKT and ERK signaling in HLE cells. Importantly, miR-449a-5p potentiated the efficacy of sorafenib in HCC cells via downregulation of PEA15, PPP1CA and TUFT1.

Conclusion: This study provides detailed insights into the targetome and regulatory network of miR-449a-5p. Our results demonstrate that targeting PEA15, PPP1CA and TUFT1 via miR-449a overexpression could have significant implications in counteracting sorafenib resistance suggesting miR-449a-5p as a promising candidate for a microRNA/sorafenib combination therapy.

References:.

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Conflict of Interest: Thea Reinkens: None declared, Amelie Stalke: None declared, Nicole Huge: None declared, Beate Vajen Deutsche Krebshilfe: 70113683, Marlies Eilers: None declared, Vera Schäffer: None declared, Oliver Dittrich-Breiholz: None declared, Brigitte Schlegelberger: None declared, Thomas Illig: None declared, Britta Skawran Deutsche Krebshilfe: 70113683.

P21.006.B Differential gene expression by sex in skeletal muscle cell types

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Background/Objectives: Skeletal muscle differs in size and fiber-type composition by sex. Identifying sex differences in cell-type gene expression will point to mechanisms underlying these differences.

Methods: We performed bulk tissue and single nucleus RNA-sequencing from skeletal muscle biopsies from live Finnish FUSION Tissue Biopsy Study donors ($n = 258$, 41.5% female). We clustered nuclei into 13 cell types. We tested for differential gene expression by sex in bulk tissue and cell types using linear regression, adjusting for age and technical covariates. We identified gene sets with higher expression levels in one sex using gene set enrichment analysis (FGSEA).

Results: Across cell types, type 1, 2A, and 2X muscle fibers had the highest proportion of genes differentially expressed by sex (9.4-16.4%), with smaller proportions in satellite cells (muscle progenitor cells) (2.0%). In bulk tissue, 32.6% of genes were differentially expressed by sex (FDR<0.05), likely reflecting greater power. Gene sets differentially expressed by sex were found in fiber types (2.4-4.3% of tested gene sets), satellite cells (0.03%), and bulk tissue (16.0%). The majority of gene sets showed consistent enrichment by sex across cell types and in bulk tissue; however, a small subset showed enrichment in opposite directions. In satellite cells, cell-cell adhesion genes were enriched for higher expression in males ($ES = 0.34, p = 8.7 \times 10^{-5}$), but enriched for higher expression in females in fiber types (min $p = 0.003$, FDR > 0.05) and bulk tissue ($ES = -0.37$, $p = 4.2 \times 10^{-12}$).

Conclusion: We identified gene sets with similarities and differences in expression by sex between muscle fibers and satellite cells, which may point to cell-type regulatory differences by sex.

References:.

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P21.007.C Profiling genome-wide DNA methylation patterns in human aortic and mitral valves

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Background/Objectives: Cardiac valves exhibit highly complex structures and specialized functions. Valvular gene expression is tightly regulated by a variety of mechanisms including epigenetic factors such as DNA methylation. To date, methylation fingerprints of non-diseased human aortic and mitral valves have not been adequately studied.

Methods: Twelve non-diseased valves free from calcification (6 aortic, 6 mitral valves, 10 males:2 females, age range 42–64 years) were de-endothelialised and subjected to reduced representation bisulphite sequencing (RRBS) on Illumina HiSeq2500. Upon FastQC, reads were trimmed via TrimGalore and mapped to hg19 using Bismark. Extracted methylation levels were analyzed using methylKit. Genes with differentially methylated (DM) promoters were categorized using PANTHER and used for network construction using NetworkAnalyst.

Results: Analysis of methylation detected at 1601 promoters genome-wide revealed 584 DM promoters, of which 13 were reported in endothelial mesenchymal trans-differentiation (EMT), 37 in aortic and mitral valve disease and 7 in ECM remodeling. Genes associated with the DM promoters were enriched for WNT-, Cadherin-, Endothelin-, PDGF- and VEGF- signaling implicated in

valvular physiology and pathophysiology and for TGFB-, NOTCH- and Integrin- signaling involved in EMT and ECM remodeling.

Conclusion: This data provides the first insight into differential regulation of human aortic and mitral valve tissue and identifies candidate genes linked to DM promoters. This study will contribute to the understanding of valve biology, valve tissue engineering approaches and to the identification of relevant drug targets.

References:.

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Conflict of Interest: None declared.

P21.008.D Sex differences in DNA methylation underlay sex differences in children's gene expression

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Background/Objectives: Sex differences in gene expression in childhood, which could explain differences in prepubertal development, behaviour and health risks between boys and girls, remain largely unknown.

Here, we aim to (i) identify genes with sex-specific gene expression in children and to (ii) determine whether these differences in gene expression can be explained by sex-specific DNA methylation patterns.

Methods: Using data from the HELIX project (823 6-9-year-old children from 6 European cohorts), we have performed transcriptome and epigenome-wide association studies of 23,054 autosomic coding genes and 386,518 autosomic CpGs on sex. The model was adjusted for cohort, age and 6 blood cell types.

Results: A total of 2,051 autosomic coding genes (8.9%) and 120,952 autosomic CpGs (31.3%) were significantly associated with sex (FDR < 0.05). 980 genes (47.8%) and 44,081 CpGs (36.4%) showed higher expression or methylation in boys. Of the 2,051 genes with sex differences, 1,339 (65.3%) could be explained by sex differences in DNA methylation. These 1,339 genes were enriched in 114 GO terms (FDR < 0.05), 48 of them directly associated with immune response.

Conclusion: Girls and boys present genome-wide differences in gene expression in autosomal chromosomes. These transcriptomic differences can be partly explained by sex differences in DNA methylation and can lead to differences in immune response and associated phenotypes.

References:.

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Conflict of Interest: Carlos Ruiz Arenas: None declared, Alejandro Cáceres: None declared, Mariona Bustamante: None declared, Martine Vrijheid: None declared, Luis Pérez-Jurado Stock options in QGenomics, Advisory Board in QGenomics, The HELIX Consortium: None declared.

P21.009.A Characterisation of new key cis-regulatory elements of the CFTR gene

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Background/Objectives: Chromatin is a dynamic structure that determine gene expression. Long-range interactions between promoter and cis-regulatory elements (CREs) regulate this expression. Dysregulation of these interactions can lead to diseases, called "enhanceropathies". Some patients with cystic fibrosis or CFTR-related disorders (CFTR-RD) have incomplete genotypes or present extreme phenotypes. This project aims to explain these unresolved cases by first identifying CREs in CFTR locus and in a second part by highlighting dysfunctions in the latter.

Methods: In order to study the regulation of the CFTR gene, reporter gene activity and chromatin immunoprecipitation tests in intestinal cells (Caco-2) allowed us to characterize new CREs. To validate the purpose, invalidation assays with CRISPR/dCas9 are developed. Then, the chromatin interactions (DNA loops) have to be confirmed by using chromatin study techniques (4C). A second part is dedicated to the detection of variants within CREs by NGS sequencing of the CFTR locus in CFTR-RD patients.

Results: CREs at introns 24 and 26 were newly identified with cooperative enhancer activity, in addition to the CREs identified in introns 1 and 12. This has allowed us to propose a three-dimensional model of CFTR gene regulation within the locus. Furthermore, an enrichment of transcription factors HNF1a, p300, FOXA1/2, and CDX2 was shown. By sequencing CBAVD patients, eight variants within CREs were identified and confirmed by functional tests.

Conclusion: Thus, this work allows a better understanding of the three-dimensional organization of the CFTR locus in order to improve the care of patients.

References:.

Grants: Vaincre la mucoviscidose, Association Gaetan Saleun.

Conflict of Interest: None declared.

P21.010.B Functional characterization of a JAG1 5'UTR variant in a patient with clinically observed Alagille Syndrome

Nicole Buhl[1;2], *Eva-Doreen Pfister*[1], *Jens Bohne*[3], *Ulrich Baumann*[1], *Björn Hartleben*[4], *Brigitte Schlegelberger*[2], *Thomas Illig*[5], *Britta Skawran*[2], *Amelie Stalke*[1;2]

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Background/Objectives: Alagille Syndrome (ALGS) is an autosomal dominant multisystemic disease predominantly affecting liver, heart, kidney, vertebrae and eyes. Two NOTCH-Signaling pathway members -JAG1 and NOTCH2- are known to be associated with ALGS. Pathogenic variants exclusively affecting the JAG1 5'UTR have not been described so far. We report on a female patient with clinically distinct ALGS carrying a 5'UTR *JAG1* variant. We performed segregation and functional analyses to assess the variant's pathogenicity.

Methods: We compared the activity of the 5'UTR variant with the wild type 5'UTR via luciferase assay after transfecting HEK293T or Huh7 cells. RNA was analyzed by northern blot. Segregation analysis was done by Sanger sequencing and short tandem repeat (STR) analysis.

Results: The patient developed neonatal cholestasis at 4 weeks of age. With cardiac defects, butterfly vertebrae and histological ductopenia, the girl was clinically diagnosed with ALGS. We identified the heterozygous *JAG1* 5′UTR variant NM_000214.3: c.-100C>T, not detected in population database gnomAD. Luciferase assays showed, compared to the wildtype, a significantly reduced luciferase activity in both cell lines. The mutation slightly decreased total luciferase RNA levels. Sanger sequencing and STR analysis revealed the patient's variant to be de novo allowing a classification as likely pathogenic.

Conclusion: To our knowledge, this is the first description of a disease-associated *JAG1* variant located in the 5′UTR in a pediatric patient with a clinically diagnosed ALGS. Going forward, genetic testing of *JAG1* or *NOTCH2* in patients with suspected ALGS should include detailed 5′UTR characterization of *JAG1* or *NOTCH2*.

References:.

Grants: DFG:#433387263.

Conflict of Interest: Nicole Buhl: None declared, Eva-Doreen Pfister Albireo, Mirum, Alexion, Orphalan, Univar, Albireo, Mirum, Alexion (Modest), Orphalan, Univar, Albireo, Mirum, Alexion (Modest), Jens Bohne: None declared, Ulrich Baumann Albireo, Mirum, Alexion, Orphalan, Albireo, Mirum, Alexion, Nestle, Vivet, Astellas (Modest), Orphalan, Albireo, Mirum, Alexion, Nestle, Vivet, Astellas (Modest), Björn Hartleben: None declared, Brigitte Schlegelberger: None declared, Thomas Illig: None declared, Britta Skawran: None declared, Amelie Stalke: None declared.

P21.012.D Methylome-wide association studies of major depressive disorder, bipolar disorder and schizophrenia spectrum disorders in the German FOR2107 cohort

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Background/Objectives: Epigenetic modifications such as DNA methylation (DNAm) are important regulators of gene expression, influenced by both genetic and environmental factors. In major psychiatric disorders, previous studies have suggested that alterations in DNAm profiles may contribute to pathogenesis and represent potential biomarkers. Thus, we here examined differential methylation in patients with major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia spectrum disorders (SSD) in comparison to healthy controls (HC) that were matched to the diagnostic groups in regard to sex and age.

Methods: Methylation profiling was conducted with the Infinium MethylationEPIC array using DNA from peripheral blood of 886 individuals (MDD: 345, BD: 100, SSD: 101, HC: 340) of the German FOR2107 cohort (https://for2107.de/). After quality control and preprocessing of the methylation data set using the minfi R package, methylome-wide association studies (MWAS) of case-control status were performed for each diagnostic group.

Results: During quality control, a total of 7 individuals were excluded. In the preliminary MWAS for MDD, no differentially methylated CpG sites could be detected that remained significant after Bonferroni correction for multiple testing. Final results for all diagnostic groups will be presented at the conference.

Conclusion: The investigation of DNAm profiles is a promising approach for understanding molecular changes in major psychiatric disorders. As the differential methylation signals typically have small effect sizes, larger sample sizes are needed to increase statistical power and any identified MWAS results should be replicated in meta-analyses of large international consortia.

References:.

Grants: NO246/10-2.

Conflict of Interest: None declared.

P21.013.A Tissue- and ethnicity- independent hypervariable DNA methylation states show evidence of establishment in the early human embryo

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Background/Objectives: Interindividual variation in DNA methylation (DNAm) has been linked to both environmental exposures and phenotypic variation. However, methylation states are generally tissue-specific, making the identification of environmentally sensitive variable DNAm states in disease-relevant tissues a challenge.

Here, we search for hypervariable CpGs ("hvCpGs") on the widely-used Illumina450K array that show high interindividual methylation variation across multiple tissues and ethnicities and test the hypothesis that hvCpGs may have been established in early development before tissue specialisation, thus showing DNAm correlation across tissues within an individual.

Methods: We leverage 30 large-scale datasets that include 19 tissues and 8 ethnicities to identify hvCpGs falling into the top 5% of interindividual methylation variation in >65% of datasets. We explore hvCpG properties, including cross-tissue DNAm correlation, using unpublished multi-tissue fetal methylation data and other resources. We also analyse genetic effects on DNAm at hvCpGs using data from a large meta-analysis of methylation quantitative trait loci (mQTL).

Results: We identify 4143 hvCpGs and show that DNAm at hvCpGs is not driven by genetic variation, probe reliability, sex or cell heterogeneity effects. Instead, hvCpGs show cross-tissue correlation and are strongly enriched for CpGs previously implicated in methylation establishment in the early embryo, including loci sensitive to periconceptional environment.

Conclusion: Our findings position hvCpGs as strong candidates for using DNAm from any tissue to study how stochastic and/or

environmental effects on DNAm in the early embryo can influence life-long health and disease.

References:.

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Conflict of Interest: None declared.

P21.014.B Novel diagnostic DNA methylation episignatures expand and refine the epigenetic landscapes of Mendelian disorders

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Background/Objectives: Overlapping clinical phenotypes and an expanding breadth and complexity of genomic associations are a growing challenge in the diagnosis and clinical management of Mendelian disorders. The functional consequences and clinical impacts of genomic variation may involve unique, disorder-specific, genomic DNA methylation episignatures.

Methods: In this study, we describe 19 novel episignature disorders and compare the findings alongside 38 previously established episignatures for a total of 57 episignatures associated with 65 genetic syndromes. New episignatures were established through bioinformatic assessment of genome-wide methylation data of patient cohorts with a molecular diagnosis for a given disorder against unaffected controls to generate a specific and sensitive classifier.

Results: We demonstrate increasing resolution and specificity ranging from protein complex, gene, sub-gene, protein domain, and even single nucleotide-level Mendelian episignatures. We show the power of multiclass modelling to develop highly accurate and disease-specific diagnostic classifiers.

Conclusion: This study significantly expands the number and spectrum of disorders with detectable DNA methylation episignatures, improves the clinical diagnostic capabilities through the resolution of unsolved cases and the reclassification of variants of unknown clinical significance, and provides further insight into the molecular etiology of Mendelian conditions.

References: Levy, Michael A et al. "Novel diagnostic DNA methylation episignatures expand and refine the epigenetic landscapes of Mendelian disorders." HGG advances vol. 3,1 100075. 3 Dec. 2021, https://doi.org/10.1016/j.xhgg.2021.100075.

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Conflict of Interest: None declared.

P21.015.C DNA methylation episignature testing improves molecular diagnosis of Mendelian chromatinopathies

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Background/Objectives: Mendelian chromatinopathies result from disruption of genes involved in chromatin structure and function. To date, over 50 chromatinopathies have been described and many exhibit unique genome-wide DNA methylation profiles known as episignatures. In this study, we evaluated the DNA methylation profile of 129 individuals with either a confirmed or suspected chromatinopathy for detection of an episignature.

Methods: DNA methylation profiles were generated with the Illumina Infinium MethylationEPIC kit on DNA specimens extracted from peripheral blood and analyzed with the EpiSign classifier for 42 episignatures.

Results: Samples were grouped into discovery, validation, uncertain and negative cohorts based on their genotype and phenotypic description. Both the discovery and validation cohorts consisted of patients carrying a pathogenic or likely pathogenic variant with a matching (discovery) or unknown (validation) phenotype. The DNA methylation profiles of all discovery and validation cases matched the expected episignature. The uncertain cohort consisted of individuals with an identified VUS by sequence analysis and 10.7% (3/28) matched a defined episignature, resulting in

variant reclassification to likely pathogenic. EpiSign analysis in the negative cohort directed a clinical diagnosis in 3/33 (9.1%) cases where molecular investigations were previously negative.

Conclusion: EpiSign analysis provides utility in the diagnosis of chromatinopathies and should be considered as part of the clinical testing cascade.

References: Kerkhof J, Squeo GM, McConkey H, et al. DNA methylation episignature testing improves molecular diagnosis of Mendelian chromatinopathies. Genet Med. 2022;24(1):51-60.

Grants: Telethon – Italy (GGP13231), LHSC Molecular Development Fund, Genome Canada Genomic Applications Partnership Program.

Conflict of Interest: None declared.

P21.016.D Investigation of SCN1A poison exons in epilepsy

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Background/Objectives: Loss of function pathogenic variants in the SCN1A gene are associated with Dravet syndrome (DS) - a devastating childhood-onset epileptic syndrome. Recent works describe several potential "poison exons" (PE) in SCN1A– an alternatively spliced in frame exons containing premature stop codon. Such PE when included to SCN1A mRNA leads to haploinsufficiency. However, a valid functional approach for SCN1A PE is not routinely available.

Methods: Bioinformatic analysis was performed using MaxEntScan and SpliceAI. All 3 PE in the SCN1A gene were cloned to pSpl3-Flu2 splicing vector. Investigated variants were introduced using site directed mutagenesis. Splicing analysis was performed using RT-PCR after transfection to HEK293T cells. Knockdown of SRSF1 and hnRNP A1 was performed using siRNA.

Results: We created a minigene systems for investigation of SCN1A PE inclusion. Wild-type constrictions showed no inclusion for PE2 and PE3 and only 8.9% inclusion for PE1. We demonstrated the pathogenic effect of PE by introduction previously reported and artificial variants into minigenes resulting in significant inclusion of PE. Analysis of 2000 WGS data revealed a potential causative variant in PE2. To investigate the regulation of PE inclusion we performed knockdown of SRSF1 and hnRNP A1 with siRNA. U7 snRNA oligonucleotides were used to correct the splicing pattern of PE in mutant constrictions.

Conclusion: We created and validated a minigene-based functional assay for investigation of inclusion and regulation of PE in SCN1A. U7 snRNA oligonucleotides can be used as a promising approach for future therapy in SCN1A-based epilepsy.

References:.

Grants: The reported study was funded by RFBR. Project number 20-315-90042.

Conflict of Interest: None declared.

P21.017.A Identifying genetic mechanisms that uncouple leanness from a favorable body fat distribution

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Background/Objectives: Leanness is associated with a favorable body fat distribution, indicated by lower waist-hip ratio (WHR). However, many genetic variants show the opposite pattern – they are associated with lower body mass index (BMI) but higher WHR. The variants also show association with an adverse cardiometabolic risk profile. The underlying biological mechanisms remain incompletely understood. We aimed to elucidate the cellular mechanisms of action of genetic variants associated with lower BMI but higher WHR, and increased risk of cardiometabolic disease.

Methods: We took forward all genome-wide significant SNPs associated with BMI, WHR or WHR adjusted for BMI in the largest GWAS published to date, and identified SNPs that are associated with lower BMI ($P < 0.05$) but higher WHR ($P < 0.05$). We prioritized candidate causal genes in the corresponding genetic regions, and assessed the genes' roles in differentiation and maturation of mouse and human preadipocytes by microscopy and quantitative methods.

Results: Altogether 252 independent loci showed an association with lower BMI but higher WHR. We prioritized six candidate causal genes for initial perturbation studies using siRNA. The studies showed an inhibition of adipogenesis for four of the six tested genes: ADAMTS9, ABHD15, EMILIN2 and COL18A1. In further experiments, we will determine the effects on lipid metabolism and insulin signalling through downregulation and dCas9 overexpression, and will examine the resulting expression profile data.

Conclusion: Our findings implicate four novel gene candidates linked to a leaner phenotype but an unfavorable body fat distribution, playing a role in adipogenesis.

References:.

Grants: Novo Nordisk Foundation (NNF18CC0034900, NNF20OC0063707).

Conflict of Interest: None declared.

P21.019.C Rare variant in CCN1 leads to a lean-to-fat switch in body composition by controlling cell differentiation

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Background/Objectives: Obesity is a major contributor to the global burden of chronic disease. Unravelling the complex aetiology of obesity is key for developing effective therapies. Genome-wide association studies provide unique opportunities for identifying novel therapeutic targets. We examined the adipose tissue mechanisms that underlie the association of Ser316Cys missense variant (MAF = 0.8%) in CCN1 with body fat percentage.

Methods: We assessed the role of CCN1-Cys316 by a phenome-wide association study (PheWAS), receptor-binding assays, in vitro gene knockdown experiments, and in vivo adipose tissue-specific gene overexpression studies.

Results: The CCN1-Cys316 allele reduced the binding of CCN1 to its integrin-avb3 membrane receptor by 50% ($p = 0.0003$). The PheWAS suggested an association with lean mass and fat mass, in opposing directions, but showed no significant association with other disease risk traits. Gene knockdown in MSCs reduced proliferation by 30% ($p = 0.0007$), whereas treatment with CCN1 reduced adipocyte differentiation significantly. In mice, adipose tissue-specific overexpression of CCN1 led to a higher BF% ($p = 0.0002$) after a high fat diet, but did not affect bodyweight, indicating a lean-to-fat switch in body composition. The mean area of mouse adipocytes increased ($p = 0.009$), suggesting an effect of CCN1 on adipocyte hypertrophy.

Conclusion: Our data indicates a key role of *CCN1* during adipocyte commitment from stem cells and an effect on adipocyte number, size, and body composition. Our ongoing experiments

using RNAseq after *CCN1* modification will further elucidate the signalling capacities of *CCN1*. The findings may open new avenues for treating obesity by lowering fat mass while retaining lean mass.

References:.

Grants:.

Conflict of Interest: None declared.

P21.020.D Coregulation within Epigenomic Systems

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Background/Objectives: Post-transcriptional and post-translational regulation by N-6-methyladenosine (m^6A) and 5-methylcytosine (m^5C) modification of ribonucleotides is essential for biological function. Dysregulation of m^6A and m^5C RNA methylation processes and changes in 'writer', 'reader' and 'eraser' effector protein abundance, is proposed to be important for the development of cancers and neurological conditions.

Methods: Here we analysed human brain tissues' m^6A and m^5C sequence datasets, as well as studied protein co-regulation of m^6A and m^5C effector proteins using a newly developed mass spectrometry protein database, ProteomeHD, which clusters proteins showing co-regulated abundance after cellular perturbations. Using a correlation cut-off ≥0.8, coregulated proteins for each set of m^6A and m^5C effector proteins was retrieved and examined by gene ontology (GO) analysis.

Results: We found co-regulation patterns between the two RNA methylation systems. For example, the m^6A mRNA writer METT14, was found to be coregulated with the m5C rRNA writers, NSUN4 [percentile score, (PS) = 0.94] and NSUN5 (PS = 0.92). Furthermore, the m^5C readers, ALYREF and YBX1 showed co-regulation with m^6A writer, reader and eraser proteins (PS > 0.9) suggesting that they are central hub proteins in such RNA regulatory systems. GO analysis indicated that specific biological functions were significantly enriched between co-regulated methylation proteins, e.g. the class of writer proteins and mitochondrial function.

Conclusion: The findings of the current study provide evidence of the existence of a co-regulatory cross talk between RNA modification systems and suggest that biological function may be context-specific to localised cellular domains.

References: Shown in poster.

Grants: Nonapplicable.

Conflict of Interest: None declared.

P21.021.A Genome wide methylation profiling in diagnostics; the results of 3 years Episign testing

Marielle Alders[1], *Jennifer Kerkhof*[2;3], *Peter Henneman*[1], *Michael Levy*[2;3], *Andrea Venema*[1], *Raissa Relator*[2;3], *Egbert Redeker*[1], *Bekim Sadikovic*[2;3], *Marcel Mannens*[1]

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Background/Objectives: A growing number of neurodevelopmental syndromes are shown to have unique genomic DNA methylation profiles, episignatures, which can be used as a diagnostic tool.

Methods: We have implemented genome wide methylation profiling as a diagnostic test, named EpiSign, in our laboratory in June 2019. The first version of this test, was able to recognize 22 conditions, which expanded to 45 conditions in v2 (2020) and to 64 conditions in v3 (2021). This latest version consist of 54 episignatures, 9 imprinting disorders and Fragile X syndrome.

Results: Between July 2019 and February 2022, 537 diagnostic EpiSign test have been requested at AUMC. In 318 cases the test was requested because the patient carried a VUS in one of the genes in the EpiSign panel. In 110 of these (35%), the methylation profile matched that of the associated syndrome, supporting pathogenicity of the variant. EpiSign was requested for 217 patients with neurodevelopmental disorder without a known cause. In 17 (8%) of these cases a positive epigenetic signature was found. These were imprinting disorders in 4 cases, FraX in one case and positive episignatures in 12 cases. In at least 6 of these episignature positive patients the causal mutation was identified after targeted and detailed (re)analysis of the associated genes.

Conclusion: These results shows that EpiSign is a powerful diagnostic tool, in particular for patients for whom conventional genetic testing was inconclusive. The number of conditions with an identified specific episignature is growing rapidly, increasing the diagnostic capabilities of this test.

References:.

Grants:.

Conflict of Interest: None declared.

P21.022.B From patient to function: modeling CRIM1 in xenopus tropicalis

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Background/Objectives: *CRIM1* is a transmembrane protein that plays a role in organogenesis, angiogenesis, and kidney disease, yet the function of *CRIM1* has not been fully elucidated especially in the context of human disease. Here,we report a patient with compound heterozygous missense variants in *CRIM1* presenting with holoprosencephaly, facial asymmetry, coloboma, cleft palate, and extremity defects. We sought to establish an animal model for these phenotypes, test whether the *CRIM1* variants are detrimental to function, and provide additional support for disease causality.

Methods: We used whole-exome sequencing and identified *CRIM1* as a candidate gene. To model, we utilized loss-of-function assays using CRISPR/Cas9 mediated genome-editing in the Xenopus model system. We designed two non-overlapping CRISPRs, generated G0 tadpoles, and verified genomic edits using CRISPR edits analysis inference. Embryos then were raised to

be evaluated for neural tube defects (NTDs), craniofacial, and eye abnormalities using brightfield/fluorescence microscopy and optical coherence tomography. To test the function of the variants, we used a gain-of-function assay with overexpression.

Results: Crim1-G0 mutant Xenopus embryos recapitulated patient phenotype closely and showed severe/moderate NTDs(50%), unilateral microphthalmia/coloboma(30%), and facial dysmorphism (20%). Cleft palate was present in 23% of the embryos with facial dysmorphism. The overexpression of wild-type human mRNA resulted in more frequent NTDs than the overexpression with the patient variants indicating that the variants are detrimental to protein function.

Conclusion: We present the first evidence that homozygous variants in *CRIM1* are associated with congenital abnormalities in humans and Xenopus tropicalis.

References:.

Grants: This study was conducted as a part of the "Fulbright Visiting Scholar Program".

Conflict of Interest: OZLEM AKGUN DOGAN Ozlem Akgun-Dogan is a Fulbright scholar in the "Fulbright Visiting Scholar Program", Stephen Viviano: None declared, Ozden Hatırnaz Ng: None declared, Nihat Bugra Agaoglu: None declared, Weizhen Ji: None declared, Lauren Jeffries: None declared, Ugur Ozbek: None declared, Saquib Lakhani: None declared, Mustafa Khokha: None declared, Engin Deniz: None declared, Yasemin Alanay: None declared.

P21.023.C Epigenomic translocation of H3K4me3 broad domains over oncogenes following hijacking of super-enhancers

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Background/Objectives: Chromosomal translocations are important drivers of haematological malignancies whereby proto-oncogenes are activated by juxtaposition with enhancers, often called enhancer hijacking. H3K4me3 is a histone mark characteristic of active promoters, but broad domains (H3K4me3-BDs) can cover entire genes, providing consistent expression. We present a new model of "epigenomic translocation", where enhancer hijacking results in the relocation of a wild-type H3K4me3-BD into the target oncogene.

Methods: We investigated 118 samples including B-cells (15 healthy, 32 malignant), T-cells (17 healthy, 4 malignant) and myeloid cells (50 healthy). Chromatin states were determined using ChromHMM (H3K4me1, H3K4me3, H3K9me3, H3K27me3, H3K27ac, H3K36me3), chromosomal translocations by targeted/genome sequencing and gene expression by RNA-seq.

Results: We studied the epigenomic consequences of *IGH-CCND1* rearrangements. We detected an H3K4me3-BD within the *IGH* locus of healthy B-cells that was absent in samples with *IGH-CCND1*. Relocation of super-enhancer from *IGH* to *CCND1* locus associated with the appearance of H3K4me3-BD over *CCND1*, resulted in overexpression and extensive chromatin accessibility. We observed similar cancer-specific H3K4me3-BDs associated with hijacking of super-enhancers of other oncogenes in B-cell (*MAF*, *MYC*, *FGFR3/NSD2*) and T-cell malignancies (*LMO2*, *TLX3*, *TAL1*). Co-occurrence of H3K4me3-BD and super-enhancer was identified genome-wide in healthy cells and associated with cell identity genes. Additionally, we predicted the 3D genome structure of the interactions between H3K4me3-BDs and super-enhancers using in-silico simulation models.

Conclusion: Our analysis suggests that H3K4me3-BDs can be created by super-enhancers and supports the new concept of epigenomic translocation, where the relocation of H3K4me3-BDs from wild-type location to oncogenes accompanies the translocation of super-enhancers.

References: PMID:34933939, bioRxiv:2021.03.12.434963.

Grants:.

Conflict of Interest: None declared.

P21.024.D Functional correlation of genome-wide DNA methylation profiles in genetic neurodevelopmental disorders

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Background/Objectives: An expanding range of genetic syndromes are characterized by genome-wide disruptions in DNA methylation profiles referred to as episignatures. Episignatures are distinct, highly sensitive and specific biomarkers that have recently been applied in clinical diagnosis of genetic syndromes. Episignatures are contained within the broader disorder-specific genome-wide DNA methylation changes which can share significant overlap amongst different conditions.

Methods: In this study we performed functional genomic assessment and comparison of disorder-specific and overlapping genome-wide DNA methylation changes related to 65 genetic syndromes with previously described episignatures.

Results: We demonstrate evidence of disorder-specific and recurring genome-wide differentially methylated probes (DMPs) and regions (DMRs). The overall distribution of DMPs and DMRs across the majority of the neurodevelopmental genetic syndromes analyzed showed substantial enrichment in gene promoters and CpG islands, and under-representation of the more variable intergenic regions. Overrepresentation analysis showed significant enrichment of the recurring DMPs and DMRs in gene pathways and networks related to neurodevelopment, including neuronal generation and differentiation and axon guidance.

Conclusion: This study expands beyond the diagnostic utility of DNA methylation episignatures by demonstrating correlation between the function of the mutated genes and the consequent genomic DNA methylation profiles as a key functional element in the molecular etiology of genetic neurodevelopmental disorders.

References: Levy, Michael A et al. "Novel diagnostic DNA methylation episignatures expand and refine the epigenetic landscapes of Mendelian disorders." HGG advances vol. 3,1 100075. 3 Dec. 2021, https://doi.org/10.1016/j.xhgg.2021.100075.

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Conflict of Interest: None declared.

P21.025.A Modelling the earliest stages of gliomagenesis using iNPCs in a three-dimensional alginate-based matrix

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Background/Objectives: The development of gliomas is believed to be triggered by isocitrate dehydrogenase (IDH1/2) mutations, which confer a new enzymatic activity that leads to production of the oncometabolite 2-hydroxyglutarate (2-HG). However, current cellular models of early gliomagenesis frequently use transformed cells, or normal stem cells with driver mutations. In the present study we aimed to develop an induced neural progenitor cell (iNPC) model to study the earliest stages of gliomagenesis in 3D.

Methods: Human induced pluripotent stem cells (hiPSCs) were differentiated into NPCs. 2-HG was applied to NPCs embedded in a alginate-based 3D matrix for 2, 7, and 14 days. RNA was isolated from NPCs after day 2, 7, 14 and key genes previously reported to be altered in IDH1-mutant gliomas were assessed by qPCR. The time distribution of changes in the expression of key genes (L1CAM, MEOX2, etc.) were determined to identify the time-dependency of 2-HG effects in this particular model. RNA-seq analysis at selected time point were performed and compared to data from other models to identify model-specific transcriptomic changes. 4C-seq analysis is under way to characterize the alterations in promoter interactions of select genes.

Results: Optimal culturing conditions were identified and expression of key genes were monitored over different time points. RNA-seq analysis identified genes that are altered in response to 2-HG in a model-dependent manner.

Conclusion: Our model for the first time characterizes transcriptomic and epigenomic changes caused by IDH1/2 mutations at the earliest stages of gliomagenesis.

References:.

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Conflict of Interest: Burcu Ekinci: None declared, Tutku Yaraş: None declared, Yavuz Oktay TUBITAK-ARDEB 117Z981 project.

P21.026.B Optimization of assay for transposase-accessible chromatin using sequencing of neutrophils

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Background/Objectives: Psoriasis is a chronic, inflammatory skin disease. Palmoplantar pustular psoriasis (PPP) is one rarer subtype of this clinically and genetically heterogeneous condition. It is characterized by hyperkeratosis, and clusters of sterile, neutrophil-filled pustules of the palms and soles. In contrast to other forms of psoriasis, there is not a single validated genetic risk factor for PPP. In our group, we aim to identify genetic risk factors for PPP. Therefore, among other things, we employ the assay for transposase-accessible chromatin using sequencing (ATAC-seq) on neutrophils, a cell type playing a crucial role in PPP. In order to do so, we developed an optimized ATAC-seq protocol.

Methods: For efficient ATAC-seq on neutrophils we combined the classic protocol[1] with a dead cell removal step and a cell fixation step[2].

Results: Using our modified ATAC protocol we were able to obtain all the desired DNA fractions including the nucleosome-free fraction.

Conclusion: Instead of using a standard procedure, one should always run proper quality controls throughout the whole ATAC-seq protocol and if necessary, optimize the workflow.

References: [1] Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol*. 2015;109:21.29.1-21.29.9. Published 2015 Jan 5. https://doi.org/10.1002/0471142727.mb2129s109. [2] Chen X, Shen Y, Draper W, et al. ATAC-see reveals the accessible genome by transposase-mediated imaging and sequencing. *Nat Methods*. 2016;13(12):1013-1020. https://doi.org/10.1038/nmeth.4031.

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Conflict of Interest: None declared.

P21.027.C Epigenomic and transcriptomic analysis of the intrinsic and extrinsic molecular mechanisms at the earliest stages of gliomagenesis in precancerous cell models

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Background/Objectives: Gliomas are the most common tumours of the brain. Point mutations in IDH1 and IDH2 genes are believed to be the earliest genetic defects in gliomagenesis and result in the production of oncometabolite 2-HG, which alters cellular metabolism as well as the epigenome. Our aim in this study is to elucidate the mechanisms underlying the genetic predisposition associated with SNP- rs55705857 in the 8q24.21 locus, both cell-intrinsic and extrinsically.

Methods: Doxycycline-inducible, immortalized human astrocyte cells (IHA) with IDH1-R132H expression were used to mimic the earliest stages of gliomagenesis. To determine which loci in the genome rs55705857 physically interacts, we used the chromosome conformation capture technique (4C-seq). Media collected from these cells at different time points were applied to primary monocytes differentiating to macrophages, and transcriptomic changes were assessed by RNA-seq and qPCR.

Results: While 4C-Seq analyses showed that the rs55705857 locus interacts with a proximal MYC enhancer that binds TRIM28, RNA-seq showed that differentially expressed genes were significantly enriched in the list of genes that were altered as a result of loss of TRIM28. RNA-seq of differentiated macrophages showed that the complement system was negatively regulated in the presence of 2-HG-containing medium.

Conclusion: For the first time, a mechanistic relationship was established between the 8q24.21 associated glioma risk locus and the control of MYC expression. Also, mutant but untransformed astrocytes have effects on monocyte differentiation, primarily via complement system and the mevalonate pathway.

References:.

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Conflict of Interest: Tutku Yaraş: None declared, Burcu Ekinci: None declared, ebru diler: None declared, Yavuz Oktay TÜBİTAK-ARDEB 214S097 and 117Z981 project.

P21.028.D Epigenetic adaptations of the masticatory mucosa to periodontal inflammation

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Background/Objectives: In mucosal barrier interfaces, flexible responses of gene expression to long-term environmental changes allow adaptation and fine-tuning for the balance of host defense and uncontrolled not-resolving inflammation. Epigenetic modifications confer plasticity to the genetic information and give insight into how tissues use genetic information to adapt to environmental factors. The oral mucosa is particularly exposed to environmental stressors such as a variable microbiota. Likewise, persistent oral inflammation is the most important intrinsic risk factor for the oral inflammatory disease periodontitis and has strong potential to alter DNA-methylation patterns. The aim of the current study was to identify epigenetic changes of the masticatory mucosa in response to long-term inflammation that resulted in periodontitis.

Methods: In an Epigenome-Wide Association Study, CpG methylation of both inflamed and clinically uninflamed solid gingival tissue biopsies of 60 periodontitis cases was analyzed using the Infinium MethylationEPIC BeadChip, applying the EpiDish algorithm for cell-type deconvolution of infiltrated immune cells, and adjustment of effect sizes of differentially methylated positions using our recently developed "intercept method".

Results: Various genes showed significantly different methylation between periodontitis-inflamed and uninflamed oral mucosa in periodontitis patients. The strongest differences were observed for genes with roles in wound healing (*ROBO2*, *PTP4A3*), cell adhesion (*LPXN*) and innate immune response (*CCL26*, *DNAJC1*, *BPI*).

Conclusion: Our results imply specific adaptations of the oral mucosa to a persistent inflammatory environment that involve wound repair, barrier integrity, and innate immune defense.

References:.

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Conflict of Interest: None declared.

P21.029.A Exploring the connections between prenatal environmental stressors and DNA methylation levels in placenta and peripheral tissues of mothers and neonates by applying Artificial Neural Networks

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Background/Objectives: Exposure to environmental stressors during pregnancy plays a powerful role in influencing later susceptibility to certain chronic diseases of the new-born through modulation of epigenetic mechanisms, including DNA methylation [1]. Our aim was to explore the connections among environmental exposures during gestation with placenta, maternal and neonatal buccal cell DNA methylation by applying artificial neural networks (ANNs).

Methods: 28 mother-infants couples have been enrolled. Information on lifestyle and environmental exposure during gestation were obtained by the administration of a questionnaire. Concentrations of heavy metals and dioxins in placenta were analyzed by means of mass spectrometry. Global and gene specific DNA methylation were performed.

Results: ANNs analysis revealed that low birth weight associated with placental H19 methylation, maternal stress during pregnancy with placental NR3C1 and maternal BDNF methylation, and exposure to air pollutants with maternal MGMT methylation. Associations among placenta concentrations of lead, chromium, cadmium and mercury with placental OXTR, maternal and neonatal HSD11B2, neonatal MECP2 and maternal MTHFR methylation, respectively, as well as among concentrations of dioxins and placental RELN, neonatal HSD11B2 and maternal H19 methylation were also observed.

Conclusion: Current results show that methylation levels of genes involved in different pathways important for the embryonic development are sensitive to various environmental stressors during pregnancy and are altered in placenta, potentially affecting foetal development, and in peripheral tissues of mothers and neonates, potentially providing peripheral biomarkers of environmental exposure.

References: Gluckman et al., N Engl J Med. 359(1):61-73, 2008.

Grants: CCM Program 2017 - CUP E72F17000390001.

Conflict of Interest: None declared.

P21.030.B A highly structured diet in Greek individuals reveals molecular signatures linked to growth hormone signalling

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Background/Objectives: Dietary restriction extends healthspan in multiple species and likely has beneficial effects for human health. To address the molecular impact of a structured diet in humans, we established the FastBio project. FastBio comprises 199 individuals following a structured diet specified by the Orthodox Christian Church (temporal abstinence from meat, dairy products and eggs for 200 days annually) and 212 individuals following a general-population, unstructured diet. FastBio explores multiple omics levels at fasting and nonfasting timepoints to capture acute and long-term effects of diet.

Methods: gDNA was genotyped and PCA on genotypes was performed to map FastBio relative to 1KG individuals. Whole blood RNA was sequenced and differentially expressed genes (DEGs) and eQTLs were detected using DESeq2 and FastQTL respectively.

Results: FastBio individuals map closest to TSI, with a gradient mapping away from 1KG populations implying additional ancestry. Most DEGs (N = 386) were detected across dietary groups during the fasting timepoint. We report enrichment of pathways linked to: a) growth hormone receptor signalling, a pathway modulated by diet, whose downregulation has beneficial effects on health, b) action of beta-blockers, c) immune and metabolic processes. We also report ~8,500 eGenes for each dietary group and timepoint.

Conclusion: We reveal key signatures driven by a highly structured diet in humans. Integration of incoming data on: 250 plasma metabolites, 1,536 plasma proteins, the gut microbiome, DNA methylation, as well as experiments in cell culture, will help us understand the molecular mechanisms through which diet shapes human health.

References:.

Grants: ERC Starting Grant to Dr Dimas.

Conflict of Interest: Anargyros Skoulakis ERC Starting Grant to Dr Antigone Dimas, Dimitris Zisis ERC Starting Grant to Dr Antigone Dimas, Vasiliki Zarkou ERC Starting Grant to Dr Antigone Dimas, Maria Anezaki ERC Starting Grant to Dr Antigone Dimas, Konstantinos Rouskas ERC Starting Grant to Dr Antigone Dimas, Antigone Dimas ERC Starting Grant to Dr Antigone Dimas.

P21.031.C Transcriptome profiling with direct-RNA long-read sequencing uncovers functional variation affecting isoform production

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Background/Objectives: RNA-seq technologies increased our knowledge of gene expression and transcript isoform signatures. However, current short-read RNA-seq methods have limitations in identifying complex transcript isoforms as full-length transcripts and base modifications are lost.

Methods: In this study, we use long-read native poly(A) RNA-seq using the Oxford Nanopore Technologies (ONT) platform, which measures the full length of mRNA molecules. We independently sequenced 60 unrelated lymphoblastoid cell lines from the 1000 Genomes/Geuvadis dataset, to investigate the effect of genetic variation on expression, splicing and structure within a population.

Results: Sequenced samples had on average 1.7 million reads with a median read length of 823 bp. We detected 11,123 protein-coding genes expressed in at least 50% of the samples. For these, we observed a good correlation of 0.61-0.79 with gene expression from the same samples sequenced with Illumina(~50M reads/sample). However, using the pi1 computed on genes with significant eQTLs in Illumina (317 samples), the estimate of Illumina-eQTLs detectable with ONT technology was only 10,7%. Isoform analysis using the FLAIR pipeline [1] identified 250,987 isoforms, of which 98,056 were not associated to any annotated gene. Overall, over 77% of isoforms associated to a gene were not previously annotated. Genetic variation analysis on isoform usage on 17,046 annotated isoforms expressed in 50% of our samples identified 70 cis-isoform-QTLs (FDR 5%), among these, nine were not previously observed cis-eQTLs.

Conclusion: Furthermore, to understand the effect of common and rare genetic variants on specific transcript alterations, we are currently evaluating allele-specific expression analysis and identifying direct-RNA modifications.

References: Tang, *Nature Communications*, 2020.

Grants:.

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P21.032.D Adenosine-to-inosine miR-200b-3p editing as a novel oncogenic process in high-grade serous ovarian cancer

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Background/Objectives: Deamination of adenosine to inosine in double-stranded microRNAs (miRNAs) affects their function as oncosupressors or oncogenes. Consequently, edition of miRNAs may play a key regulatory role in multiple signalling pathways altering cancer cell growth and survival. Therefore, edited miRNAs represent a novel and promising therapeutic strategy, especially for traditionally undruggable cancer's targets.

Methods: Here, we investigated a profile of miRNA editing through small RNA sequencing followed by miRge 2.0 analysis of 36 high-grade serous ovarian cancer (HGSOC) samples paired with 34 normal ovarian tissues. For functional studies, human ovarian cancer OVCAR3 and CAOV3 cell lines were treated with siADAR1 and 8-azaadenosine to inhibit miRNA editing or transfected with wild-type/edited miRNAs mimics and subjected to RNA-seq and proteomics analysis to identify altered biological pathways and the critical targets of edited miRNAs.

Results: We found that the tumour suppressor miR-200b-3p was overedited in HGSOC samples compared to the normal ovary tissue. Mechanistically, edited miR-200b-3p promoted cell proliferation, colony formation, migration and formation of 3D spheroids in ovarian cancer cell lines. RNAseq and GSEA analysis revealed that whereas wild-type miR-200b-3p, consistent with its tumour suppressor function, induced apoptotic signalling pathways (NES = 2.4; FDR = 0), edited miR-200b-3p significantly inhibited G1 to S cell cycle control (NES = -1.89; FDR = 0.01) and cell cycle checkpoint (NES = -0.89; FDR = 0.002) pathways.

Conclusion: Our results suggest that edited miR-200b-3p can be a novel therapeutic target for the treatment of HGSOC.

References: Not included.

Grants: The study was supported by the funds of the Ministry of Education and Science within the project "Excellence Initiative - Research University".

Conflict of Interest: None declared.

P21.033.A Epigenetic study: an aid in the diagnostic impasse of Kabuki syndrome

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Background/Objectives: Kabuki syndrome (KS) is a rare genetic disorder with a prevalence of 1/32,000 births. Clinical KS diagnosis is difficult in the first year of life or with atypical facial features. Currently, only molecular confirmation by identification of de novo pathogenic variants in *KMT2D* and *KDM6A* allow to confirm KS. Our laboratory offers a NGS targeted analysis of genes involved in DNA methylation pathway, including KS genes. We confirmed KS diagnosis in 29/41 cases (70.7%). However, the absence of molecular diagnosis in 12 / 41 (29.3%) individuals raises the question of a possible non-detected variant.

Methods: After a careful clinical data review, we classified these patients into two groups: a "typical KS" (n = 3) and "atypical KS" (n = 8). We obtained DNA methylation profiles for 11 "negative" by a collaboration with Dr B.Sadikovik, (EpiSign-CAN).

Results: Unlike "atypical KS" patients, "typical KS" displayed KS epi-signature as previously established for *KMT2D* or *KDM6A* patients. Thanks to a more efficient bioinformatic analysis, we identified *KMT2D* variants and confirmed molecular diagnosis for two "typical SK" with KS epi-signature: a 20 nucleotides deletion and a deletion of exon 35. An extended molecular analysis of the deep intronic and regulatory regions would help to identify the causal variant in the third patient.

Conclusion: It would be interesting to replicate this result in a larger sample. Such approach would provide a valuable and complementary aid to NGS, and would allow the exit from diagnostic impasse for KS patients and probably for patients with other chromatinopathies.

References: Aref-Eshghi, 2020, https://github.com/mobidic/MobiDL.

Grants:.

Conflict of Interest: None declared.

P21.034.B Artificial intelligence reveal connections among sex, gene methylation, maternal risk factors and disease severity in Autism Spectrum Disorder

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Background/Objectives: Increasing evidence points to a contribution of environmental and epigenetic factors in autism spectrum disorder (ASD), but their connections are still largely unexplored [1-3]. In the present study we used machine learning tools to unravel connections among ASD-related gene methylation levels, maternal ASD risk factors and ASD severity.

Methods: The methylation levels of MECP2, OXTR, RELN, BDNF, EN2, BCL2 and HTR1A genes have been assessed in blood DNA samples of 58 ASD children (23 males and 35 females). We then used machine learning approaches (Auto-CM) to connect gene methylation levels with maternal ASD risk factors and with disease severity (ADOS-2 score).

Results: Sex differences were observed in DNA methylation levels of the studied genes, with MECP2, HTR1A, and OXTR methylation connected to females, and EN2, BCL2, and RELN methylation connected to males. BDNF methylation was not linked to sex, but rather to maternal risk factors. Maternal pre-pregnancy BMI, gestational weight gain and living context were among factors linked to disease severity.

Conclusion: The present study highlights the power of artificial intelligence tools to unravel connections among different variables in complex disorders, revealing links among maternal risk factors and disease severity or gene methylation levels, as well as sex differences in gene methylation levels that warrant further investigation in ASD.

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Grants: University of Pisa Grant (PRA 2017-61).

Conflict of Interest: None declared.

P21.035.C A CRISPR and FACS-based assay to assess allele-specific gene regulation by transcription factors

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Background/Objectives: GWAS-identified variants are often found in large intergenic or noncoding regions. Understanding the regulatory effects of such variants is key to understanding their functional role in a given phenotype. Allele-specific transcription factor (TF) binding could explain the association of a noncoding variant with a given phenotype as a result of differences in gene expression.

Methods: We elaborated an extensive and curated list of 1719 TF and produced a lentiviral transcription factor knock out (TF KO).

We used fluorescence-activated cell sorting (FACS) to examine which TF were key for the expression of our gene of interest.

We did that by staining cells transfected with the TF KO library with a specific antibody against the protein of interest and then sorted the 5% lowest expressing cells. We then sequenced the sorted cells to find which TF KO caused loss of expression of the gene of interest.

Examining cell lines with opposite genotype provides insight TFs that affect gene expression differentially according to genotype.

Results: We applied our library to two different phenotype models: a) key transcription factors for a known gene in Multiple myeloma, *ELL2*[1]; and b) regulators in a hematopoietic stem cell model by studying the transcriptional regulators of recently discovered *ITGA9*[2].

Conclusion: We provide with a CRISPR-based library that is easy to use in in vitro cellular models and can be used to study key transcription regulators of cell surface proteins as well as intracellular and nuclear proteins.

References: 1. https://doi.org/10.1038/ncomms8213.
2. https://doi.org/10.1182/blood.2021013220.

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Conflict of Interest: Laura Duran-Lozano: None declared, Jenny Mattsson employed by BioInvent International AB, Caterina Cafaro: None declared, Maroulio Pertesi: None declared, Ram Ajore: None declared, Ludvig Ekdahl: None declared, Aitzkoa Lopez de Lapuente Portilla: None declared, Björn Nilsson: None declared.

P21.036.D Epigenome-wide association study of gestational age at birth using DNA methylation data measured on the Illumina MethylationEPIC BeadChip microarray

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Background/Objectives: Although shorter gestational age (GA) has been linked to increased risk of disease early and later in life, the mechanistic underpinnings are not well understood. Previous studies have shown a strong association between DNA methylation (DNAm) and GA. However, these studies analyzed DNAm measured on the Illumina HumanMethylation 450K array. The more recent 850K ('EPIC') array covers almost twice as many CpGs as the 450K array and includes more CpGs in regulatory regions that may be more relevant to GA. We therefore wanted to investigate the relationship between GA and CpGs on the EPIC array.

Methods: We conducted an epigenome-wide association study (EWAS) of GA using EPIC-derived DNAm data from cord blood of 953 randomly selected newborns from a sub-study of the population based Norwegian Mother, Father and Child Cohort (MoBa) study. Adjustments for child sex, maternal age and smoking, cell type proportions and batch effects were included in the analysis.

Results: 13,660 CpGs were associated with GA at Bonferroni significance, 7639 of which were specific for the EPIC array. Functional enrichment analysis further revealed that these CpGs were located near genes playing a role in development and regulation of the cytoskeleton.

Conclusion: We identified numerous differentially methylated CpGs that were associated with GA and specific for the EPIC array.

References:.

Grants: Research Council of Norway (grant 262700) and National Institutes of Health (NIH) (grant R01 1HL134840-0).

Conflict of Interest: None declared.

P21.037.A The impact of functional characterization of variants in calcium sensing receptor gene (CASR) on the clinical diagnosis

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Background/Objectives: The human calcium-sensing receptor (CaSR) is a G-protein-coupled receptor that signals *via* intracellular calcium mobilization and MAPK pathway to regulate extracellular calcium homeostasis. Loss- or gain-of-function heterozygous *CASR* variants lead to familial hypocalciuric hypercalcemia type 1 (FHH1) or autosomal-dominant hypocalcemia (ADH), respectively.

Methods: We analysed the *CASR* gene in ten unrelated families presenting abnormalities of the calcium and phosphate metabolism. The missense variants of unknown significance were induced by site-directed mutagenesis into a wild-type (WT) CaSR-expressing plasmidic vector. HEK293 cells were transfected with either the WT, the variant or cotransfected with both CaSR-containg vectors. We conducted two luciferase reporter gene assays using NFAT and SRE response elements to measure the function of CaSR on the two major CaSR-associated pathways.

Results: Eleven heterozygous *CASR* genetic variants were identified in eight different FHH1 families, one ADH family and

one patient presenting nephrolithiasis and hypercalciuria. In two families we identified two different variants in *cis* (complex allele) and in trans (compound heterozygote), respectively. The functional assays allowed to classify the eleven variants as gain of function (1/11), loss of function (8/11) and without impact (2/11) and to do a genotype-phenotype association.

Conclusion: We report and characterize eleven *CASR* variants in ten families with various clinical presentations. We were able to resolve two complex cases, with two variants presented in cis and in trans respectively and hypothesize on their implication in the observed phenotype. Our work enlarged the spectrum of pathogenic variants of *CASR* gene.

References:.

Grants: This research received a grant from Normandy region (RIN Normandy Genomic Medicine).

Conflict of Interest: None declared.

P21.038.B Analysis of the effect of thiopurine drugs on the DNA methylation profile in IBD patients

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Background/Objectives: Thiopurines, used in immunosuppressive treatment i.a. of inflammatory bowel disease (IBD), are purine analogs that via incorporation into the DNA strands, inhibit replication. It is hypothesized that thiopurines may cause epigenetic effects. Our aim was to analyze the impact of azathioprine (AZA) on the DNA methylation using the in vitro model of T lymphocytes and NK cells obtained from peripheral blood samples from 15 IBD patients and subjected to 6-day culture with 10 μM AZA addition. The control group consisted of 12 healthy individuals.

Methods: DNA methylation profile analyzes were carried out including 1) CpG islands by using bisulfite conversion and pyrosequencing of mitochondrial D loop sequence and ALU repetitive sequence, 2) global methylation analysis by detection of 5-methylcytosines using enzyme-linked immunosorbent assay (ELISA).

Results: Pyrosequencing results show no statistically significant differences in the methylation level of the D-loop mitochondrial DNA fragment and ALU sequence between in vitro cell cultures with and without 10 μM AZA supplementation, and also between patients and controls. However, for both *loci*, there were differences in the methylation level of the individual CpG positions ($p < 0.0001$). ELISA results showed that the average 5-mC level was at 4.35% for the cultured cells of patients without AZA supplement and 4.15% for those with AZA, and it did not differ significantly, even compared to the results for the control group (3.95% and 4.23% respectively).

Conclusion: The presence of 10 μM AZA addition in 6-day T lymphocytes and NK cells culture does not influence DNA methylation.

References: https://doi.org/10.1016/j.crohns.2012.06.020.

Grants: Polish National Science Centre, grant-no. 2016/23/D/NZ2/01620.

Conflict of Interest: None declared.

P21.039.C DNA methylation in 22q11.2 deletion syndrome and risk to develop schizophrenia

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Background/Objectives: Schizophrenia is a severe psychiatric disorder resulting from the interaction of genetic and environmental factors. One of the highest genetic risks is 22q11.2 deletion. 25% of 22q11.2 deletion syndrome (22q11.2DS) carriers will develop schizophrenia in their lifetime. We hypothesized that environmental factors such as stress increase the risk of schizophrenia for 22q11.2DS carriers through epigenetics.

Methods: We explored the DNA methylation difference in nine 22q11.2DS carriers affected by schizophrenia and five carriers without schizophrenia. One pair of discordant monozygotic twins was included. Besides, 30 mice were separated into four groups: wildtype (WT), Df(h22q11)/+ (G), WT_stress (E), and Df(h22q11)/+_stress (GxE). Blood samples from humans and prefrontal cortex samples from mice were sequenced by RRBS. Differentially methylated probes (DMPs) and regions (DMRs) were detected (FDR<0.05 & Δm>25%).

Results: A total of 3,895 DMPs and 1,562 DMRs were significant, corresponding to 3,773 unique genes. They were highly expressed in the brain and significantly enriched in schizophrenia GWAS hits. The most significant pathways were neuroactive ligand-receptor interaction, calcium, and focal adhesion. Meanwhile, 10,076 DMPs and 167 DMRs (Δm>50%) were identified in twins, 3,030 genes were colocalized. For mice, more genes were detected in stress influence on Df(h22q11)/+ than WT. Same groups of pathways were enriched. In total, 42 overlapped genes were highly expressed in neurons and astrocytes.

Conclusion: Stress matters in the emergence of schizophrenia for 22q11.2DS carriers. These target genes can help us to study the mechanism and therapy further.

References: J.R. Zinkstok et al., 2019, The Lancet Psychiatry.

Grants: ANR EPI-YOUNG, and Fondation Bettencourt Schueller.

Conflict of Interest: None declared.

P2NEW TREATMENTS FOR GENETIC DISORDERS

P22.001.D Papillon-Lefevre syndrome: Alveolar bone regeneration using customized 3D printed poly-l-lactic acid scaffold and autologous bone marrow mononuclear cells

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Background/Objectives: Papillion LeFevre syndrome (PLS) is an autosomal recessive disorder that is characterized by early-onset severe periodontitis and consequently premature loss of teeth. Early teeth loss is followed by progressive bone resorption,

atrophied ridges, and reduced vertical dimension of occlusion that will hinder the construction of a suitable prosthesis. Tissue engineering is a field of medicine that uses a combination of; cells, engineered biomaterials, and suitable biochemical factors to replace biological tissues. Bone monocular cells (BMMNCs) have been used for bone regeneration as they contain a fraction of stem cells. Recent technologies, such as 3D printing, have revolutionized the field of tissue engineering and provided the ability to design and fabricate patient-specific complex 3D scaffolds. Here, an innovative regenerative approach is proposed to provide a step forward towards regenerative therapy.

Methods: This study included 3 patients diagnosed with PLS. Autologous BMMNCs were seeded on a patient-specific 3D printed poly-l-lactic acid (PLLA) scaffold. A combination of; autologous platelet-rich fibrin (PRF) and nano-hydroxyapatite was used for bone regeneration. Clinical and radiographic assessment was be performed at 6 and 12 months postoperatively.

Results: All patients healed probably without any complications. During the 12-month follow-up, no donor site morbidities have been reported. Cone beam radiographs showed successful bone regeneration in all cases.

Conclusion: This proposed approach may help to improve the lifestyle and health of patients with PLS.

References:.

Grants: This work was supported by the Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33438, 2019).

Conflict of Interest: ahmad abd Elazeem Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33438, 2019)., hatem mattar Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33438, 2019)., mohamed elmasry Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33438, 2019)., mohsena abderazik Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33438, 2019)., Nermeen Ahmed Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33438, 2019).

P22.002.A Periodontal management for patients suffering from Papillon–Lefevre syndrome

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Background/Objectives: Papillon–Lefevre syndrome (PLS) is an autosomal recessive disorder caused by Cathepsin C gene mutation. Patients suffer from palmoplantar keratosis, periodontitis, and early loss of teeth. Few periodontists have attempted to preserve natural dentition and avoid total clearance of both primary and permanent teeth which causes severe psychological trauma, indigestion problems and sabotages future attempts for prosthetic intervention. As a continuation of a previous study, this study aims to assess the best intervention to preserve natural dentition and delay bone loss in patients with PLS.

Methods: 5 patients aged 8 to 14 years diagnosed with PLS by molecular and biochemical analysis. All patients received 1 year treatment divided into 2 successive phases as in the following table

Treatment	1st phase (6month)	2nd phase (6month)
Scaling and root planning	Every 3 month	Every 3 month
Diode laser pocket disinfection	Twice per month	Once per month
Local chlorohexidine gel application	non	Once per month

Periodontal condition was assessed clinically and by cone beam x-ray at the beginning and the end of each phase.

Results: Gingival indices showed marked stability during both phases. No teeth were lost or showed increased mobility. Bone level and density were more stable only during the second phase.

Conclusion: Oral hygiene measures aided by diode laser can stabilize the clinical dental condition in PLS patients. Adding chlorohexidine gel was more effective in controlling bone loss.

References:.

Grants: This work was supported by the Science and Technology Development Funding Authority, young researcher grant CALL 10 Grant number 33438,2019.

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P22.003.B L-Serine treatment is associated with improvements in behavior, EEG and seizure frequency in individuals with GRIN-related disorders due to null variants

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Background/Objectives: Pathogenic missense variants in GRIN2A and GRIN2B may result in gain or loss of function (GoF/LoF) of the N-methyl-D-aspartate receptor (NMDAR). This observation gave rise to the hypothesis of successfully treating GRIN-related disorders due to LoF variants with co-agonists of the NMDAR.

Methods: In this respect, we describe a retrospectively collected series of ten individuals with GRIN2A- or GRIN2B-related disorders who were treated with L-serine, each within an independent n-of-1 trial.

Results: Our cohort comprises one individual with a LoF missense variant with clinical improvements confirming the above hypothesis and replicating a previous n-of-1 trial. A second individual with a GoF missense variant was erroneously treated with L-serine and experienced immediate temporary behavioral deterioration further supporting the supposed functional pathomechanism. Eight additional individuals with null variants (that had been interpreted as loss-of-function variants despite not being missense) again showed clinical improvements. Among all nine individuals with LoF missense or null variants, L-serine treatment was associated with improvements in behavior in eight (89 %), in development in four (44 %) and/or in EEG or seizure frequency in four (44 %). None of these nine individuals experienced side effects or adverse findings in the context of L-serine treatment.

Conclusion: In summary, we describe first evidence that L-serine treatment may not only be associated with clinical improvements in GRIN-related disorders due to LoF missense but particularly also null variants.

References:.

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Conflict of Interest: None declared.

P22.004.C Development of a method for permanent skipping of exons 11 and 12 in the DMD gene for the treatment of Duchenne Muscular Dystrophy

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Background/Objectives: Duchenne muscular dystrophy (DMD) is X-linked recessive disease with mutations in DMD gene, coding dystrophin. Skipping one or more exons is a widely used approach for frame restoration in the DMD gene. Mutations in exons 11 and 12 of DMD are typical for 1% of patients. Purpose of the work: to evaluate the possibility of 11-12 exon skipping in the DMD using CRISPR-Cas9.

Methods: Eight sgRNAs (4 for SpCas9 and 4 for SaCas9) for introns 10 and 12 of DMD were cloned into plasmids with eSpCas9(1.1) (Addgene #71814) or SaCas9 (Addgene #61591). Plasmids were individually or pairwise transfected into HEK293T (lipofection) and immortalized healthy myoblasts (electroporation), DNA and RNA were isolated 72 hours after transfection, and PCR was performed to detect editing products. Amplified fragments were sequenced according to Sanger and analyzed by TIDE method.

Results: Experiments on HEK293T made it possible to identify two of the most active sgRNAs for SaCas9 (with efficiency 20% and 8%) and SpCas9 (with efficiency 30% and 25%). This pairs of sgRNAs were capable to create a target deletion in HEK293T and myoblasts, which confirmed by MLPA and Sanger sequencing. In addition, the formation of an inversion of exons 11-12 and a circular DNA consisting of deleted product were confirmed in both cell lines. At the RNA level, only 2 products were detected: a normal transcript and a transcript with a deletion.

Conclusion: The study shows the possibility of skipping exons 11 and 12 of the gene DMD in several cell lines.

References:.

Grants:.

Conflict of Interest: None declared.

P22.005.D The Dutch Center for RNA Therapeutics: a center to develop antisense oligonucleotide therapies for patients with nano-rare mutations

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Background/Objectives: Antisense oligonucleotides (AONs) offer the potential to treat patients with genetic diseases. Notably, for tissues allowing local injection, such as the brain and eye where high local exposure can be achieved with 3-4 infusions of low amounts of AONs annually. Proof-of-concept has been shown for example in spinal muscular atrophy and Leber congenital amaurosis. This approach can also benefit patients with private mutations, as was recently evidenced by the development of the custom-made AON milasen for a patient with Batten's disease. This underlines the potential of AONs as personalized medicines, specifically for patients with private mutations that are associated with brain or eye phenotypes. However, pharmaceutical companies are usually not interested in the development of such approaches, due to the extreme rarity of these variants.

The Dutch Center of RNA Therapeutics (DCRT) is a collaboration of Dutch academic centers with a track record in AON development that aims to develop therapies for patients with nano-rare variants and to offer these therapies in a not-for-profit manner. The DCRT works in alignment with the N-of-1 collaborative (global) and the 1 mutation 1 medicine (1M1M, European) initiatives. In the first two years, we have identified several patients with mutations that are suitable for splice modulation by AONs. Here, we outline the pre-clinical development of AON-based splice correction for a cryptic splicing mutation underlying Stargardt disease affecting the eye, and Beta-propeller Protein-Associated Neurodegeneration (BPAN) affecting the brain. We describe the Dutch roadmap towards clinical implementation, highlighting also the efforts to align developments internationally.

Methods:.

Results:.

Conclusion:.

References:.

Grants:.

Conflict of Interest: None declared.

P22.006.A The Abcc6a Knockout Zebrafish Model as a Novel Tool for Drug Screening for Pseudoxanthoma Elasticum

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Background/Objectives: Pseudoxanthoma elasticum (PXE) is a currently intractable ectopic mineralization (EM) disorder due to bi-allelic ABCC6 mutations. PXE patients have multisystemic EM, while heterozygous carriers present mainly cardiovascular EM. Rapid, cost-effective discovery of therapeutic drugs can be done by compound screening in zebrafish, but this approach is unvalidated in PXE. We validated a stable CRISPR/Cas9 *abcc6a* knockout zebrafish model – which has spinal column hypermineralization as primary phenotypic feature – as a model system for compound screening in EM.

Methods: We evaluated the anti-mineralization potential of five compounds, which has (anecdotal) positive effects reported in *Abcc6-/-* mice or PXE patients. *Abcc6a-/-* zebrafish larvae were treated from 3 to 10 days post-fertilization with vitamin K1, sodium thiosulfate (STS), etidronate, alendronate or magnesium citrate and compared to untreated fish. Following alizarin red staining, alterations in spinal hypermineralization were semiquantified.

Results: Vitamin K1 (80μM), etidronate (100μM) and alendronate (100μM) reduce hypermineralization by 42%, 33% and 39% respectively compared to untreated fish ($P < 0.05$). We show for the first time in a PXE model that 20μM STS reduces mineralization by 55%, but higher doses paradoxically result in EM. Magnesium citrate (10mM) reduces mineralization by 45% and, as only compound, also has an anti-mineralizing effect in heterozygous zebrafish (hypermineralization reduction of 77%). Physiological bone mineralization was not affected by any of the compound screens.

Conclusion: We demonstrate that the use of our *abcc6a-/-* zebrafish model is a promising strategy for drug discovery against EM.

References: N/A.

Grants: GOA019-21 grant – Ghent University.

Conflict of Interest: None declared.

P22.007.B Towards a home management solution for phenylketonuria patients

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Background/Objectives: We aim to measure Phenylalanine using a machine-learning-based chemometric calibration model to process Near-Infra-Red (NIR) spectra from a Dried-Blood-Spot (DBS) card [1, 2]. Here we present the results obtained with our new prototype.

Methods: All data were obtained using a Texas-Instruments NIRScan-Nano-EVM, bound in a custom-built 3d printed case run by a proprietary software built to manage the spectra. We used DBS-calibrators to obtain samples matched with values provided by an independent third party and established a spectral pre-analytical data treatment. A dataset of 101 samples was populated with at least 6 scans from each sample. After scrubbing, 75 spectra were used to train a machine-learning-based chemometric model and 26 as a Test-set. The model was tested on a New-set of 30 samples.

Results: For this application, the optimal optical calibration is 990-1368 nm, with Digital resolution of 150 and auto-correction gain of 64. The pre-analytical data treatment pipeline was optimized to: Kubelka-Munk function, SNV, Smoothing_Detrend, and 2nd_derivative.

Tested against the new set, the accuracy of our model is +/- 1,34 mg/dL with a Non-Error-Rate of 70%.

	Training-set (n = 75)	Test-set (n = 26)
R	0,96	0,96
R2	0,93	0,92
SPD	3,75	3,43
RMSEC	1,48	1,46
SEC	1,49	1,47

Conclusion: An RPD of 3,43 in the Test-set means a good rating for real-world applications.

An RMSE of 1,46 explains the lack of measurement accuracy found when analyzing the new set, and gives room for improvement.

These findings support the feasibility of a NIR-based home Phenylalanine measurement system.

References: ESHG2019, Poster-ID 14.090.A.
ESHG2020, Poster-ID P16.57.C.

Grants: -.

Conflict of Interest: Giuseppe Bonapace lightScience srl - Modest, lightScience srl - Modest, Ennio Tasciotti lightScience srl - Modest, Antonio Valentini lightScience srl - modest, lightScience srl - modest, lightScience srl - modest, Joseph Toaff lightScience srl - modest, Mauro Nasini lightScience srl - modest, lightScience srl - modest, Vittorio Rossetti lightScience srl - modest, lightScience srl - modest, lightScience srl - modest, Chiara Settanni lightScience srl - Modest, Stefano Alessandro Vismara lightScience srl - Modest, Azzurra Matarazzo modest, lightScience srl, Nicole Vignaroli lightScience, modest, Emiliano Binotti lightScience srl - Modest, Antonio Maiolo lightScience srl - Modest, Luca De Angelis lightScience s.r.l., modest, Maria Teresa Moricca: None declared, Giovanna Scozzafava: None declared, Onorina Marasco: None declared, Maria Pittelli: None declared, Teresa Greto: None declared, Nicola Perrotti: None declared, Daniela Concolino: None declared, Marco Flavio Michele Vismara lightScience s.r.l. - Modest, lightScience s.r.l. - Modest, lightScience s.r.l. – Modest.

P22.008.C Genome editing for beta-haemoglobinopathies without double-strand DNA cleavage

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Background/Objectives: Beta-thalassaemia, is marked by low adult haemoglobin ($\alpha_2\beta_2$), owing to defective β-globin (HBB) expression. Increased foetal haemoglobin ($\alpha_2\gamma_2$/HbF) can ameliorate the severity of the disorder and may be achieved by erythroid reduction of γ-globin repressor. This can be achieved by catalysing base editors (BEs), which are safer and likely more efficient than DSB-dependent CRISPR/Cas technology. The project aims to adopt the newest generation of genome editors for application to targets of relevance for β-haemoglobinopathies. Moreover, multiple editing targets are evaluated for induction of higher γ-globin levels.

Methods: The current project performed in silico design of guide RNAs to apply BE technology by nucleofection in erythroid

cells, to modify targets of relevance for β-haemoglobinopathies. Editing efficiency and functional studies at the DNA, RNA and protein level were carried out.

Results: Initial plasmid-based delivery of BEs resulted in high toxicity and poor performance, prompting us to establish in vitro mRNA synthesis for mRNA/gRNA-based delivery of BEs instead. Resulting precision editing with up to 86% bulk efficiency indicated differential same-target efficiency of different BEs for the clinically relevant BCL11A target. Finally, DSB-independence prompted us to evaluate duplex base editing of both, *trans*-acting factors and corresponding *cis*-regulatory elements, which resulted in elevated γ-globin induction compared to single edits and to DSB-based disruption that is currently in clinical trials.

Conclusion: The present study demonstrates, superior editing outcomes based on BEs compared to DSB-based editing for a clinically relevant target, and superior, therapeutically relevant γ-globin induction by duplex compared to simplex BE application.

References:.

Grants: RPF - Excellence /1216/0092.

Conflict of Interest: None declared.

P22.009.D MEK-inhibitor successful treatment on a patient with NRAS-related cutaneous skeletal hypophosphatemia syndrome

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Background/Objectives: Cutaneous Skeletal Hypophosphatemia Syndrome (CSHS) is a RASopathy caused by postzygotic somatic activating variants in *HRAS, NRAS*, or *KRAS* genes. We treated a patient with *NRAS*-associated CSHS with the MEK inhibitor trametinib.

Methods: A 5-year-old boy with lipomatous nevus of the head, eye choristoma, multiple hamartomas, epidermal nevus, hypotrophy of the left hemisphere, pleural lymphangioma and relapsing chylothorax, developed in the last year severe hypophosphatemic rickets unresponsive to phosphate and calcitriol treatment. DNA testing from nevus biopsy showed a somatic c.182A>G, p.(Gln61Arg) variant in *NRAS*. We administered trametinib, a RAS/MAPK pathway inhibitor, for six months. Transcriptome analysis from patient's PBMCs was performed at time 0, week 1, 4 and 12 from treatment start.

Results: Trametinib administration led to a prompt normalization of phosphatemia and phosphaturia. During the follow-up, we noted a growth improvement, the regression of the lymphangioma and chylothorax, and increase in bone mineral density with enhanced bone architecture; we also observed reduction of the epidermal nevus and hamartomas. The sole adverse events were a mild and asymptomatic increase in serum potassium and CPK. RNA-seq analysis indicated a down-modulation of the upregulated RASopathy-specific transcriptional signatures.

Conclusion: Our CSHS case is the first effectively treated with a MEK inhibitor allowing an almost complete recovery of the metabolic bone disease with practically no complication. Transcriptome data provide evidence that the altered RAS/MAPK pathway of the disease is reversed under the MEK-inhibitor treatment.

References:.

Grants:.

Conflict of Interest: None declared.

P22.010.A maxillofacial regeneration using customized 3D printed patient specific poly-l-lactic acid scaffold and autologous bone marrow mononuclear cells

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Background/Objectives: We report using patient specific 3D printed scaffolds for two applications in two cases of craniofacial disorders: clef lip and palate patient with persistant alveolar fistula and 1 case of ectodermal dysplasisa with progressive bone resorption, atrophied ridges, and reduced vertical dimension of occlusion. Using a combination of; cells, engineered biomaterials, and suitable biochemical factors to replace biological tissues combined with recent technologies, such as 3D printing revolutionized the field of tissue engineering and provided the ability to design and fabricate patient-specific complex 3D scaffolds.

Methods: This study report 2 patients with two craniofacial deformity,First a case diagnosed ectodermal dysplasia, and the other with unilateral cleft lip and palate with persistant alveolar deformity causing oronasal fistula.. Autologous BMMNCs were seeded on a patient-specific 3D printed poly-l-lactic acid (PLLA) scaffold. A combination of; autologous platelet-rich fibrin (PRF) and nano-hydroxyapatite was used for bone regeneration. Clinical and radiographic assessment was be performed at 6 and 12 months postoperatively.

Results: Both patients healed probably without any complications. During the 12-month follow-up, no donor site morbidities have been reported. Cone beam radiographs showed successful bone regeneration in those cases.

Conclusion: This proposed approach may help to improve the complex maxillofacial repair for patients with Maxillofacial anomalies.

References:.

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Conflict of Interest: ahmad abdelazeem This work was supported by the Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33494, 2019)., hatem mattar This work was supported by the Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33494, 2019)., mohsena abderazik This work was supported by the Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33494, 2019)., Inas Sayed This work was supported by the Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33494, 2019)., Nermeen Ahmed This work was supported by the Science and Technology Development Funding Authority

(STDF), young Researcher Grant (STDF - YRG– Call 10, Grant number 33494, 2019).

P22.011.B New CDKL5 KI-humanized mouse model and gene editing

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Background/Objectives: Mutations in *CDKL5* cause an incurable neurodevelopmental disorder (CDD,OMIM#300203). CRISPR/Cas9 allows genetic manipulation to either insert or correct a mutation. We here aim to generate a new CDKL5 mouse model and demonstrate the feasibility of correction by gene editing.

Methods: The mouse model was generated by CRISPR/Cas9-based Homologous Recombination. Behavioral characterization of the mouse model was performed by standard tests. The CRISPR/Cas9-based correction tool in human cells was built as described (1). An isogenic cell line was generated from iPSCs derived from patient's fibroblasts.

Results: A knock-in mouse model harboring a 40 bp humanized region surrounding the c.1090G>T (p.Glu364*)) mutation was generated. Motor and behavioral anomalies compatible with CDD were found. Brain transcriptome profile revealed a lower expression of GABRA5, suggesting an impairment of GABA circuits, in line with the reduced GAD1 expression in patient-derived neurons. In parallel, correction of the mutation was achieved in both patient-derived fibroblasts and iPSCs: neurons from mutant iPSCs confirmed the absence of CDKL5 protein, which was restored following correction. A reduced expression of *GAD1* was also observed.

Conclusion: We have generated a new Cdkl5 mouse model available for the scientific community. We achieved correction and biological rescue using CRISPR/Cas9 in vitro. Experiments are ongoing for the in vivo correction.

References: 1-Croci S et al, **High Rate of HDR in gene editing of p.(Thr158Met) MECP2 mutational hotspot** *Eur J Hum Genet 2020*.

Grants: CDKL5–associazione di volontariato onlus.

Conflict of Interest: None declared.

P23 GENETIC COUNSELLING/SERVICES/EDUCATION

P23.002.D Spectrum of medical care for pediatric patients with neurogenetic disorders – early experiences of a syndrome ambulance within a social pediatric center

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Background/Objectives: Our social pediatric center provides medical services for ca. 9.000 children pro year, mainly patients with neurodevelopmental disorders. We perform genetic analysis for around 250 patients/year, incl. >200 trio-WES-analysis/year, making us one of the largest neurogenetic diagnostic centers in the state of Bavaria, Germany.

Establishing a state-of-the-art care for our patients with classical syndromic disorders as well as rare and ultra-rare neurogenetic diseases we started our interdisciplinary syndrome ambulance 3,5 years ago. The rapid development of this ambulance caring for over 200 patients 3 years after its foundation mirrors the recent large development in the human genetics.

We present our experience in three distinctive areas:.

1. The different interdisciplinary structures for appropriate patient care depending on the age of the patient as well as the type and severity of the underlying neurogenetic condition.

2. The set-up of genetic counselling in straight-forward diagnostic cases through qualified pediatricians in collaboration with geneticist.

3. The search for approved, on-trial and novel therapeutic approaches through scheduled literature researches for all patients, and subsequently offering support to receive approved treatment or to be admitted in a clinical-trial as well as initiating compassionate-use drug-repurposing.

The goal of our presentation is to facilitate the communication and collaboration between pediatricians, geneticists and researchers on the field of neurogenetics.

In case of an invitation to an oral presentation we would also present unpublished cases of compassionate-use drug-repurposing.

Methods:.
Results:.
Conclusion:.
References:.
Grants:.
Conflict of Interest: None declared.

P23.003.A Telemedicine versus traditional consultations in clinical genetics: comparison of patient satisfaction rates

Uri Hamiel[1;2], *Audelia Eshel Fuhrer*[1;2], *Nitsan Landau*[2], *Hagit Baris Feldman*[1;2], *Daphna Marom*[1;2]

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Background/Objectives: Telemedicine is a service delivery model in which credentialed specialists provide care remotely to clients. Data regarding patient satisfaction with telemedicine consultations in the field of clinical genetics, and specifically paediatric genetics is lacking. We aimed to compare patient satisfaction rates from telemedicine versus traditional, face-to-face genetics consultations during the COVID-19 pandemic.

Methods: A cross-sectional survey, sent to 1672 parents of minors (patients<18years), or adult patients, who received counselling through the Tel-Aviv Souraski Medical Center Genetics Institute between 1/1/2020-1/6/2020. Data were collected through REDCap and converted to Microsoft EXCEL Database Program(v16.0) and STATA(v14.1).

Results: Full responses were collected from 457 patients (27.3%). Of them, 330 patients (72.2%) had face-to-face consultations, 80 (17.5%) were counselled through telemedicine, and 47 had both (10.3%). Satisfaction or high satisfaction were reported in

82.1% in the face-to-face consultation group, while 6.3% were unsatisfied or unsatisfied at all, compared with 82.5% and 11.2% in the telemedicine group, respectively. Differences were insignificant statistically between the two groups. Data were further stratified according to subspecialties. Of total consults, 58 (12.7%) were in paediatric genetics. None of the patients who received paediatric genetics counselling solely through telemedicine were unsatisfied. Seventy-six percent of all patients who were counselled through telemedicine would want to use telemedicine services in the future, while 18.7% are undecided, and 5% do not.

Conclusion: Telemedicine consultations in the genetics clinic during the COVID-19 pandemic, and specifically in paediatric genetics, were associated with high satisfaction rates, non-inferior to traditional consultations satisfaction rates.

References: N/A.

Grants: N/A.

Conflict of Interest: Uri Hamiel: None declared, Audelia Eshel Fuhrer: None declared, Nitsan Landau: None declared, Hagit Baris Feldman HBF has received honoraria for scientific talks, grants, and consults from: Sanofi-Genzyme, Protalix, Pfizer, and Takeda-Shire. She serves on scientific advisory boards of Sanofi-Genzyme and Igentify and in the past also in Shire and Regeneron., HBF has received honoraria for scientific talks, grants, and consults from: Sanofi-Genzyme, Protalix, Pfizer, and Takeda-Shire. She serves on scientific advisory boards of Sanofi-Genzyme and Igentify and in the past also in Shire and Regeneron., HBF has received honoraria for scientific talks, grants, and consults from: Sanofi-Genzyme, Protalix, Pfizer, and Takeda-Shire. She serves on scientific advisory boards of Sanofi-Genzyme and Igentify and in the past also in Shire and Regeneron., Daphna Marom: None declared.

P23.004.B First German Language Master of Science in Genetic and Genomic Counselling

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Background/Objectives: While Genetic Counsellors (GCs) are well-established around the globe, they have met resistance in the German language countries due to a perceived conflict of competencies with medical geneticists (Schwaninger et al. 2021, PMID: 33797821). In 2019, the Medical University of Innsbruck, Austria introduced the first MSc programme in Genetic and Genomic Counselling for the German-speaking countries.

Methods: Here we report the experiences from the first cohort graduating in spring 2022.

Results: The Innsbruck MSc programme follows the European standards and is EBMG accredited. This allows graduates with an additional two year training to apply for registration as a European Certified Genetic Counsellor, granting international reciprocity and freedom to move workplace within Europe and abroad. The five-semester part-time programme is currently graduating the first cohort and has started a second year group with twelve students from Germany, Austria and Switzerland.

The implementation of the master programme has commenced a lively discussion about the future of genetic counselling in all German-speaking countries. The programme team, in conjunction with the first graduates, works to establish an evidence based implementation of the occupational profile for the region. Research projects on the professional scope of practice, the separation of the professional role of genetic counsellors and the service of genetic counselling, issues around the job title, remuneration concepts and the integration of GCs into genetic services are undertaken.

Conclusion: Most crucial will be a close integration of GCs into teams together with medical geneticists, building trust in their education and skills from genetics knowledge to psychological attending.

References:.

Grants:.

Conflict of Interest: None declared.

P23.005.C Reasons for forgoing carrier testing among relatives of pathogenic variant carriers of Hereditary Breast and Ovarian Cancer and Lynch Syndrome

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Background/Objectives: Cascade screening of Hereditary Breast and Ovarian Cancer (HBOC) and Lynch Syndrome (LS) is important for prevention and clinical management of hereditary cancer syndromes. The study aims to find out reasons for forgoing carrier testing among relatives of known carriers of HBOC- or LS-associated pathogenic variants, and compare factors at the personal, family, and healthcare system levels between relatives who did not have genetic testing (GT(-)) and those who had genetic testing (GT(+)).

Methods: Data are part of the Swiss CASCADE cohort and were collected with self-administered surveys collected between September 2017 and December 2021.

Results: In total 115 relatives submitted baseline data and 38% did not have carrier testing. Compared to the GT(+) group, the GT(-) group included a higher proportion of males, who were significantly less likely to have a personal cancer diagnosis, fewer number of relatives tested in the family, less care from specialist and more likely to receive care by multiple (≥3) healthcare providers. Being female (OR:2.77, 95%CI:1.10 – 7.10), having had personal history of cancer (OR:4.47, 95%CI:1.03-19.42) and higher number of family members tested (OR:1.42, 95%CI:1.09-1.83) had higher odds of getting carrier testing.

Conclusion: Carrier testing promotion among individuals with cancer, and men and family focused interventions may help increase carrier testing.

References:.

Grants: The Swiss Cancer Research Foundation KFS-5293-02-2021, the Swiss Cancer League – KLS-4294-08-2017 and by the University of Basel, Office of the Vice Rector of Research (2016), and the Department of Clinical Research (2021).

Conflict of Interest: None declared.

P23.006.D Differences in intention to inform relatives and rates of cascade testing in families concerned with hereditary breast and ovarian cancer and Lynch syndrome: The Swiss CASCADE cohort

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Background/Objectives: Cascade testing and identification of individuals carrying pathogenic variants in causative genes associated with hereditary breast and ovarian cancer (HBOC) and Lynch syndrome (LS) are significant public health interventions. However, cascade rates of LS are much lower than HBOC, while a pronounced gender disparity exists for HBOC. We describe cascade testing in relatives from HBOC and LS families, and compare predictors of intention to invite relatives to a family-based cohort.

Methods: The Swiss CASCADE is a family-based, open-ended cohort, including carriers of HBOC- and LS-associated pathogenic variants and their relatives. Participating index cases are asked to invite their relatives.

Results: Currently 304 index cases and 115 relatives provided baseline information. We identified on average 10 relatives per index case potentially eligible for cascade testing. The family pathogenic variant was excluded in 14.7% of relatives (true negatives), while 13.9% are untested. Approximately 65% of respondents intend to invite relatives. Intention is higher for first- compared to second- and third-degree relatives, but is not different between syndromes or based on relative gender. The family environment predict intention to invite relatives for both syndromes, while additional predictors were identified for HBOC.

Conclusion: Information can help optimize delivery of genetic services.

References:.

Grants: Swiss Cancer Research Foundation KFS-5293-02-2021, the Swiss Cancer League – KLS-4294-08-2017 and by the University of Basel, Office of the Vice Rector of Research (2016), and the Department of Clinical Research (2021).

Conflict of Interest: None declared.

P23.007.A APOGeE (A Practical Online Genetics e-Education): a European medical genetics e-textbook by ERN-ITHACA

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Background/Objectives: APOGeE (A Practical Online Genetics e-Education) will be launched in 2022 as a free online interactive medical genetics textbook built with Moodle, written by various authors from the ITHACA network and other ERNs. APOGeE will cover topics about biological genetics, formal genetics, a clinical and physiological approach to genetic diseases, precision medicine, and treatment of genetic diseases.

Methods: Whilst original content is being written by authors from various European countries and is produced by a renowned international editorial committee, the book will be further

enriched with existing up-to-date documents and courses in the network.

Results: As a companion for the European Certificate in Medical Genetics and Genomics (ECMGG) examination organised by the European Union of Medical Specialists (UEMS), the main content will be supplemented by almost 200 medical vignettes of diseases as pedagogical references, all linked to their relevant chapters. Moreover, the Moodle platform will include links to external reference materials such as articles and guidelines for further reading.

Conclusion: By contributing to a structured postgraduate education programme in the field of human genetics and rare diseases, the project aims to provide a free and open source of interactive and asynchronous medical genetics learning materials: doctors and researchers from Europe and beyond, from all socioeconomic backgrounds, will be able to access the online platform with different learning modules, self-evaluation tools and discussion forums.

References:.

Grants: The project is co-funded by the CEF, Connecting Europe Facility of the European Union, under the action number 2020-FR-IA-0128.

Conflict of Interest: None declared.

P23.009.C Genetic first results: Lessons learned from pediatric secondary findings

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Background/Objectives: As clinical genomic testing becomes more common, so will receiving "genetic-first" results, defined as pathogenic/likely pathogenic variant(s) in a gene that is unrelated to the primary indication for testing, including a secondary finding (SF) recommended by the American College of Medical Genetics. A SF management framework has been articulated for adults; however, there is little, and often conflicting, guidance for how SFs should be managed in minors.

Our tertiary care sequencing program returns SF from exome and genome sequencing in minors by default. Here we synthesize our findings and present cases emphasizing the nuances of such results.

Methods: A total of 864 minors received exome or genome sequencing from 2017-2021.

Results: Seventeen SFs were returned in 16 minor participants (1.9%). Most SFs (13/17, 76.5%) were associated with potential childhood-onset of disease.

SF Risk Category	Frequency	Genes
High or near complete penetrance	4/ 17, 23.5%	*FBN1*, *RET*
Low or moderate penetrance	8/ 17, 47.1%	*BRCA2*, *DSC2*, *MHY7*, *PKP2*
Low intensity interventions	4/ 17, 23.5%	*LDLR*
Typical childhood onset	1/17, 5.9%	*OTC*

Illustrative cases emphasize the following themes:

1. SFs do not necessarily lead to clinical diagnosis or active disease mitigation. Follow-up is uncertain and at clinician discretion.
2. Considerations for results disclosure: family understanding and adaptation.
3. Age-related penetrance and cascade testing opportunity for at-risk relatives.

Conclusion: These findings highlight opportunities and challenges of genetic first results. As genomic approaches become better integrated into clinical practice, thoughtful evaluation and tailored follow-up will be essential in translating such findings into meaningful recognition and mitigation of disease.

References:.

Grants:.

Conflict of Interest: None declared.

P23.010.D Role of the genetic counsellors in oncology settings, an European survey

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Background/Objectives: For several decades now, the inclusion of non-physician genetic counsellors (GC) has been shown to be an effective way to deliver genetic counselling services in many countries including USA, Canada, UK and France. The need for appropriately trained GC to support genetic healthcare is acknowledged all around Europe and Master Degrees specialized in genetic counselling are available across Europe (eg: UK, Spain, France). Although the profession is gaining recognition globally, associations and institutions are still needed to achieve true recognition of the practice itself (billing for consultation, recognized professional title, ability to work autonomously). This is especially true in the field of oncology genetics, where the practice of the profession is often regulated by national and international guidelines.

Methods: Based on review of the academic and grey literature, we discuss the barriers, challenges and facilitators to the integration of GC in oncogenetics as well as priorities for further action.

Results: While GC provide a valuable service within an oncology practice, playing a pivotal role in bringing the benefits of oncogenetics and precision medicine, a concerted global effort is required to transform GCs' policy and practice.

Conclusion: This discussion is critical to inform professionals in genetics of the work still needed to ensure that GC are appropriately involved in the offer of oncogenetics for the best care of patients. This work will also form the basis for further empirical studies and we hope to obtain input from 2022 ESHG conference attendees in real-time through mentimeter questions.

References:.

Grants: No grants.

Conflict of Interest: None declared.

P23.011.A De Novo Experience of Individuals with Li-Fraumeni Syndrome: "Sometimes the people in your family are never going to be the ones who understand best"

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Background/Objectives: Li-Fraumeni Syndrome (LFS) is a rare hereditary cancer predisposition syndrome characterized by high lifetime risks for multiple primary malignancies. Although most individuals with LFS inherit a pathogenic *TP53* variant from a parent, approximately 20% have de novo variants with no suggestive family cancer history. This may result in an LFS experience distinct from individuals with affected relatives. This multi-case study report examines the unique psychosocial experiences of three young adults with *de novo TP53* variants.

Methods: The National Cancer Institute's LFS study (NCT01443468) recruited adolescents and young adults (AYAs; aged 15-39 years) with LFS for qualitative interviews. Three participants had a *de novo TP53* variant and a personal cancer history. An interprofessional team analyzed interview data using extended case study and narrative methods.

Results: De novo participants lacked familiarity with LFS to situate a cancer diagnosis, interpret genetic test results, or adjust to chronic cancer risk. Communicating with and receiving support from family was challenged by their lack of common experience. De novo participants experienced socioemotional isolation, which was amplified during the COVID-19 pandemic. To cope, they sought support in online rare disease communities or through mental health providers.

Conclusion: Individuals with de novo variants may lack familial guides and familiar providers to address disease management and uncertainty. Specialty health and mental health providers may support de novo patients across hereditary cancer syndromes by validating their uncertainties and connecting them with disease-specific patient advocacy groups that support adjustment to chronic cancer risk.

References:.

Grants:.

Conflict of Interest: None declared.

P23.012.B Blood chimerism – more common than you would think: Practical implications in genetic counseling

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Background/Objectives: Blood chimerism (BC) result from blood transfusion between dizygotic twins through a shared placenta. Such chimerism is usually limited to blood cells, and doesn't involve other cell types or organs. In the past, chimerism was estimated to be extremely rare, but nowadays it's believed that BC exists in approximately 8% of dizygotic-monochorionic twins. We referred a 27-years old female for oncogenetic gene panel due to breast cancer diagnosis. Two blood samples were sent to the lab consecutively, yielding no results. Existence of different blood cells in the samples was offered as an explanation. In combination with the fact that the patient has a twin brother, the suspicion of chimerism arose.

Methods: QF-PCR (using Aneufast quantitative flouresent PCR kit) was performed on DNA from the patient's blood, buccal cells and fibroblasts, and compared to her male twin and parents' blood samples.

Results: We found 70% chimerism (XY markers) in the patient's blood, 30% in her buccal cells and no chimerism in fibroblasts. Her twin's blood sample had 50% chimerism (XX markers).

Conclusion: These results support the hypothesis of chimerism, confined to blood cells, most likely due to placental blood transfusion. The diagnosis of blood-confined chimerism enabled completing the genetic testing by using DNA from fibroblasts (no known pathogenic variants were detected). In addition, her fibroblast sample may be used in future genetic testing, such as prenatal carrier screening. Since chimerism can have medical, social, forensic, and legal implications, raising awareness to the issue is highly important.

References:.

Grants:.

Conflict of Interest: None declared.

P23.013.C Additive diagnostic yield of homozygosity regions identified during chromosomal microarray testing in children with developmental delay, dysmorphic features or congenital anomalies

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Background/Objectives: Chromosomal microarray (CMA) has emerged as a robust tool for identifying microdeletions and microduplications, termed copy number variants (CNVs). Nevertheless, data regarding its utility in different patient populations with developmental delay (DD), dysmorphic features (DF) and congenital anomalies (CA), is a matter of dense debate. Although regions of homozygosity (ROH) are not diagnostic of a specific condition, they may have pathogenic implications. Certain CNVs and ROH have ethnically specific occurrences and frequencies. We aimed to determine whether CMA testing offers additional diagnostic information over classical cytogenetics for identifying genomic imbalances in a pediatric cohort with idiopathic DD, DF, or CA.

Methods: 169 patients were offered cytogenetics and CMA simultaneously for etiological diagnosis of DD ($n = 67$), DF ($n = 52$) and CA ($n = 50$).

Results: CMA detected 61 CNVs [21 (34.4%) pathogenic, 37 (60.7%) variants of uncertain clinical significance and 3 (4.9%) benign] in 44 patients. CMA identified one or more ROH in 116/169 (68.6%) patients. When considering pathogenic CNVs and aneuploidies as positive findings, 9/169 (5.3%) received a genetic diagnosis from cytogenetics, while 25/169 (14.8%) could have a genetic diagnosis from CMA. The identification of ROH was clinically significant in two cases (2/169), thereby, adding 1.2% to the diagnostic yield of CMA (16% vs. 5.3%, $p < 0.001$).

Conclusion: CMA uncovers additional genetic diagnoses over classical cytogenetics. Our findings convincingly demonstrate the additive diagnostic value of clinically significant ROH identified during CMA testing, highlighting the need for careful clinical interpretation of these ROH.

References:.

Grants:.

Conflict of Interest: None declared.

P23.014.D Patient Journey Common Needs: Rare congenital malformations syndromes with intellectual and other neurodevelopmental disorders affect one child in a million

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Background/Objectives: The heterogeneous nature of clinical presentation and huge variation in the onset of the first symptoms can, as early as the neonatal period, lead to misdiagnosis, thus to the absence of early treatment. With over 5000 rare and complex syndromes, how can ERN ITHACA meet their diverse needs? In response, patient representatives (ePAGs) have described the individual needs of their children in a syndrome-specific "patient pathway", which serves as a personal testimonial and describes the natural history of each of these rare syndromes Rett, Williams, Prader Willi, Spina Bifida and Pitt-Hopkins.

Methods: Patient representatives presented their syndrome-specific 'patient journeys' (Bolz-Johnson et al. 2019) in a workshop and identified the characteristics and needs that were common. A position paper describing relevant elements for all syndromes was presented to the Board in Düsseldorf, 2019.

Results: Genetics is the key for many ITHACA syndromes. However, it is only one aspect of child's situation and ERN ITHACA needs to go beyond genetic diagnosis and think about the holistic needs of patient communities.

Conclusion: An agreement with experts on introducing common needs in all the work packages of ERN ITHACA have been achieved, as well as developing a standard of care in a Clinical Pathway.

New patient journeys will be developed in the future.

References: Bolz-Johnson, M., Meek, J., & Hoogerbrugge, N. (2020). "Patient Journeys": improving care by patient involvement. *European Journal of Human Genetics*, *28*(2), 141-143.

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P23.015.A Views and experiences of clinical geneticists concerning unsolicited findings in next-generation sequencing: "a great technology creating new dilemmas"

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Background/Objectives: Unsolicited findings (UFs) from diagnostic genetic testing are subject of debate. There is emerging consensus that UFs from genetic testing should be disclosed, but recommendations on UF disclosure generally leave room for practice variation. This study aimed to explore the views and experiences of clinical geneticists concerning counseling UFs pre-test and UF disclosure.

Methods: We interviewed 20 medical specialists certified in clinical genetics and residents in clinical genetics working in seven national genetic centers.

Results: Participants expressed that discussing the probability of detecting UFs is an integral part of pre-test counseling. However, they had doubts about to what extent and about what to inform patients. They argued that the contents of their counseling ought to depend on the individual patient's capacity to understand information. While 'medical actionability' is broadly accepted as an important criterion for UFs to be disclosed, participants experienced substantial uncertainty regarding the concept.

Conclusion: These results direct towards tailored pre-test counseling in order to optimize genetic consultations. This study underscores the need for further demarcation of what exactly constitutes medical actionability. Installation of an expert panel to help to decide which variants to disclose, will support genetic counselors to face the dilemmas UFs present.

References:.

Grants: Not applicable.

Conflict of Interest: None declared.

P23.016.B The role of the Genetic Counsellor in the multidisciplinary team: the perception of geneticists in Europe

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Background/Objectives: Genetics is becoming fundamental in the understanding and management of pathologies; thus, it is necessary to understand what is the most effective way of taking care of people affected by or at risk of genetic pathologies. Indeed, the team dealing with such patients has evolved especially with the emergence of the Genetic Counsellor figure. This profession is still much debated and not yet recognized in all European countries. Thus, the aim of this research is to investigate both how a team should be composed in the care of patients affected by or at risk of genetic pathologies and what the role of the Genetic Counsellor should be – his field of action and competences.

Methods: A research at European level was carried out, submitting an online questionnaire, over a period of six months, to geneticists who, expressing their opinion, can identify strengths and potential areas for improvement in genetic care.

Results: 147 responses were collected from all over Europe, highlighting the importance of the counsellor's presence in the multidisciplinary team and what the counsellor's skills and qualifications should be. It was also possible to study differences between different parts of Europe.

Conclusion: Although this new profession has difficulties in recognition in some countries, it seems clear that these highly competent professionals are essential in-patient care. To the authors' knowledge this is one of the few – if not the only – study on the subject and we hope that it can paves the way to the recognition of the Genetic Counsellor profession.

References:.

Grants:.
Conflict of Interest: None declared.

P23.017.C Mainstreaming genomic testing for children with undiagnosed inborn errors of immunity

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Background/Objectives: Genetic diagnosis of paediatric of inborn errors of immunity (IEI) influences management decisions, and can alter clinical outcomes through hematopoietic stem cell transplantation (HSCT).[1,2] Historically, children with undiagnosed IEI in Queensland, Australia were referred to a state-wide clinical genetic service, delaying access to genomic testing and increasing the burden on the genetic clinic. To address this issue, we developed and evaluated a mainstreamed model of care for genomic testing for paediatric IEI.

Methods: This state-wide program included a genetic counsellor embedded within the paediatric immunology service, fortnightly multidisciplinary team meetings (MDT), and variant prioritisation meetings. Informed by the literature, 21 different virtual gene lists were developed for whole-exome sequencing. Additionally, parents completed pre-and post-testing survey assessing understanding of, and impact of genomic testing. Recruitment occurred between Nov 2020-Sep 2021.

Results: Of the 34 children with results, nine received a genetic diagnosis, and four accessed HSCT. Five children were also referred for further investigations of suspicious VUS. On average, 14 healthcare providers attended the state-wide MDT, including adult and paediatric immunologists, genomic pathologists, genetic healthcare professionals, and other non-genetic-healthcare providers. Parents demonstrated understanding of the implications of testing and reported minimal decisional regret.

Conclusion: Genomic testing can be mainstreamed for paediatric IEI. Our program improved access to genomic testing, facilitated treatment decision-making, and was acceptable to parents and clinicians alike.

References: [1.]Tangye SG, et al. J Clin Immunol. 2020;40(1):24-64; [2.]Bousfiha A, et al. J Clin Immunol. 2020;40(1):66-81.

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Conflict of Interest: None declared.

P23.018.D Models of communication for polygenic scores and associated behavioural and psychological outcomes: a systematic review

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Background/Objectives: Polygenic scores (PGS) are rapidly moving into clinical care (1). However, no guidelines exist for communicating PGS effectively, and psycho-behavioural outcomes are debated (1). We aimed to systematically review current models for communicating PGS and psycho-behavioural outcomes of receiving PGS.

Methods: Original research, communicating PGS and reporting on psycho-behavioural outcomes, was included. Search terms were applied to five databases and limited by date (2009-2021).

Results: Of 3215 articles, 28 articles, representing 17 unique studies were identified. Studies provided PGS in various settings (cancer, cardiac, and others) to inform screening and intervention. There was limited consistency in PGS communication, evaluation of psycho-behavioural outcomes, and outcomes reported. Most studies ($n = 13$) presented both numeric and visual representations of risk, and only three provided personalised lifestyle advice or additional resources. No studies used behaviour change theories to inform PGS delivery. Eight studies found no evidence of long-term negative psychological impacts, up to 12 months post-result. Of 16 studies reporting on behaviour, 10 found at least one preventative health behaviour change associated with receipt of PGS including screening ($n = 2$), lifestyle changes ($n = 8$), and medication use ($n = 2$), especially for high PGS. Low PGS was not associated with uptake of harmful health behaviours.

Conclusion: PGS has potential to personalise health interventions. High variability among studies emphasises the need for developing standardised guidelines for communicating PGS and evaluating outcomes. Our findings call for the development of best communication practices and high-quality interventions for PGS, informed by behaviour change theories.

References: (1) Yanes T, et al. Hum Mol Genet. 2020;29(R2):R165-R176.

Grants: TY funded by NHMRC(APP1194646).

Conflict of Interest: Courtney Wallingford: None declared, Hannah Kovilpillai: None declared, Chris Jacobs: None declared, Erin Turbitt: None declared, Clare Primiero: None declared, Deanna Brockman Full time employment at Color - a company in the United States that is returning PRS information to clients, Hans Peter Soyer HPS is a Medical Consultant for Canfield Scientific Inc, MoleMap Australia Pty Ltd, Blaze Bioscience Inc, Revenio Research Oy and a Medical Advisor for First Derm., HPS is a shareholder of MoleMap NZ Limited and e-derm consult GmbH, and undertakes regular teledermatological reporting for both companies., Aideen McInerney-Leo: None declared, Tatiane Yanes: None declared.

P23.019.A What does a genetic counsellor do in the university hospital of Liege?

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Background/Objectives: Lately, the University Hospital of Liège has faced a growing demand for genetic consultations. In addition to increasing the number of clinical geneticists, the University Hospital opened a first position of Genetic Counsellor (GC) in 2013, followed by a second one in 2017 and a third one in 2020. The GC profession, although recognized in many countries around the world, is not official yet in Belgium. Since 2015, a national working group has been set up and a procedure is underway to create an MSc in genetic counselling. The aim of this study was to characterize and evaluate the activities of GCs in our hospital.

Methods: We performed a retrospective observational study of the patients from the University Hospital of Liège who had consulted with a GC between 2016 and 2021. We also analyzed the other interventions/tasks of the GCs. We performed a descriptive statistical analysis.

Results: The main task of the GCs is to receive patients in consultation either in pair with a geneticist or in "genetic counseling" consultation under the supervision of a geneticist. Other tasks are mainly administrative. The number of consultations has increased by 707.5% between 2016 and 2021 and the number of administrative interventions has also increased by 5740% between 2013 and 2020.

Conclusion: The number of specific "genetic counseling" consultations and the administrative interventions have increased significantly between 2016 and 2021. The tasks of the GCs are varied and essential for the overall care of the patient, in collaboration with the rest of the (para)medical team.

References:.

Grants:.

Conflict of Interest: None declared.

P23.020.B Awareness of prostate cancer risk associated with deleterious mutations in DNA damage repair genes: a "reverse" gender gap?

*Monica Zuradelli[1;2], Carla Barbara Ripamonti[2], Monica Barile[2], Ilaria De Simone[1;2], Vittorio Fasulo[1;2], Giuseppe Chiarelli[2], Rodolfo Hurle[2], Alberto Saita[2], Giovanni Lughezzani[1;2], Nicolò Maria Buffi[1;2], Giorgio Guazzoni[1;2], Paolo Casale[2], Armando Santoro[1;2], Rosanna Asselta[1;2], Massimo Lazzeri[2], **Giulia Solda'**[1;2]*

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Background/Objectives: Germline pathogenic mutations in DNA damage repair genes (DDRG) are found in 5% of localized and up to 16% of metastatic Prostate Cancer (PCa) patients. BRCA1/BRCA2 are the most frequently mutated and associate with aggressive disease and poorer outcome. Despite available European guidelines for BRCA/DDRG testing, marked country-to-country differences exist in implementation in clinical practice, potentially excluding a not negligible number of PCa patients from genetic analysis. Here we investigate existing gaps in early diagnosis for PCa patients with familial history of DDRG mutations.

Methods: BRCA1/2+ families were selected from women with breast/ovarian cancer who attended our Genetic Counselling Clinic from January 2016 to May 2021. All healthy men, aged 35-75, were proposed genetic counselling and testing, and subsequent dedicated "enhanced" screening based on digital rectal examination, PSA/Prostate Health Index (PHI) blood test, and multiparametric MRI plus biopsy in suspected cases.

Results: In more than half of 139 families (of >1000 retrospectively analyzed) positive for DDRG mutations, the male member(s) rejected to be tested. In 28 families (20%) at least one male relative was interested in DDRG screening. Overall, 86 men performed genetic analysis, of which 35 tested positive and started the annual "enhanced" screening, together with additional 20 DDRG carriers recruited from other hospitals. About one third (16/55) had a PHI ≥20 and underwent MRI; one patient required biopsy (negative) based on imaging results.

Conclusion: Surprisingly, awareness in the male population about the risk for PCa in DDRG mutation carriers, and possible implications for early diagnosis/therapy, seems low.

References:.

Grants:.

Conflict of Interest: None declared.

P23.021.C GeNotes: A pan-specialty, pan-profession "just in time" genomic education tool for clinicians

*Amy Frost[1], Aine Kelly[1], **Michelle Bishop**[1;2], Kate Tatton-Brown[1;3]*

[1]Health Education England, Genomic Education Programme, London, United Kingdom; [2]Wellcome Genome Campus, Wellcome Connecting Science, Cambridge, United Kingdom; [3]St George's University Hospitals NHS Foundation Trust, Medical Genetics, London, United Kingdom.

Background/Objectives: Genomic medicine is at the vanguard of healthcare but many healthcare professionals lack the knowledge and skills to integrate genomic data into their clinical practice. It is therefore imperative that we upskill this workforce at scale and pace.

Methods: Health Education England's Genomic Education Programme (GEP) has developed a flagship, two-tier "just in time" online educational tool to meet the genomic educational needs of the healthcare workforce. Tier 1 ('In the Clinic', requesting tests and receiving results) resources are specialty-specific, succinct, written to a strict template and aligned to England's Genomic Test Directory. 'In the Clinic' links to tier 2 ('Knowledge Hub'). The pan-specialty 'Knowledge Hub' offers extended genomic learning opportunities from 'In the Clinic' and comprises numerous individual resources (each representing 15-20 minutes of learning) that can be accessed standalone or assembled to create bespoke learning journeys (which can be aligned to curricula/training needs).

Following discovery and alpha phase testing, GeNotes has recently completed a private beta phase qualitative (n = 11) and quantitative (n = 21) evaluation.

Results: GeNotes received excellent feedback across the four tested domains: expected use, ease of access, navigation, and resource content. It scored 90/100 in system usability scale (SUS) testing (compared with an average score for digital services of 68)[1].

Conclusion: GeNotes fulfils a previously unmet genomic educational need and will launch for oncology (as public beta) in the first quarter of 2022. It will be available for paediatrics soon after and for all working group specialties by summer 2022.

References: [1]Lewis et al, International Journal of Human-Computer Interaction, 2018.

Grants:.

Conflict of Interest: Amy Frost: None declared, Aine Kelly: None declared, Michelle Bishop: None declared, Kate Tatton-Brown KTB is a principal investigator of a research study to investigate new mechanisms of the overgrowth-intellectual disability syndromes. She is in receipt of two grants, KTB has many times been asked to speak at conferences and received honoraria for these. This is not applicable for this conference.

P23.022.D Assessing risk in genetics services: developing and piloting a clinical genetics-specific risk assessment tool

***Deborah Lambert**[1], Alana J Ward[1], Deborah Behan[2], Dearbhla Butterly[1], sally ann Lynch[1;3]*

[1]University College Dublin, School of Medicine, Dublin, Ireland; [2]Children's Health Ireland, Quality, Safety and Risk Management, Dublin, Ireland; [3]Children's Health Ireland - Crumlin, Department of Clinical Genetics, Dublin, Ireland.

Background/Objectives: Whilst using the national hospital assessment process to evaluate risks arising from long waiting lists in Clinical Genetics, it became apparent that adaptation was required for out-patient clinical genetic use.

Methods: The ISO3001-based Irish Health Service Executive's risk assessment matrix[1] was reviewed and the domains of risk impact adapted. Genetics context-specific criteria were established for impact severity scores of negligible(1) to extreme(5) across domains. The new matrix was piloted to chronicle consequences to 20 women who became pregnant while awaiting appointments to discuss diagnosis/recurrence risk because of family history or previous affected child.

Results: 10 domains of risk impact were identified: Harm to the patient and family; Harm to staff; Service user experience, Compliance with professional guidance and SOPs; National strategic objectives, Service disruption; Adverse publicity; Financial cost to hospital; Financial cost to patient; Environmental impact. Clinic waiting times in pilot charts (n = 20) ranged from 2-35 months; 4/20 had a recurrence of the condition in subsequent child while on the waiting list. All charts showed risks in multiple domains (range 1-10), with 17/20 rated moderate (3) to extreme (5) in patient/pregnancy harm.

Conclusion: The risk tool was usable by both clinical genetics and non-genetic personnel. Domains of impact for patient harm, service disruption, and financial cost to hospital varied independently between situations. Use of the new tool standardises risk assessment and clarifies areas where control measures could be implemented to improve care.

References: 1. Health Service Executive Integrated Risk Management Policy 2017 https://bit.ly/3J4mNoh.

Grants: Adelaide Health Foundation R22808.

Conflict of Interest: None declared.

P23.023.A An online genetic counselling narrative group as a tool for supporting patients with hereditary ataxias in Portugal

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Background/Objectives: Genetic counselling interventions aimed to provide psychosocial support to individuals at-risk or with hereditary ataxias are very scarce. No interventions of this kind have been reported in Portugal so far. We aimed at implementing a service improvement intervention offering a group intervention of a structured narrative exercise (already implemented with people who tested gene-positive for Huntington disease).

Methods: Tree of life intervention was delivered remotely. In parallel, it was conducted a study to explore, respectively, i) the experiences of participants in the narrative intervention, and ii) to assess the impact of the intervention in their psychological well-being. Data collection involved observations and a post-intervention focus group.

Results: The Portuguese Hereditary Ataxias Association advertised the narrative group and 13 individuals stated their interest in participating. Of these, 9 people took part in preliminary session aimed to present the goals and nature of the intervention. Six of those participants (mean age 48; 39-53, 3 men) attended the narrative session (2,5 hours), with different types of ataxia (mostly Machado-Joseph Disease). A focus group took place 2 weeks after the narrative session aiming to collect the participants' views and experiences with the intervention. Participants reported high levels of satisfaction as they had the opportunity to build new social relations that brought them different perspectives, and gained new insights to understand their biopsychosocial circumstances.

Conclusion: This intervention has potential to be extended and adapted for other types of genetic conditions and populations, and may be used as follow-up support in genetic counselling protocols.

References:.

Grants: AM holds FCT-CEECIND/02615/2017 grant.

Conflict of Interest: Milena Paneque: None declared, Mariana Policarpo: None declared, Maria Barbosa: None declared, Marina Lemos: None declared, Álvaro Mendes FCT-CEECIND/02615/2017.

P23.024.B "I got this letter" At-risk individuals experience of healthcare-assisted disclosure on hereditary cancer

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Background/Objectives: When a patient has hereditary cancer he/she is recommended to disclose information to healthy at-risk relatives (ARRs), who can then themselves ask for assessment, eventually access surveillance and even risk-reducing surgery. The Swedish multicenter randomized controlled trial DIRECT evaluates weather information letters directly from healthcare may increase the proportion of ARRs contacting an oncogenetic unit. This study explores how healthy ARRs perceive receiving such a healthcare-mediated letter.

Methods: Patients with hereditary cancer included in the DIRECT study identified ARRs to whom we sent letters with an invitation to genetic counselling. ARRs who contacted an oncogenetic unit was invited to qualitative in-depths interviews. The interviews (n = 13) were recorded, transcribed, coded, categorized and analyzed inductively.

Results: The ARRs receiving a letter thought of it as tolerable, however when contacting the oncogenetic clinic, they wanted prompt accessibility and familiarity with the matter. They expressed ambivalence around their right to know, empowerment regarding one's own health, versus the right not to know and true benefits of surveillance. They wanted a shared responsibility between the healthcare system and patients; neither an authoritarian praxis of disclosure nor that an individual could keep the information solely to himself.

Conclusion: Healthcare -assisted disclosure of hereditary risk for cancer by sending a letter to ARRs is a tolerable method. However, the corresponding clinic has to be highly accessible for re-contact and the ARRs expressed ambivalence regarding benefits and disadvantages from being contacted.

References:.

Grants: Swedish Research Council for Health, Work life and Welfare grant 2018-00964.

Conflict of Interest: None declared.

P23.025.C The emerging role of the European Society of Human Genetics-Young Committee

Elena Avram[1;2], *Juliana Xavier de Miranda Cerqueira*[2;3;4], *Can Ding*[2;5], *Mridul Johari*[2;6;7], *Ana Raquel Gouveia Freitas da Silva*[2;8], *Ileana Delia Sabau*[2;9], *Nuru Noor*[2;10], *European Society of Human Genetics - Young Committee*[2]

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Background/Objectives: The purpose of the European Society of Human Genetics - Young Committee (ESHG-Y) is to support the next generation of geneticists. Under the guidance of the ESHG, our committee aims to develop strategies and programs for a better education of human genetics and the creation of a strong network of geneticists in all European countries. We are focused on: organizing scientific events, achieving equal access to educational opportunities and promoting collaboration and leadership. We present all the projects that the ESHG-Y has been involved in since its conception in 2019.

Methods: We assessed all the ESHG-Y projects between 2019 and 2022. The role of the ESHG-Y was categorized as decisive, coordinating or supportive.

Results: Since 2020 ESHG-Y has created its own educational sessions at the ESHG Annual Conference. The ESHG-Y social media platforms have regularly promoted relevant activities like a virtual session in collaboration with the European Board of Medical Genetics (EBMG). A scientific event in which we had a coordinating role is the European Dysmorphology Meetings. In the last 4 years our representatives have had a supportive role in the ESHG Board, the ESHG Scientific Programme Committee, the ESHG Education Committee and the EBMG. We have also supported organizations like: ERN-Ithaca, Unique, Orphanet, EuroGEMS and MOOC BIG.

Conclusion: In the short span of almost 4 years the ESHG-Y has successfully implemented projects that have engaged young geneticists across Europe. For the near future, we will be seeking to build on and develop further activities such as the "ESHG Observership for Young Geneticists".

References:.

Grants:.

Conflict of Interest: None declared.

P23.026.D Good enough embryo: Prioritization of Preimplantation genetic testing (PGT) embryos in the era of genomic medicine

Shachar Zuckerman[1], *Orit Freireich*[1], *rahel beeri*[1], *Pinhas Renbaum*[1], *elinor Hakam*[1], *shira Shaviv*[1], *elina farhi*[1], *anat yadin*[1], *meirav ben shlomo*[1], *tali azar*[1], *gheona Altarescu*[1]

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Background/Objectives: New technologies of expanded carrier screening (ECS), chromosomal microarrays (CMA) and whole-exome sequencing (WES) produce extensive information regarding variants' carrier status, complicating the decisions of couples and professionals for testing and prioritizing embryos in PGT.

Methods: We describe the challenges of transferring embryos for multiple conditions:.

Results: A woman carrier of two X-linked disorders; her spouse carries AD PAX9-hypodontia; both carry AR deletion. Couple decided to transfer an embryo with PAX9-hypodontia and freeze female embryos carriers of the X-linked disorders.

- Couple carriers of Canavan and each parent a different VUS microduplication, detected by CMA in amniocentesis. They opted of transferring only non-carrier embryo for the three conditions.
- Woman carrier of GREB1L mutation, detected after WES done for a fetus with Potter, was found out to be a carrier of ACAN mutation causing short stature. Couple decided to transfer WT GREB1L but ACAN mutant embryo.
- Woman with Laing myopathy caused by AD MYH7 mutation; both partners detected by ECS as carriers of congenital adrenal hyperplasia (CAH). Linkage for paternal allele was impossible since both his parents were carriers. The couple opted to transfer WT of MYH7 and carrier of maternal CAH mutation without knowing the status of paternal allele.

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Conclusion: These cases demonstrate the challenges couples and professionals are faced with multiple genetic variants when unaffected embryos for all the tested genetic diseases are unavailable. Appropriate genetic counseling discussing medical and bioethical consideration and guidelines for PGT prioritization is crucial before decisions are being made regarding tests and embryos' selection.

References:.

Grants:.

Conflict of Interest: None declared.

P23.027.A Rare disease families' experience of accessing genetic testing and clinical genetics services in the Republic of Ireland

Alana J Ward[1], *Deborah Lambert*[2], *Vicky McGrath*[3], *James O'Byrne*[2;4;5], *Dearbhla Butterly*[2], ***sally ann Lynch***[1;6]

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Background/Objectives: National waiting times for Clinical Genetics approach three years. We aimed to identify consequences to families accessing services.

Methods: An online survey was distributed via Rare Diseases Ireland patient organisation alliance social media.

Results: Of 144 respondents 90% (129/144) were people with a rare disease and 10% (15/144) relatives. 95% (128/135) had genetic testing, 70% (88/125) leading to a diagnosis. Those where a diagnosis was made by the Clinical Genetics appointment reported higher levels of satisfaction ($z = 3.94$; $p < 0.01$).

77% (99/128) of participants had their genetic test arranged by a non-Genetic healthcare professional. The 24% (31/111) who had their genetic test result explained by a different physician/team than the one who arranged the test were more likely to report dissatisfaction ($z = 3.07$; $p < 0.01$). 55% (70/127) of respondents waited >3 months for genetic test results.

81% (80/99) of respondents accessed public appointments and 18% (18/99) private. 82% (93/114) of respondents indicated that being on the waiting list impacted their personal life and plans.

32% (37/114) of respondents said waiting for a Clinical Genetics appointment placed tension on relationships with partner/family members/friends. 23% (26/114) indicated wider impact on relatives' family planning/relationships/education/employment plans. 25% (28/114) delayed future pregnancies and 15% (17/114) expressed impact on decisions around education/employment/mortgage.

Conclusion: Longer waiting times to access genetic services have significant impact on patients' and carers' personal lives and plans. This highlights that current national waiting times increase the disadvantage already experienced by families with rare diseases.

References:.

Grants: Adelaide Health Foundation R22808.

Conflict of Interest: None declared.

P23.028.B Experiences and preferences of Healthcare Professionals using telegenetics in a UK Clinical Genetics centre during the COVID-19 pandemic

Beatriz Bustillo[1;2], *stephanie oates*[1], *kathryn lubasch*[1], *charlotte tomlinson*[1], *vishakha tripathi*[1], *sarah rose*[1]

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Background/Objectives: The changes and restrictions precipitated by the COVID-19 pandemic have led to innovation in Clinical Genetics service delivery worldwide. At the Guy's and St Thomas' (GSTT) Clinical Genetics Service, telegenetics was implemented at the beginning of the pandemic using the AttendAnywhere videoconferencing platform. We subsequently designed a qualitative study to capture experiences and preferences of Healthcare Professional's (HCP) using this service delivery model.

Methods: We conducted semi-structured interviews with seven HCPs working at the GSTT Clinical Genetics Service, including Genetic Counsellors, Clinical Geneticists and a Clinical Psychologist. Interview content was analysed using a thematic analysis approach.

Results: We present HCPs' experiences of transitioning between virtual and in-person appointments and their appraisal of the technical and practical aspects of telegenetics. We also present themes that emerged about how HCPs' clinical practice has changed to adapt to telegenetics, as well as differences in both patients' and HCPs' attitudes towards virtual appointments when compared to in-person encounters. Future considerations will be shared regarding the suitability of telegenetics for Clinical Genetics appointments.

Conclusion: Based on their experience at GSTT, HCPs interviewed would welcome the addition of telegenetics to the Clinical Genetics toolkit beyond the COVID-19 pandemic, and we will provide considerations for future guidelines.

References:.

Grants: This work was conducted as part of the UK NHS Scientist Training Programme.

Conflict of Interest: None declared.

P23.029.C Implementation of newborn screening for spinal muscular atrophy in Poland – one year experience

Monika Gos[1], *Magdalena Frączyk*[1], *Joanna Wasiluk*[2], *Aleksandra Landowska*[1], *Mariola Jurzyk*[2], *Katarzyna Durda*[3], *Natalia Szczerba*[3], *Wioletta Wawer*[2], *Paulina Kubiszyn*[2], *Maria Jędrzejowska*[4], *Mariusz Ołtarzewski*[2]

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Background/Objectives: Spinal muscular atrophy (SMA) is a severe genetic condition that leads to significant motor impairment and respiratory problems, or even death. A personalized treatment with Nusinersen, ZolgenSMA or Risdiplam is available for patients. Herein, we present a schema and our first experience with the newborn screening for SMA (NBS-SMA).

Methods: The SMA was added to the National Newborn Screening Programme in April 2021. Standard dried blood spots are used for DNA extraction for a genetic test that allows to identify homozygous deletion of exon 7 of the *SMN1* gene. The SALSA MC002 SMA Newborn Screen test is used as a screening method. All legal guardians has to sign an agreement for genetic testing.

Results: Since April 2021, the SMA-NBS was implemented subsequently in 13 districts in Poland. All likely positive samples were checked with alternative method (PCR-RFLP or MLPA) and information about confirmed cases was sent to the hospital responsible for SMA treatment. During the visit, clinical examination was performed and a blood was collected for a MLPA verification test. If the result of the verification test was positive, the personalized treatment was applied. Till now, about 140.000 newborns were screened and SMA was diagnosed in 21 (prevalence: 1/6700). The median time since birth till first positive result and verification result were 9 and 14.5 days, respectively.

Conclusion: The PCR-HRM method is useful in NBS-SMA and allows successfully to identify positive patients that can be treated with available therapies.

References:.

Grants: The project was partially supported from Institute of Mother and Child intramural grant 510-18-17.

Conflict of Interest: Monika Gos Modest, Magdalena Frączyk: None declared, Joanna Wasiluk: None declared, Aleksandra Landowska: None declared, Mariola Jurzyk: None declared, Katarzyna Durda: None declared, Natalia Szczerba: None declared, Wioletta Wawer: None declared, Paulina Kubiszyn: None declared, Maria Jędrzejowska Modest, Mariusz Ołtarzewski: None declared.

P23.030.D Clinical description of families with Li-Fraumeni syndrome in a third level hospital in Barcelona

Lorena Moreno Calle[1], *Belen Pastor Piñera*[1], *Elia Grau Garces*[1], *Aurora Sánchez Díaz*[2], *Miriam Potrony*[2], *Josep Oriola*[2], *Maria Isabel Alvarez*[2], *Celia Badenas*[2], *Lydia Gaba*[3], *Adela Rodriguez*[3], *Barbara Adamo*[3], *Aleix Prat*[3], *Francesc Balaguer*[1]

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Background/Objectives: Li-Fraumeni Syndrome (LFS) is a rare cancer predisposition syndrome that increases multiple cancers risk. Recent studies suggest an overestimation in LFS penetrance due to identification of patients with *TP53* pathogenic variants without fulfilling clinical criteria. It is imperative to understand risk-modifying factors, genotype-phenotype correlation and clinical effect of variant type to offer a correct follow-up and genetic counselling.

The study objective is to describe clinical and genetic LFS families' characteristics and assess clinical criteria compliance.

Methods: It is a descriptive, observational and retrospective study of genetically diagnosed LFS families between 2014-2021. Clinical manifestations, variant type and accomplishment of clinical criteria are described.

Results: Case	Cancer(s) proband, diagnosis age	Study– clinical criteria	Genetic diagnosis age	Pathogenic variant	Variant type
1	Osteosarcoma,9	LFS–classic	9	c.637C>T	LF
2	Stomach,34	HDGC	35	c.6365_366delTG	LF

Results: Case	Cancer(s) proband, diagnosis age	Study– clinical criteria	Genetic diagnosis age	Pathogenic variant	Variant type
3	Breast,34	HBOCS	45	c.754_762delCTCACCAT	LF/ND
4	Breast,32	LFS–Chompret	33	c.733G>A	LF/ND
5	Breast,30 & 50 Sarcoma,44 & 50	LFS–like	51	Gene deletion	LF
6	Breast,47	HBOCS	49	c.472C>T	LF/ND

NG: negative dominance, LF: loss of function.

Conclusion: Our results agree with latest research that reconsiders LFS penetrance and expressivity. It is not always possible to identify the variant type and, therefore, to use this data to personalize follow-up. Although our data is limited, it reveals the high phenotypic variability and the need to manage LFS families within a multidisciplinary team, as well as to rethink the LFS study criteria and the follow-up offered. It would be interesting to carry out multicenter studies to obtain more robust conclusions.

References:.

Grants:.

Conflict of Interest: None declared.

P23.031.A Observational study on lifestyle behaviors and nutritions in individuals undergoing genetic counselling for breast or colorectal cancer risk

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Background/Objectives: Healthy lifestyles are associated with cancer risk reduction. We aimed at exploring modifiable lifestyle behaviours among individuals at increased risk of breast/colorectal cancer.

Methods: We carried out a cross-sectional study of patients undergoing breast or colorectal cancer risk assessment.

A questionnaire was administered, including: Italian validated MEDI-Lite and International Physical Activity questionnaires, items on socio-demographic and physical features and questions on risk perception and smoke and alcohol habits.

Results: Fourty-two women and 8 men (age: 19-80 years) were recruited; 19 were affected by breast cancer, 4 by colorectal cancer, 27 were unaffected but had a family history of breast (23) or colorectal (4) cancer.

Fourty-four respondents (88%) perceived their general lifestyle as very or enough healthy. However, 16 (32%) were smokers (current or former), 37 (74%) drank alcohol and 18 (36%) were overweight or obese; 21 (42%) showed low adherence to mediterranean diet and 7 (14%) reported low exercise. Low adherence to mediterranean diet was significantly more frequent in participants of low education level (67%) compared to those with high education level (17%; $p = 0.044$). Participants with higher cancer risk were significantly more likely to smoke: 63% of those at high risk, 40% of those at intermediate risk and 14% of those at standard risk were current or former smokers ($p < 0.05$).

Conclusion: The results of this pilot study support a need for raising awareness on the role of the lifestyle in cancer risk modulation among counselees, possibly tailoring information according to their literacy.

References:.

Grants: None.

Conflict of Interest: None declared.

P23.032.B Non-genetic health professionals' knowledge, attitudes, and behaviors towards medical genetics in Croatia

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Background/Objectives: The main objective of this study was to examine the knowledge, behavior, and attitudes toward medical genetics among physicians who encounter the largest number of patients with specific genetic disorders, in their daily practice.

Methods: The cross-sectional study involved 175 residents and specialists in the Republic of Croatia, who completed the online questionnaire anonymously and voluntarily. The validated questionnaire consisted of five groups of questions: general information, knowledge, behavior in practice, attitude toward genetic testing, and additional education in medical genetics.

Results: The median score of total knowledge in medical genetics was 70.2% among obstetrician-gynecologists, 80.5% among pediatricians, and 76.7% among neurologists. A statistically significant difference was found between the groups, with obstetrician-gynecologists having higher total knowledge than the other physicians ($P < 0.001$). Many patients with genetic disorders remain undiagnosed in respondent's daily practice and not recognizing such patients is the main reason why patients are not referred for genetic testing often enough. In addition, the respondents showed a positive attitude toward genetic testing, but they do not feel educated enough to interpret the results of genetic testing.

Conclusion: The results obtained highlight the need for further education of non-genetic health professionals in medical genetics, which would lead to greater confidence and ability of physicians to recognize patients with genetic disorder, choose the right genetic testing method and to clearly convey information.

References:.

Čargonja et al. The impact of needs-based education on the change of knowledge and attitudes towards medical genetics in medical students. *Eur J Hum Genet*. 2021;29(5):726-735.

Grants:.

Conflict of Interest: None declared.

P23.033.C Art & genomics: teaching next generation scientists how to connect science and society by using art & design

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Background/Objectives: The potential large societal impact of developments in the field of genomics asks for a new generation of scientists who are not afraid to step out of their ivory tower and into society. For most scientists (in training) this is a step out of their comfort zone, requiring new skills and tools. The course *Art & Genomics* (A&G) was developed by two PhD students and two designers. It aims to provide students with the skills and tools that help them connect (with) a broad public, in dialogue about the societal impact of genomic science, on an emotional and experiential level.

Methods: Students go through the non-linear process of design thinking. Although this process asks for continuous iteration, the following successive steps can be distinguished: empathetic understanding, defining the problem, coming up with creative ideas, developing prototypes, and testing. At the end of this process, students showcase their dialogue-provoking art/design piece at an exhibition.

Results: Six students from the minor *Genetics in Society* participated in a pilot. They indicated that A&G required them to get out of their comfort zone at first, but then gave them the tools they needed to spark broad public dialogue about the societal impact of scientific research. Exhibited art/design pieces included a new fashion clothing line with your personal DNA printed on it, and a game revealing who has your genetic data.

Conclusion: Using art/design methods can build academic students' competency and confidence to connect science and society.

References:.

Grants: Impact at the Core.

Conflict of Interest: Diewertje Houtman Part-time employment at Erasmus MC, Educational support and educational materials by Impact at the Core, a program of the Erasmus University stimulating impact-driven education., Mirte de Wit Educational and financial support and educational materials from impact at the core, a program of the Erasmus University stimulating impact-driven education., Sam Riedijk Fulltime employment at Erasmus MC, PI in prenatal genetics and social genomics, Bertrand Burgers Educational and financial support and educational materials from impact at the core, a program of the Erasmus University stimulating impact-driven education., Boy Vijlbrief Fulltime employment at Erasmus MC, Educational support and educational materials by Impact at the Core, a program of the Erasmus University stimulating impact-driven education.

P23.034.D Worldwide use of EuroGEMS.org, the ESHG's guide to online educational resources, and its new full Spanish translation

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Background/Objectives: Access for individuals at all educational levels to reliable online educational genomics-related resources has been increasingly required, worldwide. A website (www.EuroGEMS.org) (1) was therefore created for the European Society of Human Genetics (ESHG), providing a user-friendly English-language guide to 110 high-quality online worldwide resources for genetics specialists, non-genetics-professionals, students and the public (2). Subsequently, its translation into other languages has been suggested by the ESHG. As Spanish is the first language of approximately 480 million people worldwide, Spanish translation was initiated (3).

Methods: EuroGEMS.org was fully translated (and carefully cross-checked) by two bilingual genetics professionals. The resulting Spanish web-pages were launched in June 2021.

Results: The www.EuroGEMS.org website has been visited from 128 countries. It has been endorsed by the ESHG Board and, in 2022, by the Human Genome Organisation (HUGO-International). The new Spanish-language pages have been accessed from 28 countries (including 12 in South and Central America) with a marked (6.3-fold) increase in the number of Spanish speakers using EuroGEMS (from January 2021 to January 2022).

Conclusion: EuroGEMS.org is used worldwide and has been endorsed by two major international societies (the ESHG and HUGO-International). Its new Spanish-language pages have greatly increased its accessibility and readership, particularly in Spain and South America but also elsewhere (including the USA). A Portuguese translation, now underway, should further increase genetics and genomics knowledge acquisition, internationally.

References: 1. https://www.eurogems.org/index.html.
2. Tobias AP and Tobias ES (December 2020). Human Mutation 41: 2021-2027. PMID: 32906220.
3. Tobias ES et al. (September 2021) Frontiers in Genetics 12: 693952. PMID: 34539735.

Grants:.

Conflict of Interest: None declared.

P23.035.A Community-driven co-creation of an educational genetics app

***Ang Davies**[1], Naz Khan[2], Alan Davies[1], Norina Gasteiger[3], Amy Vercell[4], Dawn Dowding[3]*

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Background/Objectives: Previous studies have found lack of access to culturally sensitive genetic information creates an unmet genetics-education need within Pakistani communities in the UK, where incidence of consanguineous marriage is high, increasing risk of rare genetic diseases. Mobile apps may help to raise genetic literacy and signposting to clinical services in a sensitive, engaging and accessible manner.

Methods: A mixed methods study was undertaken to co-create a genetics app with the South Asian Pakistani community (Blackburn with Darwen, UK). Including: semi-structured interviews with community members (7), exploring understanding of genetics; systematic review of current genetics apps using the Mobile App Rating Scale (MARS); and systematic literature review of genetics apps.

Results: During the interview phase participants indicated use of the potential app either alone or with family members to educate themselves/others and make informed choices. Other preferences included: privacy, lack of connection to genetics in the app logo and requirement for high quality/trusted branding (e.g. NHS logo). Participants indicated a strong preference for video content of both patients and genetics health-professionals from the same ethnicity as themselves. During the app review phase, 22 apps met eligibility criteria, all intended to inform/educate users, 32% analysed genetic data and 18% helped to diagnose genetic conditions, though readability scores were poor.

Conclusion: Community-driven co-creation allowed the collection of users' requirements and prioritisation of features,

creating a prototype genetics app. The community were integral to the design process, working together with researchers, app developers and health-professionals.

References:.

Grants: Funding from Blackburn with Darwen Council.

Conflict of Interest: Ang Davies University of Manchester, principal investigator, Naz Khan full-time, collaborator, Alan Davies full-time, collaborator, Norina Gasteiger collaborator, Amy Vercell full-time, collaborator, Dawn Dowding full-time, collaborator.

P23.036.B Development of the CME training course in clinical laboratory genetics in Georgia

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Background/Objectives: A number of genetic diagnostic laboratories have been established in Georgia in recent years, offering different types of genetic tests including diagnostic testing for monogenic and common complex diseases, predisposition testing for cancer causing genes, and reproductive genetic testing [1]. The growing availability of genetic tests in Georgia raises the question regarding a shortage of laboratory geneticists and technicians. The first CME course in Laboratory Genetics has been developed at the Tbilisi State Medical University for healthcare professionals.

Methods: The aim of this postgraduate-level course entitled "Molecular genetic testing in a clinical practice" is to provide laboratory training in the field of molecular genetics for MDs. The course has been approved by the Ministry of Education and Science of Georgia for 15 CME category I credits.

Results: The course format is hands-on laboratory exercises including, but not limited to, molecular diagnostic techniques with standard and real-time quantitative PCR and application of these techniques in various areas of molecular genetic testing. A typical class module begins with a lecture on a specific laboratory topic (e.g. DNA chemistry, gene expression, PCR methodology, 1 hour), followed by lab exercises to demonstrate principles discussed in the lecture (2 hours). The course ends with a written multiple choice exam and post-course evaluation survey.

Conclusion: We believe that this course will help healthcare professionals to understand genetic technologies, and their application to clinical practice.

References: 1. Kvaratskhelia, E., Chokoshvili, D., Kvintradze, M. et al. Public attitudes towards the genetic testing in Georgia. J Community Genet 12, 407–414 (2021).

Grants:.

Conflict of Interest: None declared.

P23.037.C The impact of psychiatric disorders on caregivers: An integrative predictive model of burden, stigma and well-being

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Background/Objectives: Looking after a family member diagnosed with a psychiatric condition, especially for those who become main caregivers, is a complex task as it involves providing dedicated and often challenging assistance and support. Caregivers are exposed to high levels of stress and many report feelings of social isolation, stigma, grief, shame, guilt, anger and helplessness. This study explores the main predictors of caregivers' experienced burden, stigma and well-being, when looking after family members diagnosed with a psychiatric disorder.

Methods: We ascertained participants who were the main caregivers of an adult or young person diagnosed with a psychiatric condition. This cross-sectional study included 168 caregivers, 98 of which were caregivers of adult patients and 70 of which were caregivers of children and adolescents with psychiatric disorders. Perceived burden, stigma, well-being, knowledge, illness perception, socio-demographic and medical variables were assessed.

Results: A number of correlates of burden, stigma and well-being have been identified and are being discussed. The integrative predictive model has shown that the caregiver's emotional representation of illness is the best predictor of burden, ($\beta = 0.38$, $p < 0.001$), stigma ($\beta = 0.53$, $p < 0.001$) and well-being ($\beta = -0.36$, $p < 0.001$).

Conclusion: Psychiatric conditions can impact both patients and their caregivers. Our findings show that the emotional representation of the psychiatric condition is the strongest predictor of caregivers' burden. Our results can enable health professionals to better tailor psychosocial interventions addressed to family members of individuals living with a psychiatric condition.

References: -.

Grants: -.

Conflict of Interest: None declared.

P23.040.B The communication chain: a qualitative analysis of patient-provider and patient-family communication in hereditary cancers

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Background/Objectives: In hereditary cancers, family-communication on genetics is crucial for family members' decision making about genetic risk assessment. Healthcare-providers have an important role in supporting family-communication but this is still limited and unclear. The study aims to explore how healthcare-providers address family-communication with mutation carriers and how this may affect mutation carriers' decision to communicate about genetic risk to family members.

Methods: In this qualitative study, data were collected with focus groups and interviews with 12 unaffected and 8 affected female carriers of hereditary breast and ovarian cancer (HBOC)-associated mutations. Data were collected in Italian, German and French in three linguistic areas of Switzerland.

Results: Data show an inconsistent management of family-communication before, during and after genetic counselling with the perception of an overall negligence of the health-system, a low level of healthcare-providers' involvement and the lack of continuity. Dynamic, contradictory, implicit logics such as responsibility, self-preservation, protection and respect of the

other, drive mutations carriers' decision to (and how) communicate or not, genetic risk to family members. Many individual and family factors influencing communication such as gender, geographical and affective distance, illness experience, interact systematically with the different logics.

Conclusion: The study innovatively frames family-communication proceeding along a communication chain from health-care providers to at-risk relatives. It highlights the need for supporting family-communication on genetic risk and potential providers' new roles.

References: Schwiter R. et al. (2018) How can we reach at risk relatives? Efforts to enhance communication and cascade testing uptake: a mini-review. Current Genetic Medicine Reports. 6:21-27.

Grants: Suisse Cancer League - KLS-4294-08-2017.

Conflict of Interest: None declared.

P24 ETHICAL, LEGAL AND PSYCHOSOCIAL ASPECTS IN GENETICS

P24.001.C Systematic reanalysis for genome diagnostic laboratories? ELSI/ethical, economical, legal and (psycho) social implications: Scoping review

Marije A. van der Geest[1], *Marielle E. van Gijn*[1], *Els Maeckelberghe*[2], *Anneke Lucassen*[3,4], *Morris Swertz*[1], *Mirjam Plantinga*[1], *Irene van Langen*[1]

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Background/Objectives: With the introduction of NGS techniques increasing numbers of disease-associated variants are being identified. This ongoing progress might lead to diagnoses in formerly undiagnosed patients and novel insights in already solved cases. Therefore, many studies suggest introducing systematic reanalysis of NGS data in routine diagnostics. Introduction will, however, also have ELSI implications that Genetic Health Professionals (GHPs) from laboratories should consider before possible implementation of systematic reanalysis.

Methods: To get a first impression we performed a scoping literature review. After searching PubMed for relevant literature between 2008 and 2021, we included 54 publications.

Results: Our findings show that for the vast majority of included articles ELSI aspects were not the main focus. However, some aspects were raised implicitly. In total, we identified nine ELSI aspects, such as (perceived) responsibilities, implications for consent and cost-effectiveness.

Conclusion: The identified ELSI aspects brought forward necessary trade-offs for GHPs to consciously take into account when considering implementation of systematic reanalysis of NGS data in routine diagnostics, balancing the various strains on their laboratories and personnel while creating optimal results for patients. Some important aspects are not well explored yet. For example, our study shows GHPs see the values of systematic reanalysis but also experience barriers, often mentioned as being practical and/or financial only, but in fact (also) being ethical and/or psychosocial. These results are in line with our previous study on recontacting (Otten et al., 2015). Engagement of these GHPs in further research on ELSI aspects is important for sustainable implementation.

References:.

Grants:.

Conflict of Interest: None declared.

P24.002.D The effect of Down syndrome on parents' daily life

Leïla Dardour[1], *Amira M'hamed*[1], *Amel Tej*[2], *Asma Ben cheikh*[3], *Hassen Kamoun*[4], *Mediha Trabelsi*[5], *Ramzi Zemni*[1]

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Background/Objectives: Down syndrome (DS) is the most common genetic cause of intellectual disability. Having a child with DS can be an unexpected event that challenges parents' ability to adapt. In Tunisia, there is no available data on the impact of DS on parents' daily life. The objective of this study is to evaluate the quality of life of these parents and determine the associated factors.

Methods: This is a cross-sectional analytical multicentric study (Medical Genetics Services of University hospitals of Tunis and Sfax, "Awladouna" Association, Tunisian Union of Assistance to Persons with Intellectual Disabilities in Sousse, and Pediatrics department of Farhat Hached hospital, Sousse, carried out between November 1 and December 31, 2018. The measurement instrument was a questionnaire: the Distress thermometer for parents.

Results: Of 131 participants, 74.8% were women. DS was diagnosed postnatally in 92% of cases, which shows the need to raise awareness among general population about prenatal screening methods. The feeling of distress was present in 57.3% of parents. Practical problems (especially professional and financial problems), emotional difficulties (depression, anxiety, and nervousness), and physical problems (fatigue, pain, and sleep disturbance) were all statistically associated with the overall distress score. There were few social problems since most of parents expressed their satisfaction with their family and friends support.

Conclusion: Almost all different aspects of daily life were associated with parents' distress, particularly the emotional and practical domains. Adapted and structured psychosocial care of these parents would allow improving their quality of life and thus that of their children with DS.

References:.

Grants:.

Conflict of Interest: None declared.

P24.003.A Ethical Issues in Prenatal Genetic Diagnosis: new guidance of the British Society for Genetic Medicine

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Background/Objectives: New British Society for Genetic Medicine (BSGM) guidance considers the ethical issues that can arise in prenatal genetic testing. Building on the Joint Committee for Genomic Medicine's Consent and Confidentiality document of 2019, it focusses on key steps of the prenatal testing pathway: and in particular in the interpretation and communication of results and subsequent clinical implications. By incorporating relevant legal principles, professional guidelines, and practical sources for support, it aims to facilitate the decision-making processes for both professionals and patients.

Methods: Based on a workshop with a multidisciplinary working group in 2019 and ongoing collaboration virtually during the pandemic, the three authors produced this report which incorporates illustrative case studies. The guidance was considered and approved by the BSGM Executive Committee in 2022.

Results: This guidance focusses on potential ethical issues related to 5 key-aspects in prenatal genetic testing: 1. Consent to investigations during pregnancy; 2. Communication and decision-making; 3. Implications of prenatal genetic testing for others; 4. Broader aspects of patient care including equal access and justice; 5. Ongoing management and post-pregnancy follow-up. The report offers case-based practical approaches and key-messages.

Conclusion: It is hoped that these clinically focused ethical guidelines are an accessible resource for any healthcare professional who utilises genetic and genomic tests.

References: N/A.

Grants: UK Economic and Social Research Council Grant (ES/T00908X/1).

Conflict of Interest: Ruth Horn UK Economic and Social Research Council (ES/T00908X/1), Alison Hall: None declared, Anneke Lucassen: None declared.

P24.004.B Trust in different offers of expanded carrier screening: a qualitative study among Dutch potential users

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Background/Objectives: Expanded carrier screening (ECS) is aimed at detecting carrier states for many, mainly autosomal recessive (AR) conditions in couples from the general population planning a pregnancy. Different ECS offers exist worldwide, amongst others in the Netherlands, Belgium and Australia. Acceptance of using new technologies requires high levels of trust. Investigating which characteristics of different ECS offers contribute to high trust levels is therefore important for implementation.

Methods: A heterogeneous sample of Dutch potential users was interviewed about trust in different ECS offers (n = 9). Subsequently they rated their level of trust in different ECS offers and ranked different aspects in terms of fostering trustworthiness (survey, n = 8).

Results: Table 1 displays which ECS aspects are associated with high and low trust.

Table 1: Trust in different ECS aspects

ECS aspect (ranking)	High trust	Low trust
Test performance (1)	High sensitivity/high specificity	Low sensitivity/ low specificity
Pre-test counselling (2)	Health care professional	Commercial company
Analysis of ECS test (3)	Scientific institute, hospital	Commercial company
Test panel (4) (number of AR conditions tested)	Limited number that can be overseen	Large (> 100)
Test with blood/ saliva (5)	Blood	Saliva
Price (6)	Expensive	Cheap
Result (individual/ couple) (7)	No differences	No differences

Trust was further found to be influenced by misunderstandings (e.g. that larger test panels have lower test performance).

Conclusion: A highly reliable ECS test-offer screening for a limited set of severe disorders with pre-test counselling conducted by a professional, scores high on trust. Reliable and neutral information is needed to avoid misunderstanding and build trust.

References:.

Grants: 250 words.

Conflict of Interest: None declared.

P24.005.C Ambivalence in the practice of advanced genetic healthcare

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Background/Objectives: The clinical implementation of next-generation sequencing (NGS) has heightened the chance of finding a genetic diagnostic answer. However, NGS results are also often more complex and uncertain and call for multidisciplinary and multifaceted decision-making. Conflicting expectations, interests, and demands in healthcare practice can fuel tension and ambivalence on both an individual and structural level. This paper uses the concept of 'ambivalence' as a lens to characterise the tensions found in dealing with NGS techniques in clinical practice (1).

Methods: We conducted extensive multi-sited qualitative research at two large European human genetics centres. Transcripts from clinical genetic consultations and semi-structured interviews with patients and healthcare professionals and field-notes from observations in multidisciplinary team meetings were inductively coded using Nvivo.

Results: Our analysis makes visible the tension, negotiation, and compromise involved in navigating the different modes of 'doing good care' at play in genetic healthcare practice (2). We show how research and clinical practice informed interests, demands, and concerns are intertwined in everyday practice and structure. We demonstrate how the experience of ambivalence creates tension in what care is offered and how care is experienced by patients.

Conclusion: Providing insight into how practices and structures are shaped by 'the dynamics of ambivalence' (1) can help to more consciously give direction to the future of genetic care.

References: 1. N. J. Smelser, American Sociological Review. 63, 1 (1998).

2. J. Pols, Sociology of Health & Illness. 25, 320–347 (2003).

Grants: KU Leuven C1 funding.

Conflict of Interest: None declared.

P24.006.D Conversational agents in genetic healthcare: an overview of ethical aspects

Marlies van Lingen[1], *Noor Giesbertz*[1], *Annelien Bredenoord*[2], *Lieke van den Heuvel*[1], *Peter van Tintelen*[1], *Karin Jongsma*[3]

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Background/Objectives: Conversational Agents (CAs) are a form of Artificial Intelligence (AI) technology and mimic human interaction using text, speech and/or virtual embodied expressions. In the medical field, CAs are attracting increasing interest because of their efficiency, scalability, and flexibility. Considering the central role of conversation in genetic care, genetic counselling is a particularly suited field for implementation. However, little is known about the ethical aspects of the introduction of CAs in genetic care. The urgency to address this issue is underlined by the recent development of several chatbot-like CAs for genetic counselling practice.

Methods: We conducted a narrative review on ethical literature related to CAs and medical AI in healthcare. Key ethical aspects were categorized by genetic counselling phase.

Results: Different phases of genetic counselling attribute different roles and responsibilities for CAs. CAs could play a role in (1) information provision, (2) guiding informed consent, (3) provide test results or (4) emotional support, and (5) support with clinical assessment of a clinician. For each phase, key ethical aspects are identified at the crossroads of ethical debates in data science and healthcare.

Conclusion: Introduction of CAs in genetic care comes with promises and challenges. CAs impact existing care relations and introduce new dimensions of interaction and governance. Careful evaluation of the impact in each counselling phase is crucial to promote a morally sound and meaningful implementation in the clinical context.

References: -.

Grants: This project is financially supported by ZonMW/IMDI and the Dutch Heart Foundation. Grant number 104021006 (ZonMW) 2019B012 (Dutch Heart Foundation).

Conflict of Interest: Marlies van Lingen: None declared, Noor Giesbertz: None declared, Annelien Bredenoord: None declared, Lieke van den Heuvel: None declared, Peter van Tintelen UMC Utrecht Fulltime, Netherlands Heart Foundation/ PLN Foundation/ Leducq Foundation, Cardiac Tissue Bank, Netherlands Heart Institute, Utrecht, the Netherlands (unpaid), Karin Jongsma: None declared.

P24.007.A Parents' views about newborn bloodspot screening (non-)participation and expansion

Jasmijn Klapwijk[1], *Sylvia Van der Pal*[2], *Sophie Wins*[2], *Tessa Van Dijk*[1], *Adriana Kater-Kuipers*[1], *Catharina P. B. Van der Ploeg*[2], *Suze Jans*[2], *Stephan Kemp*[3], *Rendelien K. Verschoof-Puite*[4], *Lion J. M. Van den Bosch*[5], *Lidewij Henneman*[1]

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Background/Objectives: Neonatal bloodspot screening (NBS) aims at early detection of treatable disorders to offer early intervention. The number of conditions being screened for is expanding worldwide, which may affect public acceptance of NBS. This study explored views of NBS participants and non-participants on (expanding) NBS in the Netherlands.

Methods: Cross-sectional survey data was collected from 804 (6051 invited; 13%) parents whose newborns participated in NBS in the Netherlands in the last two weeks of December 2019, and 48 (1162 invited; 4%) NBS non-participants in 2019 and 2020.

Results: For NBS participants, the most important reason to participate was preventing health problems. For NBS non-participants, the most important reasons to decline were belief that the heel prick would be painful for the child and parents' viewpoint on life (more actively religious, considered alternative medicine/lifestyle more important, less inclined to vaccinate their child). NBS participants made an informed decision more often than NBS non-participants (83% vs 44%), with NBS participants showing more knowledge regarding NBS than NBS non-participants (86% vs 62%). Ninety-five percent of NBS participants were positive about NBS expansion, agreeing to include conditions that were incidentally found in the mother instead of the child (86), late-onset (84%), or untreatable (61%).

Conclusion: Most parents made an informed decision about NBS participation and were positive about NBS and its expansion. Insights into parents' views on (non-)participation and expansion of NBS can help to ensure a program that suits population needs while safeguarding ethical principles for screening.

References:.

Grants: ZonMW Netherlands (no. 543002006).

Conflict of Interest: Jasmijn Klapwijk Employed part-time on Netherlands Organisation for Health Research and Development (ZonMw) grant no. 543002006)., Sylvia Van der Pal Partly supported by Netherlands Organisation for Health Research and Development (ZonMw) grant no. 543002006)., Netherlands Organisation for Health Research and Development (ZonMw) grant no. 543002006); collaborator., Sophie Wins Partly supported by Netherlands Organisation for Health Research and Development (ZonMw) grant no. 543002006)., Tessa Van Dijk Partly supported by Netherlands Organisation for Health Research and Development (ZonMw) grant no. 543002006)., Adriana Kater-Kuipers Partly supported by Netherlands Organisation for Health Research and Development (ZonMw) grant no. 543002006)., Catharina P. B. Van der Ploeg: None declared, Suze Jans: None declared, Stephan Kemp: None declared, Rendelien K. Verschoof-Puite: None declared, Lion J. M. Van den Bosch: None declared, Lidewij Henneman Netherlands Organisation for Health Research and Development (ZonMw) grant no. 543002006); Principal Investigator.

P24.008.B Ethical issues raised by prenatal genetic testing in England, France and Germany: findings from a comparative study

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Background/Objectives: Recently, a number of countries such as England, France and Germany, have started offering NIPT as a publicly funded second tier test for common chromosomal aneuploidies (trisomy 21, 18 and/or 13). Despite the benefits, the implementation of NIPT in healthcare systems raises a number of ethical issues. These issues are discussed differently across countries, echoing different political particularities and value-systems. Our study aims to shed new light on different meanings of reproductive autonomy within various socio-cultural contexts and how this impacts on the use and regulations around prenatal genetic testing.

Methods: As part of a wider research project, which is involving semi-structured interviews with women, health professionals and policy-makers, we conducted a comprehensive literature review to compare arguments about, and regulations governing NIPT in the three countries. Between December 2020 and April 2021, we reviewed approximately 250 sources in legal and regulatory texts; public reports; parliamentary debates; medical press; academic literature, bioethics, social sciences; and daily press.

Results: Although reproductive autonomy is valued in each country, it is understood and implemented differently depending on the socio-cultural context and on what other principles (e.g. disability rights, human dignity, or professional duties to promote health and reduce technology-related risks) are evoked, and how they are defined and weighed against each other.

Conclusion: To further unpack these values and principles, further qualitative research is required to investigate various stakeholders' positions and motivations. Such an in-depth understanding will contribute to both the ethical and societal debates on NIPT and other new prenatal genetic technologies.

References:.

Grants: ES/T00908X/1.

Conflict of Interest: Adeline Perrot Full time, Ruth Horn Full time, PI - ES/T00908X/1 (Economic and social research council): Non-invasive prenatal genetics and genomics in England, France and Germany - Exploring practical ethical issues 'on the ground', Ruth Horn is a member of Oxford Tropical Research Ethics Committee, the French CNRS Ethics Committee, and of the scientific advisory board of the German Human Genome-Phenome Archive. She is President of the European Association of Centre of Medical Ethics (EACME).

P24.009.C Awareness, expectations, and concerns regarding whole-genome sequencing studies: a survey of cancer patients, families and the public in Japan

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Background/Objectives: Whole-genome sequencing (WGS) is being increasingly used in research and clinical settings in cancer genomics. Studies show that cancer patients generally have positive attitudes toward genomic tumor profiling tests (Shirdarreh et al. 2021); however, few works have revealed patients' attitudes toward WGS. This study clarifies cancer patients', families', and public awareness, expectations, and concerns regarding WGS studies in Japan.

Methods: Cross-sectional anonymous online surveys were distributed to Japanese subjects, aged 20–69 years in March 2021, that registered in the survey company panel.

Results: The 10,077 respondents (response rate: 26.5%) were divided into those with a history of cancer (CPs, n = 1,204), those with family history (FMs, n = 5,968), and general adults (GAs, n = 2,915); 56.6% of CPs, 61.2% of FMs, and 70.6% of GAs had never heard of the WGS study. CPs had the highest expectations among the three groups; over 70% evaluated that it would lead to diagnosis, cure, or development of medicine by building a large-scale database. FMs had a higher level of concern than CPs; half were concerned about the accuracy or utility and the possibility of being treated unfavorably if germline findings were detected. About 60% of both CPs and FMs were concerned about the privacy of their genetic information.

Conclusion: Despite low awareness toward WGS studies, CPs had high expectations from it. Since a national project to sequence 80,000 cancer patients started in Japan, measures to prevent issues arising from misuse of genetic information and to protect genetic privacy are urgently needed.

References:.

Grants: AMED JP21cm0106001, MHLW JPMH20AD0101, JSPS KAKENHI JP18K09940.

Conflict of Interest: None declared.

P24.010.D The Australian moratorium on genetics and life insurance fails to meet Parliamentary recommendations against genetic discrimination

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Background/Objectives: Genetic discrimination is an issue of international concern to clinicians, researchers, and policy-makers. In Australia, genetic discrimination in life insurance is legal. In 2018, following a Parliamentary Joint Committee Inquiry into the Life Insurance industry, the Committee recommended an urgent ban on the use of genetic test results in Australian life insurance underwriting, in a form similar to the UK Code on genetic testing and life insurance. The Australian government has not responded to the recommendations. In 2019, the Australian life insurance industry implemented a self-regulated moratorium that applies only to policies up to certain financial limits until 2024. The moratorium will be reviewed by the industry in 2022, but has no government oversight.

Methods: We analysed the Australian moratorium from a policy perspective to measure its adherence to the Parliamentary recommendations.

Results: The Australian moratorium has failed to follow a number of Parliamentary recommendations. Importantly, it differs from the UK Code in a number of key areas (Table 1), including the financial limits, the tests included, the involvement of government, the end date, and the inclusion of research results. On each of these points of difference, the Australian moratorium offers less protection to consumers than the UK Code.

Conclusion: While the FSC moratorium is a step forward for Australia, it fails to meet the expectations of the Parliamentary recommendations. This analysis is instructive for Australian stakeholders, as well as stakeholders in other jurisdictions who are interested in the impact of genetic discrimination and the mechanisms for regulation that are being implemented internationally.

References:.

Grants:

Table 1: Comparison of UK code and Australian moratorium

	UK Code on genetic testing and insurance	*FSC moratorium on insurance and genetics*	*Consistent?*
Financial limits	The only limits on the moratorium are for life cover applications over £500,000 (approx $935,000)	The moratorium only applies up to the monetary limits on life cover and total/permanent disability cover ($500,000), income protection ($4000/month or $48,000pa), and trauma/critical illness cover ($200,000)	✗
Tests included	Only Huntington disease predictive results must be disclosed above the monetary limits for life cover. Currently, no genetic test results must be disclosed for any other type of policy	All genetic test results must be disclosed once the monetary limit is reached for all types of life insurance policies	✗
Regulation/ government involvement	A formal agreement between the UK government and the Association of British Insurers.	Industry-led and self-regulated, without any agreement or involvement with the Australian government	✗
End date	No end date (athough it is reviewed periodically)	Currently due to end in 2024 (may be extended following review in 2022)	✗
Ability to choose to disclose negative genetic test results	Yes	Yes	✓
Research results excluded from disclosure	Yes	No, unless the applicant does not receive the result	✗

Conflict of Interest: Jane Tiller JT is project manager of a project supported by a grant from the Australian Government's Medical Research Future Fund (MRFF), ref 76 721, monitoring the effectiveness of the Australian life insurance and genetics moratorium, Paul Lacaze PL is CIA of a project supported by a grant from the Australian Government's Medical Research Future Fund (MRFF), ref 76 721, monitoring the effectiveness of the Australian

life insurance and genetics moratorium, Margaret Otlowski MO is an investigator of a project supported by a grant from the Australian Government's Medical Research Future Fund (MRFF), ref 76 721, monitoring the effectiveness of the Australian life insurance and genetics moratorium.

P24.011.A To share or not to share: public perspectives on genomic data sharing

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Background/Objectives: Storage and sharing of genomic data following diagnostic sequencing is critical to the future of genomic medicine. Data sharing increases the chance of finding a diagnosis for both current and future patients and can benefit clinical and pharmaceutical research. Yet, few studies have explored public perspectives on how and where data should be stored, with whom it should be shared, and how to obtain meaningful consent to do so.

Methods: To address this issue, we conducted 7 online focus group with 39 members of the Australian public. Focus groups were recorded, transcribed, and analysed using inductive content analysis.

Results: Participants (mean age = 37 years; range 18-67) were overall in favour of storing genomic data, although they raised several concerns, particularly relating to data security and ensuring adequate regulation and standardised application processes to gain access to the data. Sharing data with clinicians and researchers was generally supported because it could provide clear medical benefit. Yet, views on sharing with pharmaceutical companies varied; some participants felt this would lead to better treatments whereas others saw companies as untrustworthy and predatory. Participants generally viewed sharing with insurance companies as inappropriate. Consent was seen as key, with many participants favouring a dynamic consent model which bolstered choice regarding the uses of the data.

Conclusion: Our findings have helped elucidate the complexities associated with developing a one-size-fits-all approach to data sharing and storage through the eyes of the Australian public. These results can be used to help guide policy development on these issues.

References:.

Grants: Australian Government GFMH#76749.

Conflict of Interest: None declared.

P24.012.B Are couples making informed choices when opting for reproductive genetic carrier screening?

Eva Van Steijvoort[1], *Hilde Peeters*[2], *Hilde Vandecruys*[3], *Jasper Verguts*[3], *Karen Peeraer*[4], *Gert Matthijs*[2], *Pascal Borry*[1]

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Background/Objectives: Reproductive genetic carrier screening (RGCS) allows to identify couples who have an increased risk of conceiving a child with a particular genetic condition. Professional organizations have emphasized that the success of RGCS should not solely be measured by the uptake of screening. An assessment of whether or not individuals are making informed choices with regard to RGCS free from coercion from others is considered to be as important.

Methods: Women visiting a gynaecologist practice were asked to consider participation in a research study where RGCS was offered for free to them and their male partner. A modified Multidimensional Measure of Informed Choice was used to determine whether couples who opted for RGCS made an informed choice. In addition, we assessed risk perception, feelings towards RGCS, anxiety and decisional conflict.

Results: A minority of participants perceived their risk to conceive a child with a hereditary condition (1.2%) to be (very) high. In total, 82% of participants (n = 77) made an informed choice with regard to RGCS according to the modified MMIC. Thirteen participants (16.9%) made an uninformed choice due to insufficient knowledge and one participant (1.3%) due to insufficient knowledge and value-inconsistency. Anxiety scores were elevated for three participants (3.8%, n = 80). Two participants (2.4%, n = 81) presented with decisional conflict.

Conclusion: Our study results show high rates of informed choice among couples who were offered RGCS in a research study where participants received up to 30 minutes of pre-test counseling.

References:.

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Conflict of Interest: None declared.

P24.013.C Mental health of adolescents and young adults with Li-Fraumeni syndrome: Results from a mixed method study

Rowan Forbes Shepherd[1], *Camella J. Rising*[1], *Patrick Boyd*[1], *Alix G. Sleight*[2], *Catherine Wilsnack*[3], *Ashley S. Thompson*[1], *Payal P. Khincha*[1], *Allison Werner-Llin*[4]

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Background/Objectives: Li-Fraumeni syndrome (LFS) is an inherited cancer syndrome with whole body cancer risks from birth. Adolescents and young adults (AYAs) with LFS may experience significant physical and psychosocial burdens during critical periods of development early in life, increasing vulnerability to poor mental health. This study examined AYAs' reported mental health and the intersection of LFS- and lifespan-related factors affecting ongoing mental health outcomes.

Methods: Eligible AYAs (aged 15-39 years) recruited from the National Cancer Institute's LFS study (NCT01443468) completed an online survey, with validated mental health measures, and/or qualitative interviews. Descriptive statistics and correlation coefficients were calculated using SPSS. An interprofessional team thematically analyzed interview data using Dedoose.

Results: Thirty-seven AYAs completed surveys (78% female) and 38 AYAs completed interviews (71% female) (11 completed both). AYAs self-reported past emotional problems ($n = 26$, 70%),

depression or anxiety diagnoses ($n = 25$, 68%), and suicidal ideation ($n = 16$, 43%). Past suicidal ideation was significantly correlated with younger age at awareness of LFS ($r = -.391$, $p < .05$). Participants described LFS-related cancer diagnoses, cancer worry, grief, and loss (e.g., family deaths, identity, occupations) that challenged their mental health. Although most ($n = 29$, 78%) reported previously receiving mental health counseling, fewer ($n = 11$, 30%) reported receiving counseling currently.

Conclusion: AYAs with LFS, especially those who learn of LFS at younger ages, may be at risk of poor mental health. These experiences may align with acute periods of LFS-related or developmental change leading to uncertainty and loss in multiple domains of life. Future studies could investigate the effect of an LFS- and youth-specific mental health support intervention.

References:.

Grants:.

Conflict of Interest: None declared.

P24.014.D Genomic research on indigenous peoples in Asia: A comparative study on Japan and Taiwan

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Background/Objectives: In 2021, the ESHG announced its opposition to the misuse of biobanks against vulnerable minorities (Forzano 2021). Many Asian jurisdictions have established biobanks emphasising the uniqueness of their genomes on the basis that genomic research has been West-biased. However, this premise can lead to the disregard of indigenous populations. The purpose of this paper is to understand the ethical considerations for genomic research on indigenous populations in Asia by focusing on major biobanks in Japan and Taiwan.

Methods: The authors conducted a literature review on the laws and guidelines regulating genomic research on indigenous populations, policies of biobanks and articles on ethical issues.

Results: The Taiwan Biobank collected nationwide biological samples, including indigenous populations. Although the group consent rule was introduced under the Free, Prior and Informed Consent (FPIC) of indigenous populations (United Nations 1997), concerns about stigmatisation remain. Biobank Japan has not mentioned the inclusion of the Ainu people, who are legally approved as the only indigenous population in Japan. Three academic societies and the Ainu Association on Hokkaido are preparing ethical guidelines on Ainu research. The Okinawa Bio-Information Bank states that Okinawans are 'genetically different from mainlanders', although Okinawans are not legally indigenous. In both jurisdictions, there were few examples or discussions on benefit sharing.

Conclusion: The legal statuses of indigenous populations in Japan and Taiwan are reflected in their positions in genomic research and in the number of ethical considerations. Further discussion of benefit sharing is required.

References:.

Grants: This research received no specific grant from any funding agency.

Conflict of Interest: None declared.

P24.015.A Building a better mobile app marketplace: an ethical and legal toolkit for app mediated genomics research

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Background/Objectives: Genomics mobile apps are set to become an important data collection tool for biomedical researchers. However, app-mediated research raises questions regarding the responsibilities of researchers, app developers, and platforms in protecting the rights of research participants. Here, we explore the specific roles and responsibilities of the two major mobile platform providers, IOS and Android, in mediating genomics research on their platforms.

Methods: We reviewed current literature and regulatory guidance addressing the status of platform providers under the GDPR and examined two new regulatory proposals, the Digital Services Act and Digital Markets Act. We analysed the impact of each on app-mediated genomics research. We then looked at the contractual obligations that mobile platforms impose on developers in the context of health and genomics research.

Results: Mobile platforms play a significant role in the ethical governance and design of app-mediated research studies, but they have few clear obligations to do so under the GDPR. As a result, research on these platforms can lead to concerns regarding privacy, transparency, conflicts of interest, and diffusion of responsibilities. New regulatory proposals are set to introduce new obligations for mobile platforms and present opportunities for promoting ethical research on platforms.

Conclusion: Taking advantage of proposed obligations for platform providers to enforce their developer policies diligently, address systemic risks, implement notice and removal procedures for illegal content, and separate various platform services, we offer a toolkit for stakeholders interested in building a better app marketplace for genomics research.

References:.

Grants: FWO Flanders-Quebec grant.

Conflict of Interest: None declared.

P24.016.B Development of a digital risk prediction tool based on family history for the general population: ethical and legal challenges

Tetske Dijkstra[1], *Irene van Langen*[1], *Boudien Sieperda*[2], *Jacoliene Zaal*[2], *Corrette Ploem*[3], ***Imke Christiaans***[1]

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Background/Objectives: Both inherited and familial cardiovascular diseases pose a risk of early and preventable cardiovascular events to relatives. Implementing a risk prediction tool to facilitate individuals from the general population to evaluate their potential cardiovascular risk based on their family history could serve as a responsible solution. However, the development and use of such a risk prediction tool gives rise to legal and ethical challenges.

Methods:.

Results: At the start of our project experts mapped its legal and ethical framework. Especially EU-regulations provided potential obstacles for development and broader availability/use of the tool. To illustrate, according to the General Data Protection Regulation, collecting health data of relatives of a tool's user -from the general population, so outside a health care environment- is only allowed after relatives' consent or anonymously. This requirement has substantial consequences for the usefulness of the tool and raises the ethical dilemma "who is the owner of family data". A second example: it follows from the Medical Device Regulation that software that generates a health risk or advice requires a CE-mark

from a 'notified body' at development, and yearly check-ups, which are expensive. In our presentation we will illustrate main bottlenecks and discuss possible solutions.

Conclusion: Besides national law, European law heavily impacts on the development of digital tools collecting family data to provide information on health risk. Direct involvement of experts in law and ethics before and during development of a tool is advised to anticipate and tackle legal and ethical challenges.

References:.

Grants: Dutch Heart Foundation 2019T111.

Conflict of Interest: Tetske Dijkstra: None declared, Irene van Langen: None declared, Boudien Sieperda: None declared, Jacoliene Zaal: None declared, Corrette Ploem: None declared, Imke Christiaans Dutch Heart Foundation (2019T111).

Netherlands Cardiovascular Research Initiative, an initiative with support of the Dutch Heart Foundation (2015-12 eDETECT).

P24.017.C ePAGs' (European Patient Advocacy Groups) role in the European Reference Network ITHACA on Intellectual disability, TeleHealth, Autism and Congenital Anomalies: How patients' voices improve care

Anne Hugon[1], *Dorica Dan*[2;3], *Ammi Andersson*[4], *Ioel Detton*[5], *Marianne Le Dref*[1], *Jill Clayton-Smith*[6], *Sofia Douzgou HOUGE*[7], *Laurence Faivre*[8;9], *Raoul Hennekam*[10], *Jean-Marie Jouannic*[11], *Tjitske Kleefstra*[12], *David Koolen*[13], *Giovanni Mosiello*[14], *Gabor Pogany*[15], *Alessandra Renieri*[16;17], *Sue Routledge*[4], *Nicholas Szeto*[1], *Marco Tartaglia*[18], *Zeynep Tümer*[19], *Birute Tumiene*[20], *Agnies van Eeghen*[10], *Lisenka Vissers**[21;22], *Klea Vyshka*[1], *Dagmar Wieczorek*[23], *Lenja Wiehe*[24], *Giuseppe Zampino*[25], *Christiane Zweier*[26], *Ernithaca consortium*[27], *Alain Verloes*[1;28]

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Background/Objectives: The importance of patients' voices is essential. However, this is the challenge for the ERN ITHACA, a patient-centred network, which aims to develop closer cooperation with ePAGs and member patient organisations. Our Patient Council is empowered through formal roles in the governance and participate through deep interaction in each or across Work Groups. All activities of ITHACA include patients, their families, and patients' organisations as equal partners. ITHACA covers more than 5000 rare and complex genetic disorders and its name is a reference to the diagnostic Odyssey on which families embark with their children affected by rare developmental diseases.

Methods: Our approach is based on patient involvement in several projects in collaboration with the EURORDIS network, in particular on the impact of patient engagement on ERNs, to highlight the value of patient-clinician partnership (Rare Dis Orphan Drugs J 2021;1:2).

Results: The expected results are, through these tools and reflections to improve the partnership involvement, a common understanding of ePAGs in the daily activities of ERN ITHACA.

Conclusion: The UN has formally adopted, on 16 December 2021 with the consensus of all 193 UN Member States, the Resolution on Addressing the Challenges of Persons Living with a Rare Disease and their Families. This recognition of the voices of patients with rare diseases requiring complex care pathways in health care systems is increasingly understood at the EU level and by patient organisations across ERNs.

References: Rare Dis Orphan Drugs J 2021;1:2.

Grants: ERN-ITHACA [EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516].

Conflict of Interest: Anne Hugon APHP Paris Nord Université Robert DEBRE - ERN ITHACA, Dorica Dan: None declared, Ammi Andersson: None declared, Ioel Detton: None declared, Marianne Le Dref APHP Paris Nord Université Robert DEBRE, Jill Clayton-Smith: None declared, Sofia Douzgou HOUGE: None declared, Laurence Faivre CHU DIJON, Raoul Hennekam: None declared, Jean-Marie Jouannic APHP Paris, Tjitske Kleefstra: None declared, David Koolen: None declared, Giovanni Mosiello: None declared, Gabor Pogany: None declared, Alessandra Renieri: None declared, Sue Routledge: None declared, Nicholas Szeto APHP Paris Nord Université Robert DEBRE, Marco Tartaglia: None declared, Zeynep Tümer: None declared, Birute Tumiene: None declared, Agnies van Eeghen: None declared, Lisenka Vissers*: None declared, Klea Vyshka APHP Paris Nord Université Robert DEBRE, Dagmar Wieczorek: None declared, Lenja Wiehe: None declared, Giuseppe Zampino: None declared, Christiane Zweier: None declared, Ernithaca consortium: None declared, Alain Verloes APHP Paris Nord Université Robert DEBRE.

P24.019.A Psychological adjustment, cancer worry and personality traits: a preliminary survey in a group of women undergoing genetic test for cancer susceptibility

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Background/Objectives: Individuals who undergo genetic test may have to deal with psychological consequences such as high levels of stress, anxiety, depression (Lombardi et al., 2019) and worries about becoming ill. Personality traits may have an influence on psychological adjustment to risk condition (Mellon et al., 2008). This study aimed to analyze if worries about cancer were associated with psychological adjustment and age. Furthermore, we investigated if personality characteristics could predict cancer worries. Finally, we evaluated differences in cancer worry and psychological adjustment between cancer affected and healthy females.

Methods: A total of 70 females (mean age = 47,19 years; s.d.=10,82; 63%, n = 44, cancer affected; 37%, n = 26, familiarity with mutation), after genetic testing, completed a set of questionnaires about psychological adjustment, personality traits and cancer worry.

Results: We found that cancer worry was positively associated with levels of anxiety ($r = 0.548$, $p = 0.000$), depression ($r = 0.503$, $p = 0.000$), stress ($r = 0.371$, $p = 0.002$), but not with age ($r = 0.160$, $p = 186$). Regarding personality traits, vulnerability predicted higher levels of cancer worries (Adjusted $R2 = 0.118$; $F = 10,258$; $P < 0.001$). No differences were found between affected and healthy females.

Conclusion: This preliminary study highlighted the importance of considering anxiety, depressive symptoms and stress during genetic counseling regardless of age and the importance of vulnerability trait in worries about cancer.

References: Lombardi L et al. 2019. Psychological aspects, risk and protective factors related to BRCA genetic testing: a review of the literature. Supportive Care in Cancer.

Mellon S et al. 2008. Risk perception and cancer worries in families at increased risk of familial breast/ovarian cancer. Psycho-Oncology.

Grants: No.

Conflict of Interest: None declared.

P24.020.B To do or not to do: Population survey on forensic familial DNA searching in the Low Countries

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Background/Objectives: Forensic familial DNA searching is a powerful tool to provide a last resort opportunity to solve criminal (cold) cases. Although the benefits are strong, strict regulations are required to address the doubts expressed by opponents concerning privacy and ethical issues. Therefore, we aimed to investigate the public opinion.

Methods: Through an online survey distributed through social media, population information of 578 participants living in the Low Countries was analysed. The survey was divided into three parts: (1) personal information to check data reliability (2) general DNA knowledge questions and (3) personal opinions on 'forensic DNA analysis'.

Results: Our population graduated cum laude with an average score of 71%. A significantly higher score was observed for highly educated participants. Surprisingly, 95% of the participants is willing to cooperate in a forensic familial search. Important factors for participation are painless sampling and privacy security. Moreover, we saw that people with a failed DNA score were significantly more afraid to cooperate in forensic DNA analysis. Nevertheless, almost all participants (96%) agreed to use online DNA databases to resolve crimes with violence, but half of them stated that member informed consents should be obtained prior to the search.

Conclusion: To conclude, the 'fear of the unknown' combined with strong feelings towards privacy are two major influencing factors to not cooperate in a forensic familial DNA search. Yet, the highly positive attitude towards forensic DNA analysis was a pleasant surprise and point towards people's high empathy and willingness to help justice.

References:.

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Conflict of Interest: None declared.

P24.021.C Rapid decisions: A qualitative analysis of health professionals' experiences using ultra-rapid genomic sequencing in critically ill children

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Background/Objectives: Although ultra-rapid genomic sequencing (urGS) can provide life-saving results for patients in neonatal and paediatric intensive care units, it can create unique ethical challenges for healthcare professionals (HPs), particularly when the diagnosis raises the possibility of redirection of care towards palliation. Few studies have explored experiences of HPs to understand the impact of urGS on decision-making in this setting.

Methods: We conducted four focus groups and two interviews online with HPs who use urGS in critically ill paediatric patients. We explored the challenges they experience when making decisions about treatment or redirection of care towards palliation following urGS. Data was analysed using inductive content analysis.

Results: Nineteen HPs participated (8 Clinical Geneticists/Paediatric Specialists with Genetic Training; 9 Genetic Counsellors; 2 Intensivists). Participants noted the increasing prioritisation of urGS over other tests to guide patient management. They expressed concern that the timing of urGS may affect parent-child bonding in this setting. Participants described the need for more complex pre-test counselling with families, to prepare for a poor prognosis. HPs found that instances where parents chose to palliate, despite a treatment being available, were particularly distressing.

Conclusion: Our findings suggest that urGS is changing clinical practice and that HPs are gradually adapting their practices to accommodate this shift. These results will assist training of the intensive care workforce to prepare for wider implementation of urGS.

References:.

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Conflict of Interest: None declared.

P24.022.D Ethical aspects of population scale reproductive genetic carrier screening: Insights from the Mackenzie's Mission project

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Background/Objectives: Genetic carrier screening to inform reproductive decisions has existed for over half a century but is now being scaled up for broader implementation. Australia is trialling a large-scale population offer of reproductive genetic carrier screening (RGCS). 'Mackenzie's Mission' is offering RGCS to up to 8,500 Australian couples. However, the move to large-scale population offers of RGCS generates numerous ethical considerations.

Methods: We analyse the overarching ethical issues in population-scale RGCS, drawing on bioethics literature and critically assessing concepts and arguments. We identify insights for policy-makers and funders to consider when delivering an ethically robust RGCS programs.

Results: A range of ethical issues arise when carrier screening moves from the clinic to the population, including: the selection of genes to screen for (e.g., how to deal with variable expressivity), the relevance of public health rather than clinical ethics approaches, and ensuring RCS programs reflect and support wider community values. An essential consideration is scalability – approaches in clinical or targeted screening are not always suitable for populations. It is also vital to maintain a constant dialogue between program-level design and how its implementation is experienced by participants.

Conclusion: Ethical population offers of RGCS require a broad public health pluralistic approach. This provides the best balance of individual and societal considerations.

References: Dive L, Newson AJ. Ethics of Reproductive Genetic Carrier Screening: From the Clinic to the Population. *Public Health Ethics* 2021; **14**: 202–217.

Grants: Mackenzie's Mission is funded by the Australian Medical Research Future Fund, grant GHFM73390.

Conflict of Interest: Ainsley Newson Professor, University of Sydney, Prof. Newson is a current recipient of grant funds from government funders in Australia: the Australian Research Council, the National Health and Medical Research Council and the Medical Research Futures Fund., Prof. Newson received a small honoraria for a talk at a bioethics department in Hong Kong in 2021., Prof. Newson sits on a range of committees. All are government or professional societies; none are commercial., Lisa Dive Research Fellow, University of Sydney (grant-funded).

P24.024.B Dynamic informed consent system, citizen science data management, quality control and integration for Latvian Genome Database

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Background/Objectives: Dynamic informed consent is used to ensure that an individual participating in biobank can modify his decision about the research that is conducted with his sample and data and actively follow research results that could be of importance to him or his immediate relatives.

Our aim is to develop a dynamic consenting and survey system prototype to integrate academic research conducted in Latvian Genome Database with citizen science initiatives maintaining data credibility via hierarchical metadata harmonization and assessing ELSA aspects.

Methods: Working groups of experts in fields of ethics, data science, biobanking and interested stakeholders from patients' organizations and the pharma industry are involved in the following tasks: (1) create "information-wise" data management algorithms for dynamic informed consent and survey system; (2) develop ELSA guidelines for implementation of "ethics by design" in the ICT system; (3) create an IT system prototype for biobank activities and citizen science initiative integration.

Results: The prototype IT design have been created that consist of registration, consent, survey, result reporting and quality control models employing different access levels of several user types – biobank donors, citizen science enthusiasts, doctors/nurses and researchers. The "ethics by design" guidelines have been generated to ensure the best ELSA compliance of ICT system prototype.

Conclusion: Integrative approach is to develop a user-centred ICT system for biobank and citizen science data integration that will enhance data utilization in various health-related areas and serve as basis for communication with participants of Latvian Genome Database.

References:.

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Conflict of Interest: None declared.

P24.025.C Exploring Ethical Futures of NIPT: Stakeholder Perspectives from Belgium

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Background/Objectives: Non-invasive prenatal testing (NIPT) is currently primarily aimed at trisomy 21, 18, and 13. Expanded NIPT could significantly increase the number of conditions and non-medical traits the fetus can be tested for. This expansion reinforces ethical concerns about what can be considered the justifiable scope of prenatal screening. In Belgium, NIPT is available to all prospective parents, genome-wide NIPT is standard, fetal sex is reported, and women only co-pay €8,68. In a context where NIPT is reimbursed by public health care, the question about the scope is especially pertinent. The general aim of this research project is to identify and review: (1) the arguments and ethical principles used to determine the scope of NIPT; and (2) the ethical challenges connected to the widening scope of NIPT.

Methods: We will address the research objectives by performing a literature review and qualitative semi-structured interview studies in Belgium with several stakeholder groups such as healthcare professionals and (expectant) parents.

Results: Expanded NIPT is a clear example of the way technological developments challenge existing norms. There is likely great diversity among and across stakeholder groups about the desirable scope of NIPT. Implementing expanded NIPT needs to be carefully deliberated and multiple stakeholder views need to be considered.

Conclusion: The current climate of rapid technological developments in prenatal screening challenge the existing framework regarding the scope of NIPT as well as the current models of consent and prompt the need to rethink them.

References: -.

Grants: -.

Conflict of Interest: None declared.

P24.026.D Exploring policy approaches to cases of nondisclosure of genetic risk

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Background/Objectives: Genetic risk information is relevant not just to patients but also their relatives. Disclosing genetic risk information to relatives can play an essential role in initiating genetic testing to determine their risk, thus enabling them to seek treatment and access reproductive screening technologies. When a patient does not inform their relatives, the interests and rights of patients and family members can come into conflict. While this ethical issue cannot easily be resolved, several countries have guidelines or legislation addressing nondisclosure. There are three main policy approaches to the disclosure: it is the obligation of the patients or clinicians, or it is within the clinician's purview to decide. More commonly, countries have no specific guideline or law, meaning it is unclear what duties patients and clinicians have towards relatives.

Methods: Using Belgium as an example, we analyzed existing national and European legislation and compared it with international precedent. We then explored ethical arguments for and against various policy approaches. This ethical analysis is supported by data from our empirical research in which Belgian clinicians were asked their opinions regarding potential national policies.

Results: There is strong ethical, legal, and empirical support for a policy that obliges patients to inform their at-risk relatives, however, there are concerns regarding the legal enforcement of such a policy.

Conclusion: Clear policy is required to address the issue of nondisclosure, although further research is required to determine which policy approach is best in a given context.

References: N/A.

Grants: N/A.

Conflict of Interest: None declared.

P25 GWAS

P25.001.A A polygenic risk score to predict sudden cardiac arrest in patients with cardiovascular disease

Eleonora Porcu[1], *Christian Thorball*[1], *Alessandra Pia Porretta*[2], *Etienne Pruvot*[2;3], *Kim Wiskott*[4], *Federica Gilardi*[4], *Aurelien Thomas*[4], *Claire Redin*[1], *Zoltan Kutalik*[5;6;7], *Tony Fracasso*[4], *Olivier Muller*[2], *Jacques Fellay*[1;5;8]

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Background/Objectives: Cardiovascular disease (CVD) is a leading health problem representing the main cause of death globally. Even when causative factors are known, we often do not understand why a cardiovascular condition causes premature death in a victim while others can live longer with the same condition.

Methods: Here we propose a combined polygenic risk score (metaPRS) based on myocardial infarction (MI), coronary artery disease (CAD) and low-density lipoprotein (LDL) to predict the risk of sudden cardiac arrest (SCA) in patients affected by severe cardiovascular conditions. For this, we collected 2,114 patients with reported history of myocardial infarction from the Centre hospitalier universitaire vaudois (CHUV) Genomic Biobank (BGC) and extracted data from the UK Biobank (UKB) on 13,696 participants with similar medical history. Among those, 303 and 932 are affected by SCA or ventricular tachycardia/fibrillation, respectively.

Results: We found that metaPRS is significantly associated with SCA in both cohorts (OR_BGC = 1.18, P_BGC = 0.006 and OR_UKB = 1.14, P_UKB = 1.1 x 10-04). Furthermore, using the diagnosis based on the International Classification of Diseases (ICD-10) codes available in the UKB, we found that our metaPRS exhibits a strong association with the presence of aortocoronary bypass graft (OR_UKB = 1.20, P_UKB = 1.04 x 10-16) and coronary angioplasty implant (OR_UKB = 1.11, P_UKB = 1.05 x 10-08).

Conclusion: These results show that genetic risk score for CVD and associated risk factors has the potential to predict the occurrence of SCA in patients with CVD, hence to identify patients who could benefit from further preventive measures.

References:.

Grants:.

Conflict of Interest: None declared.

P25.002.B Genome-wide association studies of childhood steroid-sensitive nephrotic syndrome in Japanese and South Korean populations

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Background/Objectives: Idiopathic nephrotic syndrome is the most common glomerular disease in children. To understand the genetics of steroid-sensitive nephrotic syndrome, we conducted genome-wide association studies (GWASs) in Japanese and Korean populations.

Methods: In total, 1,018 children with SSNS and 3,331 healthy adults with Japanese ancestry, 249 children with SSNS and 4,041 adult controls with Korean ancestry were recruited. Japanese samples were genotyped using Affymetrix Japonica Array. Whole-genome imputation was performed using a phased reference panel of 2,036 healthy Japanese individuals. Korean patients and healthy controls were genotyped using Axiom Genome-Wide ASI array and HumanOmni1-Quad BeadChip, separately. Whole-genome imputation was performed using 1000 Genome project phase 3 as reference panel.

Results: After quality control steps, Japanese GWAS was performed in 987 childhood SSNS patients and 3,206 controls (6,834,340 variants). HLA-DR/DQ (rs6901541, P = 2.80E−33, odds ratio [OR] = 2.49), NPHS1-KIRREL2 (rs56117924, P = 4.94E-20, OR = 1.90) and TNFSF15(rs6478109, P = 2.54E-8, OR = 0.72) regions achieved genome-wide significance. Korean GWAS was conducted in 243 SSNS patients and 4,041 controls after QC processes (2,912,342 variants). HLA-DR/DQ region (rs9272518, P = 1.04E-14, OR = 2.63) and NPHS1-KIRREL2 (rs412175, P = 4.65E-08, OR = 1.83) achieved genome-wide significance. Meta-analysis was conducted under fixed effects by "METAL". Four loci showed

genome-wide significance including HLA-DR/DQ (rs114685974, P-meta = 5.26E-39, OR = 3.67), NPHS1-KIRREL2 (rs412175, P-meta = 1.32E-24, OR = 1.90), TNFSF15 (rs7848647, P-meta = 5.20E-12, OR = 1.43) regions and a novel locus within CD28-CTLA4 (rs1181388, P-meta = 3.84E-10, OR = 1.37).

Conclusion: GWASs in Japanese and Korean datasets reveal novel loci associated with childhood SSNS.

References: Jia, X. et al. Common risk variants in NPHS1 and TNFSF15 are associated with childhood steroid-sensitive nephrotic syndrome. Kidney Int 98, 1308-1322, https://doi.org/10.1016/j.kint.2020.05.029 (2020).

Grants:.

Conflict of Interest: None declared.

P25.003.C Analysis of exome-wide copy number variation for association with asthma in UK Biobank

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Background/Objectives: The role of copy number variants (CNVs) in asthma susceptibility is not well understood. This study aimed to analyse exome-wide CNVs for association with asthma in UK Biobank.

Methods: We called common CNVs from UK Biobank whole-exome sequencing and tested them for association with asthma in a stage 1 cohort of 7,098 cases and 36,578 controls. Nominally-associated CNVs were meta-analysed in the stage 1 cohort and an additional 17,280 cases and 115,562 controls.

Results: Five of 189 high-quality CNVs were associated with asthma at a Bonferroni-corrected P-value threshold, including a deletion overlapping the HLA-DQA1 and HLA-DQB1 genes, a duplication of CHROMR/PRKRA, deletions within MUC22 and TAP2, and a duplication in FBRSL1. In silico analyses indicated that the deletion overlapping HLA-DQA1 and HLA-DQB1 is an artefact arising from under-mapping of reads from non-reference HLA haplotypes, and that the CHROMR/PRKRA and FBRSL1 duplications represent presence/absence of pseudogenes within the human leukocyte antigen (HLA) region. Bayesian fine-mapping of the HLA region suggested that there are two independent asthma association signals, and that single-nucleotide missense changes in the HLA-DQB1, HLA-DQA1 and/or HLA-DRB1 genes are likely to account for these. No CNVs were present in the credible sets.

Conclusion: These results suggest that CNVs in the HLA region are associated with asthma, but that the association is driven by amino acid changes. Identification of artefactual CNVs in the HLA region provides a cautionary tale for future analyses of sequencing data.

References:.

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P25.004.B Germline variants associated with immunotherapy-related adverse events

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Background/Objectives: Immune checkpoint inhibitors (ICIs) have yielded remarkable responses in patients across multiple cancer types, but often lead to immune related adverse events (irAEs). Although a germline cause for irAEs has been hypothesized, no individual variants have been identified, which is the focus of our study.

Methods: We carried out a Genome-Wide Association Study (GWAS) of 1,751 patients on ICIs across 12 cancer types, with replication in an independent cohort of 196 patients and independent clinical trial data from 2275 patients. We investigated two irAE phenotypes: (i) high-grade (3-5) events defined through manual curation and (ii) all detectable events defined through electronic health record (EHR) diagnosis followed by manual confirmation.

Results: We identified three genome-wide significant associations ($p < 5 \times 10\text{-}8$) in the discovery cohort associated with all-grade irAEs: rs16906115 near IL7 (combined $p = 1.6\text{x}10\text{-}11$; hazard ratio (HR) = 2.1), rs75824728 near IL22RA1 (combined $p = 6.6 \times 10\text{-}9$; HR = 1.9), and rs113861051 on 4p15 (combined $p = 1.3 \times 10\text{-}8$, HR = 2.0); with rs16906115 replicating in two independent studies. The association near IL7 colocalized with the gain of a novel cryptic exon for IL7, a critical regulator of lymphocyte homeostasis. Patients carrying the IL7 germline variant exhibited significantly increased lymphocyte stability after ICI initiation than non-carriers, and this stability was predictive of downstream irAEs and improved survival.

Conclusion: We carried out a GWAS of irAEs and identified a genome wide association replicating in two independent cohorts. We hypothesize a functional relationship with irAEs through a change in IL7, which is predictive of patient outcome.

References:.

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Conflict of Interest: Stefan Groha: None declared, Sarah Abou Alaiwi: None declared, Wenxin Xu: None declared, Vivek Naranbhai: None declared, Amin Nassar: None declared, Ziad Bakouny: None declared, Elio Adib: None declared, Pier Nuzzo: None declared, Andrew Schmidt: None declared, Chris Labaki: None declared, Talal El Zarif: None declared, Biagio Ricciuti: None declared, Joao Victor Alessi: None declared, David Braun LM Education/Exchange Services, Bristol Myers Squibb, MDedge, Exelixis, Octane Global, Defined Health, Dedham Group, Adept Field Solutions, Slingshot Insights, Blueprint Partnerships, Charles River Associates, Trinity Group, and Insight Strategy, Sachet Shukla Agenus Inc., Agios Pharmaceuticals, Breakbio Corp., Bristol-Myers Squibb and Lumos Pharma, Bristol-Myers Squibb, Tanya Keenan: None declared, Eliezer Van Allen: None declared, Mark Awad

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P25.006.B Shared genetic aetiology of osteoarthritis and type 2 diabetes

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Background/Objectives: Osteoarthritis (OA) and type 2 diabetes (T2D) are two of the most prevalent chronic health disorders worldwide. Observational studies report a positive epidemiological association between the diseases beyond their common risk factors, such as obesity and increasing age. Taking into consideration that the world's obesity rates, and average age are rising, this comorbidity pair can be considered an increasing global health challenge. Thus, in this research project, we aim to disentangle the genetic correlation between OA and T2D.

Methods: Using summary statistics of large-scale GWAS from T2D (n = 898,130) and knee or hip related OA phenotypes (n = 490,345), we investigate the genetic intersection between the traits by performing statistical colocalization analysis of established association signals. For colocalizing regions, we derive a set of high confidence likely effector genes based on biological lines of evidence, including colocalization with molecular QTLs from disease-relevant tissues. Additionally, for each of those genes, we perform Mendelian randomization analyses between expression QTLs and each disease.

Results: 18 genome regions show robust evidence of colocalization between T2D and at least one OA phenotype. 27 genes were defined as high confidence likely effector genes, including TCF7L2 and the obesity-related FTO gene. TCF7L2 is among the leading signals for T2D risk but had not been identified as associated with OA at genome-wide significance levels yet. Genetic variants associated with expression levels of TCF7L2 in pancreatic islets are causal for T2D and protective for knee-related OA phenotypes.

Conclusion: Our shared common effector genes support the epidemiologically known link between BMI and the investigated comorbidity.

References:.

Grants:.

Conflict of Interest: None declared.

P25.008.D Genome wide association study of long-term toxicity 2 years following radiotherapy for breast cancer– results from the REQUITE cohort study

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Background/Objectives: Up to a quarter of breast cancer patients treated by surgery and radiotherapy experience clinically significant toxicity. This study was designed to identify common single nucleotide polymorphisms (SNPs) associated with toxicity 2 years following whole breast radiotherapy.

Methods: A genome-wide association study (GWAS) was performed in 1,635 breast cancer patients with complete SNP, clinical, treatment and toxicity data, recruited across 27 centres in Europe and the US into the prospective REQUITE study (www.requite.eu). All patients were genotyped using Illumina OncoArrays with ~600,000 SNPs. Datasets were imputed according to OncoArray Network methods. A total of 7,097,340 SNPs with minor allele frequency >0.05 and imputation score >0.3 were tested for association with the residuals of toxicity endpoints at 2 years.

Results: Quantile-quantile plots for association with toxicity showed more associations above the $p < 5 \times 10^{-4}$ level than expected by chance. Five SNPs reached genome-wide significance. Nipple retraction (10.0%≥G1) was associated with the rs188287402 intron variant on the X chromosome ($p = 2.80 \times 10^{-8}$); breast oedema (11.1% ≥G1) with the Chr5 rs12657177 ($p = 1.12 \times 10^{-10}$) and the Chr13 rs61966613 variants($p = 1.06 \times 10^{-9}$); induration (6.5% ≥ G2) with Chr1 rs77311050 ($p = 2.54 \times 10^{-8}$); and arm lymphoedema (3.4% ≥ G1) with the Chr1 rs643644 variant ($p = 3.54 \times 10^{-8}$). Heritability estimates across different endpoints ranged from 13% to 39%. Several previously significant SNP associations with late breast toxicity were validated at the nominal 0.05 significance level.

Conclusion: This largest GWAS to date for long-term breast radiation toxicity provides evidence for genome-wide significant association of common SNPs with distinct toxicity endpoints.

References:.

Grants:.

Conflict of Interest: Harkeran Jandu: None declared, Colin D Veal: None declared, David Azria: None declared, Jenny Chang-Claude: None declared, Ananya Choudhury: None declared, Alison M Dunning: None declared, Dirk R. de Ruysscher: None declared, Laura Fachal: None declared, Sara Gutiérrez-Enríquez: None declared, Philippe Lambin: None declared, Tiziana Rancati: None declared, Barry S Rosenstein: None declared, Maria C de Santis: None declared, Petra Seibold: None declared, Elena Sperk Dr Elena Sperk: none related to the current manuscript. Outside the current manuscript: General speakers bureau Zeiss Meditec, travel support Zeiss Meditec., R Paul Symonds: None declared, Ana Vega: None declared, Liv Veldeman: None declared, Adam Webb: None declared, Catharine West: None declared, Christopher J Talbot: None declared, Tim Rattay: None declared.

P25.009.A Gene-based burden scores to improve polygenic models for blood and urine biomarkers

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Background/Objectives: Integration of common and rare functional variants for a more comprehensive genetic risk modelling in complex phenotypes.

Methods: We analyzed the genetic component of 32 blood and urine biomarkers in 200,000 samples with exomes and genotyping data from UKBioBank. We computed: 1. polygenic risk score (PRS) based on the effect of common variants as previously obtained using multivariate snpnet multivariate framework; 2. gene-based risk scores (GBRS) for each biomarker obtained by weighting each gene-based burden score in a training dataset according to the association signal obtained in a test dataset. The GBRS and PRS were then used to generate prediction models, both individually and in combination.

Results: PRS are generally more associated with the phenotype than GBRS corroborating a predominant role for common variants in the genetic liability for complex traits as metabolic biomarkers levels. However, GBRS are also strongly associated with blood and urine biomarkers suggesting that the burden of rare deleterious variants in coding regions can also substantially influence the biomarkers levels and interestingly the combination of PRS and GBRS improved the prediction performance as measured by the R^2 for 25 out of 32 biomarkers.

Conclusion: Our findings suggest the analysis of exome data and the computation of gene-based risk scores assessing the burden of rare deleterious variants can improve the current PRS model predictions.

Conflict of Interest: None declared.

P25.010.B Novel loci and Loss-of-Function genes associated with prediabetes provide insights into biological mechanisms leading to diabetes

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Background/Objectives: Prediabetes is a precursor of type 2 diabetes (T2D), and while still reversible, up to 70% of patients eventually develop diabetes. People with prediabetes have elevated glycated haemoglobin (HbA1C) and plasma glucose concentrations, and are often overweight and hypertensive. In this study we explored genetic and biological mechanisms responsible for prediabetes.

Methods: We included up to 7,748 prediabetics, 23,626 diabetics, and 286,132 controls from UK Biobank. Prediabetes and diabetes patients were identified using HbA1C values and a previously published algorithm. We tested prediabetes (vs diabetes and vs healthy controls) in a GWAS setting including >38 million variants, and examined ~8600 Loss-of-Function genes using the Loss-of-Function toolkit (LoFTK).

Results: The GWAS analyses using healthy controls identified thirty prediabetes loci. Of these, 26 had been previously associated with diabetes. Out of the 4 loci specific to prediabetes we found support from previous GWAS on HbA1c for three, and one novel locus near *GLI3*. We identified eleven loci associated with prediabetes vs diabetes, including ten known diabetes loci and a novel locus in *LPCAT1*. Loss-of-Function analyses highlighted *SHPK*, a novel gene associated with both prediabetes vs controls and vs diabetes.

Conclusion: We identified novel genes associated with prediabetes, which may lead to new insights into the biological mechanisms involved in prediabetes and diabetes.

References:.

Grants:.

Conflict of Interest: None declared.

P25.011.C Dissecting the genetic bases of Color Vision Defects (CVDs) through Genome-Wide Association Study (GWAS) in Silk Road populations

Giuseppe Giovanni Nardone[1], *Maria Pina Concas*[1], *Domenico Loperfido*[2], *Anna Morgan*[1], *Maurizio Battaglia Parodi*[3], *Paolo Gasparini*[1;4], *Giorgia Girotto*[1;4]

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Background/Objectives: Color perception relies on the brain's response to the stimuli produced by light-activated cone photoreceptors. To date, apart from three major genes (OPN1LW,OPN1MW,OPN1SW) involved in Mendelian CVDs, nothing is known for multifactorial CVDs forms.

Methods: 520 individuals from Silk Road populations were genotyped and phenotypically characterized for CVDs using the Farnsworth D-15 color test and results interpretation was performed by a certified ophthalmologist. CVDs analysed traits were the following: Deutan-Protan (DP) and Tritan. GWAS for both traits was performed; results were filtered for MAF≥5%. Gene expression of final candidates was defined using a published human eye dataset.

Results: GWAS analysis revealed several interesting hits within genes involved in eye physiology and pathologies that show high expression levels in several eye tissues. As regards DP, LRRC8D gene resulted significantly associated (p-value = 3,41*10-8) whose protein is expressed in Müller cells of the retina. Concerning Tritan, three genes (MYL3, p-value = 1,77*10-8; PDE3A, p-value = 6,64*10-9; MC5R, p-value = 6,17*10-10) were associated. MYL3 is a gene modulated by MERTK, whose mutations have been associated with retinitis pigmentosa; PDE3A encodes phosphodiesterase 3A, a protein involved with hypertension and MC5R encodes melanocortin receptor type 5, a receptor involved in ocular immunity regulation. Interestingly, drugs targeting phosphodiesterases and melanocortin receptors are known to cause temporary alterations in color vision.

Conclusion: We described, for the first time worldwide, a GWAS on multifactorial CVDs identifying four genes potentially playing a crucial role in CVDs pathogenesis. Next step will include external replication in independent populations and functional in vitro/in vivo studies to define the mechanisms underlying these clinical traits.

References:.

Grants:.

Conflict of Interest: None declared.

P25.012.D Genome Wide Association Studies of severe mental disorders reveal shared and disorder specific risk loci

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Background/Objectives: Mental disorders (MD) are pleiotropic, sharing genetic origin[1–3], likely expressed in early brain development. Descriptions of this genetic overlap are not consistent across studies. Several genetic loci associated with MDs have been identified through genome wide association studies (GWAS)[4–6], but loci delineating individual disorders from shared liability are not well described. We aim to understand the genetics underlying the similarity as well as the differences among MDs.

Methods: We use the iPSYCH2012 and 2015i cohorts, independent samples from the population of Denmark born between 1980 and 2007 with follow up until 2015. Cases are ascertained for diagnosis of at least one of six severe MDs. Genetic correlations (rG) and heritabilities are calculated using phenotype correlation genotype correlation. GWAS are performed using mixed linear models (MLM) and genomic structural equation modeling (GSEM).

Results: rG between MDs estimated within iPSYCH are consistently higher than external estimates. GWAS identify novel loci associated with the shared and individual risk of MDs. GSEM reveals structure of rG between MDs, replicates findings from MLMs, and estimates heterogeneity in associations between the shared and disorder specific risk.

Conclusion: Higher rG between iPSYCH cohorts for MDs suggest diagnoses obtained from national registers are meaningfully different from those in meta-analyses pooling data from diverse, non-uniformly ascertained studies. Biological follow up of loci delineating disorder specific and shared risk factors can reveal genetic causes for differences among MDs.

References: 1. Schork 2019.
2. Gandal 2018.
3. Brainstorm 2018.
4. Demontis 2019.
5. Grove 2019.
6. Howard 2019.

Grants:.
iPSYCH.
Lundbeck foundation fellowship R335-2019-2318.

Conflict of Interest: None declared.

P25.013.A Genome-wide meta-analysis identifies novel loci for positive thyroid peroxidase and thyroglobulin antibodies

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Background/Objectives: The presence of antibodies to thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) is an early indicator of autoimmune thyroid disease (AITD). However, thyroid antibodies are not only frequently detected in patients with AITD but also in patients without diagnosed thyroid dysfunction. This study aimed to replicate the significant findings from our discovery genome-wide association study (GWAS) and to increase statistical power to detect novel genetic variants associated with plasma antibody positivity by including over 1,500 new individuals.

Methods: Participants with elevated plasma TPOAb and/or TgAb were defined as cases (N = 341) and those with TPOAb and TgAb within reference values were defined as controls (N = 1253). We performed individual-level genome-wide association mapping using a linear mixed model (LMM) of 7,609,054 variants, imputed using the Haplotype Reference Consortium (HRC) panel, in 1,594 European-ancestry participants of the Croatian Biobank. In the meta-analysis, we combined these data with our discovery GWAS from three independent cohorts consisting of 2,613 individuals. In these 4,207 individuals, analysis was performed using the random-effects method.

Results: Meta-analysis identified ERBIN gene intron variant rs34540954 (p = 5.3 x 10-8) as the top association signal, including another 122 variants passing the suggestive genome-wide significance threshold of 5 x 10-6.

Conclusion: This study adds to the knowledge of genetic background of thyroid antibodies and will lead to better understanding of biological pathways and clinically relevant disorders related to their function.

References:.

Grants: This work has been supported by the Croatian Science Foundation grant No. 2593.

Conflict of Interest: None declared.

P25.014.B Rare variant associations and novel loci for eosinophil count - a UK Biobank whole-exome sequencing study

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Background/Objectives: Eosinophils play important roles in the release of cytokine mediators in response to inflammation. Many

associations between common genetic variants and eosinophils have already been reported, using SNP array data. However, there is a need to further investigate the role of rare variants altering eosinophil count as this might have considerable implications in the pathogenesis of inflammatory diseases.

Methods: Here, we have utilized 200 000 whole exome sequences (WES) from the UK Biobank cohort to perform gene-based analyses of eosinophil count. We defined five different variant weighting schemes to incorporate information on both deleteriousness and frequency.

Results: A total of 220 genes in 55 loci, were found to be associated with eosinophil count, of which seven genes (ALOX15, CSF2RB, IL17RA, IL33, JAK2, S1PR4, and SH2B3) are driven by rare variants, independent of common variants identified in genome-wide association studies. Two additional genes, NPAT and RMI1, have never been associated with eosinophil count before and are considered novel eosinophil loci.

Conclusion: The genes driven by rare variants can be of importance for further studies identifying therapeutic targets or biomarkers for eosinophil related traits. Additionally, rare variants with larger effects may have a large impact at an individual level. These results increase our knowledge about the effect of rare variants on eosinophil count.

References:.

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Conflict of Interest: None declared.

P25.016.D Benchmarking of univariate pleiotropy detection methods, with an application to epilepsy phenotypes

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Background/Objectives: Complex traits have been found to share genetic relationship in separate GWAS studies and the joint identification of these loci or variants via pleiotropy analysis improves gene discovery. Pleiotropy is the phenomenon of a hereditary unit affecting more than one trait. Joint analysis of sub-phenotypes improve power to discover association however, due to separate individual data collection for each trait, application of multivariate approaches is often not feasible. Therefore, to identify these switch-like or shared loci in more than one trait, we applied univariate pleiotropy detection approaches to epilepsy sub-phenotypes.

Methods: Firstly, we benchmarked five pleiotropy detection classical meta-analysis, cFDR, ASSET, CPBayes and PLACO, via simulation to identify the most powerful approach while keeping type 1 error low.

Results: ASSET method which gave a good trade-off between power and false -positive rate (FPR) is then applied to summary statistics of focal and genetic generalized epilepsies provided by the ILAE Consortium on complex epilepsies, thereby identifying a new putative pleiotropic locus at 17q21.32 and confirmed locus 2q24.3 which had already been identified via mega-analysis. Classical meta-analysis performed poorly in terms of FPR.

Conclusion: ASSET method yielded good power while keeping FPR low but, classic meta-analysis is not recommended.

References: International League Against Epilepsy Consortium on Complex Epilepsies (2018). Genome-wide mega-analysis identifies 16 loci and highlights diverse biological mechanisms in the common epilepsies. Nat Commun 9, 5269. https://doi.org/10.1038/s41467-018-07524-z.

Grants: This study received support from the Research Unit FOR-2715 of the German Research Foundation and the 710 Fonds National de la Recherche.

Conflict of Interest: None declared.

P25.018.B Genetic overlap study between idiopathic pulmonary fibrosis and COVID−19

Beatriz Guillen-Guio[1], *Richard Allen*[1], *Emma Croot*[2], *Luke M Kraven*[1], *Samuel Moss*[3], *Iain Stewart*[3], *Gisli Jenkins*[3], *Louise Wain*[1;4]

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Background/Objectives: Genetic factors influence COVID-19 susceptibility and outcomes, including the development of pulmonary fibrosis (i.e. lung scarring). Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease and the most common cause of pulmonary fibrosis in the general population. Genome-wide association studies (GWAS) of COVID-19 and IPF revealed genes associated with both diseases, suggesting these share genetic risk factors. Here we performed a genetic overlap study between COVID-19 and IPF.

Methods: Summary statistics from an IPF 5-way meta-GWAS and from the COVID-19 Host Genetics initiative GWAS meta-analysis (v6) were used. We performed genetic correlation analyses and assessed individual genetic signals to identify those variants shared between both traits. We conducted colocalisation analyses to determine whether the same causal variant was driving both traits. Finally, the association of overlapping variants with gene expression was assessed and a phenome-wide association study was performed.

Results: There was a positive genetic correlation between severe COVID-19 and IPF. We found four genetic loci with likely shared causal variants between both traits, including one novel risk locus at 7q22.1 that colocalised with decreased *ZKSCAN1* and *TRIM4* expression in blood. The other three loci colocalised with *MUC5B*, *ATP11A* and *DPP9* expression. The locus associated with increased *ATP11A* expression was also associated with higher Hb1AC levels, a biomarker used in diabetes.

Conclusion: Results suggest there are shared biological processes driving IPF and severe COVID-19 phenotypes.

References:.

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Conflict of Interest: Beatriz Guillen-Guio B Guillen-Guio is supported by Wellcome Trust grant 221680/Z/20/Z, Richard Allen R Allen is an Action for Pulmonary Fibrosis Mike Bray Research Fellow, Emma Croot: None declared, Luke M Kraven: None declared, Samuel Moss: None declared, Iain Stewart: None declared, Gisli Jenkins G Jenkins is a trustee of Action for Pulmonary Fibrosis and reports personal fees from Astra Zeneca, Biogen, Boehringer Ingelheim, Bristol Myers Squibb, Chiesi, Daewoong, Galapagos, Galecto, GlaxoSmithKline, Heptares, NuMedii, PatientMPower, Pliant, Promedior, Redx, Resolution Therapeutics, Roche, Veracyte and Vicore., Louise Wain L Wain holds a GSK/British Lung Foundation Chair in Respiratory Research (C17-1), L

Wain reports research funding from GSK and Orion and consultancy for Galapagos, outside of the submitted work.

P25.019.C Value of individual population studies to characterize loci associated with complex traits

Dariush Ghasemi-Semeskandeh[1;2], *David Emmert*[1], *Eva König*[1], *Luisa Foco*[1], *Martin Gögele*[1], *Laura Barin*[1], *Ryosuke Fujii*[1], *Christian Fuchsberger*[1], *Dorien Peters*[2], *Peter Pramstaller*[1], *Cristian Pattaro*[1]

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Background/Objectives: Genome-wide association study (GWAS) meta-analyses of complex traits have identified thousands of loci, whose biological characterization is underway. In meta-analyses, variant effect sizes vary across contributing studies due to environmental or genetic differences. However, how much study-specific features can be exploited to elucidate the underlying mechanisms is unclear. As a case study, we compared a GWAS of estimated glomerular filtration rate (eGFR) in a population-based study from the Alps, where thyroid disease is common, versus a meta-analysis on >1 million individuals that previously identified 147 kidney function relevant loci.

Methods: We performed a GWAS of age- and sex-adjusted ln(eGFR) on 10,146 participants in the Cooperative Health Research in South Tyrol (CHRIS) study, accounting for genomic kinship (genomic inflation $\lambda = 1.02$). We assessed direction-consistent replication at any variant strongly correlated with the locus lead variant ($r^2 \geq 0.8$), accounting for multiple testing. Replicated variants underwent phenome-wide mediation analysis across 72 health biomarkers.

Results: We replicated 10 loci. The variants' effect magnitudes were 1.3-to-5.8 times larger than in the meta-analysis, at similar minor allele frequencies (MAF). Free triiodothyronine and thyroxine, measured in individuals with low or high thyroid stimulating hormone levels, resulted as potential mediators for most loci. We observed potential interactions with hyperthyroidism at SHROOM3 ($p = 0.03$) and with hypothyroidism at PIP5K1B ($p = 0.03$) and GAB2 ($p = 0.04$).

Conclusion: Similar MAF, same ancestry and loci independency suggest the larger effects on eGFR in the CHRIS study being probably of environmental origin. The potential modifier role of thyroid disease warrants independent replication.

References:.

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Conflict of Interest: None declared.

P25.021.A Genome-wide association analysis and genomic prediction of thyroglobulin plasma levels

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Background/Objectives: Thyroglobulin (Tg) is a 660 kDa iodoglycoprotein produced by thyroid follicular cells. Together with iodide and peroxide, it acts as an essential substrate for thyroid hormone synthesis. So far, only one genome-wide association study (GWAS) of plasma Tg levels was performed by our research group.

Methods: Utilizing recent advancements in computation and modelling, in the current study, we apply a Bayesian approach to the probabilistic inference of genetic architecture of Tg. We fitted a Bayesian sparse linear mixed model (BSLMM) and a frequentist LMM of 7,289,083 variants, imputed using the Haplotype Reference Consortium (HRC) panel, in 1096 healthy European-ancestry participants of the Croatian Biobank. Additionally, we estimated the heritability of Tg and constructed a polygenic score (PGS). We meta-analysed these results with our previous results from two independent cohorts of 605 and 489 individuals, resulting in a total of 2,109 individuals.

Results: Meta-analysis identified 83 significantly associated variants within the ST6GAL1 gene and replicated the discovery single nucleotide polymorphisms (SNPs). Multi-SNP BSLMM analysis revealed additional association signals on chromosomes 1, 8, 10, and 14. We found that all available variants explain 17% of the variance in Tg levels and that 52% of this variation is due to a small number of 16 variants that have a major effect on Tg levels.

Conclusion: These results suggest that the genetic architecture of plasma Tg is not purely polygenic, but rather sparse, i.e. influenced by a few genes with major effects.

References:.

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Conflict of Interest: None declared.

P25.022.B Trait selection strategy for improving SNPs discoverability in multi-trait GWAS with JASS

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Background/Objectives:.

As a profusion of GWAS summary statistics became publicly available, investigators explored this data with various multi-trait GWAS methods (TATES, MTag, MostTest, JASS[(1)], …). Yet, the joint analyses of GWASs comports many challenges: data harmonisation, computational cost, and missing values. While the combinatorial possibilities are overwhelming, there is no established strategy to astutely select sets of traits to analyse.

Methods: To solve these challenges, we implemented JASS[(1)] with a specific attention to computational efficiency (on 62 traits, JASS runs in 17 minutes). Leveraging a database of 178 curated summary statistics spanning several ancestries (https://jass.pasteur.fr/index.html), we conducted multi-trait GWASs on 1219 sets of 2 to 63 traits with varying characteristics.

Results: While JASS expectedly detected more associations as set traits were more heritable, we found more exclusive associations (i.e. not significant in univariate tests) when the set of traits has a low mean heritability ($h^2 < 0.1$). This gain of exclusive associations was also significantly improved by adding more traits to the set. Sets based on clinically related or highly genetically correlated traits yield only few new associations.

Conclusion: To differentiate from univariate GWAS findings, we advise investigators to include numerous complementary traits in their joint analysis to discover up to ~30% unprecedented associations.

References: [(1)]Julienne, Hanna, et al. "JASS: command line and web interface for the joint analysis of GWAS results." *NAR genomics and bioinformatics* 2.1 (2020): lqaa003.

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Conflict of Interest: None declared.

P25.023.C A novel multi-components mixed model based bacterial-GWAS method and its application to Listeria monocytogenes

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Background/Objectives: Genome-wide Association Studies (GWAS) have been central to study the genetics of complex human phenotypes, and there is now tremendous interest for implementing GWAS-like approaches to study pathogenic bacteria. However bacterial genomes harbour complex structures making such analyses extremely challenging. Diverse and heterogeneous strategies have been proposed to define genetic variants (kmers, snp,…) and account for population structure (phylogeny, mixed model,…), but their relative performances have not been studied, and fundamental genetic modelling aspects have been surprisingly seldom discussed.

Methods: We used real bacterial sequence data and simulated phenotypes to conduct a formal comparison of alternative methods for modelling genetic structure, estimating heritability, and testing association. We leverage those results to develop a robust and powerful approach which we applied to the MONALISA cohort, a national prospective repository that systematically collects *Listeria Monocytogenes* strains in France, currently including whole genome sequencing of 3718 strains.

Results: We demonstrate that the classic human heritability model, commonly assumed in existing bacterial GWAS methods, is strongly unadapted to study highly structured organisms such as Listeria. The most efficient approach consists in a multi-component linear mixed model applied to unitigs, where components are inferred from a hierarchical clustering of the genetic relatedness matrix. Our analyse of virulence biomarkers in hypervirulent strains from MONALISA was able to identify promising targets, along clone-specific estimates of heritability.

Conclusion: We demonstrate that our refined genetic structure modelling can greatly improve power and robustness of bacterial GWAS.

References:.

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Conflict of Interest: None declared.

P25.024.D The genetics of Parkinson's Disease and the loss of dopaminergic neurons

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Background/Objectives: Polygenic risk scores (PRS) for Parkinson's Disease (PD) based on case/control genome-wide-association study (GWAS) statistics show a low predictive value, possibly due to the high heterogeneity within PD or misdiagnoses. As quantitative traits achieve better reproducibility in genetic studies and better capture the phenotypic heterogeneity within a disorder, exploring the genetics of a biomarker could improve our understanding of the genetics of disease mechanisms. Striatal binding ratio (SBR) as measured through DaTscan could be such a marker. SBR is a good diagnostic biomarker and is a proxy for the loss dopaminergic neurons, a neuropathological hallmark of PD. We explore to what extend SBR is related to the genetics of PD and what the genetic basis of SBR is.

Methods: We calculate PD PRS and cell specific PD PRS, compare their performances, and correlate them with SBR. We investigate differences in SBR progression and severity between PD mutation carriers. We perform a GWAS for SBR and evaluate the predictive performance of SBR PRS in an independent cohort.

Results: SBR and PD PRS are correlated regardless of the applied p-threshold for PRS computation. The dopaminergic neuron specific PRS for PD is significantly associated with SBR and has the highest predictive performance. SNCA carriers have lower SBR at diagnosis. Some loci are associated with SBR and the predictive performance of SBR PRS on the independent cohort looks promising.

Conclusion: SBR is not only a good diagnostic biomarker for PD, but it also allows us to study the genetic basis of dopaminergic neuron loss across disorders.

References:.

Grants:.

Conflict of Interest: Ann-Kathrin Schalkamp PhD studentship funded by Health and Cares Research Wales, Cynthia Sandor UK Dementia Research Institute which receives its funding from DRI Ltd, funded by the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK.

Ser Cymru II programme which is part-funded by Cardiff University and the European Regional Development Fund through the Welsh Government.

P25.025.A The genetic architecture of skull shape

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Background/Objectives: The human skull forms an essential layer of protection for craniofacial areas, yet the genetic basis of skull shape remains largely unknown. Here, we perform the first genome-wide association study (GWAS) seeking to find genetic determinants underlying different skull shape dimensions.

Methods: We defined 19 MRI-derived linear and ratio skull shape measures including cranial length and width and a variety of novel (geometrically derived) measures. We estimated family-based heritability (ASPS-Family, N = 368) and single-nucleotide polymorphisms-based heritability (UK Biobank, N = 38,029), and performed a GWAS (UK Biobank, N = 38,029) controlling for batch effects (study center and genotyping array) and covariates (age, sex, height, intracranial volume, and 20 principal components).

Results: Skull shape measures were heritable to varying extents, ranging from 3% to 26% (standard error = 1-2%) for SNP-based heritability, and 10% to 81% (standard error = 12-13%) for family-based heritability. We identified 59 genome-wide significant loci for 15 skull shape measures including loci nearby genes involved in embryonic development (e.g., BMP3, FGF8) and craniofacial abnormalities (e.g., craniosynostosis, JAG1, KANSL1; Coffin-Siris syndrome, ARID1B).

Conclusion: Individual skull shape measures contain genetic information that could aid the understanding of the complex mechanisms underlying craniofacial development.

References:.

Grants: Table 1 Number of loci and heritability estimates per skull shape measure

Table 1 Number of loci and heritability estimates per skull shape measure

Measure			Heritability	
Linear	Ratio (measure1:measure2)	Number of loci[a]	SNP-based (SE)	Family-based (SE)
Front-Back		7	0.24 (0.02)	0.53 (0.13)
Front-Mid		4	0.23 (0.02)	0.47 (0.13)
Back-Mid		7	0.17 (0.02)	0.39 (0.12)
Right-Left		15	0.26 (0.02)	0.75 (0.13)
Right-Mid		5	0.17 (0.02)	0.75 (0.14)
Left-Mid		11	0.19 (0.03)	0.32 (0.14)
(FrontRight+FrontLeft):2		19	0.26 (0.02)	0.33 (0.14)
(BackRight+BackLeft):2		12	0.21 (0.02)	0.39 (0.14)
(TopRight+TopLeft):2		9	0.23 (0.02)	0.81 (0.12)
Height		6	0.23 (0.02)	0.71 (0.12)
Front-Top		3	0.26 (0.02)	0.78 (0.12)
Back-Top		11	0.20 (0.02)	0.26 (0.12)
	Front-Mid:Back-Mid	4	0.16 (0.03)	0.34 (0.12)
	Right-Mid:Left-Mid	0	0.02 (0.01)	0.13 (0.13)
	Right-Left:Front-Back	10	0.26 (0.02)	0.79 (0.12)
	Front-Right:Front-Left	0	0.04 (0.02)	0.39 (0.13)
	Back-Right:Back-Left	0	0.03 (0.01)	0.11 (0.14)
	Front-Top:Back-Top	6	0.16 (0.02)	0.34 (0.12)
	Right-Top:Left-Top	0	0.03 (0.01)	0.10 (0.13)

SE, standard error. [a]Loci overlap between measures

SE, standard error. [a]Loci overlap between measures.

Conflict of Interest: None declared.

P25.026.B Inclusion of sequencing GWAS (seqGWAS) in GWAS Catalog, data format and future challenges

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Background/Objectives: Since its inception in 2008 as a resource collating top associations from genome-wide array based studies (GWAS), the GWAS Catalog has adapted to the changing genomics landscape by expanding scope to incorporate targeted arrays, summary statistics and pre-published datasets.

As of February 2022, the Catalog contains 33,162 GWA studies, including 22,675 with full p-value summary statistics and more than 325,000 curated top associations from 5,595 publications. Pre-published datasets are also available and regularly updated as they acquire published status. All data is freely available and frequently updated, based on clear principles of open access and data sharing.

Methods:.

Results: The next expansion in our scope has been the inclusion of sequencing based studies, as the decreasing cost and advances in analytical methods have made them feasible alternatives to traditional array-based GWAS. In 2021 we began to routinely triage the literature for seqGWAS publications and make these available for author submission of summary statistics. Since the beginning of this year, we are also curating and extracting top associations from the single variant analyses.

Conclusion: Gene-based analyses are of high interest to the community but present additional challenges in standardisation. One of the challenges is the lack of a standard format following FAIR principles. In consultation with stakeholders we have developed a new standard format with additional mandatory fields, suitable for single variant array and sequencing GWAS. Greater availability of summary statistics for gene based analyses will enable the development of an appropriate standard format for sharing & interoperability of this data type.

References:.

Grants:.

Conflict of Interest: None declared.

P25.027.C Liabilideep: a flexible, deep neural network approach for constructing genetic liabilities and conditioning on covariates without the need for prevalence or heritability estimates

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Background/Objectives: Recent GWAS methods use family history and population-level estimates of prevalence and heritability to improve power[1]. While there have been extensions to include covariates such as sex, age, and age-of-onset, these approaches require explicit changes to the generative model and additional prevalence information per covariate[2].

Methods: We develop a neural network—Liabilideep—with a flexible input layer that takes any set of covariates (including family history, related diseases, age, sex, etc.) and outputs a genetic liability that considers all covariates at the step of liability generation. The method optimizes a loss function that uses known GWAS SNPs and does not require estimates of heritability or prevalence.

Results: In simulations, Liabilideep reached over a median 3.5% increase in causal GWAS association statistics. On real data, using only age, sex and BMI, we observe an increase in chi-square statistics as high as 10% in GWAS of UKBB traits. Further, in simulations, we show that Liabilideep can optimally condition on covariates in ascertained data, leading to a 9.5% increase over previous approaches. Notably, aside from age and sex, current approaches cannot condition on covariates at the liability generation step, and therefore lose power in ascertained data when the covariate is causal and included in the association framework.

Conclusion: We present a flexible framework to generate genetic liabilities while leveraging covariates. Our approach is fast and can handle any number of covariates. Studies with ascertained data and GWAS with more extensive family history available may benefit from Liabilideep without needing to adjust models.

References:.

[1] https://doi.org/10.1038/s41588-020-0613-6.

[2] https://doi.org/10.1101/2021.04.20.440585.
Grants: NIH T32HG002536.
Conflict of Interest: None declared.

P25.029.A Multi-tissue TWAS identifies 137 novel gene associations with ageing outcomes in humans

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Background/Objectives: Previous genome-wide association studies (GWAS) have identified multiple genetic variants associated with human lifespan, however, only a few genome-wide significant ($P \leq 5 \times 10^{-8}$) loci, affecting lifespan have been pinpointed. An alternative approach to study human lifespan is through a gene-based association analysis called transcriptome-wide association study (TWAS), which integrates GWAS with gene expression data (eQTL), to boost statistical power and help narrow down GWAS loci, to identify causal gene candidates.

Methods: In this study, we performed a multi-tissue TWAS on human lifespan, using > 1 million parental lifespans from the UK Biobank and the LifeGen Consortium as our discovery study, and two replication TWASes on human longevity and healthspan (morbidity-free lifespan) traits, using S-MultiXcan software.

Results: Here we show the successful application of the TWAS approach to validate previous GWAS findings, and to identify several novel genes associated with ageing: 137 novel replicating genes (replication threshold $p < 0.05$) associated with either longevity or healthspan and 9 novel genes, including HTR3B, VARS, VWA7 and COASY associated with all the three ageing outcomes. We identified 109 unique gene clusters, of which 16 gene clusters were successfully fine-mapped (using FOGS), identifying the putative causal gene in the region.

Conclusion: Our results support the role of TWAS in uncovering novel trait-associated genes, and through our prioritised, putatively causal gene set, we provide novel insights into the mechanisms of human ageing.

References:.

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Conflict of Interest: None declared.

P25.030.B Polygenic scores for multiple definitions of depression explain independent variance in iPSYCH MDD

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Background/Objectives: Major Depressive disorder (MDD) is a complex, heterogeneous mental disorder with contributions from multiple genetic and environmental factors. Gene-mapping efforts have accelerated thanks to the rapid expansion of sample sizes enabled by including lightly phenotyped cases. This approach has implications for both gene-mapping and trait prediction, and debates about the best approaches for each goal are on-going.

Methods: We collected published GWAS for multiple definitions of MDD. We then computed polygenic scores (PGS) using each MDD GWAS in N = ~60,000 individuals genotyped as a part of the iPSYCH case-cohort study. We applied two PGS algorithms, pruning and thresholding (P+T) and SBayesR. We then used logistic regression to predict MDD in two iPSYCH cohorts, ranking the performance of each MDD GWAS + algorithm combination. Finally, we jointly fit all PGS in one multivariate logistic model to predict iPSYCH MDD.

Results: Consistent with previous reports, we saw clinical definitions of MDD predict less well out of sample, perhaps owing to their reduced sample sizes. Broad, shallow phenotyping and augmented phenotyping out-performed clinical MDD GWAS, perhaps because of expanded sample sizes. Importantly, when fit jointly, multiple PGS explain significant independent variance.

Conclusion: Multiple MDD PGS may capture independent components of etiology and can be combined to improve predictions. When performing gene-mapping it is important to identify definitions of MDD that are not 'too sticky' - they separate populations on core disease etiology. Strict predictions, however, can relax this requirement and should consider multiple, integrated predictors.

References:.

Grants: Lundbeck fellowship R335-2019-2318.

Conflict of Interest: None declared.

P25.032.D Non-coding rare variant trait associations in 127,724 UK Biobank genomes

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Background/Objectives: The recent growth of available whole genome sequences (WGS) offers an unprecedented opportunity to assess the contribution of rare genetic variation to a multitude of human traits and diseases. Although rare variation in exonic regions has been associated with hundreds of traits, the study of rare variation in non-coding regions – where most GWAS hits are found – is lagging behind. Indeed, as non-coding regions represent ~98% of the genome, the identification of genomic regions to focus on is crucial. In this respect, recent studies identified regulatory domains (e.g. groups of enhancers) orchestrating the expression of nearby genes, which could be key in aggregating the signal across multiple pertinent genomic regions.

Methods/Results: Here, we develop new approaches to perform rare variant association tests on non-coding regions of 127,724 genomes from the UK Biobank. In practice, we perform rare variant (MAF<0.1%) collapsing analyses on a plethora of regulatory element annotations on a per gene basis, matching cell-type-specific annotations with the relevant traits, such as blood cell-types with blood biomarkers. To further refine our search for relevant regulatory regions per gene, we use public multimodal single cell data (scRNA-seq + scATAC-seq in the same cells) to identify >5000 groups of regulatory elements that synchronously regulate gene expression.

Conclusion: By utilising functionally-relevant genomic annotations we are able to scan the non-coding human genome and pinpoint context-specific genetic variation that contributes to human traits and disease and aid the development of pertinent therapeutic agents.

References: Delaneau et al. 2019 Science.

Grants: Marie Sklodowska-Curie nº885998, SNF PP00P3_176977.

Conflict of Interest: None declared.

P25.034.B Identification of shared molecular signatures of ageing and metabolic diseases using multi-omic data

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Background/Objectives: Many of the well-defined hallmarks of ageing are often observed in age-related diseases such as Type 2 Diabetes (T2D). By utilising multi-omic data from human tissues and clinical datasets we can identify molecular phenotypes associated with ageing towards better understanding the link between ageing and disease.

Methods: We utilised data from the DIRECT consortium including genotypes (N~9.2e+10^6), transcriptomic (16,209 genes), targeted proteomic (373 proteins) and metabolomic (116 targeted metabolites) data from whole blood and plasma (3,029 participants). We evaluated the influence of age, T2D status, sex and BMI on each of the molecular phenotypes along with shared effects.

Results: We found 11,938 genes (73.7%) and 287 proteins (60.6%) differentially expressed with age (Qvalue < 5%). Of those, 163 transcripts and their corresponding proteins were associated with age, of which 78 pairs (~50%) demonstrated an opposite direction of effect of age. For example, *REG4* expression significantly decreases with age (Pval = 3.85e-50) while increasing in its protein abundance (Pval = 4.49e-14). However, the molecular phenotypes most affected by age were metabolites (81% of targeted metabolites). We additionally evaluated that sex had the greatest influence in gene expression (13,669 associated genes), while BMI greatly influenced protein abundance (304 proteins). Significantly associated proteins such as *IGFBP1* and *FABP4* were also independently associated with age, sex and T2D.

Conclusion: Most measured phenotypes are associated with age and sex in an independent manner. We are currently evaluating the changes of molecular phenotypes over time with longitudinal measurements spanning up to 48 months.

References:.

Grants:.

Conflict of Interest: None declared.

P25.035.C Estimation of efficiency low-pass whole genome sequencing on Indian population

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Background/Objectives: Several studies have suggested that low coverage whole genome sequencing (lcWGS) represents a powerful alternative to genotype arrays (GA) in traditionally under-studied populations. Moreover, methods to impute lcWGS using existing reference panels became recently available. Our aim is to compare lcWGS vs GA in their ability to capture common and population-specific variants in the Indian population. To do so we leveraged data from 2,768 individuals from the LASI-DAD study.

Methods: We consider 372 individuals for which 30X WGS (our gold standard) and GA (Illumina GSA) was available. We downsampled the 30X WGS data to simulate 0.5X,1X,2X, and 4X coverage using GATK. GLIMPSE was used for genotype refinement and imputation of lcWGS using 1) 1000genome reference panel and 2) ad-hoc reference panel built from 30x WGS of the remaining 2396 LASI-DAD individuals. GA were imputed using 1000genomes, TOPMed and the ad-hoc reference panel.

Results: lcWGS data imputed using 1000 genome as reference panel outperformed GA for all tested coverages (0.5X,1X,2X and 4X) both in terms of mean imputation quality and non-reference concordance with the gold standard (30X WGS). 11.14% more common and 15.55% more rare (AF < 1%) variants were discovered at 4x lcWGS compared to GA. We are current performing analysis using ad-hoc Indian-specific reference panel.

Conclusion: Our results so far demonstrate that low-coverage WGS is a cost-effective and viable alternative to genotyping arrays in non-European populations.

References: Martin et al., 2021.

LASI Wave 1, 2017-18, India Report, IIPS, Mumbai.

Rubinacci et al., 2021.

Grants: U01 AG064948, R01 AG05112.

Conflict of Interest: Ankita Srivastava Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland, Yuk Yee Leung Penn Neurodegeneration Genomics Center, Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Gerald Schellenberg Penn Neurodegeneration Genomics Center, Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Jinkook Lee University of Southern California, Los Angeles, CA, USA, Samuel Jones Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland, Andrea Ganna Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland.

P25.036.D A Genome Wide Association Study to investigate genetic risk modifiers of leg ulcers in adults with sickle cell disease

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Background/Objectives: Sickle cell disease (SCD) is an inherited recessive disorder that leads to abnormal sickle-shaped red blood cells. There are a range of clinical manifestations that result from this disease, including the leg ulcers sub-phenotype, which negatively impacts the quality of life of SCD patients. There may be genetic variants that modify the risk of developing SCD associated leg ulcers. SCD patients develop leg ulcers at a higher rate than those in the general population. However, not every patient with SCD will develop leg ulcers. The INSIGHTS study seeks to understand what

environmental or genetic factors are contributing to the increased risk of developing this sub-phenotype. The current project aims to detect modifier loci that may affect the risk of developing leg ulcers in a dataset of 264 SCD patients from the INSIGHTS study.

Methods: Whole genome sequencing was completed at NHGRI using the Illumina NovaSeq 6000 platform. Variant calling using GATK and association testing using PLINK has been completed on the first 121 samples (N = 53 with leg ulcers, N = 68 without leg ulcers).

Results: The strongest interim associations were on chromosome 21 near the TIAM1 gene (P-val = 1.01E-4) and on chromosome 20 near the ZFP64 gene (P-val = 1.08E-4).

Conclusion: Results from processing an additional 143 samples with this same pipeline will be reported in order to gain further insight into genetic variants that may modify the risk of developing leg ulcers in patients with SCD.

References:.

Grants: This work was supported by the Intramural Research Program of NIH/NHGRI.

Conflict of Interest: None declared.

P25.037.A An innovative approach to link genomic data with electronic administrative health databases, focused on the investigation of shared genetics among autoimmune diseases

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Background/Objectives: Multiple Sclerosis (MS) is a complex inflammatory-degenerative disease. GWASes identified >200 susceptibility loci, but they have poorly contributed to understand how genetics influences the phenotype as the reported comorbidity with other autoimmune diseases (ADs). Several AD loci were reported.

Aims:.

a) explore an innovative approach of linkage of genomic data with a large array of electronic administrative health databases (EAHDs) to test if genotype/phenotype associations can be successfully analyzed using EAHDs as well as in traditional studies.

b) test the hypothesis that MS patients share a common genetic susceptibility with other ADs, leading to the presence of comorbidity with several of them.

Methods: We constructed several algorithms to identify MS or other ADs patients from EAHDs. To explore the common genetic susceptibility among ADs we are comparing a battery of weighted genetic risk scores (wGRSs) constructed using known genetic susceptibility variants for several ADs in MS patients with or without autoimmune comorbidity.

Results: We created: 1)an algorithm capable of ascertain prevalent MS cases, validated by matching with a clinical cohort of 685 genome-wide genotyped MS patients with confirmed diagnosis (95.9% sensitivity, 99.97% specificity); 2) algorithms to identify other 14 ADs and evaluated their co-morbidity with MS.

We identified 8850 MS cases, 16.2% show a comorbidity with at least one AD: eight of these are significantly associated with MS ($P < 0.05$). Linkage with genotypic data and the AD wGRs construction is ongoing.

Conclusion: EAHDs can be successfully used to test associations in complex diseases. MS patients show comorbidity with other ADs.

References:.

Grants: Italian Ministry of Health (RF-2016-02361294 grant).

Conflict of Interest: None declared.

P25.038.B Deconstructing the genetic component of reported trauma with genomic structural equation modelling

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Background/Objectives: Self-reports of trauma are partly heritable. Heritable behaviours also influence reports of or exposure to environmental factors. Identifying heritable behavioural factors of self-reported trauma could improve our understanding of the interpretation of life events.

Methods: We calculated genetic correlations of childhood maltreatment (reporting of emotional, sexual, and physical abuse, and emotional, and physical neglect) and >700 traits to identify phenotypes that might explain the heritability of reported childhood maltreatment. We retained traits with $|r_g|>0.25$. We used genomic structural equation modelling to detect residual genetic variance in childhood maltreatment once genetically correlated traits were accounted for.

Results: More than half of the genetic component of childhood maltreatment could be accounted for by genetic correlations with twelve health traits (59%) and nine psychiatric disorders (57%). General risk tolerance, subjective well-being and autism spectrum disorder were the only traits independently associated with the genetic component of childhood maltreatment. These three traits alone explained 50% of the SNP-heritability of childhood maltreatment.

Conclusion: Our findings offer an explanation of the heritability of childhood maltreatment. The key contribution of three independently associated traits to the SNP-based heritability of reported trauma suggests that aspects of these phenotypes may be involved in gene-environment correlations or the retrospective reporting of trauma. Elucidating how these are linked with vulnerability or the subjective experience of trauma has the potential to improve trauma prevention and posttraumatic intervention strategies.

References:.

Grants:.

Conflict of Interest: None declared.

P25.039.C Understanding the genetics of autoimmune hypothyroidism

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Background/Objectives: Autoimmune hypothyroidism is common, with a prevalence of 3-5% in the general population[1]. Hypothyroidism is associated with an increased risk of various diseases and mortality[1]. Despite its estimated high heritability, little is known about the genetic basis of autoimmune hypothyroidism[1]. For this reason, we have performed the largest GWAS meta-analysis of autoimmune hypothyroidism to date.

Methods: We obtained GWAS summary statistics from nine cohorts, where cases and controls were defined using ICD9/10 codes. Reference genomes were used to impute missing genotype data[2,3]. We adjusted for covariates including age, sex, population structure, and related individuals. METAL was used to perform the meta-analysis[3].

Results: We performed the largest GWAS meta-analysis to date on autoimmune hypothyroidism, including 64,545 cases and ~500,000 controls. We identified more than 350 independent SNPs passing the GWAS significance threshold ($p = 5x10E-8$). Pathway analyses suggest involvement of known immune pathways as well as enrichment of associations in chromatin structure regulation.

Conclusion: Identification of novel genetic determinants is a key step in advancing the understanding of this common disease. These data will be a rich resource for future projects focusing on disease prediction, and personalized management, and treatment of autoimmune hypothyroidism.

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Conflict of Interest: None declared.

P25.041.A The joint phenotypic and molecular genetic architecture of major depressive disorder and anxiety disorders

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Background/Objectives: Anxiety and mood disorders occur comorbid and are main contributors to the burden of disability worldwide. By using factor analytical methods, we characterise latent genetic constructs underpinning anxiety and mood disorders.

Methods: We performed genomic structural equation modelling (gSEM) on European ancestry genome-wide association study (GWAS) summary statistics of 20 behavioural traits. To retain genetic signal we 1.) generated expanded linkage disequilibrium (LD) scores from the European subset of 1000 Genomes Phase 3 (excluding Finnish ancestry) genetic reference panel, and 2.) imputed missing genetic effect estimates based on LD structure in summary statistics. We obtained GWAS with each latent genetic factor by regressing individual variant effects on factors in a synthetic GWAS using gSEM, including 3,935,493 individuals and more than 7.7M genetic variants per putative factor trait. We annotated genes and performed pathway analysis, using MAGMA and FUMA.

Results: We identified six correlated latent genetic factors ($AIC = 3591$, $SRMR = 0.053$), explaining 59% of the variance. We identified 1,296 novel genetic variant associations and 281 gene associations not previously detected in univariate GWASs. Factor neuroticism and factor depression were both enriched in genes expressed in brain tissue: frontal cortex, anterior cingulate cortex, hypothalamus, putamen, nucleus accumbens, hippocampus, caudate nucleus, cortex, and amygdala. Factor neuroticism was additionally enriched in genes expressed in substantia nigra.

Conclusion: Our work uncovers specific biological processes, such as chromatin organisation, gene regulation, synaptic signalling, neuronal development and neurogenesis, potentially involved in anxiety and affective aetiology.

References:.
Grants:.
https://www.guysandstthomasbrc.nihr.ac.uk/researchers/funding-acknowledgement-policy/.

Conflict of Interest: None declared.

P26 COVID-19

P26.002.C Germline rare variants of lectin pathway genes predispose to asymptomatic SARS-CoV-2 infection in elderly individuals

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Background/Objectives: Emerging evidence suggests that complement system infection-dependent hyperactivation may worsen COVID-19 outcome. We investigated the role of predicted high impact variants - referred as Qualifying Variants (QVs) - of complement system genes in predisposing asymptomatic COVID-19 in elderly individuals, known to be more susceptible to severe disease.

Methods: Exploiting Whole-Exome Sequencing (WES) data and 56 complement system genes, we performed a gene-based collapsing test between 164 asymptomatic subjects (age ≥ 60 y.o.) and 56,885 European individuals from the gnomAD database. We replicated this test comparing the same asymptomatic individuals with 147 hospitalized COVID-19 patients.

Results: We found an enrichment of QVs in three genes (MASP1, COLEC10 and COLEC11), which belong to the lectin pathway, in the asymptomatic cohort. Moreover, individuals with QVs showed lower serum levels of Masp1 and of prothrombin activity compared to controls while no differences were observed for CH50 and AH50 levels that measure the activity of classical and alternative complement pathways, respectively. Finally, integrative analyses of genome-wide association study and expression quantitative loci traits data showed a correlation between polymorphisms associated with asymptomatic COVID-19 and decreased expression of MASP1, COLEC11 and COLEC10 genes in lung tissue.

Conclusion: This study suggests that rare genetic variants can protect from severe COVID-19 by mitigating the activation of lectin pathway and prothrombin activity.

References:.

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Conflict of Interest: None declared.

P26.003.D SARS-CoV-2 sequencing: A comparison of high-throughput methods

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Background/Objectives: The COVID-19 pandemic continues to threaten public health and burden healthcare systems worldwide. Whole SARS-CoV-2 genome sequencing has become essential for epidemiological monitoring and identification of new variants, which could represent a risk of increased transmissibility, virulence, or resistance to vaccines or treatment. In this study, we assess the performance of various target enrichment methods for whole SARS-CoV-2 sequencing.

Methods: We applied three target enrichment methods – two multiplex amplification methods and one hybridization capture – to the same set of nasopharyngeal patient samples (N = 93) in high-throughput mode. SARS-CoV-2 genome was obtained using short-read next-generation sequencing.

Results: All three methods provided excellent breadth of coverage of SARS-CoV-2 genome (above 99%), albeit with vastly different sequencing depth (5-fold difference) and uniformity of coverage (20% difference in coefficient of variation). Poor local coverage has negative impact on variant calling in the concerned region, leading to an occasional allele drop-out (1.2% SNPs affected for one method).

Conclusion: We discuss the performance of each target enrichment method and their potential for scaling up, in order to promote prospective programs of large-scale genomic surveillance of SARS-CoV-2 worldwide. Genomic surveillance will be crucial to overcoming the ongoing pandemic of COVID-19, despite its successive waves and continually emerging variants.

References:.

Grants: LabEx GENMED (grant number ANR-10-LABX-0013).

Conflict of Interest: None declared.

P26.005.B Design of a cost-effective diagnosis tool for SARS-Cov-2 variant detection through a next generation sequencing (NGS) based strategy

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Background/Objectives: Current pandemic situation, together with the continuous emergence of new SARS-CoV-2 variants reveal the need to develop a more versatile tool than PCR-based methods that allows both high throughput COVID-19 diagnostic and specific variant detection at reduced cost and fast turnaround times. Thus, with the aim of overcoming current test limitations and providing a strategy with these characteristics arises our novel next generation sequencing based approach.

Methods: The developed strategy works with RNA samples obtained from nasopharyngeal swabs. RNA samples are processed with our custom laboratory protocol and can be sequenced with any Illumina platform to generate results within a 24h timeframe. A tailored bioinformatic pipeline analyzes the data and generates a clinical-level report.

Results: Clinical validation results have shown that the designed solution, sensitively and specifically identifies negative

and positive samples that display a broad range in viral loads and readily identifies the following major SARS-CoV-2 variants of concern (VoC): Alpha, Beta, Gamma, Delta, Lambda and Omicron (BA.1 and BA.2).

Conclusion: The versatility of our solution allows the capability of identifying the presence of other common respiratory viruses as well as identifying patients at risk through the identification of susceptibility human variants in the host. This, together with the possibility of easily adding new VoC as they emerge, will make VoC monitoring in entire populations feasible, providing a new perspective on the application of NGS methods in the field of clinical microbiology.

References:.

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Conflict of Interest: Mª Mercedes Montero-Vale Pre-doc student, full-time, qGenomics, Pau Rodriguez-Sodupe Pre-doc student, full-time, qGenomics, Marina Alguacil-Guillén: None declared, Mateu Espasa-Soley: None declared, LLuís Armengol CSO, full-time, qGenomics, Juan Ramón González: None declared, Jairo Rodríguez R&D director, full-time, qGenomics.

P26.006.C A survival genome-wide association study to identify variants associated with COVID-19 risk of death

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Background/Objectives: SARS-CoV-2 infection clinical manifestations hugely vary among patients, ranging from no symptoms, to life-threatening conditions. This variability is also due to host genetics: COVID-19 Host Genetics Initiative identified six loci associated with COVID-19 severity in a previous case-control genome-wide association study. A different approach to investigate the genetics of COVID-19 severity is looking for variants associated with mortality, e.g. by analyzing the association between genotypes and time-to-event data.

Methods: Here we perform a case-only genome-wide survival analysis, of 1,777 COVID-19 patients from the GEN-COVID cohort, 60 days after infection/hospitalization. Case-only studies has the advantage of eliminating selection biases and confounding related to control subjects. Patients were genotyped using Illumina Infinium Global Screening Arrays. PLINK software was used for data quality check and principal component analysis. GeneAbel R package was used for survival analysis and age, sex and the first four principal components were used as covariates in the Cox proportional hazard model.

Results: We found four variants associated with COVID-19 patient survival at a nominal P < 1.0 x 10-6. Their minor alleles were associated with a higher mortality risk (i.e. hazard ratios (HR)>1). In detail, we observed: HR = 1.03 for rs28416079 on chromosome 19 (P = 1.34 x 10-7), HR = 1.15 for rs72815354 on chromosome 10 (P = 1.66 x 10-7), HR = 2.12 for rs2785631 on chromosome 1 (P = 5.14 x 10-7), and HR = 2.27 for rs2785631 on chromosome 5 (P = 6.65 x 10-7).

Conclusion: The present results suggest that germline variants are COVID-19 prognostic factors. Replication in the remaining HGI COVID-19 patient cohort (EGAS00001005304) is ongoing at the time of abstract submission.

References:.

https://doi.org/10.1038/s41586-021-03767-x.
https://doi.org/10.1038/s41431-020-00793-7.

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Conflict of Interest: None declared.

P26.007.D Transcriptomic analysis in patients with SARS-CoV-2 virus infection. Identification of the association of risk/protective profiles against the disease

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Background/Objectives: SARS-CoV2 causes the COVID-19 disease, capable of producing a severe acute respiratory syndrome. Several clinical variables and genetic variants have been related to a worse prognosis. The aim of this study is to measure if difference in the gene expression are associated with COVID-19 severity.

Methods: We performed RNA-seq Transcriptome in RNA extracted from lymphoblastoid cell line in 20 patients who require hospitalization (10 from the intensive care unit) in a GeneStudio S5 Plus Sequencer (Ion Torrent Technology). FASTQ files were obtained and trimmed using BBtools, BBduk for cutting, filtering and masking the data, and Dedupe for the elimination of duplicates.

Mapping and counting matrix was done in bash using the Salmon program. Differential expression analysis and subsequent functional enrichment was performed using Rstudio (DESeq2, ClusterProfiler, GO and KEGG).

Results: We observed that 2042 differentially expressed genes (1996 overexpressed, LFC>0 and 406 underexpressed, LFC<0) were obtained between patients who require hospitalization versus those in the intensive care unit. We found some genes previously SARS-CoV-2 associated (PGLYRP1, HDAC9 and FUT4). Furthermore, genes involved in the activity of the immune system and in inflammatory processes showed significant differences between cohorts (ABCF1 (LFC = -25.14, padj = 1.05e-13), ABHD16A (LFC = 25.00, padj = 1.05e-13) and IER3 (LFC = -24.45, padj = 2.43e-13)).

Conclusion: We described differential expression in genes of the immune system and inflammatory processes that might be have a role in the risk of develop severe symptoms of COVID-19, including admission in the intensive care unit. This results should be validated by additional functional studies.

References:.

Grants:.

Conflict of Interest: None declared.

P26.008.A A prospective study to evaluate the role of cholesterol metabolism related genes expression in predicting clinical outcome of patients with severe COVID-19

Katerina Basharova[1], *Tatiana Usenko[1;2], Anastasia Bezrukova[1], Daria Bogdanova[1], Michael Nikolaev[2;3], Artem Izumchenko[1], Elena Gavrilova[2], Irina Shlyk[2], Yuri Polushin[2], Petr Slominsky[4], Sofya Pchelina[1;2]*

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Molecular Genetics of National Research Center "Kurchatov Institute", Moscow, Russian Federation.

Background/Objectives: Latest studies have stressed the relevance of cholesterol metabolism with susceptibility to COVID-19 and its severity. We have previously shown downregulation of low-density lipoprotein particle receptor pathway in severe COVID-19 patient surviving compared to non-surviving(1). We aimed to assess the over-time expression changes of its relevant genes in severe COVID-19 patients with different outcomes (survivors/non-survivors).

Methods: Blood samples were taken from 39 severe COVID-19 patients without chronic diseases twice: on the day of admission to the intensive care (T1) and in one week (T2). Within 30-day follow-up 18 patients recovered and 21 patients died. 20 individuals never previously infected with COVID-19 were also enrolled. Expression levels of studied genes in peripheral blood lymphocytes were analyzed by real-time PCR with TaqMan assay.

Results: Increased expression of *STAB1* at T2, *PPARγ* at T1 and *CD36* at T1 and T2 were revealed in COVID-19 patients regardless of the outcome compared to controls ($p < 0.05$). Interesting, that in respective groups of COVID-19 patients increased *STAB1*, decreased *PPARγ* and in survivors decreased *LRP6* expression were revealed at T2 compared to T1 ($p < 0.01$). Also, *STAB1* expression was decreased in survivors compared to non-survivors at T2 ($p = 0.017$).

Conclusion: Our study revealed, that patients with severe COVID-19 are characterized by increased expression of cholesterol metabolism related genes, with more pronounced decrease of expression of these genes over time for survivors. Increased *STAB1* expression may be considered as a predictor of poor COVID-19 prognosis.

References: 1. Vlasov,I. et al. Transcriptomic Profiles Reveal Downregulation of Low-Density Lipoprotein Particle Receptor Pathway Activity in Patients Surviving Severe COVID-19. *Cells* 10,3495 (2021).

Grants:.

Conflict of Interest: None declared.

P26.009.B Persistent chemosensory dysfunction in COVID-19 patients: a deep dive into the psychophysical and genetic characterisation of an Italian cohort

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Background/Objectives: Chemosensory dysfunction is a hallmark of SARS-CoV-2 infection; nevertheless, the genetic factors predisposing to long-term smell and taste loss are still unknown.

This study aims to identify candidate genes possibly involved in persistent smell/taste loss through Whole Genome Sequencing (WGS) analysis of a large cohort of 130 fully characterised Italian individuals, previously diagnosed with COVID-19.

Methods: DNA of all analysed patients was used to perform WGS analysis, and a detailed personal anamnesis was collected. Moreover, orthonasal function was assessed through the extended Sniffin' Sticks test, retronasal function was tested with 20 powdered tasteless aromas, and taste was determined with validated Taste Strips. Self-reported smell and taste alterations were assessed via Visual Analog Scales plus questionnaires.

Results: The clinical evaluation allowed to classify the patients in two groups: 88 cases affected by persistent smell dysfunction (median age, 49) and 42 controls (median age, 51). Among cases, 26.1% ($n = 23$) were functionally anosmic and 73.9% ($n = 65$) were hyposmic. Within cases, 77 underwent the taste strip test: 53.2% ($n = 41$) presented hypogeusia and 46.8% ($n = 36$) were normogeusic. Preliminary WGS results on a first subset of 76 samples confirmed the important role of UGT2A1 gene, previously described as involved in smell loss. Interestingly, we identified a nonsense variant (rs111696697, MAF 0.046) significantly associated with anosmia in males (p-value: 0.0183).

Conclusion: Here, for the first time a large cohort of patients, fully characterised through a comprehensive psychophysical evaluation of smell and taste, have been analysed to better define the genetic bases of COVID-19-related persistent chemosensory dysfunction.

References:.

Grants:.

Conflict of Interest: None declared.

P26.010.C Mid-density Gene Expression profiling of SARS-COV-2 infected samples using Applied Biosystems™ TaqMan™ Flexible Array Panels

rui yang[1], Kelly Li[1], allan chen[1], phillip kilgas[1], katherine kirchmeier[1], ***Archana Gupta***[1]

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Background/Objectives: The broad spectrum of clinical manifestations from SARS-COV-2 infection and observed risk factors for severe disease highlight the importance of understanding molecular mechanisms underlying SARS-CoV-2 associated disease pathogenesis. Research studies have identified a large number of host proteins that play roles in viral entry, innate immune response, or immune signalling during infection. The ability to interrogate subsets of these genes simultaneously within SARS-COV-2 infected samples is critical to understanding how their expression contribute to phenotypic variability of the disease caused by the pathogen.

Methods: 30 Nasopharyngeal swab were obtained and included SARS-CoV-2 infected and control samples. RNA was extracted, reverse transcribed and loaded onto flexible TaqMan array panels designed specifically for targeting the most cited genes related to SARS-COV-2 entry and restriction factors as well as cytokines, chemokines, and growth factors involved in the pathogenesis.

Results: Our data indicated that not only were the levels of several of these host factors differentially modulated between the two study groups, but also that SARS-CoV-2 infected subjects presented with greater frequency of several important inflammatory cytokines and chemokines such as CCL2, CCL3, IFNG, entry receptors such as ACE2, TMRPS11A, and host restriction factors including LY6E and ZBP1.

Conclusion: TaqMan array plates provide a fast, mid-throughput solution to determine the levels of several virus and host-associated factors in various cell types and add to our understanding of how the pathogenesis may vary depending on gender, age, infection site etc.

References:.

Grants:.

Conflict of Interest: rui yang Thermo Fisher Scientific, Kelly Li Thermo Fisher Scientific, allan chen Thermo Fisher Scientific, phillip kilgas Thermo Fisher Scientific, katherine kirchmeier Thermo Fisher Scientific, Archana Gupta Thermo Fisher Scientific.

P26.011.D Gain and loss of function CFTR alleles modulate COVID-19 clinical outcome

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Pastorino[5], Mirella Bruttini[1;2;3], Nicola Picchiotti[6;7], Simone Furini[2], Gen-Covid Multicenter Study[1], Chiara Gabbi[8], Alessandra Renieri[1;2;3]

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Background/Objectives: We previously demonstrated that carrying a single pathogenic CFTR allele increases the risk for COVID-19 severity and mortality rate. We now aim to clarify the role of several uncharacterized rare alleles, including complex (cis) alleles, and in trans combinations.

Methods: LASSO logistic regression was used for the association of sets of variants, stratified by MAF, with severity. Immortalized cystic fibrosis bronchial epithelial cell lines and Fischer Rat Thyroid cells were transfected by plasmid carrying specific *CFTR* mutations. YFP-based assays were used to measure CFTR activity.

Results: Here we functionally demonstrate that the rare (MAF = 0.007) complex G576V/R668C allele mitigates the disease by a gain of function mechanism. Several novel *CFTR* ultra-rare (MAF <0.001) alleles were proved to have a reduced function; they are associated with disease severity either alone (single or complex alleles) or with another hypomorphic allele in the second chromosome, with a global reduction of CFTR activity between 40 to 72%.

Conclusion: *CFTR* is a bidirectional modulator of COVID-19 outcome. At-risk subjects do not have open cystic fibrosis before viral infection and therefore are not easily recognisable in the general population unless a genetic analysis is performed. As the CFTR activity is partially retained, CFTR potentiator drugs could be an option as add-on therapy for at-risk patients.

References: Baldassarri M et al. Severe COVID-19 in Hospitalized Carriers of Single CFTR Pathogenic Variants. JPM..11,6 558. 15 Jun. 2021.

Grants: FISR 2020/Tuscany Region COVID-19/INTERVENE - GA No. 101016775/Soka Gakkai PAT-COVID.

Conflict of Interest: None declared.

P26.013.B COVID-19: How to analyze data from whole-exome sequencing?

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Background/Objectives: COVID-19 can affect anyone with the disease's symptoms ranging from mild to very severe. Although environmental, clinical, and social factors play an important role in the disease process, host genetic factors are not negligible either. In the present article, we attempted to elaborate on the spectrum of risk variants and genes identified in different ways and their possible relationship to COVID-19 severity and/or mortality.

Methods: We present three different approaches to search host genetic risk factors that influence the development of COVID-19 disease. First, we analyzed the exome sequencing data obtained from Slovak patients who died of COVID-19. Second, we selected risk factors/genes that were associated with COVID-19. Finally, we compared each group of found risk variants with data from dead patients and two control groups, worldwide public data of the Non-Finnish European population from the gnomAD database, and genetic data from Non-invasive prenatal testing in the Slovak population.

Results: We illustrate the utility of genomic data showed strong association in meta-analyses conducted by the COVID-19 HGI Browser.

Conclusion: To our knowledge, the present study is the first population analysis of COVID-19 variants worldwide and also in the Slovak population that provides different approaches to the analysis of genetic variants in whole-exome sequencing data from patients who have died of COVID-19.

References:.

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Conflict of Interest: None declared.

P26.014.C Identification of genetic determinants of COVID-19 severity by exome sequencing

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Background/Objectives: The severity of the symptoms of coronavirus disease 2019 (COVID-19) has been associated to age, comorbidity, and male sex. Besides virus characteristics, host genetic factors influence the infection outcome. Different genome-wide association studies and meta-analyses investigated the contribution of common variants, whereas the role of rare variants just started to be elucidated. Our goal is to determine the contribution of rare variants to the development of severe COVID-19 in the Italian population.

Methods: We compared the genetic background of 215 severe COVID-19 patients with 1764 individuals from the general population. Rare variants (minor allele frequency <1%) from whole-exome sequencing data were retrieved using a bioinformatics variant discovery pipeline. We tested the impact of rare variants (classified according to their predicted effect on the gene product) both using a burden test design, and an iterative machine learning (ML) approach.

Results: We identified a total of 690,000 rare variants in the entire examined population. Among them, 250 were associated with COVID-19 severity at a nominal $P < 0.05$. Gene-based burden test revealed a gene with an excess of loss-of-function mutations

at P < 0.05. Finally, the ML approach, analysing all the 690,000 rare variants, identified the best combination of variants that is able to predict COVID-19 severity in our cohort.

Conclusion: Our work provides new insights on the genetic signature of COVID-19 in the Italian population. The most informative rare variants could be exploited to define individuals' risk profiles to COVID-19 severity for the Italian population.

References:.

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P26.016.A Plasma cytokine profile in patients with severe COVID-19

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Background/Objectives: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease (COVID-19) enters the lung tissue through exocytosis, leading to the release of a large amount of pro-inflammatory cytokines called 'cytokine storm'. The aim was to provide more insight into relationship between plasma cytokines profile and fatal outcome of COVID-19.

Methods: Plasma cytokines (IL-17F,GM-CSF,IFNg,IL-10,CCL20/MIP3a,IL-12P70,IL-13, IL-15,IL-17A,IL-22,IL-9,IL-1b,IL-33,IL-2,IL-21,IL-4,IL-23,IL-5,IL-6,IL-17E/IL-25,IL-27,IL-31,TNFa,TNFb,IL-28A) were detected in 30 patients with severe COVID-19 by a Luminex assay system with Milliplex Human Th17 Magnetic Premix 25 Plex Kit (HT17MG-14K-PX-25, Merk-Millipore, USA) according to the instructions. Patients were followed up for 30 days since admission to intensive care. 18 patients died and 12 patients survived during the period of observation. The control group comprised 10 individuals who had never been diagnosed with COVID-19.

Results: IL-10 and CCL20/MIP3a plasma levels were elevated in non-survivors patients with COVID-19 compared to controls ($p = 0.0027$, $p = 0.012$, respectively). IL-15, IL-6, IL-27 plasma levels were higher in survivors with COVID-19 compared to controls ($p = 0.049$, $p = 0.026$, $p = 0.00032$, respectively). Interestingly, IL-15, IL-27 plasma levels were increased in non-survivors with COVID-19 compared to controls and survivors with severe COVID-19 (IL-15: $p = 0.00098$, $p = 0.00014$, respectively; IL-27: $p = 0.011$, $p < 0.0001$, respectively). Receiver operating characteristic (ROC) analysis has been conducted for IL-15 and IL-27. Cut-off value was estimated as 25.50 pg/ml for IL-15 and 1.51 pg/ml for IL-27.

Conclusion: Our study demonstrated a more pronounced immune response in non-surviving patients with severe COVID-19. IL-15, IL-27 could be considered as a sensitive biomarker of the fatal outcome from COVID-19.

References:.

Grants:.

Conflict of Interest: None declared.

P26.017.B Whole genome sequencing of a German cohort to understand host genetics in COVID-19

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Background/Objectives: The disease course upon SARS-CoV-2 infection is highly variable and comprises a range from asymptomatic infection to severe (and even lethal) COVID-19. Genetic factors substantially contribute to this variability, as evidenced by epidemiological studies and recent results from genome-wide association studies (GWAS) as well as sequencing-based approaches. The host genetics group of the German COVID-19 OMICs Initiative (DeCOI) has been founded with the aim to identify additional genetic variants that influence COVID-19 severity through whole genome sequencing (WGS) analyses.

Methods: Until January 2022, WGS has been performed on approximately 1200 individuals affected by COVID-19.

Results: The most recent data freeze comprised 952 individuals. In this dataset, no carrier of a deleterious protein-altering variant has been detected in *TLR7*, which is the only conclusive risk gene for severe COVID-19. Applying a gene-based association test of rare variants to the subcohort of European individuals ($n = 752$, mean age: 56 years, females: 44%), including 199 severely affected individuals, we did not observe any significant association after correction for multiple testing. Exome-wide association analysis of common variants in this subcohort replicated the GWAS-locus on chromosome 3.

Conclusion: With this ongoing work, we are contributing to international efforts to elucidate the host genetics of COVID-19, also by sharing our summary statistics for meta-analyses. Currently, we are sequencing additional severely affected individuals and we are refining analytical strategies, which will also include the joint analysis of common and rare variants at genome-wide scale.

References:.

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P26.018.C Strong impact of the COVID-19 pandemic on fetal development and chromosomal abnormalities

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Background/Objectives: To date, not many studies have been conducted to examine the role of COVID-19 on gestation and fetal development. During COVID-19, pregnant women had difficulty accessing prenatal screening and care due to pandemics restrictions and lockdowns. In this retrospective study we aimed to assess the effect of the SARS-CoV-2 outbreak on fetal development in both prenatal and postnatal outcomes pre- and pre-COVID-19 pandemics in Northern Cyprus.

Methods: A total number of 61 aborted materials were karyotyped during the pre-pandemic period (January 2017 and March 2020) whereas 24 samples were analysed during the peri-pandemic period (March 2020-November 2021). On the other hand, 25 new-borns blood samples during the pre-pandemic and 44 samples during the pre-pandemic period were analysed.

Results: No statistically significant difference found in health and abnormal aborted material karyotypes between two periods. On the other hand, a statistical significance was observed in postnatal chromosomal abnormalities ($P = 0.04$) after two long pandemic lockdowns, which are known as the first and the second waves, dramatically indicating that no baby with Down syndrome was between 2017-2020 whereas seven babies with Down Syndrome were born as consequences of without taking precaution against lockdowns.

Conclusion: Overall, prenatal care is failed which resulting increased postnatal chromosomal abnormality due to heavy flight restrictions, economic inflation instability, limited access to medical services during COVID-19 pandemic lockdowns in Northern Cyprus.

References:.

Grants:.

Conflict of Interest: None declared.

P26.019.D Carnosic acid as potential anti-SARS-CoV-2 therapeutics: a mechanistic insight

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Background/Objectives: Rosmarinus Officinalis L.(Rosemary) extract Carnosic acid(CA) has been investigated for its antimicrobial and antioxidative properties(1). Only limited number of publications reported the utilization of this extract in SARS-CoV-2 infection. Also, the mechanistic understanding of CA remains to be determined. Our goal was to elucidate the potential role of CA in COVID19. To obtain mechanistic insight of pharmacogenomic action of CA, comprehensive in silico analyses were performed. Further in vitro experiments were done to illustrate the cytotoxicity of CA and confirm in silico findings.

Methods: CA was extracted from Rosmarinus Officinalis L. by HPLC. Stimulation assays were performed using the COVID19 samples. In silico pharmacogenomic properties of CA were performed by using SwissADME. SwissTargetPrediction tool was utilized to define the possible targets. SARS-CoV-2-interacting proteins were evaluated using STRING(2). To verify in silico findings, gene expression levels were analyzed using qPCR.

Results: Among the top 15 SwissTargetPrediction target molecules(out of 100), Prostaglandin E synthase(PTGES) had the highest probability for CA. Among 332 proteins identified using the STRING, PGES2 was found to be interacting with the nsp7, important molecule for viral replication. The stimulation assays and gene expression analyses confirmed the viral inhibitory role of CA through PTGES pathway.

Conclusion: To our knowledge, our work is the first to reveal the inhibitory role of CA in COVID19 through PTGES pathway. Given the crucial role of PTGES in inflammation, it is noteworthy to examine CA as potential anti-SARS-CoV2 therapeutics.

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Grants:.

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