ABSTRACTS COLLECTION

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

e-Posters

EP01 Reproductive Genetics

EP01.001 Correlations between cytogenetic findings and spermatogenic failure in Bulgarian infertile men

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Background/Objectives: Chromosomal aberrations have a great impact on spermatogenesis, semen quality, and successful conception. The objective of our study was to determine the type and frequency of chromosomal aberrations and polymorphisms in men with different degrees of spermatogenic failure in comparison to men with normozoospermia, in order to find some correlations between cytogenetic findings and the abnormal results of semen analysis.

Methods: In our study, we have performed cytogenetic analysis in 901 infertile men, divided into 5 groups according to semen analysis—normozoospermia, asthenozoospermia, oligoasthenozoospermia, severe male factor and azoospermia.

Results: The frequency of polymorphisms was similar in all groups (11–16%, without significant differences). The frequency of numerical and structural aberrations increases with the degree of the spermatogenic failure (3.5% in normozoospermia, 5.6% in asthenozoospermia, 9.8% in oligoasthenozoospermia, 9% in severe male factor and 13.5% in azoospermia). We have found significantly higher incidence of numerical chromosome aberrations in severe male factor (7%) and azoospermia (9.3%). Oligoasthenozoospermia was associated with chromosomal translocations, as it occurs in 45% of cases with translocation, compared to 20% in the group with normal karyotype.

Conclusion: We revealed that chromosomal translocations are significantly associated with oligoasthenozoospermia, whereas numerical chromosomal aberrations—with severe male factor and azoospermia. These are important aspects of genetic counseling for those cytogenetic findings. Chromosome polymorphisms don't seem to disturb significantly spermatogenesis and their impact should be studied in regard to unsuccessful pregnancy achievement, even in patients with normozoospermia.

References:

Grants:

Conflict of Interest: None declared.

EP01.002 Comparison of carrier status among patients with or without family history of disease using targeted and expanded panels

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Background/Objectives: Targeted carrier screening (CS) based on ethnicity or family history (FH) is common. However, many families lack background ethnic or genetic information. In this study, we compared the impact of using expanded versus targeted disease panels on patients with and without patient reported FH.

Methods: Results from Horizon™ CS with 27-genes ($N = 19,397$) or 274-genes ($N = 58,327$) performed between January 2020–June 2021 were analyzed. FH among carriers was determined by ICD-10 code Z84.81. Cases without an ICD10 code were excluded.

Results: Among 77,724 individuals undergoing CS, those with FH were more likely to be carriers (59.3%; 2598/4,379) than those without FH (55.4%; 40,623/73,345). However, the 274-gene panel was more likely to identify carriers with FH (67.6%; 2236/3309) or without FH (65.0%; 35,745/55,018) than the 27-gene panel (33.8%; 362/1,070 and 26.6%; 4,878/18,327, respectively). Interestingly, patients without FH were more likely to be carriers of variants in a single gene (34.3%; 18,862/55,018) than patients with FH (31.9%; 1055/3309) ($p < 0.05$).

Conclusion: In this cohort, FH was associated with an increased positive rate. However, the expanded panel was better at identifying carriers than the targeted panel regardless of FH, and was more effective at identifying variants in a single gene without FH. Taken together, these results indicate that the use of expanded CS is more effective than targeted CS.

References:

Grants:

Conflict of Interest: Karen Phaik Har Lim Full-time employee of Natera Inc., Option to own stock in Natera Inc., Bridgette Meyers Full-time employee of Natera Inc., Option to own stock in Natera Inc., Ezen Choo Full-time employee of Natera Inc., Option to own stock in Natera Inc., Trevor Smart Full-time employee of Natera Inc., Option to own stock in Natera Inc., Sindhu Arun Full-time employee of Natera Inc., Option to own stock in Natera Inc., Nina Sanapareddy Full-time employee of Natera Inc., Option to own stock in Natera Inc., Dianne Keen-Kim Full-time employee of Natera Inc., Option to own stock in Natera Inc.

EP01.003 Chromosomal heteromorphisms as a risk factor for reproductive failure

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Background/Objectives: Chromosomal heteromorphisms are normal variations, which could be detected at specific chromosomal regions. Despite being considered normal findings, it is possible that these heteromorphisms are associated with reproductive failure (RF).

Methods: A total of 661 women with unexplained RF (primary infertility and recurrent miscarriages) and 139 female control subjects, who had at least one child, were investigated by conventional cytogenetic analysis. GTG differential banding technique was applied, and approximately 10 metaphases were analyzed per patient. A t-test was performed.

Results: Mean age of the patients with RF was 33.00 years, and of the control subjects—29.95 years. 61 (9.38%) of the case subjects had a chromosomal heteromorphism, and of the control subjects—seven (5.03%). The distribution of the chromosomal heteromorphisms between the two groups showed a statistically significant difference ($p \leq 0.05$).

The most common heteromorphism among the women with RF was 21ps+ - 12 (19.67%), followed by inv(9)(qh) – 11 (18.03%), 16qh+ - 10 (16.39%), 9qh+ - 7 (11.47%), 22ps+ - 7 (11.47%). The rest of the polymorphisms involved the heterochromatin of chromosomes 1, 13, 14, and 15. For the control group, 21ps+ was also the most common polymorphism – 3 (42.86%), followed by 9qh+ - 2 (28.57%). There was one inv(9)(qh) and one 1qh+.

Conclusion: Our results showed a statistically significant difference between the distribution of chromosomal heteromorphisms among the two groups. These chromosomal heteromorphisms could be considered a risk factor for reproductive failure. This illustrates the importance of cytogenetic analysis in the investigation of patients with infertility.

References: Not applicable.

Grants: Not applicable.

Conflict of Interest: None declared.

EP01.004 Development of a nationwide study to identify monogenic causes of female idiopathic reproductive failure

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Background/Objectives: In Europe, the mean maternal age at the time of childbirth has increased and the proportion of women experiencing reproductive failure is growing. Premature ovarian insufficiency (POI) refers to menopause before the age of 40 due to diminished ovarian reserve. Often, recurrent miscarriages (RM) represent a prelude to POI due to possible overlapping molecular etiologies[1]. Although rather common conditions (~1% of women), knowledge on (mono)genic causes of POI/RM is limited, hindering patient management.

Methods: We aim to develop a nationwide cohort of idiopathic POI and RM cases to be systematically analyzed for genetic causes, using exome sequencing of patients and their affected family members. Inclusion criteria follow international guidelines: menopause <40 yrs/3 or more miscarriages; anamnesis, family history, questionnaire, relevant laboratory findings (e.g. FSH > 25 IU/l).

Results: The study pipeline to recruit idiopathic POI/RM patients has been developed at the Women's Clinics of Tartu University Hospital and East-Tallinn Central Hospital. Additional affected cases were identified from the Estonian Biobank (EstBB) database. From 768 EstBB participants with the ICD-10 diagnosis of POI, only 21 fit our stringent study criteria.

Conclusion: Research of idiopathic POI and RM is challenged due to insufficient clinical data to distinguish genetic *vs* non-genetic causes, and highly intimate nature of these conditions. Genetic research is alerted as timely counselling of women with high risk to POI/RM will have immediate impact to their reproductive decision making.

References: 1. Dean et al. (2018) J Assist Reprod Genet 35:2121–8.

Grants: PRG 1021 (Estonian Research Agency).

Conflict of Interest: None declared.

EP01.005 Aberrant hypomethylation of imprinted differentially methylated regions is involved in biparental placental mesenchymal dysplasia

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Background/Objectives: Placental mesenchymal dysplasia (PMD) is often associated with androgenetic/biparental mosaicism (ABM), and is frequently complicated by Beckwith–Wiedemann syndrome. These phenomena suggest an association between PMD and aberrant genomic imprinting, likely involving CDKN1C and IGF2. Another type of PMD involving the biparental genome has also been reported. However, the frequency and etiology of biparental PMD are still not fully understood.

Methods: Twenty-five macroscopic PMD specimens were genotyped by DNA microarray or short tandem repeat analysis. DNA methylation analysis using bisulfite pyrosequencing was performed on 15 placenta-specific imprinted differentially methylated regions (DMRs) and 36 ubiquitous imprinted DMRs. Allelic expression of imprinted genes was examined by RT-PCR using single nucleotide polymorphisms. Whole exome sequencing (WES) was performed on four biparental PMD specimens.

Results: Genotyping revealed that approximately 30% of macroscopic PMD specimens were biparental, while the rest exhibited ABM. DNA methylation of most DMRs in PMD specimens with ABM displayed the paternal epigenotype. Importantly, biparental PMD specimens exhibited aberrant hypomethylation at six placenta-specific DMRs. Five imprinted genes associated with these DMRs were biallelically expressed. Aberrant hypomethylation was also observed at eight ubiquitous DMRs, including GRB10, but not ICR1 or ICR2, which regulate the expression of IGF2 and CDKN1C, respectively. WES did not reveal any pathological genetic abnormalities.

Conclusion: Our data clarify the prevalence of biparental PMD and ABM-related PMD. The results strongly implicate that the hypomethylation of DMRs, including placenta-specific DMRs and GRB10, but not ICR1 or ICR2, plays a role in the pathogenesis of biparental PMD.

References:

Grants: JSPS, AMED, MHLW.

Conflict of Interest: None declared.

EP01.006 Different IVF culture media do not affect the methylome of IVF children

Rebekka Koeck[1;2], *Jorg Tost*[3], *Florence Busato*[3], *Dimitri Consten*[4], *Jannie van Echten-Arends*[5], *Sebastiaan Mastenbroek*[6], *Yvonne Wurth*[4], *Heleen Zandstra*[2], *Ron van Golde*[2], *John Dumoulin*[2], *Han Brunner*[2;7], *Aafke van Montfoort*[2], *Masoud Zamani Esteki*[1;2]

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Background/Objectives: A growing number of children born are conceived through in vitro fertilization (IVF) procedures that have been linked to increased risks of adverse perinatal outcomes and altered growth profiles in the resultant children. These outcomes are also influenced by the media used for embryo culture and this effect is hypothesized to be mediated epigenetically, e.g. through DNA methylation. Therefore, we investigated the methylome in IVF offspring who underwent embryo culture in different media.

Methods: We profiled the umbilical cord blood (UCB) methylome of 106 IVF-neonates cultured in G5 or HTF, and the saliva methylome of 120 9-year-old IVF children, cultured in G3 or K-SICM, using the Infinium Human Methylation EPIC BeadChip. Analyses were carried out separately on UCB and saliva samples using empirical Bayes moderated mixed effects linear models adjusted for potential confounders. Methylation outliers represent values more than three interquartile ranges from the upper or lower quartiles.

Results: In both comparisons (UCB and saliva) we identified no significant methylation differences between the culture medium groups in terms of: (i) systematic differences at single CpG sites or regions, (ii) imprinted sites/genes or birth weight associated sites, (iii) stochastic differences presenting as DNA methylation outliers or differentially variable sites, and (iv) epigenetic gestational age acceleration (UCB samples only).

Conclusion: The IVF culture media investigated did not lead to methylome difference in the resultant neonates/children, suggesting that any culture medium induced epigenetic alterations resolve prenatally. To investigate environmental-epigenetic interactions occurring earlier during human development, epigenetic profiling of preimplantation embryos is required.

References:

Grants: March of Dimes (6-FY13-153).

Conflict of Interest: None declared.

EP01.007 Mosaic runs of homozygosity in first trimester spontaneous abortions with normal karyotype

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Background/Objectives: Runs of homozygosity (ROH) are considered as pathogenic factor for human health. It's deleterious effect can be realized through the homozygotization of recessive mutations or imprinting disorders, highlighting the need to map such regions. Given the high frequency of mosaicism in embryos and the limitations of aCGH in detecting it, we decided to analyze different tissues of embryonic and extraembryonic origin, including parental samples.

Methods: Eleven trios (mother, father, SA) were investigated by SurePrint G3 Human CGH+SNP 4 × 180K microarrays (Agilent Technologies, USA). Euploid placental tissues were separated into chorionic villi (CV) and extraembryonic mesoderm (EM).

Results: Twenty-four ROHs were found in 10 samples. Seventeen ROHs were present in both tissues, while two were confined to CV (5q14.3, 8p23.3-23.1) and five to EM (4q28.2-28.3, 8q11.21-q11.23, 8q24.11-q24.12, 18q12.3, 18q12.1-12.2). Since none of the ROHs were inherited, we hypothesized that they resulted from a combination of identical parental haplotypes. SNP-analysis showed no uniparental disomy within homozygous regions. We hypothesize that a trisomy rescue could have occurred before divergence of embryonic and extraembryonic lineages. As a result, in one cell lineage a chromosome with a different haplotype was lost leading to ROH, and in another a chromosome with the same haplotype was lost keeping heterozygosity within the region. Therefore, ROH would be found in one tissue and heterozygosity of the same region in another.

Conclusion: This study highlights the underestimated level of mosaic ROHs in abnormal human reproduction.

References: non.

Grants: Russian Science Foundation №21-65-00017, https://rscf.ru/project/21-65-00017.

Conflict of Interest: None declared.

EP01.008 A significant drop in sperm DNA fragmentation in a complete teratozoospermic patient post smoking cessation and its impact on ART outcome: case report

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Background/Objectives: We assessed Sperm DNA Fragmentation in patients with 100% abnormal sperm morphology and its correlation with live birth rate post Intracytoplasmic Sperm Injection. One of our initial cases did not conceive after treatment. During his first visit, we advised him to quit smoking in addition to a previous treatment that included oral antioxidant was already being administrated. After three months of this combined approach, Sperm DNA fragmentation and semen parameters were re-evaluated. A second round of ICSI was performed that resulted in a healthy live birth. We are reporting this finding as a case report.

Methods: We evaluated the Sperm DNA fragmentation using sperm chromatin dispersion test and sperm structure using MSOME test prior the second round of Intracytoplasmic Sperm Injection.

Results: Sperm DNA fragmentation dropped after smoking cessation from 49% to 29%. Abnormal sperm morphology changed from complete teratozoospermia (100%) to severe teratozoospermia (97%). A live birth was achieved.

Conclusion: Although routine Sperm DNA fragmentation testing is not yet recommended in clinical practice, we concluded that it is of critical value in complete teratozoospermia patients. A combined approach of antioxidants and life style changes has a significant impact on sperm morphology, sperm DNA integrity and live birth post Intracytoplasmic Sperm Injection.

References:

Grants:

Conflict of Interest: None declared.

EP01.009 FOXL2 gene deletion in a patient from Assisted Reproduction Unit detected by the a-CGH technique

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Background/Objectives: Assisted reproduction guidelines consider the genetic evaluation of patients with reproductive difficulties by karyotyping, however, after a first evaluation of other techniques in patients with striking characteristics, a-CGH can be a technique of great value. We present the case of a 19-year-old woman with primary infertility of 5 years of evolution, with low ovarian reserve detected by transvaginal ultrasound and biochemical determination for AMH of 0.94 ng/ml (0.96–13.34 ng/ml).

Methods: GTG-banding karyotype was performed according to standard protocols. Twenty metaphases chromosomes were examined. PCR screening of CGG repeats in *FMR1* gene. Array-CGH analysis was performed by a CGXTM HD v1,1 4-plex array 180k (PerkinElmer), with an average resolution of 40 kb in the backbone and 20 kb in the regions of interest.

Results: Karyotype result: female, 46,XX. CGG repeats in FMR1 gene normal profile. A-CGH: arr[GRCh37] 3q22.3(138662657_138665307)x1; 2.65 kb deletion in chromosomal region 3q22.3 that includes the *FOXL2* gene described as crucial in ovarian development. Mutations in this gene, such as truncating mutations, have been associated with female infertility with an autosomal dominant inheritance pattern.

Conclusion: a-CGH can be considered as a screening technique for genome analysis in patients whose gynaecological and biochemical parameters may indicate the presence of genetic factors not assessed by karyotyping and can assist patients and physicians in making more appropriate and effective reproductive decisions.

References:

Grants:

Conflict of Interest: None declared.

EP01.010 A novel heterozygous variant in the DNA binding DM domain of the DMRT1 associated with Sertoli cell-only syndrome

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Background/Objectives: Sertoli cell-only syndrome (SCOS) is the most severe form of male infertility accompanied by complete spermatogenic failure. It may arise as a consequence of genetic abnormalities, especially if detected in the family. The aim of this study was to determine the potential genetic cause of infertility in two SCOS brothers that had no common infertility-related genetic abnormalities.

Methods: Two brothers were subjected to testicular biopsy and diagnosed with SCOS. DNA was isolated from the peripheral blood and whole-exome sequencing was performed. Variants were called, annotated, while those variants with a frequency higher than 1% in databases, intron, and synonymous were removed. The remaining variants were assessed by the Exomiser software and researched in literature. Selected variants were confirmed in brothers and their parents by Sanger sequencing.

Results: A novel heterozygous missense variant was found and confirmed in the DNA binding DM domain of the DMRT1 gene (p.Pro74Leu; chr9:g.842059C>T) shared by brothers. Their mother was heterozygous for the same variant, while the father was not.

Conclusion: The DMRT1 gene is already associated in the literature with gonadal dysgenesis and male infertility. However, the novel P74L variant shared by the brothers is the first one reported in a familial case of male infertility and in the DM domain that directly interacts with the DNA.

References: Murphy MW, Lee JK, Rojo S, Gearhart MD, Kurahashi K, Banerjee S, et al. An ancient protein-DNA interaction underlying metazoan sex determination. Nature structural & molecular biology. 2015;22(6):442-51.

Grants: No. KK.01.1.1.01.0008.

Conflict of Interest: None declared.

EP01.013 Validation analysis of a non-invasive pre-implantation genetic test for aneuploidy (niPGT-A)

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Background/Objectives: Invasive techniques as polar body-, blastomer-, trophectodermbiopsy or blastocentesis are used in preimplantation genetic diagnosis to obtain embryonic DNA. The finding of cell-free embryonic DNA (cfDNA) in culture media samples from blastocysts (BCM) opened new possibilities, since invasive interventions on the embryo could be avoided and false findings due to mosaic constellation could be reduced.

Methods: Non-invasive PGT-A test based on MALBAC amplification, Analyses of 1.10 genomic DNAs with known complete and partial aneuploidies diluted to single cell level, 2.5 prenatal samples from native amniotic fluid supernatants, 3.20 BCM samples.

Results: In genomic DNAs as well as in amniotic fluid supernatants all aberrations could be confirmed. The concordance rate was 100%. In the analysis of BCM, the cfDNA showed a complete / partial concordance rate of ~88% with regard to the aberrant chromosomes to the corresponding DNA from biopsied TE cells.

Conclusion: The technical and analytical reliability of the analysis platform, kit and software have been tested. In the first analyses (genomic DNA and amniotic fluid), all aberrations could be reliably detected. The final aim was to determine the efficiency and concordance rate between the cfDNA from BCM samples and the DNA from TE cell biopsies of the same embryo. Our analyses results show a high level of concordance in comparison to results of TE analysis with regard to the affected chromosomes. niPGT-A is a promising new method for non-invasive aneuploidy screening in the context of artificial reproductive treatment.

References:

Grants:

Conflict of Interest: None declared.

EP01.015 Telomere length in spermatogenic cells from azoospermic patients is characterized by intercellular, but not interindividual variability

Mikhail Krapivin[1], *Olga Efimova*[1], *Yanina Sagurova*[1], *Irina Aleksandrova*[2], *Irina Mekina*[1], *Evgeniia Komarova*[1], *Aleksandr Gzgzyan*[1], *Igor Kogan*[1], *Anna Pendina*[1]

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Background/Objectives: To analyze the intercellular and interindividual variability of telomere lengths (TLs) in the chromosomes from human spermatogenic cells: mitotic spermatogonia and meiotic spermatocyte I at the diplotene/diakinesis stage.

Methods: Testicular samples were obtained by open biopsy from 22 azoospermic patients aged 24–60 years, mean 38.5. TLs were measured in the chromosomes from 353 mitotic spermatogonia and 281 meiotic spermatocytes I at the diplotene/diakinesis stage. For chromosome preparations, testicular tissues were treated with colchicines, hypotonic solution and then fixed on the glass slides. TLs were assessed using quantitative fluorescence in situ hybridization (Q-FISH) as relative values: by dividing the telomeric fluorescence by the subtelomeric fluorescence measured in ImageJ 1.51. Intercellular variability was estimated as the fold change between the TL measured in each spermatogonia/spermatocyte and the mean TL value across all of the studied spermatogonia/spermatocytes of an individual. Interindividual variability was estimated as the fold change between the mean TL in all of the studied spermatogonia/spermatocytes of every individual and the mean TL in the whole group of patients (22 individuals).

Results: Intercellular TL variability both among spermatogonia and among spermatocytes I significantly exceeded interindividual variability ($p = 0.03$ and $p = 0.01$, respectively, Mann-Whitney U-test).

Conclusion: High intercellular TL variability both among spermatogonia and spermatocyte I could be explained by spermatogenic stem cell generations with different TLs in each testis. These cells differentiate and give rise to spermatozoa with different TLs. Spermatozoa with different TLs, in turn, could contribute to developmental potential of embryos.

References:

Grants:

RSF №18-75-10046.

Conflict of Interest: Mikhail Krapivin RSF №18-75-10046 (collaborator), Olga Efimova RSF №18-75-10046 (principal investigator), Yanina Sagurova RSF №18-75-10046 (collaborator), Irina Aleksandrova: None declared, Irina Mekina: None declared, Evgeniia Komarova RSF №18-75-10046 (collaborator), Aleksandr Gzgzyan: None declared, Igor Kogan: None declared, Anna Pendina RSF №18-75-10046 (collaborator).

EP01.016 Immunological incompatibility of a married couple as a cause of repeated abortions

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Background/Objectives: Intricate KIR–HLA interactions occur between maternal NK cells and fetal extravillous trophoblasts in the decidua and are key for maintaining tolerance to the fetus, implantation and placentation. We assume that the immunological incompatibility of a married couple with *KIR/HLAC* genes leads to implantation and fetal development disorders. The aim of this study was to perform *KIR/HLA-C* genotyping to determine "good" compatibility between uterine KIR receptors and embryonic HLA-C.

Methods: *KIR/HLA-C* genotyping was performed by SSP-PCR. The experimental group consisted of 117 couples with ≥2 consecutive miscarriages and/or unsuccessful IVF.

Results: The spectrum of *KIR* genes was analyzed and the frequency of *KIR* genotypes in women with recurrent spontaneous abortion (RSA) was established. The *AB* genotype is the most common (70.09%), the *AA* genotype was established in 33 women out of 117 surveyed (28.21%), and the *BB* genotype was established in 1.71% women. *HLAC* genotyping of couples with RSA showed the *C2/C2* genotype of the *HLAC* gene in 17.95% of women, 20.51% of men and 33.33% of embryos. According to the results of *KIR-HLAC* analysis of genotyping of couples with RSA, a significant risk of reproductive losses of immunological origin was found in 52.99% of cases.

Conclusion: The immunological incompatibility of the couple affects reproductive processes and causes recurrent miscarriages and *KIR-HLAC* genotyping is allows to assess the risks of the embryo being rejected by the maternal immune system.

References:

Grants:

Conflict of Interest: None declared.

EP01.017 Title: Mosaic mutation in androgen receptor gene in a patient with partial androgen insensitivity syndrome

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Background/Objectives: Androgen insensitivity syndrome (AIS) is the most frequent etiology of 46, XY disorders of sex development (DSDs). It is characterized by evidence of feminization of the external genitalia at birth, abnormal secondary sexual development in puberty, and male infertility. AIS patients classified into complete, partial, and mild forms. It is caused by hemizygous pathogenic variant in Androgen receptor gene (*AR)*.

There are more than 500 different *AR* gene allelic variants reported to be linked to AIS, but the mosaic mutations have been rarely identified.

Here, we describe the clinical and molecular features of male patient presented with Partial androgen insensitivity syndrome.

Methods: A 36-year-old male presented with primary infertility, sever oligospermia, bilateral gynecomastia, decreased body hair distribution, normal male external genitalia except for hypospadias. Whole exome sequencing with deletion/duplication analysis was performed.

Results: hemizygous likely pathogenic variant c.649del was found in AR gene with significantly reduced allele ratio compared with a normal hemizygous allele in male. This result is consistent with mosaicism in this patient.

Conclusion: AIS with AR gene sequencing can be considered in male patients with infertility. Mosaic mutations in AR are rare and their detection is still a great technical challenge. WES offer an opportunity to detect lower levels of mosaicism more readily than other traditional methods. This will improve the clinical management, and counselling.

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Grants:

Conflict of Interest: Mohammed Almatrafi: None declared, Zohor Azher full, medical genetics, abdulaziz baazeem full, urology, Abdullatif Almarashi full, phd.

EP01.018 The Digital Genetic Assistant for expanded carrier screening: efficiency and effectiveness

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Background/Objectives: Paucity of genetic counsellors led to the development of the Digital Genetic Assistant (DGA) by Igentify. In enables online enrolment, education and consent for expanded carrier screening (ECS). Triaged low versus high-risk results are returned by personalized videos. Usability of DGA was ascertained for ECS.

Methods: Couples undergoing ThermoFisher CarrierScan® by ECS (1487 variants, 357 genes) at Genetics Institutes of Tel-Aviv and Sheba Centers from 12.2020 to 8.2021 utilizing DGA were included. Comprehension was assessed by interactive questions, followed by online consent. Results of the DGA algorithm were approved or rejected by the medical team. Low risk couples without mutations in the same gene received an personalized video and report, while high risk participants, carriers of a mutation in the same gene or x-linked conditions, underwent a face-to-face (F2F) counselling.

Results: 197 couples underwent ECS via DGA. Eight high-risk couples (4%) underwent F2F counselling. Of the low-risk couples (189, 96%), 46 (23%) did not carry any mutation. DGA saved traditional F2F or telephonic counselling in 143 (73%) of couples that were not carriers of a mutation in the same gene.

An online survey provided feedback, completed by either one or both partners in 136 couples (69%). The consent video was clear for 91% of responders and 95% of felt comfortable receiving their results this way and were satisfied (88%) with the DGA.

Conclusion: Digitalized platform saved 72.6% of F2F interactions. Understanding and Satisfaction were high. This platform may be utilized to facilitate accessibility for further genetic services.

References:

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Conflict of Interest: adi reches fugene genetics, Michal Berkenstadt: None declared, Vered Ofen Glassner: None declared, Yael Furman: None declared, Galit Delmar: None declared, Amit Weinstein: None declared, Doron Behar: None declared, Nurit Goldstein: None declared, Liat Abu-Gutstein: None declared, Karin Alperin: None declared, haike reznik wolf: None declared, Nofar Mimouni: None declared, Odeya Kazimirski: None declared, Anat Bar-Ziv: None declared, Shlomit Eisenberg-Barzilai: None declared, yuval yaron medical consultant to Genoox, elon pras: None declared, Hagit Baris Feldman Sanofi Genzyme, Sanofi Genzyme Protalix Pfizer Taked Shire, Sanofi-Genzyme Igentify, in the past Shire and Regeneration.

EP01.019 The ability of human oocytes to develop into blastocysts depends on telomere length and TERT content in cumulus cells

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Background/Objectives: The present study has examined whether the ability of human oocytes to undergo preimplantation development depends on the content of catalytic telomerase subunit (TERT) and telomere length (TL) in cumulus cells.

Methods: The studied sample included cumulus cells obtained from 32 oocyte-cumulus complexes retrieved from 31 patients in IVF cycles. TERT content and TLs were measured in 32-50 cumulus cells from each studied oocyte-cumulus complex using immunohistochemistry with specific antibodies, and Q-FISH, respectively. A total of 1342 cumulus cells were analyzed.

The oocytes from the studied oocyte-cumulus complexes were fertilized using standard IVF procedures and then were cultured

individually. The oocytes were divided into two groups: the first group included oocytes that developed to the blastocyst stage and the second group included those that demonstrated developmental arrest. The TERT content and TLs were compared between the cumulus cells of the oocytes from two groups.

Results: Both TERT content and TLs were significantly higher in cumulus cells of oocytes that were able to develop into blastocysts compared to those of the arrested ones (Mann-Whitney *U* test, $p = 0.0052$ and $p = 0.0058$, respectively). TERT content and TLs correlated positively in cumulus cells ($r = 0.4577$).

Conclusion: The ability of human oocytes to develop into blastocysts depends on TERT content and TLs in cumulus cells.

References:

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Conflict of Interest: Irina Aleksandrova: None declared, Mikhail Krapivin RSF № 18-75-10046 (collaborator), Olga Efimova RSF № 18-75-10046 (principal investigator), Irina Mekina: None declared, Evgeniia Komarova RSF № 18-75-10046 (collaborator), Mariia Ishchuk RSF № 18-75-10046 (collaborator), Aleksandr Gzgzyan: None declared, Igor Kogan: None declared, Anna Pendina RSF № 18-75-10046 (collaborator).

EP01.020 A retrospective overview in female patients prediagnosed with FMR1 associated disorders

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Background/Objectives: Repetitions of trinucleotide "CGG" in FMR1 gene account for diseases such as Fragile-X syndrome (FXS), Fragile-X Tremor Ataxia Syndrome (FXTAS), Fragile-X associated primary ovarian insufficiency (FXPOI) & diminished ovarian reserve(FXDOR), intellectual disability, subfertility or infertility. In this study, we aim to make retrospective reviews on FMR1 mutation in female patients forwarded to our clinic for genetic analysis between 2014 & 2021.

Methods: PCR based fragment analysis of genomic DNA from a total of 401 patients' blood samples was performed. Resulting data and collected clinical information were matched.

Results: Only 3 patients with prediagnosis of FXPOI & FXDOR, out of 64, was identified with premutations in FMR1 gene (%4.7) with no full mutation found. These patients were %20 of the total amount(15) of patients with premutation.

1 patient out of 28 with prediagnosis of FXTAS was found to carry premutation (%3.6).

13 of patients with developmental delay out of 138 (%9.4), were carrying mutations, 9 full mutation(%6.5), 3 intermediate form(%2.1), 1 grey zone(%0.7).

Abnormal chromosomal analysis results were found in 2 of the patients prediagnosed with FXPOI (47,XXX & 46,X,del(X)(q26)). Noting that 88 out of 203 patients with FMR1 associated disorders prediagnosis' weren't forwarded to cytogenetics evaluations.

Conclusion: Female patients reported with POI & DOR must be evaluated for mutations in FMR1 gene. We suggest the cut-off limits for repeat size length responsible for FXPOI & FXDOR should be further investigated as they might show differences from FXS and FXTAS. Cytogenetic testing shouldn't be neglected with patients of FMR1 associated prediagnosis.

References: https://doi.org/10.1002/ajmg.a.36511.

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Conflict of Interest: None declared.

EP01.021 CYP21A2 gene pathogenic variants in the women of reproductive age with hyperandrogenism

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Background/Objectives: One of the causes of hyperandrogenism in women of reproductive age is a non-classical form of congenital adrenal hyperplasia (NC CAH). NC diagnosis presents difficulties due to the absence of specific clinical manifestations.To determine the significance of the using CYP21A2 gene analysis of the in women with symptoms of hyperandrogenism.

Methods: DNA from 82 patients with hyperandrogenism and 43 controls was analyzed for the presence of pathogenic variants in the CYP21A2 gene using the PCR-RFLP, NGS, real-time PCR.

Results: The compound mutations were identified in five patients (6%), and the diagnosis of NC was confirmed. Heterozygous carriage of pathogenic variants was revealed in 28 patients (34%). Both "heavy" and "light" variants are identified. Deviations of the CYP21A2 gene genotype from the "wild" type were detected in 40% of patients. The 2 substitutions of unknown significance were also identified. In controls the heterozygous pathogenic variants were detected in two patients (4.5%). The frequency of pathogenic variants in patients' groups with infertility, miscarriage and without them was almost the same (45%, 37%, and 37.5%, respectively).

Conclusion: The frequency of pathogenic variants in the CYP21A2 gene is significantly higher in individuals with hyperandrogenism compared to controls. This fact requires a personalized approach to therapy and further examination of spouses at the planning stage of pregnancy for estimation of the risk of CAH in the child.

References:

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Conflict of Interest: None declared.

EP01.023 Genetic map of reproductive health like a base for restoring demographic balance in Ural population

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Background/Objectives: In the last decade in Russia, the general trend is the expansion of the geography of reproductive centers and the growing of services Assisted Reproductive Technologies. However, the use of ART cannot prevent giving birth to ill children automatically. In our region we know 2 cases of this sad scenario at least: a child with cystic fibrosis and a child with phenylketonuria were born after ART. Since 2022 we have been ready to propose for future parents a carrier testing for severe inherited pathology as an optimal solution.

Methods: By our research, we plan to test 1000 Ural family couples for spinal muscular atrophy (SMA)-carrier by multiplex ligase amplification probes and carrier some monogenic diseases by next generation sequencing (NGS).

Results: We have the first 200 samples of DNA. It is couples who came for preconception genetic consultation and have karyotype normal. Our actual genetic map of reproductive health includes 52 genes associated with known inherited diseases, among them are *CFTR, PAH, SMN1, GALT, LAMA2, MMUT, BTD*, and others.

Conclusion: The widespread introduction of such a genetic map into medical practice can be of decisive importance in reducing child and infant disability and mortality in the region, in the long term - restoring the demographic balance of the population of the Middle Urals.

References:

Grants:

Conflict of Interest: None declared.

EP01.025 Assessment of spermatogenic progression in men with structural chromosomal aberrations

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Background/Objectives: Cytogenetic aberrations are well-established causes for male infertility. Besides aberrations of sex chromosomes, structural autosomal aberrations are associated with infertility, i.e. low sperm counts. Meiosis in translocation carriers results in chromosomally normal/balanced or unbalanced gametes. There is evidence that presence of translocations impairs spermatogenetic/meiotic processes. However, studies on spermatogenic progression in translocation carriers are scarce.

Methods: In order to investigate associations between cytogenetic aberrations and spermatogenesis status, we retrospectively analysed 4663 males from one fertility centre and assessed cytogenetics, semen parameters, and testicular histology.

Results: We found cytogenetic aberrations in 9.3% (432/4663) with 1.3% (60/4663) being structural autosomal aberrations. Carriers of reciprocal translocations (RT, $n = 28$) and Robertsonian translocations (ROB, $n = 15$) showed large variation in sperm counts, ranging from normozoospermia to azoospermia. Testicular histology in azoospermic patients with RT ($n = 5$) and ROB ($n = 2$) revealed marked differences in spermatogenic progression. We observed that some RT and ROB carriers showed meiotic arrest in most seminiferous tubules and a smaller proportion with full spermatogenesis. Interestingly, in other carriers with the same aberration type, we found in the majority of seminiferous tubules a complete absence of germ cells. To exclude further genetic causes for the spermatogenic failure in translocation carriers, we currently perform exome sequencing.

Conclusion: Translocation carriers, even of the same aberration, i.e. the common der(13;14)(q10;q10), show large variation in sperm counts and spermatogenic progression. Whether additional genetic variants influence the testicular phenotype, remains to be elucidated.

References:

Grants: This work was supported by the DFG Clinical Research Unit 326 'Male Germ Cells'.

Conflict of Interest: None declared.

EP01.026 Deep RNA sequencing of decidual cells identifies widespread isoform diversity and alternative splicing

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Background/Objectives: Alternative splicing (AS) is crucial for the regulation of human genes expression. Global screening for changes in AS has not been previously carried in the placental cells despite a comprehensive analysis of pre-mRNA AS in many human tissues and cells in normal and pathological conditions. The decidual stromal cells (DSCs) play a key role in maintaining the function of placenta during human pregnancy. The aim of this study was to investigate genes and isoforms involved in functioning of the human DSCs during uncomplicated pregnancies.

Methods: We applied deep RNA sequencing of DSCs obtained by Laser capture microdissection. AS events are identified in existing genome annotations using the R-package "SGSeq".

Results: Analyses of AS isoforms detected 151233 isoforms in DSCs. 373 genes with two or more transcripts have been identified. Of these, 130 genes meet criteria (CPM > 10; the proportion of transcript ranges from 0.5 to 0.95). GO enrichment analysis revealed that these genes are associated with canonical wnt-signaling pathway, regulation of substrate-dependent cell migration, mRNA splicing, stem cell proliferation. The network of gene interactions revealed a cluster of co-expression of 24 genes. The *CTNNB1, EIF4A2, EIF4G1, EIF4G2, FLNA, FN1, HNRPA2B1, HNRNPH1, HNRNPK, HNRNPU, RBFOX2, RBM25* and *SRSF5* genes are central in the network with the largest number of interactions. A maximum of 10 of alternative transcripts corresponds to the FN1 gene.

Conclusion: Obtained data confirm the importance of AS in DSCs, which significantly increases transcriptional diversity and plays a key role in regulatory functions.

References:

Grants: The reported study was funded by RFBR №18-29-13045.

Conflict of Interest: None declared.

EP01.027 MCM-domain containing 2 (MCMDC2) - a novel candidate gene for male infertility

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Background/Objectives: Infertility affects 10–15% of all couples, and although frequently suspected, underlying genetic causes often remain unidentified. Recently, *Mcmdc2*, a minichromosome maintenance (MCM) paralog, was published in the context of murine sterility and meiotic arrest. Members of the MCM family are important for DNA replication, repair, and meiotic recombination, which suggests that this also applies to human MCMDC2.

Methods: Whole exome sequencing data from the MERGE cohort of >1600 men, mostly affected by azoospermia and infertility, was screened for bi-allelic rare (MAF <1%, gnomAD) coding variants in *MCMDC2*.

Results: Two azoospermic, otherwise healthy men from non-consanguineous parents were identified with bi-allelic, loss-of-function variants in *MCMDC2*. Hormonal parameters (FSH, LH, testosterone) and testicular volumes were within reference ranges for M1226, who carried two compound heterozygous frameshift variants inherited from each parent. His fertile sister was wildtype

for both variants. M1762 was homozygous for a stop-gain variant likely disturbing the MCM family domain. His parents were heterozygous carriers. Decreased testosterone and increased FSH levels indicate testicular disturbance, accordingly, M1762's histology revealed meiotic arrest on spermatocyte level, with apoptotic spermatocytes and occasional round spermatids; testicular sperm extraction (TESE) was negative.

Conclusion: This is the first report of recessively inherited *MCMDC2* deficiency in the context of male infertility. The testicular phenotype of meiotic arrest seen in mutation carriers is in accordance with the phenotype observed in two independently described infertile *Mcmdc2* knockout mice and further supports a function of MCMDC2 in meiosis.

References: PMID 27986806/27760146.

Grants: This work was supported by the DFG Clinical Research Unit 326 'Male Germ Cells'.

Conflict of Interest: None declared.

EP02 Prenatal genetics

EP02.001 Prenatal tests and pregnancy termination amongst Moslem women living at home with a child afflicted with a genetic disease or syndrome

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Background/Objectives: According to the Islamic religion, Moslem women are not permitted to terminate a pregnancy after the first 120 days. Prenatal diagnosis tests are divided into screening and invasive tests which are low risk for abortions. Consanguinity is very common in Moslem society. Herein, we raised the question as to whether a Moslem woman with a child afflicted with a genetic disease, living at home, would perform more prenatal tests and pregnancy terminations versus women with normal children living at home.

Methods: 771 Moslem women participated in the study; 37.1% had a child afflicted with a genetic disease; 62.9% did not; 52% were city-dwellers and 48% lived in villages.

Results: Moslem women with an abnormal child afflicted with a genetic disease living at home will undergo more prenatal tests including invasive tests and will undergo more pregnancy terminations ($p < 0.001$). More city-dwellers underwent different prenatal tests and pregnancy terminations. Village-dwellers were more religious and counselled more by a religious authority. City-dwellers received more emotional support from their husbands, family, and friends. Non-invasive prenatal testing (NIPT) was performed in direct correlation with a higher socioeconomic status and academic education and was performed more in the cities ($p < 0.001$).

Conclusion: Families with a child afflicted with a genetic disease received more genetic counseling. Families living in cities received more genetic counselling. Women living in the villages were more religious and abided with religious rulings. NIPT was performed more in the cities due to higher economic status and academic education.

References:

Grants:

Conflict of Interest: None declared.

EP02.002 Type and frequency of copy number aberrations in prenatal samples detected by karyotyping and array CGH analysis in Bulgarian patients

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Background/Objectives: Chromosome analysis by microarrays (array CGH) is a high-resolution technology capable of detecting microdeletions and microduplications throughout the genome that are missed by conventional karyotyping. In the present study we aimed to summarize the results of prenatal diagnosis by karyotyping and chromosome microarray analysis to make a comparison and determine the frequency of aberrations in different indications, which will allow us to create an algorithm for array CGH analysis in prenatal diagnostics.

Methods: The study included 63 amniotic samples subjected to conventional karyotyping and 145 prenatal samples for microarray analysis.

Results: Pathogenic aberrations were found in 9.5% of karyotyping cases, as 83.3% of them could be diagnosed by QF-PCR for the most common aneuploidies (rapid aneuploid test - RAT). Array CGH analysis revealed 23 pathogenic aberrations (15.75% of cases after RAT). In 5 cases (21.7%) the size of the found aberrations is below 1 Mbp, in 11 cases (47.8%) the size is between 1 and 6 Mbp. Seven of the pathogenic aberrations (30.4%) could be diagnosed by karyotyping due to size over 10 Mbp—4.8% of all samples. We found variants of unclear significance (VOUS) in 19% of cases—between 8% and 28% in different indications.

Conclusion: Our results show that array CGH should be used after the exclusion of common aneuploidies by QF-PCR and it increases the diagnostic rate of karyotyping more than three times—15.75% vs 4.8%. The lowest was the diagnostic rate in isolated elevated nuchal fold, and the highest—in a family history for disorder.

References:

Grants:

Conflict of Interest: None declared.

EP02.003 AnDDI-prenatome - the French national project of prenatal trio exome sequencing: 43% of diagnostic yield in 28 days with 80% pregnancy care changes

Frédéric Tran Mau-Them[1;2], *Anne-Sophie Denommé-Pichon*[1;2], *Hana Safraou*[1;2], *Ange-Line Bruel*[1;2], *Antonio Vitobello*[1;2], *Sophie Nambot*[3], *Julian Delanne*[3], *Aurore Garde*[3], *Caroline Racine*[3], *Arthur Sorlin*[1;2;3], *Sebastien Moutton*[1;2;3], *Chloe Quelin*[4], *Marine Legendre*[5;5], *Cindy Colson*[6], *Anne-Claire Brehin*[7], *Alban Ziegler*[8], *Audrey Putoux*[9], *Alinoe Lavillaureix*[4], *Anne-Marie Guerrot*[7], *Jeanne Amiel*[10], *Caroline Rooryck-Thambo*[11], *Carine Abel*[12], *Patricia Blanchet*[13], *Magali Gorce*[8], *Godelieve Morel*[4], *Alice Goldenberg*[7], *nicolas gruchy*[14], *Melanie FRADIN*[4], *Agnes Guichet*[15], *Odile Boute*[6], *Elise Schaefer*[16], *Gabriella Vera*[7], *Catherine Delorme*[6], *Rodolphe Dard*[17], *Christine Francannet*[18], *Estelle Colin*[15], *Emilie Tisserant*[2], *Marie Vincent*[19], *Bertrand Isidor*[19], *Sylvie Odent*[4], *Marie-Laure Humbert*[20], *Yannis Duffourd*[1;2], *Christinne Binquet*[20], *Christophe Philippe*[1;2], *Laurence Faivre*[2;3], *Christel Thauvin-Robinet*[1;2;3]

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Background/Objectives: Etiological prenatal diagnosis (PD) of congenital abnormalities is a real challenge since abnormal ultrasound signs are discovered in 5–10% of pregnancies. The large deployment of exome/genome sequencing (ES/GS) appears as a strong opportunity to improve PD but faces the difficulties of returning diagnosis in a short turnaround time and interpreting genomic data with incomplete clinical/imaging data. We present the French multicentric AnDDI-prenatome study of prenatal trio-ES.

Methods: Rapid trio-ES (delay maximum 42 days) was performed, in parallel or after array-CGH, in pregnancies (10–34 weeks of gestation) with 2 ultrasound abnormalities. Only ACMG class 4 and 5 variants were considered for positive diagnosis.

Results: 19 different centers included 150 pregnancies. ES was performed in first intention in 81/150 and after normal array-CGH in 60/150. The median delay was 28 days for positive diagnosis and 26 days for negative ones. The diagnostic rate was of 42% in first intention and of 27% after normal array-CGH. The concordance rate between ES and array-CGH was of 100% to detect CNV. Altogether, a causal diagnosis was identified in 50/150 foetuses, rising to 54/150 after VUS investigation. The pregnancy management could have been modified in 79% of the cases.

Conclusion: The AnDDI-prenatome study shows that prenatal ES is feasible with acceptable turnaround in France, with a major interest in pregnancy management. Despite prenatal incomplete clinical/imaging data, its diagnostic yield appears similar to postnatal ES. Its application on a larger scale, notably its impact on healthcare system and organization, should now be carried to consider its routine implementation.

References:

Grants:

Conflict of Interest: None declared.

EP02.005 Noninvasive prenatal testing for cystic fibrosis using circulating trophoblasts in maternal blood

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Background/Objectives: Cystic fibrosis is the most common autosomal recessive disorder and prenatal screening has been recommended (1). This study aimed to develop cell-based non-invasive prenatal testing (NIPT) for cystic fibrosis using circulating trophoblasts in maternal blood.

Methods: Blood samples were collected from pregnant women with either high risk for CF (group 1, $N = 8$) or invasive sampling performed for other reasons (group 2, $N = 21$). Circulating trophoblasts were enriched and isolated by FACS single cell sorting. The cell origin was identified by DNA profiling using STR analysis. Cystic fibrosis analysis for detection of 50 pathogenic variants in CFTR was conducted by fragment analysis or next-generation sequencing and the results were compared between trophoblasts and invasive samples.

Results: Circulating trophoblast cells (range 1–5, median 4) were tested from eight pregnancies at risk of cystic fibrosis in group 1. The cell-based NIPT results were in concordance with the result of invasive testing in seven cases but one cell-based NIPT gave an inconclusive result. From the second group, an average of circulating trophoblast cells (range 1–10, median 5) were tested from 21 pregnancies. Among these, two previously unidentified maternal carriers of cystic fibrosis were found and all cell-based NIPT results were in concordance with those of invasive testing for the 50 CFTR variants. In one sample, no trophoblast cells were found.

Conclusion: This study provides a proof-of-concept for detection of cystic fibrosis using cell-based NIPT.

References: (1) ACOG, webpage accessed 9th Feb 2022, https://www.acog.org/womens-health/faqs/cystic-fibrosis-prenatal-screening-and-diagnosis.

Grants: IV: Novo Nordisk Foundation (NNF16OC0018772), LJ: Innovation Fund Denmark (0153-00004B).

Conflict of Interest: Line Dahl Jeppesen Full time employed by Arcedi, Lotte Hatt Full-time employed by Arcedi, Dorte Lildballe: None declared, Ida Vogel: None declared.

EP02.006 Can the phenotype of Down syndrome be predicted at the combined first trimester screening for trisomy 21?

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Background/Objectives: The ability of the combined first trimester screening (cFTS) to identify Down syndrome pregnancies is well established, but can cFTS for trisomy 21 predict the Down syndrome phenotype? We aimed to investigate the potential association between cFTS result and phenotype in Down syndrome.

Methods: We performed a register based cohort study including all cases of trisomy 21 in Denmark during 2005-2018 diagnosed in pregnancy or before 13.5 years of age. We compared screen negative (<1:300) and screen positive (≥1:300) trisomy 21 cases with respect to phenotypic characteristics: congenital malformations, anthropometry, and childhood morbidity.

Results: Of 2,167 trisomy 21 cases (pre- or postnatally detected), 1,672 (77%) were screen positive, 242 (11%) screen negative and 253 (12%) not screened. Prenatally, more screen positive cases had a recorded malformation (table); potentially due to detection bias. In liveborn children, comparable proportions of screen negative and positive cases had severe congenital heart disease (CHD) and non-CHD malformations (table).

	Screen positive, % (95% CI)	Screen negative, % (95% CI)
Prenatal CHD	28.6% (20.0-38.6%)	10.0% (6.2-14.9%)
Prenatal non-CHD malformation	32.7% (23.5-42.7%)	9.5% (5.8-14.4%)

	Screen positive, % (95% CI)	Screen negative, % (95% CI)
Postnatal severe CHD	24.1% (15.4-34.7%)	20.5% (14.8-27.2%)
Postnatal non-CHD malformation	8.4% (3.5-16.6%)	13.1% (8.5-19.0%)

Fetal and newborn anthropometry as well as duration of childhood hospital admissions was similar among screen negative and positive cases.

Conclusion: In a 14-year nationwide cohort, we did not observe a consistent phenotypic difference between screen negative and screen positive trisomy 21 cases.

References:

Grants: Aarhus University. Health Research Foundation of Central Denmark Region (A2602). Health Foundation (20-B-0065).

Conflict of Interest: None declared.

EP02.007 Undiagnosed arthrogryposis: further expanding the molecular and phenotypic spectrum

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Background/Objectives: Arthrogryposis multiplex congenita (AMC) is a phenotypic descriptor implying congenital contractures in two or more body areas. Exome sequencing provides an effective approach in elucidating the molecular basis, considering the presence of genetic and phenotypic heterogeneity. We aimed to identify the molecular etiology in undiagnosed fetuses within the AMC spectrum, using exome sequencing.

Methods: 14 patients from ten families displaying AMC were enrolled. Array-CGH analysis was performed in all fetuses, followed by exome analysis. Sanger sequencing was performed to validate variants.

Results: We identified known or novel candidate variants in COG6, NEB, RAPSN, KIAA1109, DOK7, HSPG2, PSAT1, and PIEZO2. Three patients were shown to harbor additional biallelic variants in VPS13B, DNAH9, and USH2A, complicating the phenotype. Moreover, we identified a frameshift variant in USP14 in three similarly affected fetuses. The resemblance of the fetal findings to the previously reported Usp14 mouse models supported that our variant likely led to a novel intrauterine-onset human AMC phenotype (1).

Conclusion: Our results provide new insights into the clinical and molecular characterization of a small AMC cohort; to contribute to phenotype-genotype correlation, expand the clinical spectrum, report novel variants, and present the first human phenotype that appears to be attributable to a truncating variant in USP14.

References:

(1) Turgut, Gozde Tutku et al. "Functional loss of ubiquitin-specific protease 14 may lead to a novel distal arthrogryposis phenotype." Clinical genetics, https://doi.org/10.1111/cge.14117. 23 Jan. 2022, https://doi.org/10.1111/cge.14117.

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EP02.008 Polymorphisms of the MTHFR gene and maternal risk of offspring aneuploidy

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Background/Objectives: Chromosomal aneuploidies cause about 50% of early pregnancy losses and affect 0.3–0.5% of childbirths, with still insufficiently understood underlying mechanisms of chromosomal malsegregation. Folate metabolism gene polymorphisms have been shown to lead to DNA hypomethylation and damage, with possible consequent abnormal chromosomal segregation during meiosis. The objective was to investigate the association between maternal 5,10-methylentertahydrofolate reductase (*MTHFR*) gene polymorphisms, crucial for DNA methylation, and risk of offspring aneuploidy.

Methods: *MTHFR* gene polymorphisms 677C>T and 1298A>C were determined by polymerase chain reaction based method, in 163 women with offspring aneuploidy and 155 women with healthy children. Five genetic models were used to assess risk, according to the type of aneuploidy and the age of women at conception.

Results: *MTHFR* 677TT genotype and T allele were significantly more prevalent among women with offspring aneuploidy, with an increased risk of aneuploidy demonstrated under a recessive (OR 3.499), homozygote (OR 3.456) and allele contrast model (OR 1.574). The more prominent association was found with sex chromosome aneuploidies and trisomy 13/18, and also in women ≤35 years at conception. No association was observed between 1298A>C polymorphism and risk of offspring aneuploidy, although synergistic effect of two polymorphisms increase the risk of aneuploidy, primarily amplifying the 677T allele effects ($p < 0.001$).

Conclusion: Maternal *MTHFR* 677C>T gene polymorphism, alone or in combination with another 1298A>C polymorphism, appears to be a substantial risk factor for offspring aneuploidy in Montenegro population, especially for sex chromosome aneuploidies and trisomy 13/18, and among younger women.

References:

Grants:

Conflict of Interest: None declared.

EP02.009 Non-invasive fetal sex determination in maternal plasma using chip-based digital PCR

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Background/Objectives: Clinical indications for fetal sex determination include risk of X-linked disorders and the management of fetuses at risk of congenital adrenal hyperplasia. Currently fetal sex diagnosis could be obtained using invasive procedures such as amniocentesis and chorionic villus sampling, which are associated with a 1% risk of miscarriage. Non-invasive prenatal diagnosis based on circulating cell-free fetal DNA created new possibilities of early detection of fetal sex. The aim of this study was to design and validate a rapid, reliable and low-cost method for non-invasive fetal sex determination based on the assessment of cell-free fetal DNA in maternal plasma.

Methods: To determine fetal sex, blood samples from 35 pregnant women at weeks 11 to 17 of gestation were analysed. Cell-free DNA was isolated from the plasma samples using a commercially available kit. A multiplex PCR was performed for the simultaneous amplification of target sequences of Y chromosome using a chip-based QuantStudio 3D Digital PCR system. The results were validated by results from invasive diagnostics.

Results: All maternal plasma samples based on the detection of Y chromosome-specific sequences were determined correctly.

Conclusion: Chip-based digital PCR is a reliable method with high accuracy for non-invasive fetal sex determination. Our results demonstrate that fetal sex determination by detecting Y chromosome sequences in maternal plasma using chip-based digital PCR platform is clinically applicable and could be used in clinical practice.

References:

Grants:

Conflict of Interest: None declared.

EP02.010 Prenatal diagnosis of holoprosencephaly associated with a de novo balanced t(4;7)(p14;q36) translocation

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Background/Objectives: Holoprosencephaly (HPE) is a complex brain malformation resulting from incomplete division of the forebrain. Approximately 25–50% of individuals with HPE have a chromosome abnormality. Analysis of recurrent chromosomal anomalies led to the identification of 12 candidate regions (HPE1 to HPE12). SHH, the major gene implicated in holoprosencephaly, was isolated from the critical region HPE3 on chromosome 7q36. We report a prenatal case with holoprosencephaly and a balanced de novo translocation t(4;7)(p14;q36) without any loss of the distal part of chromosome 7q confirmed by array-CGH.

Methods: Chorionic villi culture and GTG banding were done following standard protocols. Array-CGH was performed using 60K KaryoNIM Prental®. Maternal contamination was ruled out prior to the study.

Results: At 12 weeks of gestation, ultrasound examination of the fetus showed holoprosencephaly. Cytogenetic studies showed a balanced translocation between the long arms of chromosomes 4 and 7, 46,XX,t(4;7)(p14;q36). Parental karyotypes were normal. CGH-array study was normal, without any loss of the distal part of chromosome 7q.

Conclusion: Two breakpoint de novo rearrangement has a 6.7% empiric risk of phenotypic abnormality. Abnormal phenotypes are thought to result from gene disruption, position effect, or deletion at breakpoints. Balanced translocations involving 7qter have been reported previously related with HPE[1]. We present a prenatal case of holoprosencephaly with altered karyotype and normal array-CGH, which highlights the importance of performing a karyotype study in cases of HPE.

References: [1] Benzacken, B. et al. Different proximal and distal rearrangements of chromosome 7q associated with holoprosencephaly. J. Med. Genet. 1997; 34: 899-903.

Grants: UMH-Citolab1.19A.

Conflict of Interest: None declared.

EP02.011 Deletion of PAX3 gene in aborted fetus with spina bifida and meningomyelocele

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Background/Objectives: *PAX3* gene is localized on chromosome 2 (q36.1) and its product (transcription factor Pax3) is a key regulator of embryogenesis involved in the development of nervous and muscle system. Heterozygous mutations of *PAX3* gene are linked to autosomal dominant Waardenburg syndrome (WS) 1 and 3, characterized by hearing loss, loss of pigmentation and eye abnormalities. We present a case of *PAX3* deletion detected in a fetus with multiple congenital abnormalities.

Methods: We examined a tissue of a fetus aborted at 20+4 week gestation for US abnormalities which included an absent nasal bone, an abnormal finding in posterior cranial fossa, dilatation of left lateral ventricle, sacral spina bifida with meningomyelocele, and left club foot.

Results: Karyotype was normal 46,XX. This deletion (size 3 Mb) on chromosome 2 including *PAX3* was detected by aCGH – 2q35q36.1(221148359_224433293) x1. A complete loss of one copy of *PAX3* was confirmed by MLPA. The CNV was not found in healthy parents.

Conclusion: Arnold-Chiari malformation (cerebellar abnormalities), spina bifida and meningomyelocele are described as rare features of WS. While most cases of spina bifida and meningomyelocele are determined multifactorially, we hypothesize that a combination of an absent nasal bone and spina bifida may indicate a loss of function of *PAX3* gene. The simultaneous presence of these abnormalities can guide investigation strategies during prenatal diagnosis.

References:

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Conflict of Interest: None declared.

EP02.012 Prenatal diagnosis of a novel pathogenic HNF1B variant: phenotypic variability between monozygotic twins

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Background/Objectives: *HNF1B* mutations are associated with autosomal dominant tubulointerstitial kidney disease (OMIM 137920). Although the severity of renal phenotype is extremely variable, *HNF1B* intragenic mutations result in more severe renal function impairment than copy number variants encompassing the entire gene (17q12 microdeletions). *HNF1B*-related disease often manifests with fetal hyperechogenic kidneys; however, reports of prenatal diagnosis of intragenic variants are rare.

Methods: We performed an NGS renal disease gene panel in a bichorial biamniotic pregnancy, in which renal anomalies were detected by prenatal ultrasounds.

Results: The analysis identified a novel de novo heterozygous splice-site variant c.1046-2A>T (IVS4) in the *HNF1B* gene in both twins. Karyotype was normal (46,XX) and QF-PCR analysis determined monozygosity. Ultrasounds revealed hyperechogenic multicystic kidneys and severe oligohydramnios in fetus 2, already at 16+2 weeks. At birth, she showed flattened head with frontal bossing, unilateral cataract, clubfeet and bilateral pneumothorax; she died on the second day of life. Instead, fetus 1 showed no abnormalities until 20 weeks, when ultrasound detected bilateral renal enlargement and hyperechogenicity with right kidney cysts; amniotic fluid was normal. She had normal birth parameters and at last assessment (5 months) creatinine was 1.34 mg/dL.

Conclusion: We have described a case of prenatal diagnosis of a novel pathogenic *HNF1B* variant associated with a severe renal disease in a twin pregnancy, confirming that intragenic variants are associated with a poorer renal prognosis, but with variability even between identical twins. We have also deeply described the prenatal and postnatal presentation of the disease, which can be useful for prenatal genetic counseling.

References:

Grants:

Conflict of Interest: None declared.

EP02.013 The diagnostic utility of genome sequencing for fetal congenital heart defects

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Background/Objectives: Compelling data in support of prenatal genetic diagnoses by exome sequencing in fetuses with structural abnormalities detected by ultrasonography is well recognized[1]. Congenital heart defects are genetically heterogeneous and contributed wide range of genetic mutation types. This is a pilot study to investigate the diagnostic utility of genome sequencing (GS) to detect a broader range of causative genetic variants in a prenatal diagnosis setting.

Methods: Genome sequencing (>30X) was prospectively performed 13 prenatal trios with heart defects for which karyotyping and/or chromosomal microarray results were undiagnostic.

Results: 4/13 (30.8%) of trios received diagnostic genetic findings by genome sequencing. They included pathogenic or likely pathogenic variants in *DNAH5*, *COL4A1*, *PTPN11*, and *KRAS*. Notably, a balanced translocation [46,XX,t(14;22)(q32.33;q13.31) mat] detected by GS in the trio with the *DNAH5* mutations. The translocation also explained the adverse pregnancy histories of presumably de novo Phelan-McDermid syndrome of this trio. GS also detected medically actionable findings in *BRCA2* and carrier statues in *GJB2*, *HBB*, *USH2A*, *HBA1* and *HBA2*.

Conclusion: Genome sequencing provided additional yield and timely results of diagnostic variants, including a wide spectrum of mutation types. Not only did genome sequencing facilitate genetic diagnoses, it also provided clinically relevant medical actionable findings and carrier statuses. We provide evidence to support the application of genome sequencing for fetuses with congenital heart defects.

References: 1. Lord, Jenny, et al. "Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study." The Lancet 393.10173 (2019): 747–757.

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Conflict of Interest: Ye Cao Research Assistant Professor, Department of Paediatrics & Department of Obstetrics and Gynecology, The Chinese University of Hong Kong, Shatin, Hong Kong, China, partially support by Funding source: CUHK Direct Grant (2020.074), Matthew Hoi Kin Chau Postdoctoral Fellow, Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, China, Yu ZHENG: None declared, Yilin Zhao: None declared, Shuk Yi, Annie Hui Consultant & Clinical Associate Professor (honorary), Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, China, Hoi Wan, Angel Kwan Resident, Clinical Tutor (honorary), Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, China, Zirui Dong Assistant Professor, Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, China, HMRF (07186576), Kwong Wai Choy Professor, Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, China.

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EP02.014 Familial form of Milroy disease with incomplete penetrance creates diagnostic challenges in a family with recurrent fetal lymphedema

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Background/Objectives: Milroy disease is an autosomal dominant condition caused by mutations in the FLT4 gene that affects the normal function of the lymphatic system resulting in congenital lymphedema. Intrafamilial variability and reduced penetrance are observed in this condition. About 10–15% of people with a mutation in the *FLT4* gene do not develop the features of

Milroy disease. Incomplete penetrance can sometimes obscure autosomal dominant inheritance pattern.

Methods: We present a family of healthy parents with two consecutive pregnancies with fetal lower limbs edema detected prenatally at second trimester ultrasound examination. Karyotyping was performed on amniotic fluid samples from the two pregnancies and blood samples of both partners. Clinical exome sequencing of DNA obtained from uncultered amniocytes from the second pregnancy was performed on MiSeq (Illumina). Sanger sequencing in fetal and parental DNA was applied for confirmation and segregation analysis.

Results: Prenatal diagnosis of the first pregnancy revealed mosaic fetal karyotype: 46,XX,-10,+mar (del10q?)[2]/46,XX[25]. The family decided to terminate the pregnancy. Both partners were carriers of normal karyotypes. Amniocentesis performed on the second pregnancy (ten years later) detected normal fetal female karyotype (46,XX) and a heterozygous pathogenic variant in *FLT4* gene - c.3341C>T (p.Pro1114Leu). This variant was inherited by the father.

Conclusion: The phenomenon of incomplete penetrance can make it challenging for genetics professionals to interpret a person's family medical history and predicting the risk of passing a genetic condition to future generations. This should be taken into account in counselling for autosomal dominant conditions.

References: None.

Grants: None.

Conflict of Interest: None declared.

EP02.015 High-resolution chromosomal microarray analysis in the prenatal setting

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Background/Objectives: Prenatal chromosomal microarray analysis (CMA), traditionally indicated for high-risk pregnancies, is nowadays frequently applied preventively. Here we report on the outcomes of the application of high-resolution CMA in a series of prenatal cases.

Methods: 219 couples were referred to the Laboratory of Medical Genetics of the National and Kapodistrian University of Athens for prenatal CMA. Indications included ultrasonographic findings, previous pregnancy or offspring with chromosomal abnormality, a parent carrier of a balanced chromosomal translocation, advanced maternal age, and preventive testing due to parental anxiety. DNA was extracted from either amniotic fluid (AF) or chorionic villus sample (CVS) (DNA mini kit, Qiagen) and was subsequently tested for chromosomal aberrations with the 4x180K G3 CGH+SNP microarray (Agilent Technologies). Maternal cell contamination was excluded for all samples.

Results: A total of 250 samples were studied (143 AF, 107 CVS). 141 of all embryos were male and 109 female. 198 embryos (79.2%) had a normal karyotype and 52 (20.8%) showed one or more chromosomal abnormalities. The findings were classified as pathogenic or likely pathogenic in 38 cases (15.2%) and as Variants of Uncertain Clinical significance (VUS) in 14 cases (5.6%).

Conclusion: High-resolution CMA is a powerful tool in the prenatal setting, with high diagnostic yield regardless of indication. VUS appear with relatively high frequency, however this is expected to further decline as knowledge on the clinical significance of findings increases.

References: Oneda B, et al. High-resolution chromosomal microarrays in prenatal diagnosis significantly increase diagnostic power. Prenat Diagn. 2014;34:525–33.

Grants: N/A

Conflict of Interest: None declared.

EP02.016 Prenatal diagnosis of Saethre-Chotzen syndrome caused by TWIST1 microdeletion and complex chromosomal rearrangement involving chromosomes 5, 7 and 11

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Background/Objectives: Saethre-Chotzen (SC) is syndromic craniosynostosis rarely diagnosed prenataly. It is caused by mutations, or less frequently deletions of TWIST1 gene leading to its haploinsufficiency. Clinical presentation is variable, with coronal craniosynostosis, facial dysmorphism, hand and foot anomalies being the most common manifestations. We present a case of prenataly diagnosed SC-like syndrome due to complex chromosomal rearrangement involving chromosomes 5, 7, and 11.

Methods: A 32-year-old women was referred to genetic counselling in 28th gestation week of her third pregnancy due to presence of ultrasound anomalies. Brachicephaly, hypertelorism, flat face, micrognathia, relative macroglossia and small posterior fossa were noted.

Results: Karyotype analysis was performed on blood sample obtained by cordocenthesis. De novo translocation involving chromosomes 7 and 11 was found- 46,XY,t(7;11)(p15.5;q21)dn. Subsequent chromosomal microarray analysis (Agilent Sureprint G3 Human 8x60K) revealed presence of three microdeletions on chromosome 7: 7p21.1-p15.3 (4,82 Mb including TWIST1 gene), 7p12.1-p11.2 (1.3Mb), 7q21.11 (2,9 Mb), and one on chromosome 5 (5p12-p11, 581Kb).

Conclusion: Complex chromosomal rearrangements involving TWIST1 gene are very rare cause of SC syndrome. Prenatal manifestations in our case were consistent with TWIST1 haploinsufficiency, however, additional clinical manifestation can be expected due to presence of multiple microdeletions in proband. This case confirms utility of chromosomal microarray in prenatal cases of suspected syndromic craniosynostosis.

References: Spaggiari E, Aboura A, Sinico M, Mabboux P, Dupont C, Delezoide AL, Guimiot F. Prenatal diagnosis of a 7p15-p21 deletion encompassing the TWIST1 gene involved in Saethre-Chotzen syndrome. Eur J Med Genet. 2012 Aug-Sep;55(8-9):498-501.

Grants: None.

Conflict of Interest: None declared.

EP02.017 Further delineation of prenatal presentation in congenital dyserythropoietic anemia type II (CDAII)

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Background/Objectives: Nonimmune hydrops fetalis (NIHF) is a fetal abnormality with high genetic heterogeneity including genes associated to congenital anemias. Congenital dyserythropoietic anemia type II (CDAII) is caused by pathogenic variants in the *SEC23B* gene following an AR inheritance. Patients usually manifest with mild to moderate anemia, although in some cases blood transfusion may be required. Prenatal clinical manifestation as hydrops foetalis and

intrauterine death is barely described. We present here two new cases of severe CDAII presenting with hydrops foetalis.

Methods: Two pregnant women were referred to the Foetal Medicine Unit of our Hospital at 26 and 18 weeks of gestation, respectively. Ultrasound findings showed fetal ascites and pericardial effusion resulting in severe hydrops foetalis in both cases. They underwent prenatal exome sequencing in fetal DNA.

Results: Molecular results showed pathogenic and probably pathogenic variants c.325G>A (p.Glu109Lys) and c.1648C>T; (p.Arg550*) in *SEC23B* (fetus 1), and c.2101C>T (p.R701C) and c.2102G>A (p.R710H) in *SEC23B* (fetus 2). The variants were in heterozygous state in their respective healthy parents. Although some of these variants have previously been reported, there is no clear genotype-phenotype correlation considering the type of variant.

Conclusion: Challenges in prenatal exome include the unknown fetal presentation of several genetic conditions that are recognized in postnatal settings. Indeed, our study provides further evidence of the presentation of CDAII as hydrops fetalis, expanding the clinical spectrum of this condition. Moreover, a categorical molecular diagnosis enables an early and adequate prenatal management to improve the prognosis of CDAII and to offer an appropriate genetic counseling to the families.

References:

Grants:

Conflict of Interest: None declared.

EP02.018 Non-indicated invasive tests yield higher percentage of Ashkenazi Jewish patients

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Background/Objectives: The risk for clinically significant copy number variants in uneventful pregnancies is estimated at about 1%. Numerous factors influence the uptake of prenatal invasive testing, and the objective of our study was to explore the effect of maternal origin on the willingness to undergo non-indicated testing.

Methods: This retrospective cohort study included data of all prenatal microarray tests during 2019–2021 in genetic laboratory of Carmel Medical Center, Haifa, Israel. The reasons for invasive testing were categorized as: 1) indicated tests (e.g., abnormal ultrasound and high-risk serum screening), 2) maternal age ≥35 years and 3) younger women. The percentage of Ashkenazi Jewish patients in each indication was compared to non-Ashkenazi Jewish and to Israeli Arab population.

Results: Of the overall 1400 prenatal microarray tests, 676 (48.3%) were performed due to a medical indication, 392 (28.0%) in women older than 35 years, and 332 (23.7%) in younger women. The percentage of Ashkenazi patients performing invasive testing due to maternal age over 35 years (76.0%) and in younger women (81.0%) was significantly higher compared to the rate of Ashkenazi patients undergoing indicated testing (61.4%). A significant decline in patients from other origins was noted in non-indicated vs. indicated tests.

Conclusion: Higher rates of Ashkenazi patients performing non-indicated invasive tests can be explained by various factors, including socio-economic status, religious beliefs, language barriers, and geographic factors. These potential influences should be taken into consideration during prenatal genetic counseling.

References:

Grants:

Conflict of Interest: None declared.

EP02.019 Retrospective analysis of fetal magnetic resonance imaging (feMRI) examinations in the last 10 years at a tertiary center: experience of a single radiologist and a single perinatologist

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Background/Objectives: To evaluate the role of fetal magnetic resonance imaging (feMRI) in high-risk pregnancies and the concordance between feMRI and ultrasonography (US).

Methods: Retrospective analysis of US and feMRI in 97 fetuses in the last 10 years at a tertiary center. US was performed by a single perinatologist, feMRI images were analyzed by a single radiologist.

Results: Mean gestational age at US was 26.0 ± 4.2 weeks, and feMRI was within 3 days after US. Indications for feMRI were CNS ($n = 80$, 82%) and non-CNS ($n = 17$, 18%). CNS-related indications, ventriculomegaly ($n = 41$, 42%) had the greatest ratio. In 51 fetuses (53%), feMRI and US were completely concordant. In 11 fetuses (12%), feMRI demonstrated extra findings. In 33 fetuses (34%), feMRI excluded US indication completely. There was no instance where US had additional value to feMRI. For fetuses without additional value, the kappa score was 0.69, indicating a substantial agreement.

Conclusion: This study is the first in the literature to present data from a single center, involving a single perinatologist and a single radiologist. We claim that feMRI and USG have low concordance in prenatal imaging (<0.8 kappa score). However, this low concordance still helps to navigate the pregnancy follow-up. The couple is counselled for invasive prenatal studies when additional findings are reported. Exclusion of the findings suspected in the US examination serves to decrease anxiety the remaining 34%.

References: Davidson J, Brennan K, Matthew J et al. Fetal magnetic resonance imaging (MRI) enhances the diagnosis of congenital body anomalies. J Pediatr Surg. 2022;57(2):239–244.

Grants:

Conflict of Interest: None declared.

EP02.021 Re-analysis of exome sequencing data of prenatal cases presenting with fetal structural anomalies

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Background/Objectives: Exome sequencing for fetuses with structural anomalies detected on ultrasound has been implemented into clinical practice in the UK Genomics Medicine Service, demonstrating diagnostic rates between 6.2% and 80%. Prenatal diagnosis provides information for prenatal and postnatal management and treatment (Best et al. 2018).

Re-analysis of exome data of previously unsolved cases can increase diagnostic yield by ~12% (Ji et al. 2021).

The objective of this study is to evaluate the diagnostic potential of exome sequence re-analysis in previously unsolved fetal anomaly cases.

Methods: DNA was extracted from CVS, amniotic fluid, fetal blood or post-mortem fetal tissue, and enriched using Agilent SureSelect CRE V2 or Nonacus ExomeCG and sequenced on Illumina NextSeq 500 or NovaSeq. Review of SNVs and CNVs was undertaken using the Congenica clinical decision platform. Re-analysis was performed using updated fetal anomaly panels and gene agnostic prioritisation.

Results: Through original exome sequencing analysis, diagnosis was achieved in 59/180 fetuses. Of 82 cases assessed for CNVs, 2 had pathogenic variants. Re-analysis was performed on 123 unsolved cases. Updated figures will presented.

Conclusion: This study illustrates the diagnostic utility of re-analysing exome sequencing data in unsolved cases with fetal structural anomalies.

References: Best, S., Wou, K., Vora, N., Ven der Veyver, I. B., Wapner, R., Chitty, L. S., 2018. Promises, pitfalls and practicalities of prenatal whole exome sequencing. Prenatal Diagnosis. 38(1):10–19.

Ji, J., Leung, M. L., Baker, S., Deignan, J. L., Santani, A. 2021. Clinical Exome Reanalysis: Current Practice and Beyond. Molecular Diagnosis and Therapy. 25(5):529–536.

Grants:

Conflict of Interest: None declared.

EP02.022 Preimplantation genetic testing of m.8344A>G MERRF mitochondrial DNA mutation: challenge and success

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Background/Objectives: Mitochondrial DNA (mtDNA) mutations cause a wide range of serious genetic diseases with high transmission risk, due to their maternal inheritance. One of the most frequent mtDNA mutation, the m.8344A>G of the *MT-TK* gene, is the cause of MERRF syndrome (Myoclonic Epilepsy with Ragged-Red Fibers). Two patients, carrier of the m.8344A>G mutation, have requested PGT in our center, but their high mutation levels in blood (75%) questioned their chance to get at least one « transferable » embryo.

Methods: Mutant loads were assessed by semi-quantitative fluorescent PCR followed by enzymatic digestion on 56 single-cells sampled from 30 embryos.

Results: Embryonic mutant loads were ranging from 10 to 97%, in favor of random segregation of mutant mtDNA molecules during oogenesis. Except in one embryo, the mutant loads were stable among the different blastomeres of day-3 embryos (±2%) supporting the feasibility of PGT by single-blastomere analysis. Furthermore, embryos with low (<30%; $n = 3/30$) or intermediate mutant loads (>30% et <70%; $n = 7/30$) were obtained. Uterine transfers of embryos were performed in 3 cycles, resulting in a single pregnancy and one healthy baby was born. The remaining 8 healthy embryos have been vitrified.

Conclusion: Our data demonstrate the clinical utility of the PGT procedure even for patients who carry high mtDNA mutation levels, and have important consequences in terms of genetic counseling.

References:

Grants:

Conflict of Interest: None declared.

EP03 Sensory Disorders (Eye, Ear, Pain)

EP03.003 Pathogenicity prediction for novel genetic variants related to Hearing Loss in a cohort of patients from Argentina

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Background/Objectives: Hearing loss (HL) is the most common disorder affecting 1:500 newborn children. Identification of causative mutations is demanding due to the large number (more than 100) genes involved. Whole-exome sequencing (WES) has become a cost-effective alternative approach for molecular diagnosis of HL. However, the follow-up of novel variants, in particular missense changes, which can lead to a spectrum of phenotypes and unequivocal genotype-to-phenotype correlations, is not always straightforward. In this study, we investigated the genetic cause of sensorineural hearing loss in patients with severe/profound deafness.

Methods: After the exclusion of frequent *GJB*2-*GJB*6 mutations by Sanger Sequencing, we performed WES in 32 unrelated Argentinean families. To predict the effect of some novel variants, protein modelling and protein stability analysis were employed.

Results: Mutations were detected in 16 known deafness genes in 20 patients: *ACTG*1, *ADGRV*1 (*GPR*98), *CDH*23, *COL*4*A*3, *COL*4*A*5, *DFNA*5 (*GSDDE*), *EYA*4, *LARS*2, *LOXHD*1, *MITF*, *MYO*6, *MYO*7*A*, *TECTA*, *TMPRSS*3, *USH2A* and *WSF*1. Notably, 11 variants affecting 9 different non-*GJB*2 genes resulted novel. Structural protein analysis in *LARS*2 and *MYO*6 proteins provided strong evidence on the impact of the mutations on protein function, as well as a novel high confident protein modelling to be used in future analyses.

Conclusion: These results highlight the value of whole exome sequencing to identify candidate variants, as well as bioinformatic strategies to infer their pathogenicity.

References:

Grants:

Conflict of Interest: None declared.

EP03.004 Clinical exome sequencing reveals pathogenic mutations in Bulgarian patients with inherited retinal degenerations

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Background/Objectives: Inherited retinal degeneration (IRD) is a group of retinopathies with more than 300 genes associated and more than 20 different clinical phenotypes described (RetNet). However, genetic diagnosis remains unclear in ~1/3 of cases. Clinical and genetic heterogeneity makes difficult identification of disease-causing mutations. Therefore, molecular diagnosis is important for genetic counseling and treatment. The aim of the present study was to identify the genetic diagnosis in a group of Bulgarian patients affected by IRDs.

Methods: In a selected group of 80 patients diagnosed with different forms of IRDs, we performed targeted sequencing of clinical exome (including 4813 OMIM genes) on MiSeq platform of Illumina. A detailed analysis, followed by Sanger sequencing and segregation analysis, was used to identify pathogenic variants.

Results: Pathogenic variants in IRD-related genes were found in 72 out of 80 patients analyzed. Seventy nine mutations were found in 33 IRD genes, explaining 90% of the cases—65 known and 14 novel. In 8 patients (10%) genetic cause of the disease was not identified, possibly due to low-covered region (ORF15) of a major gene for X-linked retinopathy, RPGR, or a mutation in an undescribed gene yet. Predominant clinical phenotype in the studied patient group was macular degeneration with mutations in the common gene responsible for this disease, ABCA4.

Conclusion: Targeted sequencing of clinical exome allowed detection of disease-causing mutations in 92% of our cases, a percentage which exceeds previously reported diagnostic yield of 60–70% for IRDs.

References: RetNet (https://sph.uth.edu/retnet/).

Grants: KP-06-N33/12/18.12.2019, D01-285/17.12.2019, D01-395/18.12.2020, D01-302/17.12.2021.

Conflict of Interest: None declared.

EP03.005 Estrogen and androgen related genes in the corneal epithelium in Keratoconus

Katarzyna Jaskiewicz[1], *Magdalena Maleszka-Kurpiel*[2;3], *Malgorzata Rydzanicz*[4], *Justyna Karolak*[5], *Rafał Płoski*[4], *Marzena Gajecka*[1;5]

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Background/Objectives: Keratoconus (KTCN) is the most common corneal ectasia, affecting 1:2000 individuals worldwide, characterized by progressive thinning of the cornea, leading to pathological cone formation(1). Hormones play a critical role in regulating cell survival, proliferation, and differentiation(2). Since sex-dependency and pregnancy-caused progression were observed in KTCN, we aimed to analyze the transcriptomic profile of sex hormones-related genes in the corneal epithelium (CE) of KTCN.

Methods: RNA samples were extracted from CE of 15 male KTCN patients undergoing cross-linking procedure and 3 male mild myopia patients undergoing refractive error correction (RNA/DNA/Protein PurificationPlus MicroKit, NorgenBiotek). The NGS libraries were prepared using TruSeq Stranded TotalRNA LibraryPrep Gold (Illumina) and sequenced on Novaseq6000 platform (100M reads/sample). RNA-seq data was analyzed implementing previously established pipelines.

Results: We haven't revealed any difference in the expression of sex hormones receptors (ESR1, ESR2, AR) between KTCN and control samples. However, the expression of genes responsible for the transduction of signals to the nucleus (RAF1, GRB2, JAK1, CREB1), and genes related to sex hormones' synthesis (HSD17B2) was changed. Moreover, in the pathway analysis we have noticed 'Activated PKN1 stimulates transcription of AR regulated genes KLK2 and KLK3 pathway' as one of the most overrepresented in the CE of KTCN.

Conclusion: As abnormalities in estrogen and androgen related genes have been identified, these features should be considered in CE transcriptomic profiling in KTCN.

References: 1.https://doi.org/10.1007/s00438-016-1283-z.
2. https://doi.org/10.1038/srep25534.

Grants: The National Science Centre grant no.2018/31/B/NZ5/03280. Co-financed by the European Social Fund, Operational Programme Knowledge Education Development, project 'International scholarship exchange of doctoral students and academic staff', No.POWR.03.03.03.00-00-00-PN13/18.

Conflict of Interest: None declared.

EP03.006 Molecular findings in a Romanian case series of retinal dystrophies

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Background/Objectives: Retinal dystrophies (RDs) are a group of heterogenous disorders with complex inheritance and variable clinical pictures, that include nyctalopia, colour blindness, central or peripheral vision loss up to complete blindness. Significant progress has been made to determine the molecular substratum, linked to more than 300 genes.

Methods: Six patients with retinal dystrophies were referred to our laboratory for WES or panel sequencing for genes associated with RDs, between November 2019–July 2021. Ion GeneStudio S5 System (Thermo Fisher Scientific) and Ion Reporter platform were used.

Three patients were clinically diagnosed with retinitis pigmentosa, two with cone-rod dystrophy, and one with unspecific retinal anomaly and cataract.

Results: Two of the patients diagnosed with retinitis pigmentosa and one of the patients with cone-rod dystrophy carried pathogenic variants in ABCA4 gene. The third retinitis pigmentosa patient had a pathogenic missense in RHO gene. An indel in CDHR1 gene and a missense variant in RGR gene were found in the other patient with the cone-rod dystrophy. Only a pathogenic variant in CRYBB2 gene, causative for cataract, was found in the last patient.

Conclusion: Molecular diagnosis of RDs is essential in order to clarify the clinical presentation and evaluate the prognosis of age-related visual impairment, as well as the genetic of these highly heterogenous group of diseases. Appropriate counselling and where available, a gene-based therapy depending on the nature of the disease pathology will help the management of the symptoms.

References:

Grants:

Conflict of Interest: Andreea Tutulan-Cunita Cytogenomic Medical Laboratory, Anca Pavel Cytogenomic Medical Laboratory,

Simona Buliga: None declared, VASILICA PLAIASU: None declared, Ina Ofelia Focsa Cytogenomic Medical Laoratory, Danai Stambouli Cytogenomic Medical Laboratory.

EP03.007 Is the decreased methylation of the PCDHA10 gene causative in high myopia in children?

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Background/Objectives: High myopia (HM), an eye disorder with a refractive error of −6.0 D or higher, has a complex etiology involving environmental, genetic, and likely epigenetic factors. Here, we report the study of differentially methylated protocadherin alpha (PCDHA) gene cluster in children with HM.

Methods: From the genome-wide methylation data of blood DNA of 18 Polish children with HM and 18 matched controls, we retrieved CG dinucleotides with lower methylation levels in HM cases. Selected sequence variants were Sanger sequenced in the studied children and members of a Polish family affected with HM.

Results: The PCDHA gene cluster, located in myopia locus MYP25 (5q31), includes the CG dinucleotide with the most decreased methylation level in HM cases versus controls. The CG site is located in transcription start site-1500 region of PCDHA10 and intronic regions of PCDHA 1–9. A previous GWAS indicated that SNV rs246073 in this cluster, was associated with refractive error in Europeans ($n = 542{,}934$, p value $= 2.0 \times 10^{-14}$). Also, an exonic nonsense variant rs200661444 (c.2017C>T, p.(Q673X)) in CpG island of PCDHA10, predicted as disease causing, was detected in our exome sequencing in the HM family, but did not completely segregate with HM. The SNV overlaps binding sites of AP-2alphaA, essential for the morphogenesis of lens vesicle and regulating the transcription of genes involved in the eye development.

Conclusion: Alterations in the methylation pattern of specific CG dinucleotides could be linked to early-onset HM and therefore be used to develop non-invasive biomarkers of HM in children and adolescents.

References: PMID:30858441.

Grants: National Science Center in Poland, 2019/35/N/NZ5/03150 (JS).

Conflict of Interest: None declared.

EP03.008 TCF4 trinucleotide repeat expansion in the Finnish patients with Fuchs' endothelial corneal dystrophy

Inka-Tuulevi Pettinen[1;2;3], *aino jaakkola*[4], *Kari Krootila*[4], *maria kaukonen*[1;2;3], *Hannes Lohi*[1;2;3], *Tero Kivela*[4], *Joni Turunen*[1;4]

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Background/Objectives: Fuchs endothelial corneal dystrophy (FECD) causes a progressive loss of corneal endothelial cells, resulting in structural changes and loss of corneal clarity. FECD is the leading cause of corneal transplantation in western countries. Multiple genetic risk factors contribute to the disease, but the best-known risk variant is an expansion of intronic CTG-repeat in the *TCF4* gene. An expansion of more than 50 repeats causes a significant risk for the disease.

Methods: One hundred and ten Finnish patients with a clinical diagnosis of FECD were collected from Helsinki University Hospital. The patients with FECD were examined by slit-lamp, corneal tomography, and endothelial specular microscopy. Short tandem repeat analysis and triplet repeat primed PCR were used to assess the repeat number in *TCF4*. The CTG-genotype was also analyzed in a control cohort of 202 individuals from the Finnish Red Cross Biobank.

Results: A CTG-expansion of >50 was observed in 92 of the 110 patients with FECD in homo- or heterozygous state (84%, 95% CI 0.75–0.90). One of the 92 cases had the expansion present in both alleles. Furthermore, only five of the 202 control individuals had >50 CTG-repeats, each in a heterozygous state (0.03%, 95% CI 0.007–0.05).

Conclusion: These results indicate that the CTG-repeat in *TCF4* is a notable risk factor for FECD also in the Finnish population with more than 80% of the patients carrying the expansion. Further analysis is performed to clarify the role of the expansion in the clinical and pathological features of these patients.

References:

Grants:

Conflict of Interest: None declared.

EP03.009 The first study of the RPE65 gene in the Russian Federation

Anna Stepanova[1], *Vitaly Kadyshev*[1], *Olga Shchagina*[1], *Alexander Polyakov*[1]

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Background/Objectives: Retinitis pigmentosa (RP) is a group of heterogeneous genetic diseases with a prevalence of ~1:4000 worldwide. LUXTURNA® (voretigene neparvovec) is a gene therapy approved for the treatment of patients with autosomal recessive retinal dystrophy caused by biallelic pathogenic variants in the *RPE65* gene. Therefore, an accurate molecular diagnosis is critical to the approval of targeted therapy.

RPE65 mutations are thought to be responsible for ~0.6–16% of all RP or Leber congenital amaurosis (LCA) cases in various populations. Studies have not yet been conducted to assess the frequency of RPE65-dependent forms of RP in Russia.

Methods: Our study included 189 unrelated patients with different forms of hereditary retinal degeneration. Patients' DNA was analyzed by targeted next-generation sequencing (NGS) using an «Ophthalmo» disease-specific panel, which included 211 genes.

Results: In 11 patients, variants in the *RPE65* gene were identified in the homozygous (4 patients) or compound heterozygous (7 patients) state. The variants were confirmed by Sanger sequencing for all patients. Segregation analysis was performed for 9 families, and the variants were proved to be biallelic. Thus, the proportion of RPE65-patients in the current study was 5.8%.

We have identified 12 different variants in the RPE65 gene, including 8 missenses (c.1024T>C(p.Tyr342His), c.1307G>A(p.Gly436Glu), c.746A>G(p.Tyr249Cys), c.1451G >T(p.Gly484Val), c.272G>A(p.Arg91Gln), c.1451G>A(p.Gly484Asp), c.1340T>C(p.Leu447Pro), c.982C>T(p.Leu328Phe), 2 nonsense (c.370C>T(p.Arg124*), c.304G>T(p.Glu102*)) and 2 splicing site mutations (c.1451-1G>A, c.11+5G>A). Two mutations were novel (c.1024T>C(p.Tyr342His),

c.1340T>C(p.Leu447Pro)). The most common mutation c.370C>T(p.Arg124*) was found on 5 chromosomes (22.7%).

Conclusion: All patients with confirmed biallelic mutations in the RPE65 gene are eligible for targeted therapy.

References:

Grants:

Conflict of Interest: None declared.

EP03.010 Leber's hereditary optic neuropathy: case report

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Background/Objectives: Leber's hereditary optic neuropathy (LHON) is an inherited mitochondrial disorder that is typically present in young males with sequential visual loss due to optic neuropathy, characterised by acute or subacute failure of central vision. LHON is maternally inherited, with variable penetrance, typically an autosomal recessive condition, but rare dominant cases have been reported.(1,2).

In 95% of cases, LHON is caused by one of three primary mutations of the mitochondrial DNA (mtDNA), m.11778G>A in the *MT-ND4* gene, m.14484T>C in the *MT-ND6* gene, or m.3460G>A in the *MT-ND1* gene.(3).

Methods: In this study, we report a Moroccan family with multiple affected individuals, suffering from visual impairment since childhood.

Results: Among the tested members of the family, 6 individuals were found harbouring one of the primary mutations, m.11778G>A in the *MT-ND4* gene, with a 99.2% heteroplasmy rate.

Conclusion: Leber's hereditary optic neuropathy is one of the most common mitochondrial diseases, secondary to three major mutations in three mitochondrial genes. In our study, we found the most common mutation, confirming its global distribution in the population.

References:

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Grants:

Conflict of Interest: None declared.

EP03.012 Variant c.394C>T in FGF3 is a recurrent cause of profound congenital deafness in Slovak Roma

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Background/Objectives: Recessive mutations of fibroblast growth factor 3 (*FGF3*) cause rare type of congenital deafness, with labyrinthine aplasia, microtia, and microdontia (LAMM syndrome, OMIM # 610706). We present two cases of unrelated Slovak children of Roma ethnicity with congenital deafness with homozygous c.394C>T p.(Arg132Trp) variant in *FGF3*.

Methods: NGS sequencing was performed (Illumina, Nextera Flex Pre-enrichment True Sight One Expanded; NextSeq 550 Illumina) with targeted bioinformatic analysis of genes associated with hearing loss (bcl2fastq v2.20 and SEQNEXT v5.2.0), ref. GRCh37/hg19.

Results: Newborn hearing screening was positive in both patients (boys) with absent OAEs and with profound bilateral mixed hearing loss confirmed by further audiology testing. In patient N°1 microtia was present bilaterally in contrast to patient N°2 without external ear abnormality. MRI examination of middle and internal ear structures was not available. Targeted NGS sequencing confirmed homozygosity for c.394C>T p.(Arg132Trp) variant in *FGF3* gene (NM_005247.2) dbSNP: rs372402801, with variant frequency in gnomAD 0,004 % (0 homozygotes), not reported in ClinVar nor in HGMD. The variant was classified as variant of unknown significance (PM2, PP2, PP3) according to ACMG criteria.

We evaluated the frequency heterozygotes for the given variant in the in-house database of 167 exomes of healthy controls of self-determined Roma persons from Slovakia and Czech republic unrelated to our patients. We found 7/167 heterozygotes with the resulting carrier frequency 4,19%.

Conclusion: Though generally very rare, LAMM syndrome might be frequent cause of profound mixed congenital deafness in Roma population in our region. Further studies are necessary to confirm founder effect.

References:

Grants:

Conflict of Interest: None declared.

EP03.013 Unexpected Array CGH result in a patient with leading symptom ocular coloboma

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Background/Objectives: Ocular colobomata are caused by a failure to close of the embryonic fissure during development. Occurrence may be isolated, but they are often associated with other systemic anomalies. Although many pathogenic variants in different genes have been identified, a large proportion of isolated cases remains idiopathic. Here we report on a 31-year-old male with bilateral retinal, choroidal and iris colobomata, mild facial dysmorphism, premature graying of the hair, aplasia of the left 12th rib and jaw cysts.

Methods: We performed cytogenetic banding and microarray CGH analyses followed by qPCR.

Results: Cytogenetic banding analysis gave a normal male karyotype (46,XY) and no evidence of an isodicentric derivative chromosome 22 (Cat Eye syndrome). Microarray CGH and qPCR analyses in the parents identified a de novo heterozygous ~3.47

Mb deletion in 6p24.3-p24.1 encompassing 25 genes, i.a. *TFAP2A*. Pathogenic *TFAP2A* mutations cause the rare autosomal dominant Branchiooculofacial syndrome (BOFS, MIM # 113620). The clinical presentation is extremely variable and typically includes branchial skin defects, eye abnormalities (e.g. colobomata), nasolacrimal duct stenosis / atresia and facial dysmorphism. Other reported abnormalities include subcutaneous cysts, dental abnormalities, and premature hair graying. Intellect is usually normal.

Conclusion: More than 95% of pathogenic variants in patients with BOFS are SNVs or indels. Here we present a de novo 6p24.3-p24.1 deletion including *TFAP2A* as a rare cause of BOFS. Our case shows the value of chromosomal microarray diagnostics in patients without the "classical" indication neurodevelopmental disorder.

References:

Grants:

Conflict of Interest: None declared.

EP03.014 Detection of two novel splicing mutations associated to deafness by whole exome sequencing

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Background/Objectives: Point mutations affecting splicing may result in the formation of aberrant transcripts of the gene. Recent research has underlined the abundance and importance of splicing mutations in the etiology of inherited diseases. The myosin gene family is associated with different diseases including deafness, although few splicing mutations in *MYO* genes have been described.

The aim of this study was to identify mutations associated with deafness by whole exome sequencing (WES).

Methods: Genomic DNA was extracted from peripheral blood leukocytes in a cohort of 12 patients. WES was performed to identify germinal mutations that could be responsible for the disorder.

Results: In one patient, we identified a mutation in heterozygosis in the *MYO7A* gene (c.4852G>A; p.Ala1618Thr, rs772542296), which affects both splicing and the protein sequence. This mutation has been classified as an uncertain significance variant in ClinVar, but it is considered likely pathogenic in Varsome. In other case, we found a splicing mutation in heterozygosis in *MYO3A* gene (c.2506-1G>A, rs201023600), reported as conflicting interpretations of pathogenicity in ClinVar but predicted as pathogenic in Varsome. *MYO7A* and *MYO3A* have been associated with nonsyndromic progressive deafness, which is compatible with both clinical histories. This is the first time that these mutations are reported as pathogenic and associated with deafness. Implications of other mutations found, mainly missense, is being investigated in the same cohort of patients.

Conclusion: WES is a useful approach to identify splicing mutations associated with deafness, as c.4852G>A; p.Ala1618Thr in *MYO7A* and c.2506-1G>A in *MYO3A*.

References:

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Conflict of Interest: None declared.

EP03.015 NTRK1-associated congenital insensitivity to pain with anhidrosis – two novel cases from Polish population

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Background/Objectives: *NTRK1*-associated congenital insensitivity to pain with anhidrosis (*NTRK1*-CIPA, OMIM #256800) is severe neurological disorder of autosomal recessive inheritance, characterized by sensory and autonomic neuropathy leading to self-injuries and hyperthermia episodes, with high risk of neurodevelopmental dysfunctions including intellectual disability and epilepsy. It is extremely rarely reported outside the Japanese and Israeli Bedouin population.

Methods: Here we report two unrelated individuals of Polish origin, presenting features consistent with hereditary sensory and autonomic neuropathy diagnosis. Individual 1: 4 years of age female, insensitivity to pain, anhidrosis, hyperthermia episodes, drug-resistant epilepsy, tendency to self-injuries, dental self-extractions, neurodevelopmental delay. Individual 2: 12 years of age male, insensitivity to pain, anhidrosis, recurrent hyperthermia episodes, palmar and plantar hyperkeratosis, recurrent joints injuries and inflammation.

Results: Direct sequencing of *NTRK1* coding region detected compound heterozygosity in both patients: individual 1: c.574+1G>A (maternal origin) and c.845delT (paternal origin), individual 2: c.574+1G>A (paternal origin) and c.850+6T>A (*de novo*).

Conclusion: c.850+6T>A is a novel variant detected in *NTRK1* gene. Both individuals share common variant c.574+1G>A, described previously and associated with *NTRK1*-CIPA. Given the shortage of data regarding the disorder outside the populations with higher prevalence, clinical description and molecular findings described here expand the knowledge of disease variability.

References:

Grants:

Conflict of Interest: None declared.

EP03.016 Novel COL9A1 mutation causes autosomal recessive isolated high myopia

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Background/Objectives: Identifying the genetic cause of isolated high myopia affecting two siblings in consanguineous Bedouin family.

Methods: Affected individuals underwent thorough ophthalmologic examination, including fundus photography and optical coherence tomography (OCT). Whole-exome sequencing (WES) of the proband and homozygosity mapping of the studied kindred

(750k SNP arrays) were done, assuming autosomal recessive heredity. WES variants which passed our filtering cascade were screened using our database of 500 ethnically-matched controls and validated using Sanger sequencing.

Results: The two siblings were affected since infancy, and recently, ages 18 and 21 years, their refractive errors ranged from -8 to -20 dioptres. One sibling had anisometropia (9 dioptres), amblyopia, and strabismus. Fundus and OCT findings were compatible with high myopia. They had no facial deformities, hearing loss or skeletal anomalies. Linkage analysis unravelled several homozygous loci shared only by two affected individuals. Using our variant analysis pipeline, all WES variants within these loci were ruled out except for one: COL9A1 (NM_001851.4): c.1550G>A, p.G517E, segregating in the kindred as expected for recessive heredity and not found in 500 ethnically-matched controls or public databases.

Conclusion: We report a novel COL9A1 mutation causing autosomal recessive high myopia without systemic abnormalities. COL9A1 takes part in assembly of type IX collagen molecules, and is expressed in various tissues including the eye. COL9A1 mutations were previously reported mainly in association with Stickler syndrome, hearing loss and multiple epiphyseal dysplasia. Our findings suggest that genetic evaluation of isolated severe myopia should include analysis for the presence of COL9A1 pathogenic variants.

References:

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Conflict of Interest: None declared.

EP03.017 Spectrum of pathogenic variants in the rhodopsin gene in Slovenian patients with retinal dystrophies

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Background/Objectives: Pathogenic variants in the rhodopsin gene (RHO) cause various forms of retinitis pigmentosa (RP), which is a progressive retinal degeneration, and less commonly congenital stationary blindness. The aim of our study is to review the spectrum of pathogenic variants in RHO in Slovenian patients with retinal dystrophies.

Methods: Study included 40 patients from 11 unrelated families harbouring pathogenic variants in RHO. Patients were identified from database of Slovenian patients with genetic eye diseases at the Eye Hospital, UMC Ljubljana. Phenotypes were classified into four categories according to fundus autofluorescence (FAF): night blindness without degeneration (normal FAF), classic RP (cRP) (hyperautofluorescent ring), sector RP (sRP) (peripheral hyperautofluorescent border extending ≤ 2 quadrants) and pericentral RP (pRP) (pericentral hypoautfluorescent area).

Results: RHO patients represented 11% (40/362) of Slovenian RP cohort (4,5%; 11/247 families) and 41% (40/97 patients) of dominant RP. Pathogenic variants included p.Gly90Asp (4 unrelated families), p.Val87Asp (4 unrelated families), p.Pro347Leu (2 unrelated families) and a novel variant p.Ser297Arg (1 unrelated family). The last two variants were associated only with cRP, whereas p.Val87Asp caused either cRP, sRP or pRP; and all four phenotypes were observed in the p.Gly90Asp cohort. Of the variants only p.Pro347Leu is frequent in other cohorts.

Conclusion: RHO variants represent a frequent cause of RP in Slovenia with specific spectrum of variants. The p.Gly90Asp and p.Val87Asp were the most frequent and displayed significant phenotypic diversity, not usually associated with RHO.

References: /.

Grants: /.

Conflict of Interest: None declared.

EP03.019 ACTG1: a spectrum ranging from non-syndromic hearing impairment to polymalfomative fetal presentations

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Background/Objectives: Pathogenic variants of *ACTG1* have been reported for 2 distinct phenotypes: Autosomal dominant isolated deafness DFNA20/26 and Baraitser-Winter syndrome 2, that associates intellectual deficiency, ocular malformations, dysmorphism, epilepsy and cerebral malformations. Surprisingly, hearing impairment is seldom associated to Baraitser-Winter syndrome 2. There is a high prevalence of DFNA20/26 patients identified through gene panel sequencing presenting with isolated sensorineural hearing impairment of dominant transmission. DFNA20/26 usually presents as non-syndromic, progressive, postlingual, hearing impairment with an onset between the first and third decade. The objective is to better characterize the phenotypes associated with *ACTG1* variants.

Methods: This is a retrospective study on a French cohort of 35 patients and 2 fetuses.

Results: Most of the patients have a typical presentation of DFNA20/26. 3 patients present with developmental delay and a recognizable dysmorphism with flat face and arched eyebrows. 4 patients present with auditory neuropathy. In the 2 fetal cases we found corpus callosum and cerebral anomalies, associated to cardiac and skeletal malformations for 1 of them.

Conclusion: *ACTG1*-associated phenotype is broader than currently described. We have identified extra-auditory symptoms and a recognizable dysmorphism in a number of patients.

References:

Grants:

Conflict of Interest: None declared.

EP03.022 Mutational analysis and genotype-phenotype correlation of 22 families with GUCY2D variants

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Background/Objectives: The *GUCY2D* gene, located in chromosome 17, encodes for the retinal-specific guanylate cyclase, which is expressed in photoreceptors. Pathogenic *GUCY2D* variants are associated with an ample range of inherited retinal dystrophies (IRD). The aim of this study is to describe the mutational and phenotypic landscape of patients with variants in this gene.

Methods: A total of 22 probands characterized with *GUCY2D* variants were selected from a cohort of more than 4500 families with IRD. Variants were identified by different NGS technologies and/or Sanger sequencing. Clinical data were obtained from questionaries and health records. A detailed ophthalmic evaluation was performed in 5 cases.

Results: Among the studied probands, 14 of them carry a dominant pathogenic variant that is associated with cone-rod dystrophy (CRD) and 8 have a recessive hereditary pattern, mostly associated with a phenotype of Leber congenital amaurosis (LCA). A total of 17 different variants were identified, including 2 novel variants. The variants showing a dominant pattern are exclusively missense, located in the exons 13-14 of *GUCY2D*. Meanwhile, the recessive-related variants are missense and truncating, distributed throughout the gene. LCA patients, especially those carrying biallelic truncating variants, show a significantly earlier age of onset and loss of visual acuity than CRD cases.

Conclusion: Our study supports the genotype-phenotype model established for *GUCY2D* variants, and broadens the knowledge of the gene's mutational diversity.

References:

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Conflict of Interest: None declared.

EP03.023 Genomic landscape of dominant retinitis pigmentosa in a cohort of 333 Spanish families

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Background/Objectives: Inherited Retinal Dystrophies (IRD) are a group of rare diseases whose prevalence is 1:3000−4000 people worldwide. Currently, the reported diagnostic rate of IRD is 50% −60%. The aim of this work is to update an overview of the molecular characterization rate and gene frequency of autosomal dominant retinitis pigmentosa (adRP) in Spain.

Methods: A cohort of 372 families with a suspected diagnosis of adRP was selected from our database, of which 39 families were excluded because they are deceased, related patients or not collaborative. So, 333 families were studied through classic genotyping methods or targeted next-generation sequencing (NGS). The last up-date of adRP data was reported on 2018 with 258 unrelated adRP families.

Results: Overall, 70% of our adRP cohort has been characterized with an increase of diagnosis rate of ~+10% from 2018. Furthermore, 7.5% of this cohort has been reclassified to other types of inheritance (autosomal recessive or X-linked). Additionally, we identified adRP-causing mutations in 4 not previously reported genes in this cohort (*GUCA1A/RP1L1/RAX2/FZD4*). Although gene frequency data have changed slightly, mutations in *RHO/PRPF31/PRPH2/RP1* continue being the most frequent.

Conclusion: Implementation of NGS has increased the characterization rate. However, there are 30% unsolved cases, which have not been studied by NGS yet or could be caused by undiscovered variants/genes, or genomic rearrangements difficult to identify through this technology. Thus, it is essential to study new molecular mechanisms that explain this disease.

References: https://doi.org/10.1167/iovs.18-23854, https://doi.org/10.1186/gm155.

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Conflict of Interest: None declared.

EP03.024 Clinical and genetic study of Waardenburg syndrome type 1 in Tunisian patients

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Background/Objectives: Waardenburg syndrome type 1 (WS1) is a rare autosomal dominant disorder associated with *PAX3* gene mutations. It is clinically characterized by sensorineuronal hearing loss and pigmentary anomalies of eyes, skin and hair. Dysmorphic features involve dystopia canthorum, synophrys, and prominent nasal root.

This study aims to present the clinical and genetic characteristics of WS1 in Tunisian patients.

Methods: We included eleven patients with clinical diagnosis of WS1. The entire coding region of *PAX3* has been sequenced in six individuals. Multiplex Ligation-dependent Probe Amplification (MLPA) was performed for two patients with normal *PAX3* sequencing.

Results: We report eleven patients from eight unrelated families. An affected first-degree relative was identified in 3/8 families. Dysmorphic features involved dystopia canthrom (11/11), prominent nasal root (8/11), synophrys (4/11) and alae nasi

hypoplasia (3/11). Clinical findings also included sensorineural deafness (8/11), hypochromic irises (7/11), hair hypopigmentation (9/11) and abnormal skin pigmentation (4/11). We noted an intrafamilial phenotype heterogeneity in our patients.

PAX3 sequencing in six individuals revealed three novel pathogenic variants in four of them: c.942delC; c.933_936dupTTAC and c.164delTCCGCCACA. MLPA, performed for two patients with normal sequencing, showed a heterozygous deletion of exons 5 to 9 in one of them (1).

Conclusion: These results highlight the clinical variability associated with WS1. The incomplete penetrance of *PAX3* variants, suggested by familial cases study, is an important parameter to consider for genetic counseling.

References: (1) Trabelsi M. et al 2017.

Grants:

Conflict of Interest: None declared.

EP03.025 Phenotype of patients harbouring p.Cys870Ter in USH2A exon 13 amenable for exon skipping therapy

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Background/Objectives: The purpose of our study was to determine the long-term natural history of disease in patients carrying a c.2610C>A; p.(Cys870Ter) variant in *USH2A* exon 13, who would be amenable to exon skipping therapy.

Methods: Study included 8 patients (3 male) from the Slovenian *USH2A* cohort, harbouring p.(Cys870Ter) in *USH2A* (2 homozygous). Median age at first exam was 42 years (range, 25-56) and the median age at last exam was 53 years (range, 25-65); with a median follow up of 10 years (range, 0-23). Phenotypic analysis included age at onset, visual acuity (VA, decimal Snellen), Goldmann perimetry (target II/4), colour vision (Ishihara), fundus autofluorescence imaging (FAF) and optical coherence tomography (OCT). Right eye was taken for analysis.

Results: Median age at onset was median 20 years (range, 8-35 years). At the first and last exam, the median VA were 0,6 (0,16-1,0) and 0,15 (range, 0,005-1); the median number of recognized Ishihara plates were 9/15 and 0/15; and the median visual field diameters were 22° (5°-114°) and 18° (5°-22°), respectively. Hyperautofluorescent ring on FAF delineating preserved photoreceptors on OCT was present in 75% at the first and 63% at the last exam. Kaplan Maier analysis showed that 50 % patients reach legal blindness based on visual acuity on the better eye (VA ≤ 0,1) at the age of 57 (95%CI 52-63) years.

Conclusion: Long term follow-up data of patients with variants in exon 13 of *USH2A* will be useful in conducting clinical trials that aim to slow down disease progression.

References: None.

Grants: ARRS J3-1750.

Conflict of Interest: None declared.

EP03.026 Pathogenic variant frequencies in autosomal dominant non-syndromic hearing loss genes

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Background/Objectives: Autosomal dominant non-syndromic hearing loss (ADNSHL) is an extremely heterogeneous sensorineural disorder, both genetically and clinically. However, the global prevalence of ADNSHL is yet unknown, as most pathogenic gene variants have a very rare allele frequency. To ascertain whether database searches could be utilized as predictors of pathogenicity, recently, nine genes (*REST*, *COL11A1*, *PTPRQ, PDE1C, MYO3A, TRRAP, PLS1, SLC12A2* and *LMX1A*) associated with ADNSHL were identified and analysed in peer-reviewed publications by the developed algorithm.

Methods: Initially, the clinical significance of variants was analysed in public sources such as the Human Gene Mutation Database and ClinVar while predictions of pathogenicity were calculated in silico. Current global and population specific allele frequencies were estimated with high quality sequencing reads in the Genome Aggregation Database (gnomAD). Minor allele frequency of ≤0.00002 defined by PM2_Moderate criteria was estimated from American College of Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/AMP) guideline scores adapted for ADNSHL interpretation.

Results: Only MYO3A, TRRAP and PDE1C gene variants were represented in gnomAD and met the PM2_Moderate criteria according to ACMG/AMP guidelines. The remaining variants were not found in any genetic variation database.

Conclusion: In this study, the algorithm may be applied to interpret the pathogenicity of variants and lead to a more precise estimation of global disease frequencies.

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Grants: Medical University of Graz.

Conflict of Interest: None declared.

EP03.028 Genetic background of adult Cochlear Implantat candidates in Czech Republic

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Background/Objectives: The role of genetics in pediatric cochlear implantation (CI) has already been repeatedly investigated, but the same knowledge in adults is still rather vague. While congenital or early onset hearing impairment show mostly autosomal recessive (AR) trait (with *GJB2* gene comprising up to 50%), postlingual demonstrate mostly autosomal dominant (AD) inheritance.

Our aim is to map genetic background of patients seeking cochlear implantation in adulthood.

Methods: We performed genetic testing in adult 210 CI users with age of onset from birth to 60 years, 55% having positive family history, mostly of an AD condition.

First we investigated all patients via Sanger sequencing and MLPA of *GJB2* gene, second MLPA of *STRC* gene deletion and sequencing of mtDNA regions m.1237 - m.1892 and m.7001 – m.7982, followed by homemade NGS panel of 174 hearing loss related genes in negatives.

Results: Mutations in *GJB2* gene were identified in 46 (22%), *STRC* deletion in 4 (2,4%) and mtDNA in 6 (3,6%).

From 154 NGS-tested patients 78 (50%) were negative, 52 (34%) VUS (ACMG class 3) and 24 (16%) ACMG class 4/5.

Surprisingly only in one third (8 patients) AD diseases were identified. The most common findings were AR Usher syndrome (thrice *USH2A*, once *USH1C*) and Pendred syndrome (thrice SLC26A4), followed by AD Stickler syndrome (twice *COL11A1*, once *COL2A1*) and nonsyndromic DFNA20 (twice *ACTG1* mutation).

Conclusion: We identified genetic background in 80 (38%) investigated subjects.

References:

Grants: NV19-06-00189, IP 9782, IP 6024, ZD-ZDOVA2-001.

Conflict of Interest: Radka Kremlíková Pourová Department of Biology and Medical Genetics, Czech Ministry of Health, Pavel Votypka: None declared, Marcela Malíková: None declared, Michaela Zelinová: None declared, Zdenek Fík: None declared, Zdeněk Čada: None declared, Jan Bouček: None declared.

EP03.029 Genomic study of non-syndromic hearing loss in unaffected individuals: frequency of pathogenic and likely pathogenic variants in a Brazilian cohort of 2097 genomes

Caio Robledo Quaio[1;2], *Antonio Victor Campos Coelho*[1], *Livia Maria Silva Moura*[1;3], *Rafael Lucas Muniz Guedes*[1;3], *Kelin Chen*[1], *Jose Ricardo Magliocco Ceroni*[1], *Renata Moldenhauer Minillo*[1], *Marcel Pinheiro Caraciolo*[1;3], *Rodrigo de Souza Reis*[1;3], *Murilo Cervato*[1;3], *Tatiana Ferreira de Almeida*[1], *Joao Bosco de Oliveira Filho*[1]

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Background/Objectives: Hearing loss is a common sensory deficit in humans and represents an important clinical and social burden. We assessed pathogenic and likely pathogenic variants associated with non-syndromic hearing loss (NSHL) in a cohort of unaffected Brazilian individuals referred to genome sequencing.

Methods: We used samples of 2097 patients from participating centers of the Brazilian Rare Genomes Project to sequence the whole genome and search for both sequence variants and copy-number variants in genes associated with NSHL.

Results: We identified 222 heterozygotes (10.59%) for relevant sequence variants, 54 heterozygotes (2.58%) for copy-number variants and four homozygotes (0.19%) for sequence variants. An important fraction of these individuals ($n = 104$) presented alterations associated with autosomal dominant forms of NSHL. Using data from the heterozygous individuals for recessive forms and the Hardy–Weinberg equation, we estimated the population frequency of affected individuals with autosomal recessive NSHL to be 1:2,222.

Conclusion: Genome sequencing identified relevant frequencies of molecular alterations associated with NSHL in a cohort of unaffected individuals and showed to be a valuable method to study the distinct molecular mechanisms underlying this heterogeneous group of conditions.

References:

Grants: This study was funded by PROADI-SUS, Brazil.

Conflict of Interest: Caio Robledo Quaio Hospital Israelita Albert Einstein, Antonio Victor Campos Coelho Hospital Israelita Albert Einstein, Livia Maria Silva Moura Hospital Israelita Albert Einstein, Rafael Lucas Muniz Guedes Hospital Israelita Albert Einstein, Kelin Chen Hospital Israelita Albert Einstein, Jose Ricardo Magliocco Ceroni Hospital Israelita Albert Einstein, Renata Moldenhauer Minillo Hospital Israelita Albert Einstein, Marcel Pinheiro Caraciolo Hospital Israelita Albert Einstein, Rodrigo de Souza Reis Hospital Israelita Albert Einstein, Murilo Cervato Hospital Israelita Albert Einstein, Tatiana Ferreira de Almeida Hospital Israelita Albert Einstein, Joao Bosco de Oliveira Filho Hospital Israelita Albert Einstein.

EP03.030 Unraveling the genetic basis of early-onset inherited retinal disease in a Saudi Arabian cohort reveals a novel RIMS2-related family

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Background/Objectives: In this study, we aimed to uncover the molecular causes of Leber Congenital Amaurosis (LCA) and early-onset retinal dystrophy (EORD) in 15 families of Saudi Arabian descent, using an integrated approach consisting of autozygosity mapping and targeted resequencing or whole exome sequencing (WES).

Methods: A total of 18 patients (7 females and 11 males ranging between 2-9 years old) from 15 Saudi consanguineous families underwent ophthalmological examinations to establish a clinical diagnosis. They underwent autozygosity mapping, targeted gene testing combined or not with whole exome sequencing (WES). Variants were validated, classified according to ACMG/ACGS guidelines, and subjected to segregation analysis if family members were available.

Results: Patients displayed decreased best corrected visual acuity, photophobia, amaurotic pupils, congenital nystagmus, and oculodigital sign. Likely pathogenic variants were found in 13/15 studied families in genes previously implicated in LCA/EORD, including 6 novel variants and a putative founder *RPGRIP1* variant (c.1107del;p.(Glu370Asnfs*5)) for the Saudi Arabia population. Interestingly, a novel homozygous *RIMS2* splice variant c.1751+1G>T was identified in an EORD patient without any signs of systemic involvement or neurodevelopmental symptoms. All variants were found in runs of homozygosity (ROH), apart from two heterozygous *GUCY2D* variants.

Conclusion: Our approach uncovered 13 distinct likely pathogenic variants in 13/15 studied families (86%), demonstrating the power of autozygosity-guided WES in a genetically heterogeneous consanguineous cohort with LCA/EORD. Finally, we report a biallelic *RIMS2* variant in a seemingly non-syndromic patient and corroborate the previous role of *RIMS2* in EORD pathogenesis.

References:

Grants: FAME Postdoctoral Fellow, FWO 1802220N.

Conflict of Interest: None declared.

EP03.031 An update on genetic background of autosomal dominant hearing loss

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Background/Objectives: Autosomal dominant hearing loss (ADHL) is the second most common form of inherited HL. It affects

mainly high frequencies and progresses over time. Autosomal-dominant genes are responsible for about 20% of cases of hereditary non-syndromic deafness, with 51 different genes identified to date.

Methods: In this study, 105 families with ADHL underwent targeted next-generation sequencing (NGS) using a cochlea-specific HL multi-gene panel (237 genes). Prior to NGS, environmental HL risk factors and DFNB1 locus (*GJB2* and *GJB6*) related hearing impairment had been excluded in all probands. Presence of the selected probably pathogenic variants and their segregation with HL within the family were confirmed by Sanger sequencing.

Results: Genetic cause of ADHL was identified in 43,8% (46/105) of the examined families. Among the 46 identified HL variants only 26% (12/46) have been previously reported and the remaining 74% are novel (34/46). We identified missense variants (27/46; 58,7%), splice site variant (9/46; 19,5%), stop-gain variants (5/46; 10,9%) and frameshift variants (5/46; 10,9%). Among the most common causative genes were *MYO6* ($n = 8$), *TBC1D24* ($n = 5$), *KCNQ4* ($n = 4$), *GSDME* ($n = 4$), *POU4F3* ($n = 4$) and *WFS1* ($n = 4$). Pathogenic variants causative of HL in the *SLC44A4, NLRP3, LMX1A, FGFR3, CD164, GRHL2, TMC1, COCH, ATP2B2* and *CEACAM16* genes were detected in single families.

Conclusion: Considering frequent identification of novel genetic variants it is necessary to perform thorough clinical examination and variant segregation analysis with ADHL in all available family members. In the largest families without genetic diagnosis of HL linkage analysis and whole genome sequencing will be performed.

References:

Grants: NCN grant 2016/22/E/NZ5/00470.

Conflict of Interest: None declared.

EP03.032 Evaluating a causal relationship between Complement Factor I protein level and advanced age-related macular degeneration using Mendelian Randomisation

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Background/Objectives: Advanced age-related macular degeneration (AAMD) risk correlates with rare Complement Factor I (CFI) genetic variants associated with low CFI protein levels(1), but the relationship is unclear.

Methods: Two-sample inverse variance weighted Mendelian Randomisation (MR) was used to evaluate evidence for relationship between CFI and AAMD risk(2), comparing genetically predicted CFI levels in AAMD and Geographic Atrophy (GA) patients and controls.

We derived genetic instruments for systemic CFI level in healthy INTERVAL study participants(3). To evaluate a genetic causal odds ratio (OR) for effect of CFI on AAMD risk, results were combined from an AAMD genome-wide association study(4) with CFI levels from SCOPE and SIGHT AAMD patients.

Results: Common CFI variant rs7439493 strongly associated with low CFI, explaining 4.8% phenotypic variance. Using rs7439493, MR estimates for AAMD odds increase per standard deviation (SD) CFI decrease were 1.47 (95% confidence interval (CI) 1.30–1.65, $P = 2.1 \times 10^{-10}$). MR using rare CFI variant p.Gly119Arg indicated a 1SD decrease in CFI led to increased AAMD odds of 1.79 (95% CI 1.46–2.19, $P = 1.9 \times^{-8}$). P.Gly119Arg explained 1.7% phenotypic variance. For benchmarking, results from a CFI-specific immunoassay on 24 p.Gly119Arg positive GA patients indicated each 1 SD (3.5 µg/mL) reduction in CFI associated with OR 1.67 of AAMD (95% CI 1.40–2.00, $P = 1.85 \times 10^{-8}$).

Conclusion: Concordance in MR calculations provide genetic evidence for a potentially causal role of low CFI increasing AAMD risk.

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Grants: Gyroscope Therapeutics.

Conflict of Interest: Amy Jones Gyroscope Therapeutics Ltd, Gyroscope Therapeutics stock options, Stuart MacGregor Australian National Health and Medical Research Council: Program, CRE, Fellowship, Gyroscope Therapeutics Ltd, Xikun Han: None declared, James Francis Gyroscope Therapeutics Ltd, Gyroscope Therapeutics stock options, Claire Harris Gyroscope Therapeutics Ltd, Ra Pharmaceuticals, Gyroscope Therapeutics stock options, Consultancy income from Q32 Bio Inc -Payment to Institution.

Consultancy income from Chinook Therapeutics -Payment to Institution.

Consultancy income from Biocryst Pharmaceuticals- Payment to Institution, Royalty income from commercialized factor I ELISA; Hycult Biotech, David Kavanagh Gyroscope Therapeutics Ltd funding source, Alexion pharmaceuticals-Personal.

Novartis-Personal, Gyroscope Therapeutics, Gyroscope Therapeutics-Personal.

Alexion pharmaceuticals- Personal & Institutional.

Novartis-Personal.

Apellis pharmaceuticals-Personal.

Sarepta Therapeutics- Personal, Andrew Lotery Gyroscope Therapeutics Ltd, Gyroscope Therapeutics Ltd, Nadia Waheed Gyroscope Therapeutics Ltd, Carl Zeiss Meditec.

Topcon.

Regeneron.

Heidelberg.

Nidek.

Optovue, Ocudyne.

Gyroscope Therapeutics stock options, Apellis.

Nidek.

Boehringer Ingelheim.

EP04 Internal Organs & Endocrinology (Lung, Kidney, Liver, Gastrointestinal)

EP04.001 Mendelian randomization of eosinophils and other cell types in relation to lung function and disease

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Health Research, Nottingham Biomedical Research Centre, Nottingham, United Kingdom; [4]National Institute for Health Research, Leicester Biomedical Research Centre, Leicester, United Kingdom.

Background/Objectives: Eosinophils are involved in airway inflammation in respiratory disease. Interleukin-5 (IL5) partly controls eosinophil production and survival; and response to anti-IL5 agents in asthma is correlated with baseline eosinophil counts. However, it is not clearly understood whether eosinophil levels are causally related to COPD and other respiratory phenotypes. We investigated causality between eosinophils and: lung function, acute exacerbations of COPD (AECOPD), asthma-COPD overlap (ACO), moderate-to-severe asthma, and respiratory infections.

Methods: We performed Mendelian randomization using 151 variants from genome-wide association studies of blood eosinophils in UK Biobank/INTERVAL, and respiratory traits in UK Biobank/SpiroMeta, using methods relying on different assumptions for validity. We performed multivariable analyses using eight cell types for traits where there was possible evidence of causation by eosinophils.

Results: Causal estimates derived from individual variants were highly heterogeneous. This may arise from pleiotropy. The average effect of raising eosinophils was to increase risk of ACO (weighted median OR per SD eosinophils 1.44 [95%CI 1.19,1.74]), and moderate-severe asthma (weighted median OR 1.50 [95%CI 1.23,1.83]), and to reduce FEV_1/FVC and FEV_1 (weighted median estimator, SD FEV_1/FVC: −0.054 [95%CI −0.078,−0.029]. Effects on lung function were more prominent in individuals with asthma.

Conclusion: Broad consistency between methods suggests eosinophils have a causal effect, though of uncertain magnitude. However, given the heterogeneity in results from individual instrumental variables, it is possible that these variants impair respiratory health via pleiotropic mechanisms, rather than by raising eosinophils. Our results suggest anti-IL5 agents may be useful in management of other respiratory conditions, including people with both asthma and COPD.

References:

Grants:

Conflict of Interest: Anna Guyatt: None declared, Catherine John: None declared, Alexander T Williams: None declared, Nick Shrine: None declared, Nicola F Reeve: None declared, Ian Sayers: None declared, Ian P. Hall Funded research collaborations with GSK, Boehringer Ingelheim and Orion, Louise Wain Funding from GSK for collaborative research projects outside of the submitted work, Nuala A Sheehan: None declared, Frank Dudbridge: None declared, Martin D Tobin Funding from GSK for collaborative research projects outside the submitted work.

EP04.002 Relationship between ADAM19, FAM13A, IREB2 genes common variants and COPD susceptibility and severity

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Background/Objectives: Chronic obstructive pulmonary disease (COPD) is a heterogenous disease characterized by persistent progressive airway limitation caused by both environmental factors and genetic predisposition. In this study, we aimed to investigate relationship between ADAM19, FAM13A, IREB2 genes previously detected common variants and COPD susceptibility and severity.

Methods: The clinical data of 110 patients having persistent airway limitation according to COPD definition of GOLD were collected. Patients were screened for ADAM19, FAM13A, IREB2 genes common variants using BigDye terminator on an ABI Prism 3500 genetic analyzer.

Results: There was a statistically significant relationship between IREB2 rs2568494 GA genotype and COPD disease. The number of patients with FAM13A rs2869967 TC genotype were lower compared to the patients without TC genotype besides; respiratory insufficiency risk was 3.758 fold increase, mMRC □ 2 risk was 2.359 fold increase. GOLD B+D rate was higher in the patients with FAM13A TC variant (74.4%) compared to the patients without FAM13A TC variant (55.2%). FEV1 measures were observed to be lower in the patients with ADAM19 rs1422795 AG genotype.

Conclusion: Our results suggest that IREB2 variants may be associated with COPD. In addition, FAM13A and ADAM19 variants were not related with the disease susceptibility but with the severity. ADAM19, FAM13A, IREB2 genes may be contributor of COPD pathophysiology. Furthermore, associations in different pathways investigated in our study are so important to identify new pathways reflecting COPD heterogeneity.

References: 1. GOLD. Global Strategy for the Diagnosis, Management and Prevention of COPD: 2020 Report. www.goldcopd.org.

Grants: This study was supported by Hacettepe University SRPC Unit (2019-17521).

Conflict of Interest: None declared.

EP04.003 Complex compound inheritance in a four-generation ACDMPV family

Esra Yildiz Bolukbasi[1], *Justyna Karolak[2], Tomasz Gambin[3], Przemyslaw Szafranski[1], Admire Matsika[4], Sam McManus[5], Hamish S. Scott[6;7;8], Peer Arts[6], Thuong Ha[6;9], Christopher P. Barnett[10;11], Jonathan Rodgers[12;13], Pawel Stankiewicz[1]*

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Background/Objectives: Lethal lung developmental disorders (LLDDs) are a rarely diagnosed group of pediatric anomalies presenting with severe progressive respiratory failure and persistent pulmonary arterial hypertension (PAH). They have been histopathologically classified as Alveolar capillary dysplasia with misalignment of the pulmonary veins (ACDMPV), Acinar dysplasia (AcDys), Congenital alveolar dysplasia (CAD), and other unspecified primary pulmonary hypoplasias (PHs). LLDDs are caused by heterozygous alterations involving FOXF1 (ACDMPV), TBX4, FGF10, or homozygous variants involving FGFR2 (AcDys, CAD, and PHs).

However, wide variable expressivity observed in carriers of FOXF1 (pLI 0.96), TBX4 (pLI 0.50), or FGF10 (pLI 0.94) heterozygous coding variants suggests that they alone can be insufficient to manifest clinically.

Methods: Using exome and genome sequencing, and functional reporter assay, we have studied a four-generation family with ACDMPV and PAH.

Results: We have identified a frameshift variant c.881_902dup (p.Gly302Profs*46) in FOXF1 in five individuals with ACDMPV ($N = 1$), PAH ($N = 2$), or unaffected ($N = 2$). Interestingly, in the proband's mother and aunt with PAH, we have found a non-coding rs560517434-A variant within the critical interval of FOXF1 lung-specific enhancer in trans to the FOXF1 coding variant that increased its promoter activity in a reporter assay 10-fold.

Conclusion: Supporting the recent data on complex compound inheritance of LLDD, our results indicate the non-coding variant in trans to the heterozygous coding mutation might have acted as a hypermorph, rescuing the lethal phenotype in the proband's mother and aunt.

References: PMID:31686214, 30639323, 35075769.

Grants: NIH-NHLBI R01HL137203.

Conflict of Interest: Esra Yildiz Bolukbasi: None declared, Justyna Karolak: None declared, Tomasz Gambin: None declared, Przemyslaw Szafranski: None declared, Admire Matsika: None declared, Sam McManus: None declared, Hamish S. Scott: None declared, Peer Arts: None declared, Thuong Ha: None declared, Christopher P. Barnett: None declared, Jonathan Rodgers: None declared, Pawel Stankiewicz NIH-NHLBI R01HL137203.

EP04.004 Autosomal recessive renal tubular dysgenesis – case report

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Background/Objectives: Autosomal recessive renal tubular dysgenesis (ARRTD, ORPHA:97369) is a rare disease characterised by absent or poor development of proximal renal tubules due to biallelic pathogenic variants in genes encoding proteins of Renin-Angiotensin-Aldosteron system. We present case of anhydramnios in two consecutive pregnancies of unrelated partners. First pregnancy was terminated for early anhydramnios and Potter sequence. Proband was born from the second pregnancy. Prenatal ultrasound revealed anhydramnios. Postnatally, severe hypotension, anuria and abnormal ossification of membranous bones were present.

Methods: Molecular genetic testing was performed by trio exome sequencing of proband and parents (FocusedExome, Agilent). Confirmation of the variants was performed by Sanger sequencing. Cosegregation analysis for assessing germline variant pathogenicity was performed on the DNA isolated from cultivated amniocytes of the fetus from the first pregnancy.

Results: NGS analysis revealed proband´s compound heterozygous status: nonsense pathogenic variant c.1486C>T p.(Arg496Ter) in the ACE gene inherited from heterozygous father and two variants in the ACE gene: in-frame deletion c.41_67del p.(Leu14_Leu22del) and missense variant c.3490G>A p.(Gly1164Arg) inherited from heterozygous mother. Both maternal variants are classified as variants of unknown significance by ACMG. The same genotype was confirmed by cosegregation analysis in the fetus from the first pregnancy.

Conclusion: Based on the molecular properties of in-frame deletions of leucine residues in signal peptide, typical phenotype and results of cosegregation analysis we proposed this in-frame deletion to be likely pathogenic. Identification of disease-causing variants allow us to offer PGT-M or targeted prenatal diagnosis.

References:

Grants:

Conflict of Interest: None declared.

EP04.005 Sex-dependent influence of GSTM1 and GSTT1 polymorphism on asthma control in children observed at the age of 8 to 10 years

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Background/Objectives: The aim of this study was to examine the association between sex and glutathione S transferase gene polymorphisms in children with asthma, aged 8-10 years. Some studies demonstrate a close association between the and clinical asthma expression. GST gene has a number of polymorphisms, including GSTM1 and GSTT1, which are thought to have a direct impact on the pathophysiology of asthma.

Methods: Blood samples were taken from 50 kids (33 boys and 17 girls born in the term) who, due to respiratory difficulties. Multiplex PCR was used to determine the presence or absence of GSTT1 and GSTM1 genes in the presence of the control β-globin gene.

Results: GSTT1 was detected in 36% (18) of boys and 14% (7) of girls, while GSTM1 was observed in 4% (2) of boys and 2% (1) of girls. GSTT1 and GSTM1 were observed in 22% (11) of boys and 14% (7) of girls. A positive correlation between allergies and GSTT1 was found as strong in boys and medium in girls. GSTM1 and allergies were negatively correlated in males, while the same polymorphism was positively correlated with asthma in females.

Conclusion: GSTT1 polymorphism appears to play a role in asthma development in children, regardless of their sex. However, GSTM1 seems to have a protective effect against allergy development in males, while it potentially increases the risk of childhood asthma in females.

References: Su X, Ren Y, Li M, Kong L, Kang J. Association of glutathione S-transferase M1 and T1 genotypes with asthma: A meta-analysis. Medicine (Baltimore). 2020;99(34):e21732.

Grants: Non.

Conflict of Interest: Mirela Mačkić-Đurović full time, Amina Aščerić full time, Izeta Aganović-Mušinović full time, Selma Dizdar full time.

EP04.007 Genetic analyses in Polish patients with lethal lung developmental disorders

Katarzyna Bzdęga[1], *Anna Kutkowska-Kaźmierczak*[2], *Izabela Plaskota*[2], *Marta Smyk*[2], *Magdalena Niemiec*[2], *Artur Barczyk*[2], *Małgorzata Nowak-Pleś*[3], *Witold Błaż*[4], *Beata Łoniewska*[5], *Andrzej Marszałek*[6], *Gail Deutsch*[7], *Tomasz Szczapa*[8], *Pawel Stankiewicz*[9], *Justyna Karolak*[1]

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Background/Objectives: Lethal lung developmental disorders (LLDDs) are rare anomalies characterized by severe respiratory failure in neonates, with lack of treatment. LLDDs include alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV), acinar dysplasia (AcDys), congenital alveolar dysplasia (CAD), and other primary pulmonary hypoplasias (PHs). They are caused by heterozygous alterations involving FOXF1 (80-90% of ACDMPV) and TBX4 or FGF10 (65% of AcDys, CAD, and PHs). The aim of our study was to identify LLDD-causative variants in Polish patients. Recently, we provided evidence for a complex compound inheritance of LLDDs.

Methods: DNA samples from two deceased neonates with clinically and histopathologically diagnosed LLDDs and from one fetus with suspected hydronephrosis were analyzed using arrayCGH and/or Sanger sequencing of TBX4, FGF10, and FOXF1.

Results: Our analyses revealed a heterozygous variant c.16G>A (p.Gly6Ser) in TBX4 (MAF < 0.001) in one patient, inherited from her apparently healthy father; further studies, including in trans non-coding variants that may have influenced the disease phenotype are pending. Prenatal testing in the mother of the fetus with hydronephrosis revealed a de novo heterozygous CNV deletion on 16q24.1, involving FOXF1 and its upstream lung-specific enhancer (chr16:86,053,209-86,705,830; hg19), enabling ACDMPV diagnosis.

Conclusion: Complementary clinical, histopathological, and genetic studies are needed for efficient diagnosis of LLDDs and to further elucidate their reported complex compound inheritance.

References: PMIDs:19500772, 30639323, 31686214, 35075769.

Grants: National Science Centre in Poland 2019/35/D/NZ5/02896.

Conflict of Interest: None declared.

EP04.009 Diagnostic yield and recognized barriers of a pediatric endogenetics clinic

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Background/Objectives: Background: The advent of molecular genetic technologies paved a path for the diagnosis of many pediatrics endocrine disorders. Joint evaluation by endocrinologists and medical genetics specialists can potentially increase diagnostic effectiveness by ensuring the exclusion of non-genetic mimics, by rationally selecting appropriate genetic diagnostic tools, by shorten the workup process and guide therapy. There is paucity data regarding the potential Diagnostic benefit of an integrated endogenetics clinic.

Objective**:** Evaluation of the diagnostic's yield of an integrated endogenetics clinic.

Methods: A retrospective review of medical records of all patients who attended the clinic between 2017 and 2021.

Results: 210 patients were evaluated. The diagnostic's yield was 20% compared to less than 10% of non-multidisciplinary genetic clinic.

Conclusion: Given the frequent futile use of diagnostic modalities, referral of non-genetic mimics among endogenetic disorders and the complexity of clinical genomic data analysis, a multi-disciplinary endogenetics clinic seems justified. Further research is needed to assess endogenetics clinic effect on larger scale data.

References:

Grants:

Conflict of Interest: None declared.

EP04.010 Diagnostic utility of NGS panel testing including non-coding and mitochondrial DNA variants in patients with monogenic diabetes

Alicia Scocchia[1], ***Johanna Känsäkoski***[2], *Kimberly Gall*[1], *Julie Hathaway*[1], *Archie Taylor*[1], *Johanna Huusko*[2], *Manuel Bernal*[2], *Pernilla von Nandelstadh*[2], *Johanna Tommiska*[2], *Inka Saarinen*[2], *Matias Rantanen*[2], *Jennifer Schleit*[1], *Massimiliano Gentile*[2], *Pertteli Salmenperä*[2], *Jussi Paananen*[2], *Samuel Myllykangas*[2], *Juha Koskenvuo*[2]

[1]*Blueprint Genetics Inc, Seattle, United States;* [2]*Blueprint Genetics, Espoo, Finland.*

Background/Objectives: Identifying the molecular etiology for diabetes can guide treatment, allow early screening and supportive therapy for associated features, and inform familial recurrence. Most panel testing historically performed has not included non-coding regions or mitochondrial genes. We retrospectively assessed the diagnostic utility of NGS panels containing both nuclear and mitochondrial genes, including selected non-coding variants, associated with monogenic diabetes.

Methods: Clinical reports of 507 patients with suspected monogenic diabetes who underwent panel testing at a CLIA laboratory were examined (MODY Panel, $n = 387$; Comprehensive Monogenic Diabetes Panel; $n = 120$). Testing included sequence and copy number variant (CNV) analyses of NGS data from a validated clinical exome assay, including established non-coding variants. Mitochondrial genome was included for 199 patients. Molecular diagnosis was defined as the identification of pathogenic or likely pathogenic variant(s) consistent with the patient's phenotype and known associated disease inheritance.

Results: A molecular diagnosis was established in 24.9% (126/507) of patients in 11 genes. Most molecular diagnoses were identified in *GCK* ($n = 78$, 61.9%), *HNF1A* ($n = 22$, 17.5%), and *HNF1B* ($n = 7$, 5.6%). Diagnostic CNVs were reported in ten patients. A diagnostic non-coding variant in *INS* was identified in one patient. The mitochondrial *MT-TL1* m.3243A>G variant was identified in six patients.

Conclusion: Nearly 25% of patients in this cohort received a molecular diagnosis, including a non-coding variant in one patient and mitochondrial variant in six patients, demonstrating the diagnostic utility of panel testing with concurrent analysis of both nuclear and mitochondrial genes including selected non-coding variants for individuals with suspected monogenic diabetes.

References:

Grants:

Conflict of Interest: Alicia Scocchia Significant; Blueprint Genetics Inc, Johanna Känsäkoski Significant; Blueprint Genetics, Kimberly Gall Significant; Blueprint Genetics Inc, Julie Hathaway Significant; Blueprint Genetics Inc, Archie Taylor Significant; Blueprint Genetics Inc, Johanna Huusko Significant; Blueprint Genetics, Manuel Bernal Significant; Blueprint Genetics, Pernilla von Nandelstadh Significant; Blueprint Genetics, Johanna Tommiska Significant; Blueprint Genetics, Inka Saarinen Significant; Blueprint Genetics, Matias Rantanen Significant; Blueprint Genetics, Jennifer Schleit Significant; Blueprint Genetics Inc, Massimiliano Gentile Significant; Blueprint Genetics, Pertteli Salmenperä Significant; Blueprint Genetics, Jussi Paananen Significant; Blueprint Genetics,

Samuel Myllykangas Significant; Blueprint Genetics, Juha Koskenvuo Significant; Blueprint Genetics.

EP04.011 TTC12 loss-of-function mutations cause primary ciliary dyskinesia and unveil distinct dynein assembly mechanisms in motile cilia versus flagella

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Background/Objectives: Cilia and flagella are evolutionarily conserved organelles whose motility relies on the outer and inner dynein arm complexes (ODAs/IDAs). Defects in ODAs/IDAs result in primary ciliary dyskinesia (PCD), a disease characterized by recurrent airway infections and male infertility. To date PCD mutations in assembly factors cause a combined ODA/IDA defect, affecting both cilia and flagella.

Methods: We identified four loss-of-function mutations in *TTC12*, which encodes a cytoplasmic protein, in four independent families in which affected individuals displayed a peculiar PCD phenotype characterized by the absence of ODAs and IDAs in sperm flagella, contrasting with the sole absence of IDAs in respiratory cilia.

We analysed both primary cells from individuals carrying *TTC12* mutations and human differentiated airway cells invalidated for *TTC12* by a CRISPR-Cas9 approach, as well as *TTC12* depletion in the ciliated model, *Paramecium tetraurelia*.

Results: Our results revealed an IDA defect restricted to a subset of single-headed IDAs different in flagella and cilia, while *TTC12* depletion in *Paramecium tetraurelia* recapitulated the sperm phenotype.

Conclusion: Overall, our study, which identifies *TTC12* as a new gene involved in PCD, unveils distinct dynein assembly mechanisms in human motile cilia versus flagella.

References:

Grants: Legs Poix and RaDiCo.

Conflict of Interest: None declared.

EP04.012 Clonal culture of renal cells from urine as a model to study somatic mutagenesis in pre-cancerous kidneys

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Background/Objectives: By performing WGS of single somatic genomes from the kidney of 6 healthy donors, we have identified a specific kidney cell subset that is prone to somatic mutagenesis during aging (Franco et al. 2019). This subset expresses markers of kidney tubule damage-repair, such as KIM1 and VCAM1. Here, we aim to better elucidate the mechanisms underlying the excess of somatic mutations in these cells.

Methods: To overcome the limitations connected with low availability of somatic genomes from normal kidneys, we developed a protocol for in vitro expansion of kidney epithelial cell clones, obtained from the urine, a non-invasive, easy-to-collect biopsy. Concomitant whole genome- and transcriptional marker-analyses of clonal populations allow the study of mutation landscapes in specific kidney cell types.

Results: An average of 2 clones per urine sample (range 0-5) expanded enough for WGS and transcriptional marker-analysis ($n = 28$ urine samples from 12 donors, aged 24–63 years). Both KIM1+VCAM1+ and KIM1-VCAM1- kidney tubule epithelial cells could be cultured. Collected DNA was of suitable quality for WGS. Furthermore, 1 in 7 clones expanded sufficiently for functional experiments aimed to dissect the molecular mechanisms leading to enhanced mutagenesis in the KIM1+VCAM1+ subset.

Conclusion: Kidney cell culture from the urine is a suitable method to profile somatic mutations in single pre-cancer genomes from human kidneys and dissect molecular mechanisms of mutagenesis.

References: Franco, I. et al., Genome Biol, 2019, **20**(1): 285.

Grants: MSCA individual fellowship SoMuKT-896832.

Conflict of Interest: None declared.

EP04.013 Molecular diagnosis of cystic kidney disease with NGS panels covering difficult-to-sequence regions

Kimberly Gall[1], ***Manuel Bernal***[2], *Julie Hathaway*[1], *Alicia Scocchia*[1], *Johanna Huusko*[2], *Inka Saarinen*[2], *Matias Rantanen*[2], *Jennifer Schleit*[1], *Tiia Kangas-Kontio*[2], *Tuuli Pietilä*[2], *Pertteli Salmenperä*[2], *Massimiliano Gentile*[2], *Jussi Paananen*[2], *Samuel Myllykangas*[2], *Juha Koskenvuo*[2]

[1]*Blueprint Genetics Inc, Seattle, United States;* [2]*Blueprint Genetics, Espoo, Finland.*

Background/Objectives: Inherited cystic kidney disease (CKD) is a heterogeneous group of conditions commonly leading to end stage kidney disease. Identifying the molecular etiology of CKD can have implications for clinical management. However, genetic testing using NGS is complicated by segmental duplication in several genes. A strategy that addresses these challenging regions is needed to maximize diagnostic potential. Here, we assessed the diagnostic yield of NGS panels in a cohort of CKD patients.

Methods: We examined test results from 1007 patients tested for CKD at Blueprint Genetics. Testing was done with the Polycystic Kidney Disease Panel (up to 12 genes) or the Cystic Kidney Disease Panel (up to 41 genes). The panels included 4 genes challenged by regions of segmental duplication. Copy number analysis was done bioinformatically using two different pipelines, including a proprietary pipeline for the detection of small CNVs.

Results: A genetic diagnosis was established in 557 patients (55%). Diagnostic variants were identified in 17 genes. The most frequently implicated genes were *PKD1* (364 patients, 65%), *PKD2* (75 patients, 13%), *PKHD1* (40 patients, 7%) and *HNF1B* (37 patients, 7%). Of patients with a diagnostic *PKD1* variant, 77% (280/364) were located within the pseudogene regions. Diagnostic CNVs were reported in 9% (49/557) of all diagnosed patients.

Conclusion: These results demonstrate the clinical utility of genetic testing for CKD using NGS panels. Most diagnoses are due to variants in *PKD1* emphasizing the importance of including tailored approaches for challenging genomic regions. Given the frequency of CNVs, including high-resolution CNV detection significantly improves the diagnostic potential.

References:

Grants:

Conflict of Interest: None declared.

EP04.014 Fatal case of congenital nephrotic syndrome in a newborn with two bialeal mutations in the NPHS1 gene

Vita Antsupova[1], *Iryna Lastivka*[2], *Ludmila Khlunovska*[2], *Anastasiya Babintseva*[2], *Larysa Sheiko*[3], *ljudmila Brisevac*[3], *Iana Ushko*[1], *Iryna Malieieva*[1], *Oleksiy Godovanets*[2], *Olha Polodienko*[4], *Volodymyr Davidiuk*[5], *Olexiy Shapovalov*[4]

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Background/Objectives: Congenital nephrotic syndrome (CNS) is an autosomal recessive kidney disease characterized by massive proteinuria, edema, and hypoproteinemia. Most cases of CNS are caused by mutations in the NPHS1 gene that encodes nephrine. Physical examination, family history and laboratory tests allow to suspect the disease.

Methods: clinical-genealogical, molecular-genetic, paraclinical, instrumental.

Results: A 28-day-old boy showed an increase in the size of the abdomen, a decrease in appetite and a decrease in diuresis. The child was born prematurely, with low body weight, from the first pregnancy against the background of chronic pyelonephritis, the risk of miscarriage and polyhydramnios. Prenatal ultrasound screening revealed an increase in the right kidney of the fetus, signs of intrauterine infection. Objectively on examination: the child's condition is severe due to edema, hypovolemia, decreased diuresis. Symptom complex of nephrotic syndrome: proteinuria, hypoproteinemia, arterial hypotension. Ultrasound examination of the kidneys revealed diffuse changes in the parenchyma. The child is suspected of congenital nephrotic syndrome of the Finnish type, a molecular genetic study was recommended. The boy was found to be homozygous for two pathological alleles of the NPHS1 gene (c.2053G>T (p.Gly685Cys та c.2746G>A(p.Ala916Thr). Symptomatic treatment of the child was ineffective, the child died. The father and mother are heterozygous carriers of both mutations in the NPHS1 gene.

Conclusion: Taking into account the genotypes of the parents, as well as the fact that recessive mutations in NPHS1 are associated with a severe course of the disease, recommendations are given for planning subsequent pregnancies using reproductive technologies and prenatal diagnosis.

References:

Grants:

Conflict of Interest: None declared.

EP04.015 Characterization of molecular diagnostic findings in an unselected cohort with suspected congenital hypothyroidism or resistance to thyroid hormone

Christele du Souich[1], *Alicia Scocchia*[2], *Kimberly Gall*[2], *Julie Hathaway*[2], *Archie Taylor*[2], *Johanna Huusko*[1], *Manuel Bernal*[1], *Inka Saarinen*[1], *Jennifer Schleit*[2], *Jussi Paananen*[1], *Samuel Myllykangas*[1], *Juha Koskenvuo*[1]

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Background/Objectives: Congenital hypothyroidism (CH) and resistance to thyroid hormone syndrome (RTH) are conditions impacting production/use of thyroid hormones. The etiology of monogenic CH is diverse and identifying a precise diagnosis and beginning early treatment can prevent significant neurologic and motor damage. We assess the diagnostic utility of panel tests for individuals with suspected CH or RTH and provide an overview of the yet uncharacterized molecular diagnostic findings identified in this population.

Methods: A retrospective review of 117 individuals who underwent panel testing for CH or RTH at Blueprint Genetics was performed. Testing included both sequencing (NGS) and copy number variant (CNV). The target region included the coding exons of up to 22 genes and up to 16 non-coding variants in these genes.

Results: A molecular diagnosis was established in 19.7% of patients. Molecular diagnoses were identified in seven genes associated with thyroid dysgenesis, dyshormonogenesis, or RTH: *THRB*, *TG*, *NKX2-1*, *DUOX2*, *PAX8*, *TSHB*, and *TSHR*. Diagnostic variants were not identified in the *SLC5A5* or *TPO* genes. No P/LP CNVs were found. Diagnostic yield for CH was significantly higher among individuals tested in their first 2 years where most P/LP variants associated were presumed truncating variants.

Conclusion: We describe the molecular findings in an unselected cohort undergoing genetic testing for suspected CH or RTH. A molecular diagnosis was found in 19.7% of individuals with a higher diagnostic yield in individuals tested at <2 years. This study reinforces the importance of genetic testing in this patient population.

References:

Grants:

Conflict of Interest: Christele du Souich Full time employee of BlueprintGenetics, Alicia Scocchia Salaried employee of Blueprint Genetics, Kimberly Gall Salaried employee of Blueprint Genetics, Julie Hathaway Salaried employee of Blueprint Genetics, Archie Taylor Salaried employee of Blueprint Genetics, Johanna Huusko Salaried employee of Blueprint Genetics, Manuel Bernal Salaried employee of Blueprint Genetics, Inka Saarinen Salaried employee of Blueprint Genetics, Jennifer Schleit Salaried employee of Blueprint Genetics, Jussi Paananen Salaried employee of Blueprint Genetics, Samuel Myllykangas Salaried employee of Blueprint Genetics, Juha Koskenvuo Salaried employee of Blueprint Genetics.

EP04.016 Molecular diagnosis of classic form of 21-hydroxylase deficiency in a Romanian pediatric group

Sorina Schipor[1], *Andrei Muresan*[1], *Ioana Nedelcu*[1], *Camelia Procopiuc*[1], *ELENA EMANUELA BRAHA*[1], *Aura Madalina Boboc*[1], *Andreea Brehar*[1], *Oana-Monica Popa*[1], *Susana Vladoiu*[1], *Dana Manda*[1], *Andra Caragheorgheopol*[1], *Iuliana Gherlan*[1;2]

[1]*"C. I. Parhon" National Institute of Endocrinology, Bucharest, Romania;* [2]*"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.*

Background/Objectives: 21-Hydroxylase deficiency (21-OHD) is an autosomal recessive disorder caused by *CYP21A2* gene mutations and is the most common form of congenital adrenal hyperplasia. Here we present the results of genetic analysis of *CYP21A2* gene in a Romanian selected group of paediatric patients with clinical signs of classic 21-OHD.

Methods: All patients were recruited in a tertiary endocrinology centre. Based on biochemical and clinical data we identified 10 patients with classic 21-OHD. The age at diagnosis varied between 5 days and 3 years. *CYP21A2* gene molecular analysis was done according to EMQN guidelines[1] including TaqI PCR-RFLP, Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) to identify gene mutations and large fragment deletions/conversions. Then, the genotypes were analysed in correlation to patients' clinical presentation (genotype-phenotype correlations).

Results: Genetic analysis confirmed classic form of 21-OHD in all 10 patients. Large deletions/conversions were detected in majority of alleles (30.77 %), followed by IVS2-13A/C>G microconversion (15.38%) and I172N (15.38%), R357W (7.69%), Q318X (3.85%) and P31L (3.85%). We also identified a plethora of polymorphisms, synonymous modifications or missense mutations with predicted benign consequences. Genotypes of all 10 patients were consistent with the suggested clinical phenotype (7 salt-wasting and 3 simple-virilising forms).

Conclusion: The molecular analysis strategy combining MLPA, PCR-RFLP and Sanger sequencing is able to identify the complex mutation spectrum of *CYP21A2* gene. In our study large deletions/conversions were dominant in classic 21-OHD patients, and the genotype-phenotype correlation is high.

References: 1.Baumgartner-Parzer S, et al. Eur J Hum Genet. 2020 Oct;28(10):1341-1367.

Grants: None.

Conflict of Interest: None declared.

EP04.018 Next generation sequencing (NGS) for the identification of mutations in genes, associated with various degrees of chronic kidney disease and hyperuricemia

Radosveta Bozhilova[1], *Olga Beltcheva*[1], *Daniela Pencheva*[1], *Kunka Kamenarova*[1], *Galia Zlatanova-Rashkova*[2], *Stephka Tsocheva*[2], *Ventzislav Shurliev*[3], *Boryana Deliyska*[3], *Maria Gaydarova*[2], *Radka Kaneva*[1]

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Background/Objectives: The aim of this study was to apply next generation sequencing (NGS) for genetic testing of hyperuricemia/gout patients with chronic kidney disease.

Methods: After obtaining informed consent, we collected samples from 5 patients. We used TSO gene panel and MiSeq platform (Illumina) for targeted sequencing of 4 813 genes. For variant confirmation and segregation analysis we applied Sanger sequencing. The pathogenicity of each nucleotide change was evaluated based on the ACMG criteria.

Results: Pathogenic or potentially pathogenic missense variants were found in several genes involved in maintaining normal kidney structure and function - *COL4A3*, *COL4A4*, *UMOD*, *GATM* and *SLC14A2*, coding for the α3 and α4 chains of type 4 collagen, uromodulin, the mitochondrial L-arginine: glycine amidinotransferase and UT2 transporter, respectively.

Two of the substitutions, in *COL4A3* and *UMOD*, were novel with unknown effect. The *UMOD* variant, p.Arg222Cys (rs1313544461), was identified in an individual with prominent family history - multiple affected members in three generations. The variant segregated with the disease, is absent in GnomAD and was judged pathogenic by multiple prediction tools. Based on the ACMG criteria p.Arg222Cys was assumed the disease-causing mutation in this family.

Conclusion: The present study is a first attempt to identify the genetic cause of hyperuricemia associated with impaired kidney function in Bulgaria. Identifying the molecular basis of the disease will guide the choice of therapy in each case and will contribute to our understanding of the biological processes governing the normal renal function.

References: None.

Grants: D-116/24.05.2020, D01-285/17.12.2019, D01-395/18.12.2020, D01-302/17.12.2021.

Conflict of Interest: None declared.

EP04.020 A unique case of severe liver disease associated with the recurrent c.187C>T, p.(Arg63Trp) variant in HNF4A

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Background/Objectives: The recurrent c.187C>T, p.(Arg63Trp) variant in *HFN4A* is associated with a multisystem metabolic disease characterized by congenital hyperinsulinism, diabetes, and renal Fanconi syndrome. Of the 15 cases reported in the literature, only 6 presented with liver involvement, which was mild and transient.

Methods: We report a 16-year-old boy born to non-consanguineous parents and presenting with lower limb deformities and short stature secondary to rickets, Fanconi syndrome, chronic liver disease with portal hypertension, esophageal varices requiring endoscopic band ligation, and early-onset diabetes. His mother had a similar phenotype, except for liver disease.

He was born preterm and hypotonia, thrombocytopenia, and cholestatic jaundice with abundant cytoplasmic glycogen on liver biopsy warranted extensive metabolic workup, but no cause was identified.

The clinical picture suggested the diagnosis of Fanconi renotubular syndrome 4 (FRS4) with maturity-onset diabetes of the young (MODY) (MIM#616026).

Results: Sanger sequencing of MODY genes identified the heterozygous variant c.187C>T, p.(Arg63Trp) in *HNF4A*, confirming the diagnosis. The variant was shown to be maternally inherited.

Conclusion: This FRS4 with MODY patient has the most severe form of liver disease reported so far, showing that, contrary to previous belief, FRS4 with MODY can be associated with liver disease that is not mild or transient. Thus, we suggest that liver function should be monitored in these patients.

The patient's mother has normal liver tests, emphasizing the variable expressivity of this disorder.

References:

Grants:

Conflict of Interest: Catarina Macedo Medical Genetics Resident, André Travessa Medical Doctor, Patricia Costa Reis Medical Doctor, Ana Fernandes Medical Doctor, João Campagnolo Medical Doctor, Mafalda Bourbon PhD.

PhD, Investigadora Auxiliar/Coordenadora da Unidade de Investigação e Desenvolvimento, Gisela Gaspar INSA Lisboa, Margarida Vaz INSA Lisboa, Ana Isabel Lopes Medical doctor, Ana Berta Sousa Medical Doctor.

EP04.021 Report of 4 patients with pulmonary hypertension and c.622G>T variant in NFU1 gene: is there a founder effect?

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Background/Objectives: Multiple mitochondrial dysfunctions syndrome 1 is A recessive disorder due to pathogenic variants in *NFU1* gene. Failure to thrive, pulmonary hypertensión and neurological regression are the most common features of this disease.

Methods: Here we report four patients from three unrelated families with homozygous variants in *NFU1* gene. In patients 1 and 2 the diagnosis was uncovered by whole-exome sequencing, and in patients 2 and 3 by direct Sanger sequencing because of clinical suspicion.

Results: The main clinical characteristics of our patients include failure to thrive (4/4), pulmonary hypertension (4/4), neurological regression (4/4) and glycine levels (3/3). All patients died in the first 6 months of life. None of the families had a history of consanguinity, but all had a known Basque Country background. Whole exome sequencing in patients 1 and 2 identified a homozygous c.622G>T(p.G208C) variant. Sanger sequencing of c.622G>T variant confirmed the diagnosis of suspect in patient 3 (sibling) and 4.

Conclusion: In infants with pulmonary hypertension, failure to thrive, neurological regression and increased glycine from the Basque country, Multiple mitochondrial dysfunctions syndrome 1 due to *NFU1* c.622G>T variant should be considered. The Basque origin of all the patients with a common haplotype suggests a founder effect in this population. Screening in Basque Country population could be performd to estimate an overall carrier rate of the mutation.

References: Navarro-Sastre, Aleix et al. "A fatal mitochondrial disease is associated with defective NFU1 function in the maturation of a subset of mitochondrial Fe-S proteins." American journal of human genetics vol. 89,5 (2011): 656-67.

Grants:

Conflict of Interest: None declared.

EP04.023 Prediction of intrahepatic cholestasis of pregnancy using a combination of clinical features and plasma cell-free RNA

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Background/Objectives: Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disease, characterized by skin pruritus and elevated total bile acids (TBA). It can induce preterm birth, fetal distress, and stillbirth. Typically, ICP was diagnosed in the third trimester of pregnancy. This study aimed at constructing a predictive model utilizing clinical features and plasma cell-free RNA (cfRNA) to predict ICP in early pregnancy.

Methods: Detailed clinical information and plasma cfRNA were collected from 149 pregnant women (ICP, $N = 43$, control, $N = 106$) between 13 and 25 gestational weeks with a bile acid measurement <10 μmol/L. A predictive model was constructed and validated in an independent cohort of 54 pregnant women (ICP, $N = 11$, control, $N = 43$).

Results: The liver function indicators, including TBA ($P < 0.001$), alanine aminotransferase ($P < 0.001$), and γ-glutamyl transpeptidase ($P < 0.001$) were elevated in women subsequently diagnosed as ICP. Consistently, liver-specific signatures in plasma cfRNA were significantly higher in pregnancies with ICP, and gene set enrichment analysis found that pathways related to bile acid transport and liver function were enriched in up-regulated genes. The integrated predictive model was more accurate, with an area under the receiver operating characteristic curve (AUROC) of 0.87 compared with the models derived for clinical features (AUROC, 0.75) and plasma cfRNA (AUROC, 0.80).

Conclusion: Altogether, the combination of clinical information and plasma cfRNA can identify liver-specific signatures in early pregnancy and effectively predict subsequent ICP. These will be benefit in ICP detection and prevention.

References: none.

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Conflict of Interest: Jinghua Sun: None declared, Songchang Chen National Natural Science Foundation of China (No.81901495) the research grant from the National Key R&D Program of China (2018YFC1004900) the Shanghai "Rising Stars of Medical Talent" Youth Development Program Clinical Laboratory Practitioners Program (201972).

Shanghai Municipal Commission of Health and family planning (202140110), Xuanyou Zhou: None declared, Tingyu Yang: None declared, Weihui Shi: None declared, Zhongzhen Liu: None declared, Lanlan Zhang: None declared, Qing Zhou: None declared, Zunmin Wan: None declared, Lin Wang: None declared, Yiyao Chen: None declared, Fang Chen the research grant from the National Key R&D Program of China (2018YFC1004900), Hefeng Huang Shanghai Municipal Health Commission (GW-10.1-XK07), Wen-Jing Wang the research grant from the National Key R&D Program of China (2018YFC1004900) the Science, Technology and Innovation Commission of Shenzhen Municipality under grant (No.JCYJ20180703093402288), Chenming Xu National Natural Science Foundation of China (Nos.81771638 and 81971344) the Shanghai Municipal Commission of Science and Technology Program (No.21Y21901002).

EP05 Skeletal, Connective Tissue, Ectodermal and Skin Disorders

EP05.001 Pathognomonic oral findings in three rare syndromes caused by KCNK4, MIA, and GZF1 gene mutations

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Background/Objectives: Orodental findings as amelogenesis imperfecta (AI), dentinogenesis imperfecta (DI) and gingival fibromatosis (GF) could be characteristic pathognomonic and diagnostic features in rare syndromes.

Methods: Three patients were recruited from the Centre of Excellence clinics, National Research Centre, Cairo, Egypt. The first patient was 7 years old male with negative parental consanguinity. The clinical examination revealed hypertrichosis and gingival fibromatosis. The second patient was 6 years old male with positive parental consanguinity. Clinically, the patient had an extreme short stature and opalescent teeth. The third patient was 13 years old female with positive parental consanguinity. The patient complained of short stature and amlogenesis imperfecta. Preliminary diagnosis went to Hypertrichosis/Gingival fibromatosis (OMIM# 135400) and Goldblatt syndrome (OMIM# 184260) for patient 1 and patient 2, respectively. The diagnosis remained unknown for patient 3.

Results: Targeted sequence for TRIP11 gene in Patient 2 was performed and was negative. Blood samples were sent for WES that revealed KCNK4, MIA, and GZF1 mutations, respectively. The diagnosis of patient 1 was Facial dysmorphism, hypertrichosis, epilepsy, intellectual/developmental delay, and gingival overgrowth syndrome (FHEIG) (OMIM# 618381), patient 2 was odontochdrondysplasia type 2 without hearing loss or diabetes (OMIM# 619269), patient 3 was joint laxity, short stature and myopia (OMIM# 617662).

Conclusion: Orodental geneticists play an important role in cooperation with the multidisciplinary team to reach the accurate diagnosis and the proper genetic counseling. Orodental findings as tooth structure defects could be pathognomonic to some rare syndromes. Moreover, a dental management plan was designated according to the confirmed diagnosis.

References:

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EP05.002 First Interim Analysis of the International X-Linked Hypophosphataemia (XLH) Registry: Family history and genetic findings

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Background/Objectives: X-Linked Hypophosphataemia (XLH) is a rare, progressive, hereditary phosphate-wasting disorder characterised by excessive fibroblast growth factor 23 activity, caused by changes in the PHEX gene. The International XLH Registry is an ongoing 10-year data collection programme. Here, we describe baseline family history and genetic data from the first interim analysis.

Methods: The International XLH Registry (NCT03193476), initiated August 2017, aims to recruit 1,200 children and adults with XLH. This analysis describes results of patients eligible as of 29/03/2021. Data are presented on XLH family history and genetic findings.

Results: Overall, 579 subjects (64.6% female) were included in this analysis. Data on XLH family history were collected for 466 (80.5%) subjects; 220 (47.2%) recorded their biological mother affected with XLH, 71 (15.2%) recorded their biological father affected. The 311 subjects reporting siblings had a total of 309 affected siblings (mean = 0.7; SD = 0.78). Information on genetic evaluation was available for 495 subjects; of them 309 (62.4%) had undergone genetic testing. Two subjects' ages were not reported; the proportion of subjects with recorded genetic testing results was higher in children (239/330, 72.4%) vs adults (68/163, 41.7%). Of these subjects, the majority had a confirmed PHEX variant (88.7% children; 91.2% adults). Non-PHEX variants were reported in the paediatric population only: FGF23 variants in 3 children, SLC4A3 in 1 child, and "other" in 7.

Conclusion: The International XLH Registry provides real-world, genetic findings and family history in people affected by XLH.

The authors acknowledge the contribution of all members of the XLH Registry Steering Committee.

References:

Grants: N/A

Conflict of Interest: Outi Mäkitie Kyowa Kirin, Alexion, Merck, Kyowa Kirin, BridgeBio, Jonathan Liu Full time employee of Kyowa Kirin International., Angela Williams Full time employee of Kyowa Kirin International., Sue Wood Full time employee of Kyowa Kirin Internationa.

EP05.003 A family with Ehlers-Danlos syndrome and novel mutation in COL5A1 gene

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Background/Objectives: Classic Ehlers-Danlos syndrome (cEDS) is a connective tissue disorder, mainly caused by heterozygous *COL5A1 or COL5A2* variants encoding type V collagen. The diagnosis is established with the minimal clinical diagnostic criteria and molecular testing. Herein, we report a family with four affected persons in three generations with identified variant of uncertain significance in *COL5A1* gene.

Case report: The index patient, a female at age of 12y, presented with mitral valve prolapse, photophoby, chronic fatigue, chronic pain in knees and ankles, epicanthus, mild scoliosis, arachnodactyly, skin hyperextensibility and joint hypermobility. Family history revealed affected father, brother and grandfather. Father at age of 47y presented with progressive and painful contractual arachnodactyly with Heberden's nodes, foot pain, easy fatigue, shortness of breath, hypertrophic obstructive cardiomyopathy and discal hernias. Brother at age of 24y presented with contractual arachnodactyly and pectus excavatum. Grandfather was expired and history of contractual arachnodactyly was given.

Methods: As the index patient fulfilled the criteria for cEDS with skin hyperextensibility and joint hypermobility **and minor criteria of** epicanthus, complications of joint hypermobility and family history **we performed** next generation sequencing (NGS) connective tissue disorders panel.

Results: A novel variant of uncertain significance, c.260_262del (p.Thr87del), was identified in *COL5A1* gene in the index patient. Family testing of the father and the sibling was performed and the same variant was identified in both family members.

Conclusion: The clinical manifestations range in severity, and families with mild to severe expression have been described. Genotype-phenotype correlations remain to be determined.

References:

Grants:

Conflict of Interest: None declared.

EP05.006 Bleeding assessment in 195 patients with osteogenesis imperfecta

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Background/Objectives: Osteogenesis Imperfecta (OI) is commonly defined as "brittle bones" disease, but there are also more characteristics like blue sclerae, hearing loss, dental problems, ligamentous laxity and a short stature. Easy bruising is also a very common feature and there are multiple case reports on haemorrhagic events in OI. Large population studies on bleeding tendency in OI are very sparse, while other connective tissue disorders with easy bruising have much more relevant research. This paper reviews the clinical aspects of bleeding and bruising in OI based on the self-bleeding assessment tool (BAT) questionnaire among a large cohort of OI patients. The aim of this study is to make a first translation to clinical consequences of bleeding due to surgery, tooth extraction, menstrual and obstetrical bleeding and to present therapeutic considerations relevant to bleeding in OI.

Methods: This explorative study was conducted at the national expert center for adults with OI in the Netherlands. The self-BAT was digitally distributed among 354 adults with different clinically confirmed types of OI.

Results: 195/354 patients with OI types 1,3 and 4 were included. Self-BAT scores were increased in 37-44%.

Conclusion: Bleeding tendency seems to be a relevant feature in OI patients. This study should be a wakeup call for all clinicians who treat OI patients to consider assessment of bleeding tendency and take the right interventions to reduce haemorrhagic symptoms and improve quality of life.

References: n/a.

Grants: None.

Conflict of Interest: None declared.

EP05.008 Likely pathogenic and known variants in EDA, EDAR and NECTIN4 in Egyptian families with different forms of Ectodermal dysplasia

Inas Sayed[1], *Ghada El-Kamah*[2], *Hoda Radwan*[3], *Eman Rabie*[3;4], *Suher Zada*[4], *Mostafa Mostafa*[1], *Nehal Hassib*[1], *mennat mehrez*[1], *Khalda Amr*[3]

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Background/Objectives: Ectodermal dysplasia (ED) is a heterogenous nosologic group of disorders characterized by primary defect in at least two of the ectodermal derived tissues, namely hair, nails, teeth and sweat glands. The most common features are teeth agenesis, absent or reduced sweating (anhydrosis or hypohidrosis), hypotrichosis and dysplastic nails. The oral and dental findings include tooth agenesis, microdontia, abnormal shaped teeth and decreased salivary flow. More than 163 ED phenotypes have been reported with different patterns of inheritance, however the X-linked hypohidrotic ED (OMIM#305100) caused by EDA gene represents the most frequent type. Only 75 ED phenotypes were linked to 77 genes which makes molecular diagnosis challenging and drives the search for potential disease-causing variants.

Methods: Fifteen ED Patients from 12 Egyptian families were subjected to oro-dental and general clinical examination focusing on the skin, and other ectodermal elements. Whole exome sequencing (WES) was performed for the probands. Familial segregation was confirmed using Sanger sequencing.

Results: We identified eight pathogenic and likely pathogenic variants in three genes (EDA, EDAR and NECTIN4) including five novel mutations. We report five variants of unknown significance (VUS) in four other genes (NFKB2, RSPO4, BTD and KRT14).

Conclusion: WES provides a valuable tool in identifying potentially disease-causing variants in a phenotypically diverse group of ED. The detection of four VUS requires further functional studies for evaluation. Recognizing the genetic causes of the condition help in genetic counselling and in possible finding of new treatments.

References:

Grants: Science and Technology Development Fund (STDF) grant no: 33494.

Conflict of Interest: Inas Sayed STDF 33494, Ghada El-Kamah: None declared, Hoda Radwan: None declared, Eman Rabie: None declared, Suher Zada: None declared, Mostafa Mostafa: None declared, Nehal Hassib: None declared, mennat mehrez: None declared, Khalda Amr: None declared.

EP05.009 A novel homozygous mutation of TCIRG1 gene in a case of infantile malignant osteopetrosis

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Background/Objectives: Osteopetrosis is a genetically heterogeneous group of skeletal disorders, which is result of differentiation or functional defects of osteoclasts. That leads to bone thickening, abnormal bone marrow cavity formation and impaired bone remodeling. The most severe form of osteopetrosis is infantile malignant osteopetrosis. It is a rare genetic disorder, the incidence is 1:250000, most often a result of mutation in TCIRG1 gene. The reported patient is a 1-year old boy presented with visual impairments, bone marrow failure, hepatosplenomegaly, hypocalcemia and supportive X-ray changes of increased bone density and bone-to-bone appearance. There is history for parental consanguinity.

Methods: Upon physical examination he was found to have impaired visual milestones, abnormal craniofacial appearance, short limbs, hepatosplenomegaly, inguinal and umbilical hernias. Laboratory results shows anemia, hypocalcemia, hypophosphatemia, hyperparathyroidism, high alkaline phosphatase, low 25(OH)D. There is MRT data for hydrocephalus. The molecular genetic analysis was performed by NGS (panel, that includes the associated with osteopetrosis genes).

Results: The clinical manifestation, laboratory tests and x ray changes make the diagnosis osteopetrosis highly likely. It was confirmed by genetic testing, that revealed a novel homozygous pathogenic variant, c.205C>T (p.Gln69*) in TCIRG1. The couple of parents are carriers of the mutation.

Conclusion: Early diagnosis is important to direct the appropriate treatment to prevent the disease progression before irreversible sequelae occur. The surveillance and treatment are managed by multidisciplinary team. The only treatment of infantile malignant osteopetrosis is haemopoetic stem cell transplantation. Our patient was referred to specialized center for treatment of osteopetrosis.

References:

Grants:

Conflict of Interest: None declared.

EP05.010 Identification of rare novel TSPEAR variants in autosomal recessive ectodermal dysplasia using whole exome sequencing

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Background/Objectives: Ectodermal dysplasia (ED) is a group of heterogenous inherited disorders due to the dysfunction of ectodermal developmental processes with subsequent defects in at least two of four ectodermal derivatives, these are hair, teeth, nails and sweat glands. ED can be classified into five clusters according to the disrupted developmental pathway. The disease-causing gene remains unknown in approximately 50% of ED. We used whole exome sequencing (WES) to identify disease causing variants in a cohort of phenotypically variable ED patients whose underlying molecular pathology could not be characterized through targeted NGS panel containing EDA, EDAR, EDARADD&WNT10A genes.

Methods: DNA was extracted from blood samples of nine ED patients from eight consanguineous families. WES was performed, followed by Sanger sequencing for segregation of variants of interest. For novel missense variants, changes in protein structure were predicted in silico.

Results: Five novel TSPEAR variants disrupting functional domains were identified. A frameshift homozygous variant was identified in one patient with severe clinical picture. An in-frame deletion was identified in seven patients with variable clinical presentations: in five patients in homozygous form, and in a compound heterozygous form with a missense variant in two patients. One patient had two compound heterozygous missense variants.

Conclusion: We expanded the clinical and molecular spectrum of TSPEAR variants which might propose an additional ED cluster since TSPEAR is known to function via Notch signalling pathway.

References: Wright et al., 2019 (PMID: 30703280).
Bowles et al., 2021 (PMID: 34042254).

Grants: The American University in Cairo research grant#R36 and Science and Technology Development Fund grant#33494.

Conflict of Interest: None declared.

EP05.012 Positive association between a common polymorphism within the GPR126 gene and idiopathic scoliosis in Bulgarian patients

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Background/Objectives: Several genome wide association studies suggested that polymorphic variants of GPR126 gene could take part in the pathogenesis of idiopathic scoliosis. The present case-control study investigated the association between a common polymorphism, GPR126 (rs6570507, A/G), and idiopathic scoliosis in Bulgarian patients.

Methods: The association study was performed on 127 patients and 254 controls after obtaining written informed consent. The mean Cobb angle was 53.8 ± 21.2°. The mean age of patients was 11.2 ± 2.9 years. The cases were divided into subgroups based on disease onset, sex, family history, and curve progression. The genotyping was carried out by TaqMan Real-Time PCR method. The statistical analysis was performed by Pearson's chi-squared test and Fisher's exact test with p-value less than 0.05 as statistically significant.

Results: The frequencies of the variant G allele and the GG genotype in the total group of patients and in the subgroup of patients with Cobb angle above 40° were significantly higher than those in the controls ($p < 0.05$). In addition, this case-control study revealed statistically significant association between GPR126*rs6570507 and primary scoliosis in females, adolescents, and sporadic cases.

Conclusion: The results confirmed previously reported associations between a common variant of GPR126 gene and idiopathic scoliosis in Caucasian and Asian populations and suggested that the molecular marker rs6570507 is an independent predisposing and modifying factor of idiopathic scoliosis in different subgroups of Bulgarian patients.

References: Kou et al. Sci Rep. 2018;8(1):11575.

Grants: MEXT/JSPS KAKENHI №T20K05260 and Jikoshunyu Kyoinhaibun-keihi №T5452.

Conflict of Interest: Svetla Nikolova: None declared, Milka Dikova: None declared, Alexandre Loukanov MEXT/JSPS KAKENHI №T20K05260 and Jikoshunyu Kyoinhaibun-keihi №T5452.

EP05.013 Targeted next-generation sequencing contributes to genetic diagnosis of osteogenesis imperfecta in Bulgarian patients

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Background/Objectives: Osteogenesis imperfecta (OI) is a systemic connective tissue disease characterized by low bone mass, bone fragility, and deficient growth. The clinical phenotype is highly variable and there is also genetic heterogeneity. Clinical exome sequencing (CES) help genetic diagnosis, counseling, and treatment of OI patients.

Methods: Ten patients with OI were directed for targeted next-generation sequencing (NGS) in Molecular Medicine Center in the period 2019–2021 year. NGS was performed on MiSeq platform. Direct sequencing by Sanger was used in order to confirm estimated pathogenic variants.

Results: Genetic cause of the disease was estimated in 5 from 10 analyzed patients. Pathogenic/probably pathogenic variants in COL1A1 were found in 4 patients and a nonsense variant in COL5A1 in one patient. The variants that we have detected in COL1A1 are one missense, one nonsense, and 2 frameshift mutations. In 1 from 5 of undiagnosed patients, we found heterozygous missense pathogenic variant in BMP1. In an OI patient girl with deafness genetic cause for the disease was not found but two missense variants in MYO7A and COCH were observed. These genes are associated with deafness and probably they could contribute to hearing loss in the patient. In another patient with not estimated genetic cause for OI we found a heterozygous

pathogenic variant in SLC26A2 gene, mutations in which are associated with autosomal recessive chondrodysplasias.

Conclusion: Targeted NGS contributes substantially to genetic diagnosis of OI and allowed detection of disease-causing mutation in 50% of the patients.

References: OMIM.

Grants: D01-285/17.12.2019, D01-395/18.12.2020, D01-302/17.12.2021.

Conflict of Interest: None declared.

EP05.014 A previously unreported de novo FBN1 missense variant associated with a severe phenotype of neonatal Marfan syndrome

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Background/Objectives: Neonatal Marfan syndrome (nMFS) is characterized by a severe neonatal or infantile phenotype. We present a patient with nMFS and lethal cardiovascular phenotype.

Methods: Fetal ultrasound performed at GA 41+3 showed pronounced tricuspid regurgitation with a dilated right atrium, atrial flutter with 2:1 conduction and atrial rate of 280. Incipient circulatory overload led to acute caesarean section. The patient was flaccid at birth and needed respiratory assistance. ECG showed sinus rhythm of 120 bpm, birth weight 3800 g, length 60 cm and head circumference 33 cm. Persistent pulmonary hypertension of the newborn and worsening cardiorespiratory distress was evident the first 24 hours. Other phenotypic features involving enlarged hands and feet with arachnodactyly, elbow and knee flexion contractures, inverted thumbs, disproportionately long forearms and lower legs, long narrow face, micrognathia, premature craniosynostosis of sutura metopica and coronalis, deep set eyes, hypoplastic ear cartilage and loose skin were striking.

Echocardiography showed that all cardiac structures were enlarged with prolapse, annular dilatation and regurgitation of both atrioventricular valves, and aortic dilatation with regurgitation. Also, a non-restrictive PFO/ASD was noted. Following medical supportive therapy and palliative care, the boy deceased from congestive heart failure at 4 months of age.

Results: Genetic analysis revealed heterozygosity for a *de novo FBN1* variant: c.3284G>C (p.Cys1095Ser).

Conclusion: The clinical and genetic findings and considerations in this case can be helpful for the prenatal and clinical diagnosis, management and genetic counseling in patients with a similar clinical picture and/or the same variant in *FBN1*.

References:

Grants:

Conflict of Interest: None declared.

EP05.015 Expanding the phenotypic spectrum of B3GAT3-associated disorders

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Background/Objectives: First trimester ultrasound on a foetus of a 33-years-old lady at her third pregnancy (previous ones ended in spontaneous abortions) identified increased NT (3.60 mm, >99th centile). A chromosomal analysis was performed, which showed a normal male karyotype (46,XY).

Ultrasound in the 17th week of gestation showed an abnormal flection with lateral deviation of both hands and bilateral talipes equinovarus, movements of all limbs were normal. A further ultrasound in the 19th week of gestation showed short ribs with abnormal curvature, abnormal conformation of the column, reduced fetal movements, amniotic fluid at the upper limit. An array-CGH-analysis (60K, AgilentTechnologies) did not show any pathogenic CNV.

Because of the prognosis, the pregnancy was interrupted. In addition to the ultrasound findings, fetal autopsy showed facial dysmorphisms (flat face, anteverted nares, long philtrum, macroglossia, short neck), arachnodactyly, platyspondyly of the thoracic vertebrae and reduced ossification of cervical vertebrae.

Methods: SureSelect-Agilent Custom Constitutional Panel 17Mb encompassing 5219 genes. Virtual panel "skeletal disorders" (342 genes).

Results: The analysis led to the identification of a homozygous likely pathogenic variant (c.668G>A, p.(Gly223Asp)) in the *B3GAT3* gene (Larsen-like Syndrome). Both parents were confirmed to be carrier of the same missense variant and it was found that they had ancestors in a small village located north-west of the Italian city of Turin. This might therefore be a founder mutation of the valleys nearby Turin.

Conclusion: The identified variant might be a founder mutation of the valleys nearby Turin. This case expands the knowledge of the *B3GAT3* phenotypic spectrum to the prenatal manifestations.

References:

Grants:

Conflict of Interest: None declared.

EP05.016 Application of aCGH technique in Ehlers-Danlos syndrome diagnostics

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Background/Objectives: Ehlers-Danlos syndrome (EDS) is a heterogeneous group of heritable connective tissue disorders. The 2017 International Classification of EDS recognized 13 subtypes caused by pathogenic variants in 19 different genes, encoding different types of collagen as well as protein involved in collagen metabolism or functioning. For all types of EDS, the genetic background was determined, except for the hypermobile type (hEDS).

Aim of our study was an evaluation by aCGH (Array Comparative Genomic Hybridization) a large alterations in genome as a potential background of hEDS.

Methods: The study group included 43 hEDS patients, 33 women and 10 men, negative for NGS-EDS panel (Illumina).

aCGH was performed using Agilent SurePrint G3 Unrestricted CGH 8 × 60K microarrays (Agilent Technologies) which provide an average resolution of 120 kb. These microarrays contain approximately 60000 probes. Results were analyzed using CytoGenomics software.

Results: In tested patients no large deletions or duplications were detected. In 42 only benign variants were found, in 2 of them any alterations in genome were observed.

Conclusion: Application of different molecular methods still did not give us an answer to the question about genetic background of hEDS. Our investigation shows that also large genome changes are not the basis of this connective tissue disorder.

References: 1. Malfait F, Francomano C, Byers P, et al.: The 2017 international classification of the Ehlers-Danlos syndromes. Am J Med Genet C Semin Med Genet. 2017;175(1):8-26.

Grants: This investigation was supported by the CM UMK grant MN-10/WL/2020.

Conflict of Interest: None declared.

EP05.018 Male-pattern hair loss: Integration of GWAS and single-cell RNASeq data to identify pathobiologically relevant hair follicle cell types

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Background/Objectives: Male-pattern hair loss (MPHL) is a prevalent and highly heritable form of hair loss. GWAS have identified >350 genomic risk loci and have implicated numerous candidate genes and pathways. However little is known about the cell types and hair-cycle stages in which these genes and pathways exert their pathobiological effects.

Methods: We used (i) a statistical model that relies on the assumption that genes with critical functions in pathogenic cell types are likely to be located within disease-associated loci (Hu et al.,2011) together with (ii) published GWAS-data (Yap et al., 2018), and (iii) a comprehensive single-cell RNASeq dataset of the murine hair follicle (HF) during hair growth and rest (Joost et al.,2020) to identify MPHL-relevant HF-cell types across hair-cycle stages.

Results: Our analyses revealed a role of different HF-cell types across and in specific hair-cycle stages. While e.g. dermal fibroblasts seemed to be of pathogenic relevance across hair-cycle stages, sebaceous gland cells and endothelial cells seemed to specifically contribute to MPHL-pathogenesis during growth or rest, respectively. Pathway-based analyses of the most specifically expressed genes in associated cell types suggest a similar picture on the molecular level, where e.g. androgen-signalling plays a role across cell types and hair-cycle stages whereas ErbB- or EDA-signalling are only active in specific cell types and hair-cycle stages.

Conclusion: Our data provide novel insight into MPHL-relevant HF-cell types and cellular processes and constitute an important basis for systematic functional follow-up of GWAS findings in relevant cell types.

References: Hu et al. (2011)-PMID:21963258.
Joost et al. (2020)-PMID:32109378.
Yap et al. (2018)-PMID:30573740.

Grants:

Conflict of Interest: Nicole Engelmann: None declared, Sabrina Henne: None declared, Stefanie Heilmann-Heimbach SHH receives a salary from the Life & Brain GmbH.

EP05.019 Biallelic copy number variations in both upstream & downstream enhancers of SHOX gene causes mesomelia and clubfoot without short stature

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Background/Objectives: SHOX-related disorders include idiopathic X-linked short stature (MIM#300582), Leri-Weill dyschondrosteosis (LWD)(MIM#127300), and Langer mesomelic dysplasia (LMD) (MIM#249700). Duplications and deletions in the upstream and downstream conserved non-coding regulatory elements (CNEs) of SHOX gene are also reported.

Methods: A 7-year-old girl, born at term with bilateral clubfeet was examined. Birth measurements were unremarkable. Mesomelic shortness of forearms and legs, Madelung deformity, ulnar deviation and cubitus valgus were noted. Her height was at −1.45 SDS. First cousin parents had proportionate average height.

Results: Xrays showed mesomelic dysplasia. Whole genome sequencing (WGS) was negative. Reevaluation of WGS for SHOX regulatory regions revealed four copies of GAIN: two upstream and two downstream. Parents were carriers (Table). The unaffected mother's X-ray showed mild Madelung deformity.

Conclusion: This is the first report of biallelic inheritance of both upstream and downstream duplicated CNEs of SHOX gene. Biallelic CNVs in regulatory regions of SHOX gene may be the cause of mesomelia resembling mild Langer Mesomelic Dysplasia without short stature.

References: 1. Spurna Z, Capkova P, Srovnal J, et al. Clinical impact of variants in non-coding regions of SHOX - Current knowledge Gene. 2022;818:146238.

2. Bunyan DJ, Baffico M, Capone L, et al. Duplications upstream and downstream of SHOX identified as novel causes of Leri-Weill dyschondrosteosis or idiopathic short stature. Am J Med Genet A. 2016;170A(4):949-957.

Grants: No grants.

	UPSTREAM	DOWNSTREAM
PATIENT	arr[GRCh37] Xp22.33(168552_451049) x4	arr[GRCh37] Xp22.33(614734_802868) x4
MOTHER	arr[GRCh37] Xp22.33(168552_451049) x3	arr[GRCh37] Xp22.33(629999_802868) x3
FATHER	arr[GRCh37] Xp22.33 or Yp11.32(168552_451049 or 118552_401049)x3	arr[GRCh37] Xp22.33 or Yp11.32(614734_802868 or 564734_752868)x3

Conflict of Interest: None declared.

EP05.020 An aberrant IRAK1 gene generates a hypersensitive and hyperactive IRAK1 protein in the synovial fibroblast, when in presence of Escherichia coli Group D lipopolysaccharides effectuates an amplified expression of IL-1, IL-6 and TNFα, resulting in seropositive rheumatoid arthritis

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Background/Objectives: Rheumatoid arthritis is postulated to be the result of a combination of genetic and environmental

influences; the mechanistic factors which remain undefined. To determine cause of RA.

Methods: Root cause analysis of genetic/cellular processes associated with seropositive rheumatoid arthritis (SPRA) demonstrates: (1) Proliferation of synovial fibroblasts and expression of inflammatory cytokines cultivates synovitis. (2) Synovial fibroblasts mount Toll-like receptor 4 (TLR4) on their surface. (3) Escherichia coli Group D (EcGD) has been strongly associated with SPRA. (4) TLR4 triggers due to EcGD lipopolysaccharides. (5) TLR4 utilizes crucial transducer Interlukin-1 Receptor Associated Kinase-1 (IRAK1) protein to generate IL-2, Il-6 and TNFα, cytokines responsible for RA. (6) IRAK1 gene located on x-chromosome, with SPRA demonstrating a preponderance for women, and variable penetrance likely facilitated by random switching on/off the x-chromosome gene.

Results: Examination of systemic cellular mechanisms and genetic factors associated with SPRA demonstrate an overactive variant of the pivotal IRAK1 protein is the root cause of SPRA.

Conclusion: An anomalous IRAK1 gene results in generation of hypersensitive and hyperactive IRAK1 protein in fibroblasts, which when the TLR4 is triggered by serum lipopolysaccharides generated by presence of EcGD, amplifies expression of IL-1, IL-6 and TNFα, which results in synovial hypertrophy; left untreated, manifests into erosive arthritis. Blocking the hyperactive IRAK1 protein, specifically in synovial fibroblast cells, with targeted therapy, would lead to optimal management of SPRA. In women, whom develop SPRA, deactivating the aberrant IRAK1 gene in synovial fibroblasts and actuating IRAK1 demonstrating normal function, would be highly curative for SPRA.

References: to provide.

Grants: not applicable.

Conflict of Interest: None declared.

EP05.022 Ethnospecific markers of osteoarthritis in women from the Republic of Bashkortostan, Russia

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Background/Objectives: Osteoarthritis (OA) is a common joint disease, with at least 30% genetic determination (Sophie C Warner, 2017). By 2020, more than 50 target genes have been identified (Ioanna Tachmazidou, 2019), but the results require validation on different ethnic group (Louise N Reynard, 2013).

Methods: DNA samples from 417 women (51.67 ± 11.5 y.o.) with OA and 161 healthy women from Ufa (Republic of Bashkortostan, Russia) were analyzed for the associations of polymorphic variants in 3′ UTR regions of COL1A1, COL11A1, ADAMTS5, MMP1, MMP13, SOX9, FGFR1, FGFRL1 and incidence of OA using kompetitive allele-specific PCR. Ethnic composition was presented as follows: 144 Russian (Slavic group of the Indo-European language family), 159 Tatar (Turkic branch of the Altai language family), 114 mixed and representatives of small ethnic groups.

Results: Identified associations are presented in Table 1. All associations remained statistically significant after Benjamini-Hochberg correction (*p**).

Table 1. Associations of the miRNA target sites loci in different ethnical groups

SNP	Gene, loci	Ethnicity	Allele	*p*	*p**	OR; 95% CI
rs6854081	*FGF2* (4q28.1)	Tatar	G	0.0001	0.0002	OR = 4.78; (1.89–12.02)
rs1061237	*COL1A1* (1p21.1)	Russian	C	0.017	0.034	OR = 1.77; (1.07–2.94)
rs229069	*ADAMTS5* (21q21.3)	Mixed	T	0.0002	0.0004	OR = 2.25; (1.30–3.89)
rs73611720	*GDF5* (20q11.22)	Mixed	T	0.004	0.008	OR = 3.02; (1.38–6.60)

Conclusion: Ethnospecific markers of OA development in the FGF2 (*rs6854081*), COL1A1 (*rs1061237*), ADAMTS5 (*rs229069*) and *GDF5* (*rs73611720*) genes were identified in women from the Republic of Bashkortostan.

References:

Grants: This work was financially supported by a grant from the Republic of Bashkortostan for young scientists SEC-GMU-2021.

Conflict of Interest: None declared.

EP05.023 Congenital defects in a patient carrying a novel homozygous AEBP1 variant could expand the phenotype of Ehlers-Danlos syndrome classical-like type 2

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Background/Objectives: In 2018 a new clinical subtype, caused by biallelic variants in the AEBP1 gene, was added to the current nosological classification of the Ehlers Danlos Syndromes (EDS). This new phenotype, provisionally termed EDS classical-like type 2 (clEDS2), has been not yet fully characterized, as only seven cases have been reported to date. Here we describe a patient, homozygous for a novel AEBP1 pathogenic variant, whose phenotype is reminiscent of classical EDS but presenting also with previously unreported clinical features.

Methods: Besides the EDS typical features, this patient presented with multiple congenital defects including cleft palate, agenesis of multiple phalanxes of the left foot, and partial agenesis of the right pectoral muscle. Cytogenomic array and targeted exome sequencing, according to the clinical features, were performed on DNA isolated from a blood sample. Full clinical examination and medical history were obtained and compared to the previously reported cases of AEBP1-related EDS.

Results: An homozygous novel AEBP1 variant, c.2123_2124delTG (p.Val708AlafsTer5), was detected by exome sequencing, segregating from heterozygous parents.

Conclusion: Our case recapitulates most clinical features previously reported in clEDS2, especially those reminiscent of classical EDS. Conversely, the additional congenital defects in this patient might be novel manifestations, expanding the phenotype of AEBP1 biallelic mutations. Although a different concomitant etiology for cleft palate and foot phalanges agenesis cannot be formally excluded, the connection of AEBP1 protein with TGF-beta and WNT pathways, for instance through the interaction with frizzled receptors, may suggest an intriguing common underlying etio-pathological mechanism.

References:

Grants:

Conflict of Interest: None declared.

EP05.024 Steroid sulfatase deficiency in Tunisian patients: awareness of STS pseudogene technical trap

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Background/Objectives: X-linked recessive ichthyosis (XLI) is a genetic disorder that affects the skin, caused by a deficiency of the steroid sulphatase enzyme encoded by the *STS* gene (OMIM # 300747). Our work aims to study the clinical and genetic characteristics of 8 Tunisian patients with XLI.

Methods: We collected eight patients with XLI, all males, from three unrelated Tunisian families from central Tunisia. Genetic diagnosis was conducted through Sanger Sequencing, haplotype analysis of STR markers, MLPA analysis, FISH and CGH techniques.

Results: Our 8 patients presented with collodion baby at birth that evolved into a thick, polygonal, dirty, dark scaly ichthyosis with a generalized distribution. Direct sequencing revealed the same 13 bp deletion in all patients. However, their mothers were not carriers of this variant and no common haplotype was shared between affected patients around *STS* gene. Sequence alignment with reference human genome revealed an unprocessed pseudogene of the *STS* gene located on the Y chromosome, on which the 13 bp deletion was actually located. *STS* MLPA analysis revealed a deletion of the entire *STS* gene for the 3 families, confirmed by FISH and CGH array techniques.

Conclusion: Geneticists must be aware of the presence of *STS* pseudogenes that can lead to misdiagnosis. Pseudogenes sequence similarities with the gene of interest must be taken into account when designing primers for sequencing to avoid mistaken results.

References: Ballabio, A. et al. Isolation and characterization of a steroid sulfatase cDNA clone: Genomic deletions in patients with X-chromosome-linked ichthyosis.

Grants:

Conflict of Interest: None declared.

EP05.025 Early onset end-stage renal disease and retinal dystrophy in a cranioectodermal dysplasia patient with WDR35 variants

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Background/Objectives: Cranioectodermal dysplasia (CED), is a clinically and genetically heterogenous disorder characterized by skeletal, craniofacial, and ectodermal abnormalities. CED belongs to a group of disorders known as ciliopathies and is associated with defective cilia function and structure. To date six genes have been associated with this syndrome (*WDR35*, *IFT122*, *IFT140*, *IFT144*, *IFT52*, and *IFT43*). Here we describe on a 4-year-old male CED patient whose features include dolichocephaly, multi suture craniosynostosis, facial dysmorphism, narrow thorax, limb shortening, and brachydactyly. The patient presented early-onset chronic kidney disease and early onset of retinal degeneration. He developed the end-stage of renal disease at the age of 11 months and has been transplanted at the age of 2 years and 5 months. Retinal dystrophy has been diagnosed at the age of 3.5 years.

Methods: The NGS custom designed SureSelect (Agilent Technologies) panel consists of 61 genes and 11 single nucleotide variants (SNVs) known to be associated with craniosynostosis has been performed in the patient.

Results: Targeted NGS detected two heterozygous variants p.(Gly303Arg) [c.907A>G] in exon 9 and p.(Leu641*) [c.1922T>G; rs199952377] in exon 18 in the *WDR35* gene. The presence of both variants was confirmed by Sanger sequencing and segregation analysis revealed that the mother and father are each carriers of p.(Gly303Arg) or p.(Leu641*), respectively.

Conclusion: CED patients with variants in the *WDR35* gene should be monitored regularly for kidney function, and standard ophthalmologic evaluation including ERG and fundoscopy should be performed to detect early signs of retinal degeneration.

References: Tan, W., Lin, A., & Keppler-Noreuil, K. (2021). Cranioectodermal Dysplasia. *Definitions*.

Grants:

Conflict of Interest: None declared.

EP05.027 Expanding the spectrum of PAX9 mutations associated with selective tooth agenesis type 3

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Background/Objectives: We present here a 10-years old girl with oligondontia and abnormal dental shape with positive family history (mother, maternal uncle and grandfather). She presented with eruption of 4 definitive elements (two peg shaped maxillary central incisors and two firsts inferior molars). Moreover, she showed a delay in the replacement of the inferior deciduous incisors. Given the suspicion of oligodontia, an Orthopantomagram was performed, which showed only 10 more still unerupted definitive elements, confirming the diagnosis. Given the positive family history, a clinical exome was performed in duo with the mother.

Methods: SureSelect-Agilent Custom Constitutional Panel 17Mb encompassing 5219 genes.

Virtual panel "oligodontia" (31 genes).

Results: The analysis led to the identification of a heterozygous likely pathogenic variant in the PAX9 gene (c.771+1G>A, p.?) in both mother and daughter. The variant, never reported, absent in the main population and mutation databases, alters the canonical donor splicing site. Skipping of exon 4 would lead to a frameshift. However in absence of functional data, the use of an alternative upstream or downstream splice site cannot be excluded.

Conclusion: PAX9 is associated with non-syndromic selective tooth agenesis type 3 (OMIM # 604625). This condition is inherited

in an autosomal dominant way and affected people mostly show lack of permanent molars and possibly of second premolars and central incisors, which may also show shape anomalies. This phenotype is highly overlapping with that of our family.

Further segregation analysis in the family and functional studies are needed in order to expand the pathogenic mechanisms underlying PAX9-associated oligodontia.

References:
Grants:
Conflict of Interest: None declared.

EP05.028 Rare association of Cone-rod dystrophy in a patient with Neurofibromatosis type 1

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Background/Objectives: Neurofibromatosis type 1 (NF1; MIM 613113) is a autosomal dominant disease, characterised by the presence of cafe-au-lait macules, Lisch nodules and neurofibromas. Cone-rod dystrophy (CORD; MIM 602225) is characterized by decreasing visual acuity, loss of color vision, decreasing peripheral vision and nyctalopia leading to blindness.

Methods: The authors present a case of a 16 year old girl diagnosed with NF1 associating Cone-rod dystrophy.

Results: The patient is in our genetics department record from the first year of life. Family history is positive for NF1 with one affected parent, the father. At the latest follow-up she presents with multiple café-au-lait macules, bilateral axillary freckles, emerging neurofibromas, strabismus and astigmatism. Molecular diagnosis confirmed the diagnosis of NF1 identifying the pathogenic variant c.3871-2A>T on *NF1* gene (17q11.2) and the c.119G>A varinat on *CRX* gene (19q13.33) classified as likely pathogenic based on ACMG guidelines.

Conclusion: NF1 associating CORD has been described only in a few cases in literature. Although positive diagnostic for NF1 is based on clinical signs, molecular analysis can confirm the diagnosis and provide additional information.

References:
Kylstra JA, Aylsworth AS. Cone-rod retinal dystrophy in a patient with neurofibromatosis type 1. Can J Ophthalmol. 1993 Apr;28(2):79-80. PMID: 8508343.

Zobor D, Kaufmann DH, Weckerle P, Sauer A, Wissinger B, Wilhelm H, Kohl S. Cone-rod dystrophy associated with amelogenesis imperfecta in a child with neurofibromatosis type 1. Ophthalmic Genet. 2012 Mar;33(1):34-8. https://doi.org/10.3109/13816810.2011.592178. Epub 2011 Jul 5. PMID: 21728811.

Grants: None.
Conflict of Interest: None declared.

EP05.029 9 new cases of spondylometaphyseal dysplasia with corner fractures: enhancement of the phenotypic spectrum of FN1 gene mutations

Caroline Michot[1], *Pauline Marzin*[1], *Genevieve Baujat*[1], *Eugénie Koumakis*[2], *Anne Dieux-Coeslier*[3], *Massimiliano Rossi*[4], *Coralie Haudry*[5], *Anne-Laure Tourre*[5], *Julie Steffann*[5], *Sophie Rondeau*[5], *Valérie Cormier-Daire*[1]

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Background/Objectives: Spondylometaphyseal dysplasia with corner fractures (SMDCF, MIM 184255) is an autosomal dominant skeletal dysplasia, characterized by irregular metaphyses, coxa vara, scoliosis and secondary ossification sites ("corner fractures"). SMDCF is linked to mutations in *FN1*, encoding fibronectin, an extracellular matrix glycoprotein. *FN1* heterozygous mutations are also involved in glomerulopathy with fibronectin deposits (GFD). GFD mutations are located in C-terminal part of fibronectin type-III domain.

We aim to describe the SMDCF natural history.

Methods: We recruited 9 cases from 5 families: 2 females and 7 males; 6 children (3 to 14 years) and 3 adults.

Results: All families harbor *FN1* heterozygous mutations: 4 in fibronectin type-I domain; 1 in N-terminal part of fibronectin type-III domain.

The 3 adult men measure 121 to 160 cm. 5 children displayed IUGR. 4 children grow between −3 and −5,5 SD (1 GH deficiency). 2 children grow at -1,5/-2 SD, one under GH therapy. Joint hyperlaxity is noted in 2 families; genu valgum is frequent. 3 cases have scoliosis; lumbar hyperlordosis is almost systematic.

Metaphyseal dysplasia and coxa vara are constant. 8 cases had hip surgeries. 4 children display corner fractures, which are no longer visible in adults. Vertebrae are: tall in adulthood, ovoid in 3 children. A child has basilar impression with posterior fossa cyst.

No patient has impaired renal function.

Conclusion: We confirm a correlation between mutation localization and clinical presentation. The basilar impression case indicates systematic spine assessment. Joint laxity assessment is recommended. We underline endocrinological management, even if the relevance of GH treatment requires further investigations.

References:
Grants:
Conflict of Interest: None declared.

EP05.030 Epidermolysis Bullosa: Literature Review and Three Case Presentation

Laura Popa[1;2], *Nicoleta Andreescu*[1;2], *Cristian Zimbru*[2], *Adela Chirita-Emandi*[1;2], *Simona Farcas*[2], *Doriana Chilom*[2], *Maria Puiu*[1;2]

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Background/Objectives: Epidermolysis bullosa (EB) is a genodermatosis in which clinical manifestations varies in severity and character according to subtype. There are three main EB categories: simplex, junctional, and dystrophic. EB can be inherited in recessive (AR) or dominant (AD) manner, AR form being the most severe.

Methods: Here we present three cases of EB, two cases of dystrophic EB (DEB) and one case of simplex EB (SEB), genetically analysed by NGS sequencing. All three patients present bullous lesions and skin exfoliation, diagnosed by dermatologist.

Results: Following sequencing, we determined three different mutations. One of the patients with DEB has been identified with a missense homozygous mutation (c.425A>G) in COL7A1 gene, also

known as the most common variant among DEB patients. For the other patient with DEB, two different heterozygote mutations have been identified, both in COL7A1 gene (frameshift c.5960del, missense heterozygote c.425A>G). The frameshift c.5960del mutation is less common; therefore, there are few information available for this mutation. The sequencing analysis for the SEB patient revealed a missense heterozygote mutation (c.1400T>C) in KRT5 gene. The two DEB variants have been associated with AR inheritance, while SEB variant has been associated with AD inheritance.

Conclusion: The first conclusion reached is that the same EB type with similar manifestations can be determined by different mutations. The second conclusion is that the mutation information and knowing the inheritance pattern, can help us predict the evolution of the disease, create the basis for an accurate genetic counselling and increase the prevalence of prenatal diagnosis among families with risk.

References:

Grants:

Conflict of Interest: None declared.

EP05.031 Two sibs with overgrowth, macrocephaly, intellectual disability and homozygous novel pathogenic FIBP variant, Thauvin-Robinet Faivre Syndrome

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Background/Objectives: Thauvin-Robinet Faivre Syndrome is a newly defined rare autosomal recessive overgrowth sydrome characterized with intellectual disability, facial dysmorphism, macrocephaly and variable congenital malformations. It is caused by homozygous *FIBP* mutations. *FIBP* gene locates on 11q13.1 and codes fibroblast growth factor intracelluar binding protein. To date only four patient has been reported with this disorder. Here we report 2 siblings born from consanginous parents.

Methods: Case 1, 14 years old boy with tall stature, macrocephaly, moderate intellectual disability. He has downslanting palpebral fissures, widely spaced/deep set eyes, thick lips, scoliosis, athrophic-dysplastic right kidney, large hands and feet, 4/5 mild motor deficit in right arm and leg. His metabolic workup chromosome analysis, 5q35 FISH analysis, CGG repeat on FMR1 gene, eye examination and echocardiography were normal. Microarray analysis was also normal. Case 2, 3 years old girl with tall stature, macrocephaly, developmental delay, round face, widely spaced eyes, epichantic folds, flat mid face, thick lips. Her methabolic workup, chromosome analysis and eye examination were normal.

Results: Whole exome sequencing analyses of case1 reveaed homozygous pathogenic c.412-3_415 dupCAGTTTG, (p. Asp139Alafs*Ter3) variant. Sanger sequencing of *FIBP* gene revealed, homozygous pathogenic c.412-3_415 dupCAGTTTG variant in in both sibs. This pathogenic variant was also confirmed heterozygous state in parents with direct sequencing.

Conclusion: Here we report a rare autosomal recessive overgrowth sydrome in two affected siblings. Reporting two new cases with pathogenic *FIBP* mutation will be support and expand the clinical spectrum of Thauvin-Robinet Faivre syndrome.

References: Thauvin-Robinet, et al.2016. *Clinical Genetics*, *89*(5).

Grants:

Conflict of Interest: None declared.

EP05.032 A long-term surveillance of a 32 years old patient with Osteogenesis imperfecta type III

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Background/Objectives: Osteogenesis imperfecta (OI) is a group of rare disorders. It is caused by mutations in the *COL1A1* and *COL1A2* genes that codify the alpha 1 and alpha 2 chains of type 1 collagen. The incidence of OI is 1:10,000. The main important signs include spontaneous fracture or fracture from minor trauma, joint hypermobility, skin fragility, easy bruising.

Methods: The authors tracked the physical, mental and social integration of a 32-year-old patient diagnosed with severe OI from birth.

Results: Phenotypically the patient presents relative macrocephaly frontal bossing, triangular face, blue sclera; He has had numerous spontaneous fractures (over 100) since birth, leading to extremely severe disharmonious dwarfism, with marked shortening and curving of the limbs, pectus deformity and binding him to a wheelchair. People with OI are usually lonely, self-conscious, sensitive, suspicious of the opinions of others, depressive, with poorer quality of life (QOL); our patient proved to be the opposite: he is cheerful, optimistic, passionate about history and politics, surrounded by friends, lives life to the fullest; he has a Master`s degree in environmentalism; currently working as a customer service operator.

Conclusion: People with OI can live a happy and satisfying life. These people need a number of specific support measures provided by an adapted lifelong support system delivered through specialized genetic disease centers.

References: Palomo T, Vilaça T, Castro ML. Osteogenesis imperfecta: diagnosis and treatment. Curr Opin Endocrin Diabetes Obes 2017 24(6):381-388.

Grants: None.

Conflict of Interest: None declared.

EP05.033 Autophagy polymorphisms are associated to hip fracture outcome

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Background/Objectives: Hip fracture in the older population is associated to high morbidity and mortality dependent on, among others, factors like genetics (*1*). Autophagy is a process involved in bone metabolism linked to aging-related diseases. Thus, we analysed the relationship of single nucleotide polymorphisms (SNPs) in autophagy related genes (ATGs) with the clinical variables and outcome of hip fracture in older patients.

Methods: 87 patients from the University Hospital of Salamanca, presenting osteoporotic hip fracture and aged 80 years or older, were included in the study after signing informed consent in compliance with the Declaration of Helsinki. Biodemographic, clinical and functional variables were recorded. Genomic DNA was extracted from peripheral blood by standard phenol/chloroform protocol and SNP genotyping was carried out using TaqMan probe-mediated qPCR. Four SNPs were analysed: rs3759601 (*ATG2B*), rs2245214 (*ATG5*), rs1864183 (*ATG10*) and rs2241880

(*ATG16L1*). Statistical analysis was carried out using Chi-square, Student-t and ANOVA tests with statistical significance considered at p-values < 0.05.

Results: rs3759601 heterozygotes showed lower 90-day survival, while rs2245214 heterozygotes, rs1864183 heterozygotes and A/A rs2241880 carriers showed higher 90-day survival. Both rs2245214 and rs1864183 heterozygotes showed better functional status and rs1864183 heterozygotes showed lower incidence of comorbidities.

Conclusion: Our results point to a relationship between autophagy and the outcome of hip fracture. This highlights an understudied area that could help assess the prognosis of these patients.

References: 1. B. Abrahamsen, T. Van Staa, R. Ariely, M. Olson, C. Cooper, *Osteoporos. Int*. **20**, 1633–1650 (2009).

Grants: This project was funded by FIS-FEDER PI20/01569.

Conflict of Interest: None declared.

EP05.034 Differential expression of TLR7 and miRNA-146a genes in peripheral blood and skin samples of patients with systemic sclerosis

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Background/Objectives: Progressive fibrosis of the skin and internal organs is one of the hallmarks of systemic sclerosis (SSc). Accumulative evidence demonstrates that Toll-like receptors (TLRs) may represent the link between immune activation and tissue fibrosis. Releasing of endogenous TLR ligands and their binding to TLR receptors complexed to autoantibodies might be one of the mechanisms that initiate fibrotic events. It arose recently that 'fine-tuning' of the TLR/NF-kB signaling pathway is taken place through down-regulation of *IRAK1* gene via miR-146a.

Methods: The expression of *TLR7* and *miRNA-146a* genes in PBMNC of 50 SSc patients and 13 healthy individuals using RT-qPCR technique was performed. Comparative analysis of these genes in affected and unaffected skin areas of the five SSc patient was performed in addition.

Results: In skin tissue samples the expression of *TLR7* gene was 56% lower in affected (mRSS score>10) compared to unaffected tissue sample. When peripheral blood samples were examined, we found that patients with severe skin involvement (mRSS score>10) showed 26% lower *TLR7* expression compared to patients with mild skin involvement (mRSS score≤10).

In addition, 19% lower level of *miR-146a* expression was detected in affected compared to unaffected skin sample. In peripheral blood samples, 37% lower expression of miR-146a was detected in patients with severe skin involvement compared to patients with mild skin involvement.

Conclusion: Synchronized expression *TLR7* and *miR-146a* genes in skin tissue samples and blood samples suggest that both of these molecules should be further investigated as non-invasive biomarkers for skin involvement in SSc patients.

References:

Grants: MESTD, RS (III41004 and 451-03-68/2022-14/200042).

Conflict of Interest: None declared.

EP05.035 Phenotype diversity associated with TP63 mutations

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Background/Objectives: We saw a 41-year old South Indian patient (index) and his 10-year old son. They reported a history of diverse cutaneous and extracutaneous symptoms affecting the ectodermal appendages. Our aim was to find the underlying genetic cause of this autosomal dominant ectodermal dysplasia associated with hypotrichosis, hyperpigmentation, hypohidrosis and syndactyly for our patients.

Methods: Detailed physical examination was done for both affected family members. Whole-exome sequencing (WES) was performed in both patients. Results were confirmed by Sanger sequencing in the patients and the unaffected parents of the index.

Results: WES identified the heterozygous variant c.1922C>T; p.(Ala641Val) in exon 14 of TP63 (NM_003722.5) in father and son. The variant could not be detected in the unaffected wife/mother. The parents of the index both did not carry the variant, which indicates a de novo pathogenic variant.

Conclusion: Since the first description of a TP63 variant, the broad spectrum of molecular defects within this gene became increasingly clear. Each patient with a TP63 mutation reported displayed a plethora of distinct cutaneous and extra-cutaneous symptoms, reflecting the clinical heterogeneity of these disorders. The symptoms of the here described family do not fit into any of the TP63-related entities. The findings of our patients and critical review of the literature point to a phenotype diversity associated with TP63 mutations. It seems therefore reasonable to critically question the old clinical classification and to overcome the historical terminology. We propose the use of the term TP63-associated disorder for the future.

References:

Grants:

Conflict of Interest: None declared.

EP05.037 A novel frameshift variant of EDAR gene in a patient diagnosed with hypohidrotic ectodermal dysplasia

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Background/Objectives: Hypohidrotic ectodermal dysplasia (HED) is a hereditary disease characterized by the triad of hypohidrosis, anodontia/hypodontia and hypotrichosis signs. In this study we present a patient with clinical diagnosis of HED. The patient had periorbital hyperpigmentation, low-set ears, short philtrum in addition to the characteristic clinical findings of the disease. His mother and father were second cousins. We aimed to analyse DNA sequence of the reported candidate genes for HED disease in order to confirm the diagnosis of the patient.

Methods: Genomic DNA was extracted from the patient's peripheral blood sample. Exons and exon-intron transition regions of the HED associated genes (*EDA, EDAR, EDARADD, EDA2R, TRAF6, IKBKG* and *WNT10A*) were investigated by next generation sequencing. Variants were analyzed using Ion Reporter Software (ThermoFisher Scientific Inc.) and SEQ (Genomize Inc.) programs. Variant information servers dbSNP, ClinVar, Ensembl and ACMG criteria were used for evaluations.

Results: In the eighth exon of the EDAR gene, c.677_678dup (p.Lys227GlyfsTer8) frameshift variant was detected in homozygous state. It was predicted as a "pathogenic" change due to premature termination of protein translation. The variant was not reported in population databases (gnomAD, ExAC) and disease-specific databases (ClinVar, OMIM).

Conclusion: The detected frameshift variant in our study is presumed a candidate variant that could be attributed to HED, considering predicted functional effects and its homozygosity. To better elucidate genotype-phenotype association, variant segregation status in the patient's family and larger population data will be examined.

References: https://doi.org/10.1111/ijd.14048, https://doi.org/10.1007/s13353-015-0307-4.

Grants: Akdeniz University Scientific Research Projects Coordination Unit, Project Number: TSA-2021-5594.

Conflict of Interest: None declared.

EP05.038 Melnick-Needles syndrome: a male with severe and perinatally lethal phenotype

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[1]*National and Kapodistrian University of Athens, Laboratory of Medical Genetics, Medical School, Athens, Greece;* [2]*National and Kapodistrian University of Athens, 1st Department of Pathology, Medical School, Athens, Greece;* [3]*National and Kapodistrian University of Athens, Research University Institute for the Study and Prevention of Genetic and Malignant Disease of Childhood, Athens, Greece.*

Background/Objectives: Melnick-Needles syndrome (MNS) is a rare X-linked skeletal dysplasia, with severe lethal phenotype in males and less than 70 cases in the literature. MNS presents with extreme clinical heterogeneity whereby phenotypes in female carriers range from severe to very mild. A pathogenic *FLNA* variant was detected in a male fetus and is presented in the context of X-Linked Otopalatodigital Spectrum Disorders.

Methods: A 45 years-old female was referred for clinical evaluation and counselling, after 4 miscarriages, a successful IVF and delivery, and 2 therapeutic abortions due to severe anomalies (IUGR, hydrops, narrow chest etc). Specialised post-mortem examination on one of the aborted foetuses with a 46,XY karyotype, indicated renal dysplasia, cleft palate, lungs hypoplasia, constructures, short neck and multiple congenital anomalies. Subsequent genetic studies on DNA extracted from frozen tissues included Whole Exome Sequencing (WES) and targeted Sanger sequencing.

Results: The NM_001110556.2:c.3562G>A missense variant detected in hemizygosity is expected to cause a p.(Ala1188Thr) substitution which based on ACMG guidelines is classified as pathogenic (PM1, PM2, PP3, PP5). Segregation analysis with Sanger sequencing verified findings and indicated that the mother is a manifesting carrier, with very mild characteristics (hoarse voice, short stature) and the female born to IVF is normal.

Conclusion: To the best of our knowledge, this is only the fifth time a male with MNS was genetically diagnosed, and the second record of this specific variant. NGS supports marked improvement in diagnosis of cases with unusual severe phenotypes even before complete clinical presentation.

References: PMID: 34008892, 29575627.

Grants:

Conflict of Interest: None declared.

EP05.039 Familial and bilateral Poland Syndrome with hepatic hemangioma

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Background/Objectives: Poland Syndrome is a rare congenital disease known as hypoplasia or absence of pectoralis major muscle and upper limb abnormalities. Most cases are unilateral and sporadic, rare familial cases show reduced penetrance. Etiology of this disorder is still unknown, however intrauterine vascular defects have been suspected. Up-to-date only one case with a hemangioma has been reported (1). We present a familial Poland Syndrome in three generations with bilateral involvement and hepatic hemangioma in the proband.

Methods: A 27-year-old male patient was referred with the complaint of inability to raise his arms. The patient who has abnormal physical examination findings was evaluated with imaging and whole exome sequencing.

Results: In physical examination, limited abduction was observed in both shoulders and bilateral pectoralis major muscles could not be palpated. Family pedigree was compatible with an autosomal dominant inheritance with variable expression. In the proband, bilateral absence of the pectoralis major muscle was confirmed with ultrasound. No abnormalities in other muscles and thoracic structures were detected with tomography, however coincidentally, hepatic hemangioma measuring 34x30 mm was revealed with abdominal MRI. No candidate gene was found in whole exome sequencing.

Conclusion: Our case is unique with autosomal dominant inheritance pattern of Poland syndrome and with the presence of additional vascular defect, a hepatic hemangioma, probably a genetic vascular disorder without known locus.

References: 1. Riyaz N, Riyaz A. Poland syndrome (anomaly) with congenital hemangioma: a new association. Indian J Dermatol Venereol Leprol. 2006 May-Jun;72(3):222-3. https://doi.org/10.4103/0378-6323.25785. PMID: 16766839.

Grants:

Conflict of Interest: None declared.

EP05.040 Novel DTDST mutation in a Moroccan patient with diastrophic dysplasia

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[1]*Mohammed VI University Hospital, department of genetics,Clinical Research Center, Marrakech, Morocco;* [2]*Cadi Ayyad University, School of Medicine, Marrakech, Morocco.*

Background/Objectives: Diastrophic dysplasia is a rare disorder marked by short stature with short extremities and joint malformations leading to multiple joint contractures, it's a rare syndrome with a prevalence estimated at 1-1.3/100,000. A hitchhiker thumbs, cleft palate and cystic ear swelling in the neonatal period may be suggestive signs of this syndrome. Diastrophic dysplasia is caused by mutations in the *SLC26A2* (*DTDST*), which encodes a sulfate transporter that is predominantly expressed in the cartilage.

Objective: Confirm the clinic diagnostic of Diastrophic dysplasia in our patient.

Methods: We studied the DNA from the family by a direct sequencing analysis of PCR amplified DNA from the proband and their parents.

Results: We report through this work a undescribed *SLC26A2* mutation, in a girl with diastrophic dysplasia.

Conclusion: Interest of genetic consultation and dysmorphology expertise in the orientation of the diagnosis for certain constitutional bone diseases.

References: Superti-Furga A, Neumann L, Riebel T, Eich G, Steinmann B, Spranger J, Kunze J (1999) Recessively inherited multiple epiphyseal dysplasia with normal stature, club foot, and double layered patella caused by a DTDST mutation. J Med Genet 36:621–624.

Superti-Furga A, Unger S, the Nosology group of the international skeletal dysplasia society (2007) Nosology and classification of genetic skeletal disorders: 2006 revision. Am J Med Genet A 143:1–18.

Mégarbané A, Haddad FA, Haddad-Zebouni S, Achram M, Eich G, Le Merrer M, Superti-Furga A (1999) Homozygosity for a novel DTDST mutation in a child with a 'broad bone-platyspondylic' variant of diastrophic dysplasia. Clin Genet 56:71–76.

Grants: No grants.

Conflict of Interest: None declared.

EP05.042 A large family with short stature and genu varum: expanding the phenotype associated with ACAN variants

André Travessa[1], *Patrícia Dias*[1], *Belinda Campos-Xavier*[2], *Catarina Ferreira*[2], *Andrea Superti-Furga*[2], *Ana Berta Sousa*[1]

[1]*Serviço de Genética Médica, Departamento de Pediatria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal;* [2]*Division of Genetic Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland.*

Background/Objectives: Since 2010, heterozygous *ACAN* variants have been found in patients with short stature (SS), advanced bone age, early-onset osteoarthritis and/or osteochondritis dissecans.

Here, we report a multigenerational family with 12 patients with SS, in whom we found an *ACAN* variant. Some of them also presented *genu varum*, thus expanding the phenotype associated with *ACAN* variants.

Methods: Two 20-year-old twin sisters were referred at the age of 2 years due to growth delay and a family history of SS. Pregnancy was unremarkable but height at birth was <5th centile. They evolved with mildly disproportionate SS (136,2 and 138,7cm at adult age) and macrocephaly, and went on to develop lumbar hyperlordosis and *genu varum* that required surgical correction.

The family history was remarkable for several relatives with SS and lumbar hyperlordosis (son, sister, nephew, father, paternal grandfather, aunt, uncle, three first cousins, and a second cousin). Some also had *genu varum* (paternal grandfather and aunt), and others had degenerative joint disease (two first cousins).

Results: Whole-exome sequencing performed in the probands' second cousin documented the presence of a heterozygous pathogenic variant in *ACAN*: c.1180C>T, p.(Arg394Ter). This variant was shown to be present in six affected relatives, including the twin sisters.

Conclusion: This case highlights the intrafamilial variability of *ACAN*-related SS and expands the phenotype to include *genu varum*. In the literature, the variant c.1180C>T, p.(Arg394Ter) was found in one patient with SS, advanced bone age, and osteochondritis dissecans, reinforcing that genotype-phenotype correlations in *ACAN*-related SS and very limited.

References: None.

Grants: None.

Conflict of Interest: André Travessa Medical Geneticist, Patrícia Dias Medical Geneticist, Belinda Campos-Xavier Laboratory Geneticist, Catarina Ferreira Laboratory Geneticist, Andrea Superti-Furga Medical Geneticist, Ana Berta Sousa Medical Geneticist.

EP05.043 Ectodermal-dysplasia-like phenotype associated with null variant in LRP6 gene

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Background/Objectives: Heterozygous pathogenic variants in the *LRP6* gene are a confirmed cause of selective tooth agenesis 7 (OMIM # 616724). Recently, an 11-year-old male with oligodontia and sparse scalp hair carrying a truncating *LRP6* variant was reported[1].

Methods: In our genetic counseling unit, we saw a 19-year-old woman with microcephaly, short stature, sparse scalp hair, pale skin, severe myopia and impressive gestalt. Her teeth were normal in quantity and morphology. We performed trio-exome-sequencing.

Results: We identified a heterozygous *de novo* variant c.4361dup, p.(Ser1455Lysfs*7) in *LRP6*. Loss of function is a known mechanism of tooth agenesis 7. Nonetheless, truncating *LRP6* variants are listed in gnomAD (pLI 0.7 and o/e 0.22). Distribution of pathogenic and gnomAD-variants in *LRP6* does not point to a genotype-phenotype-correlation. There is evidence for an incomplete penetrance. However, our patient's characteristics show hardly any overlap with the established *LRP6*-associated phenotype.

Conclusion: Although our patient does not show the typical symptoms of tooth agenesis 7, we still consider the null variant in *LRP6* to be a candidate explaining her phenotype. The variant occurred *de novo* and an overlap of the pathomechanisms of an ectodermal-dysplasia-like phenotype and oligodontia seems plausible. Also, there is a recent report of a patient with a null variant in *LRP6* and sparse hair. However, larger cohorts are needed to clarify if there is a broader phenotypic spectrum including ectodermal dysplasia.

References: 1.Yu, M. et al. Lrp6 Dynamic Expression in Tooth Development and Mutations in Oligodontia. J. Dent. Res. 100, 415–422 (2021).

Grants:

Conflict of Interest: Franziska Roessler Institute of Human Genetics, University of Leipzig Medical Center, Rami Abou Jamra Institute of Human Genetics, University of Leipzig Medical Center, Principal investigator of "Genetics of rare diseases based on Next Generation Sequencing", Vincent Strehlow Institute of Human Genetics, University of Leipzig Medical Center.

EP05.044 Genetic variants in epidemolysis bullosa patients in Lithuania

Zivile Zemeckiene[1;2], ***Rimvydas Jonikas***[1], *Kristina Aleknavičienė*[1;2], *Marius Šukys*[1;2], *Inga Poceviciene*[1], *Rasa Traberg*[1;2], *Rasa Ugenskiene*[1;2]

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Background/Objectives: Epidermolysis bullosa (EB) is a group of genetic skin diseases that cause fragile, blistering skin. Genetic testing of these diseases is important to better management and future predictions.

Methods: Whole exome sequencing and skin disease virtual gene panel analysis was performed for seven patients who were referred to clinical geneticist due to clinical diagnosis of epidermolysis bullosa in The Hospital of Lithuanian University of Health Sciences in 2021. All patients developed symptoms soon after birth or in early infancy.

Results: Likely pathogenic variants in *KRT5* (NM_000424.4) gene were found for two patients with no family history of epidermolysis bullosa, one patient with EB dystrophica had novel variant c.1423G>T and other with EB simplex – known variant c.74C>T. Two other EB simplex patients with negative family history had known heterozygous pathogenic c.927+1G>A and likely pathogenic c.1162C>G variants in *KRT14* (NM_000526.5) gene. Three patients had heterozygous variants in *COL7A1* (NM_000094.3) gene. First EB dystrophica patient had known likely pathogenic variant c.6119G>A. Another patient with EB dystrophica was heterozygote for two likely pathogenic variants – one known maternal c.933C>G, and other novel paternal variant –c.6449G>A. As parents are healthy, we suspect autosomal recessive inheritance. Third patient with undefined EB type had novel VUS-leaning pathogenic variant in *COL7A1* c.7886G>T and likely pathogenic variant in *FLG* gene, his family had similar symptoms in four generations.

Conclusion: Eigth different pathogenic/likely pathogenic variants in three different genes were determined in seven patients. Three of the variants were novel.

References:

Grants:

Conflict of Interest: None declared.

EP05.045 High prevalence of scoliosis in Koolen-de Vries syndrome: An international observational retrospective cohort study

Arianne Bouman[1], *Romy Bouwmeester*[2], *David Koolen*[1], *Joyce Geelen*[2], *Willemijn Margriet Klein*[3], *Leo van Vlimmeren*[4], *Marinus de Kleuver*[5], *Pauline Burger*[6;7;8;9], *Jean Louis Mandel*[6;7;8;9;10]

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Background/Objectives: The Koolen-de Vries syndrome (KdVS, OMIM #610443), a rare neurodevelopmental disorder secondary to 17q21.31 microdeletion or mutation in *KANSL1*-gene, is associated with scoliosis. We describe the prevalence, clinical and radiological characteristics of scoliosis in KdVS.

Methods: In this international retrospective cohort study, 54 participants with KdVS were included. Mean age of participants was 13.6 years (SD 8.4). We retrospectively analyzed participants' spine radiographs, MRI's and corresponding radiology reports for scoliosis and additional anomalies. Presence of scoliosis-related clinical conditions were assessed in medical records and patient surveys.

Results: Scoliosis was present in 30/54 participants (56%). Prevalence increased with age, from 36% at age 10 to 67% at age 18. Mean age at diagnosis was 10.6 years. During follow-up, the number of coronal curves increased and the curve magnitude progressed. At the time of inclusion, most curves (60%) were below 30°. Participants with scoliosis received less often physiotherapy (p 0.002). Bracing therapy was received in 7/24 participants (29%), and surgical spinal fusion in 3/30 participants (10%).

In KdVS, we found that scoliosis was radiologically associated with hyperkyphosis (47%) and hyperlordosis (50%). A majority of the patients had (mild) hypotonia (83%) and all patients (100%) were able to walk.

Conclusion: Prevalence of scoliosis in KdVS is high: 56%. Scoliosis in KdVS cannot be included in one of the existing scoliosis' categories, therefore we label it as scoliosis due to neurodevelopmental disorder. We advise routine coronal and sagittal full-standing spine X-ray screening before the start of the growth spurt and at the age of 18 years.

References:

Grants:

Conflict of Interest: Arianne Bouman full-time, Romy Bouwmeester full-time Radboudumc, David Koolen full-time Radboudumc, Joyce Geelen full-time Radboudumc, Willemijn Margriet Klein full-time Radboudumc, Leo van Vlimmeren full-time Radboudumc, Marinus de Kleuver full-time Radboudumc, Pauline Burger: None declared, Jean Louis Mandel: None declared.

EP05.048 Craniosynostosis as an additional feature of rare genetic syndromes

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Background/Objectives: Craniosynostosis (CS) represents a highly heterogeneous genetic condition whose genetic background has not been fully revealed yet. The abnormality occurs either as an isolated form or syndromic, an element of hundreds of different inborn syndromes. Consequently, CS may often constitute a challenging diagnostic issue.

Methods: gDNA samples were subjected to whole-exome sequencing. The coding and flanking intronic regions were enriched using a custom-designed in-solution exome enrichment (TWIST bioscience, San Francisco, USA) and sequenced using the Illumina NovaSeq system (Illumina, San Diego, USA).

Results: We revealed seven heterozygous variants in the seven patients in the following genes – *ARID1A* (linked to Coffin-Siris type 2 syndrome), *KMT2A* (linked to Wiedemann-Steiner syndrome), *KMT2D* (linked to Kabuki type 1 syndrome), *MN1* (linked to MN1 C-terminal truncation syndrome; MCTT syndrome, and CEBALID syndrome), *NSD1* (linked to Sotos type 1 syndrome), and *FAM111A* (linked to Gracile bone dysplasia, and Kenny-Caffey syndrome type 2).

Conclusion: We have shown that CS may be an additional feature of different, not associated earlier with it, syndromes. We have also pinpointed the possible underestimated co-occurrence of CS and intellectual disability, suggesting it may be overlooked when intellectual disability constitutes a primary clinical complaint.

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Grants: None.

Conflict of Interest: None declared.

EP05.049 The role of rare variants in male-pattern hair loss: Analysis of whole-exome sequencing data in the UK Biobank

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Background/Objectives: Male-pattern hair loss (MPHL) is a highly heritable and prevalent form of hair loss. Genome-wide association studies (GWAS) have identified more than 350 genomic risk loci, providing insights into the contribution of common genetic variants (MAF>1%) to MPHL etiology. However, systematic studies on the relevance of rare variants (MAF < 1%) in MPHL development are lacking. Using a tranche of 200,629 exomes from the UK Biobank, we tested for a contribution of rare coding variants to MPHL etiology.

Methods: 2,632,801 nonsynonymous variants (MAF < 1%) in protein-coding genes were tested for association with MPHL using SKAT-O gene-based and GWAS-style single-variant analyses in a case-control and extreme phenotypes setting. Association testing was followed by data interpretation, integration with MPHL GWAS data, and testing for enrichment in biological pathways.

Results: Both our single-variant and gene-based analyses identified significant associations of MPHL and rare variants in the X-chromosomal HEPH and EDA2R genes. Nominally significant associations were identified for an additional 17,892 single variants and 3,119 genes. This included associations in previously implicated candidate genes (e.g. WNT10A) and novel candidate genes at and beyond known GWAS loci (e.g. LAMA5, HOX13). Notably, nominally significantly associated genes were enriched for genes causative for monogenic trichoses as well as for genes located within topologically associated domains of MPHL-GWAS loci.

Conclusion: Our data point to a contribution of rare coding variants to MPHL etiology, broaden the MPHL-associated allelic spectrum at and beyond known risk loci, and suggest a biological association between monogenic trichoses and the common MPHL phenotype.

References:

Grants:

Conflict of Interest: Sabrina Henne: None declared, Sugirthan Sivalingam: None declared, Lara Hochfeld: None declared, Carlo Maj: None declared, Oleg Borisov: None declared, Andreas Buness: None declared, Markus M Nöthen Professor Markus M. Nöthen receives a salary from the Life & Brain GmbH., Peter Krawitz: None declared, Stefanie Heilmann-Heimbach Stefanie Heilmann-Heimbach receives a salary from the Life & Brain GmbH.

EP06 Cardiovascular Disorders

EP06.001 Aspirin reverses Fibronectin-mediated inhibitory effect on trophoblast invasion through Akt and ERK signaling in preeclampsia

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Background/Objectives: Preeclampsia is a severe gestational hypertensive disorder that represents a major threat to mortality and morbidity in maternal and fetal health worldwide. The pathoetiology of preeclampsia is believed to have defects of trophoblast differentiation and trophoblast invasion in placenta. Recent clinical trials showed that aspirin is an effective agent for preeclampsia prevention but the drug acting mechanism needs further investigation. Elevated Fibronectin (FN) expression in maternal circulation and placenta has been shown to be associated with preeclampsia, however, the role of FN in human pregnancy and its pathoetiology in preeclampsia is unclear.

Methods: FN knockdown (si-FN) and exogenous treatment of recombinant FN protein in HTR-8/SV neo cells.

Results: In our presenting study, FN was upregulated in the placenta of preeclamptic patients and aspirin suppressed trophoblast FN expression in a dose-dependent way. FN was shown to inhibit trophoblast migration and invasion abilities without affecting cell proliferation. Moreover, FN activated signaling pathway of ERK and Akt. Aspirin was demonstrated to not only suppressing FN expression but also reversing FN-mediated inhibitory effect on cell motility and signaling pathway.

Conclusion: In conclusion, FN inhibited trophoblast invasion and could be a potential biomarker for preeclampsia. Aspirin may exert its prevention effect from preeclampsia development through suppressing FN expression and alleviating FN-mediated inhibitory effect on trophoblast invasion.

References:

Grants: National Cheng Kung University Hospital, Tainan, Taiwan (NCKUH-11102045); Ministry of Health and Welfare, Tainan, Taiwan (TNHPA11001).

Conflict of Interest: Meitsz Su National Cheng Kung University Hospital, Tainan, Taiwan (NCKUH-11102045); Ministry of Health and Welfare, Tainan, Taiwan (TNHPA11001).

EP06.002 Biology of telomeres in patients with coronary heart disease without acute cardiovascular events

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Background/Objectives: Cardiovascular disease is the leading cause of death worldwide. Decrease or loss of function of myocardial cells or blood vessels is the cause of coronary heart disease. Telomeres are repetitive DNA sequences located at the ends of chromosomes that maintain genetic stability. The biology of telomeres is associated with several human disorders and diseases, especially cardiovascular diseases.

Methods: The study included 30 patients with ischemic disease and 30 healthy people. The standard phenol-chloroform extraction method was used to isolate the DNA. RNA isolation was carried out by the standard method using TRIzol. There was qPCR for measuring relative telomere lenght (RTL) and hTERT gene expression.

Results: The results of the study showed that the RTL of patients before surgery is 2 times shorter compared to this indicator of healthy people living in the same area ($p < 0.01$). The area under the ROC curve is 0.667 ± 0.045 ($p = 0.01$). Through a systematic analysis, it was found that the OCT in patients with coronary artery disease was significantly shorter than in the control group, while inversely correlated with the severity of coronary artery disease. The level of hTERT expression in both groups was extremely low, slightly higher in the control group ($p > 0.05$).

Conclusion: Presumably, inflammatory processes and oxidative stress, complementing each other, are the causes of irreparable damage to telomeres, accelerating the aging process and

leading to irreversible consequences in atherogenesis. More research is needed to elucidate the biology of telomeres in atherogenesis.

References:

Grants: Program of Basic Research of Siberian Branch of the Russian Academy of Sciences (0419-2022-0001).

Conflict of Interest: None declared.

EP06.004 Combined exosomal and plasma non-coding RNA signature associated with urinary albumin excretion in hypertension

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Background/Objectives: Non-coding RNA (ncRNA), released into circulation or packaged into exosomes, play important roles in many biological processes in the kidney. The purpose of the present study is to identify a common ncRNA signature from exosomes, urine and plasma associated with early renal damage and its related molecular pathways by constructing a RNA-based transcriptional network.

Methods: This is an observational case-control study which included twenty-one patients with essential hypertension ($n = 21$) and twenty-two without persistent elevated urinary albuminuria (UAE) (≥30 mg/g urinary creatinine). Three individual libraries (plasma and urinary exosomes and total plasma) were prepared from each hypertensive patient for ncRNA sequencing analysis. Next, a RNA-based transcriptional regulatory network was constructed.

Results: The three RNA biotypes with the greatest number of differentially expressed transcripts were long-ncRNA (lncRNA), microRNA (miRNA) and piwi-interacting RNA (piRNA). We identified a common 24 ncRNA molecular signature related to hypertension-associated albuminuria, of which lncRNA was the most representative. In addition, the transcriptional regulatory network analysis showed five lncRNA (LINC02614, BAALC-AS1, FAM230B, LOC100505824 and LINC01484), and the miR-301a-3p to play a significant role in network organization and to target critical pathways regulating filtration barrier integrity, tubule reabsorption and systemic endothelial dysfunction.

Conclusion: Our study found a combined ncRNA signature associated with albuminuria, independently of biofluid origin identifies a handful of potential targets involved in filtration barrier, tubule reabsorption and endothelial function that may be utilized to treating hypertension-associated albuminuria and cardiovascular damage progression.

References:

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Conflict of Interest: None declared.

EP06.005 Expression of innate immune response genes in the native heart valves obtained from patients with infective endocarditis

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Background/Objectives: Invective endocarditis (IE) is an inflammatory disease characterized by a dysfunction of heart valves and associated with high level of in-hospital mortality. Innate immune response playing the important role in the IE pathophysiology is genetically determined [1]. This study aimed to access the expression of TLR1, TLR2, TLR4 and TLR6 genes in the native heart valves obtained from IE patients.

Methods: The expression of TLR1, TLR2, TLR4 and TLR6 genes has been investigated in biopsies of native heart valves obtained from 26 patients with IE and from 12 patients who underwent surgical correction of non-infectious heart defects (control) using RT-qPCR with TaqMan probes. ACTB, GAPDH and B2M were used as a reference gene. The expression level was calculated by ΔCt method.

Results: We discovered no differences in the mRNA level of TLR2 in the native heart valves obtained from IE patients and from patients involved in the control group (fold-change was 0.97). At the same time, the expression of TLR4, TLR1 and TLR6 was significantly decreased in IE patients (fold-change was 0.65, 0.56 and 0.38, respectively) compared to the control.

Conclusion: A decreased activity of TLR-receptors leads to atypical forms of inflammation due to a failure of the innate immune response and an increased susceptibility of valve tissue to bacterial invasion.

References: 1. Weinstock M, Grimm I, Dreier J, et al. Genetic variants in genes of the inflammatory response in association with infective endocarditis. PLoS One. 2014; 9(10):e110151.

Grants: The fundamental research project of Research Institute for Complex Issues of Cardiovascular Diseases No. 0419-2022-0001.

Conflict of Interest: None declared.

EP06.006 Diagnostic yield of genetic testing in an unselected cohort of patients with congenital heart disease

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Background/Objectives: Chromosomal microarray analysis is typically the first genetic test offered to patients with congenital heart disease (CHD), particularly in syndromic cases where copy number variants (CNVs) and aneuploidies are more common. Few studies describe the genetic findings in CHD cohorts referred for Next Generation Sequencing (NGS) panel testing. We present the diagnostic yield of >200 patients with CHD receiving NGS panel testing to inform the utility of this test in these patients.

Methods: We undertook a retrospective review of 204 consecutive CHD patients who underwent testing for the Congenital Structural Heart Disease panel. Patients were considered syndromic if their requisition described ≥1 extracardiac anomaly. The identification of a pathogenic (P) or likely pathogenic (LP) variant(s) (with a modified ACMG/AMP classification scheme) was considered diagnostic. Chi-square analysis and Fisher's exact test determined statistical significance (P value < 0.05).

Results: In this cohort, 14.2% of patients ($n = 29$) had a diagnostic test result. P/LP CNVs were identified in 3.4% ($n = 7$) of patients; two of these were intragenic. The diagnostic yield was significantly higher in patients with a syndromic presentation ($n = 17$, 20.7%) compared to an isolated presentation ($n = 10$, 9.5%) ($P = 0.015$). There was no significant difference in diagnostic yield by age group at the time of testing ($P = 0.8737$).

Conclusion: One in seven patients in this cohort received a diagnostic test result. CNVs were common; more than one quarter of these were intragenic. This work demonstrates that: high resolution CNV detection capabilities are important and NGS panel testing should be considered for patients with CHD.

References:

Grants:

Conflict of Interest: Julie Hathaway Blueprint Genetics, Marcos Cicerchia Blueprint Genetics, Johanna Tommiska Blueprint Genetics, Saija Ahonen Blueprint Genetics, Eija H. Seppala Blueprint Genetics, Kimberly Gall Blueprint Genetics, Alicia Scocchia Blueprint Genetics, Inka Saarinen Blueprint Genetics, Matias Rantanen Blueprint Genetics, Jennifer Schleit Blueprint Genetics, Tiia Kangas-Kontio Blueprint Genetics, Massimiliano Gentile Blueprint Genetics, Pertteli Salmenperä Blueprint Genetics, Jussi Paananen Blueprint Genetics, Samuel Myllykangas Blueprint Genetics, Juha Koskenvuo Blueprint Genetics.

EP06.008 Cardiomyopathies (CMs): from clinical characterization to Whole Exome Sequencing (WES) analysis in Italian patients

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Background/Objectives: CMs are functional and structural abnormalities of the heart muscle characterised by a high clinical and genetic variability with more than 70 genes so far described. The main aim is to define the genetic cause of CMs in patients carefully clinical selected.

Methods: 200 Italian cases (68 familial and 132 sporadic) were analysed by WES through the Illumina NextSeq 550 platform focused on an in-silico panel of 66 genes related to cardiac diseases and distinguished into the three categories of CMs (structural, channelopathies (CAPs) and aortopathies (AORTO)).

Results: Among the enrolled patients, 87.5% were clinically classified as CMs, 7% as CAPs, and 5.5% as AORTO. WES allowed the molecular characterization of 21.5% patients with the highest detection rate in familial cases (34% vs 15% sporadic ones). TTN, MYH7, MYBPC3, PKP2 and FLNC are the most mutated genes explaining 70% of the CMs cases. Three main findings are the identification of 1) two novel gross deletions in the PKP2 gene confirmed by MLPA and SNPs array 2) a novel missense variant in the KCNH2 gene in a family affected by Long-QT Syndrome and 3) a non-canonical splicing variant in the ACVRL1 gene in a woman affected by Rendu-Osler-Weber Syndrome.

Conclusion: This study highlighted the importance of a multidisciplinary approach for the characterization of CMs patients. The advent of next-generation sequencing technologies facilitated the molecular diagnosis of the CMs and shed light on the complexity of this class of diseases, providing important information for clinical management and recurrence risk estimation.

References: NA

Grants: NA

Conflict of Interest: None declared.

EP06.009 Genetic findings in a family with hereditary spherocytosis, haemolytic anaemia and pulmonary hypertension

Memoona Shaukat[1;2;3], *Mareike Iankeit*[4], *Ding Cao*[1;2;3], *Nicola Benjamin*[2;3], *Ekkehard Grünig*[2;3], *Michal Zawada*[1], *Katrin Hinderhofer*[1], *Christina Eichstaedt*[1;2;3]

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Background/Objectives: Genetic defects are known in up to 18 genes to cause pulmonary arterial hypertension (PAH). However, in many cases the genetic background is unclear. The objective of this study was to identify the genetic background of a family with several cases of hereditary spherocytosis (HS), cholelithiasis and pulmonary hypertension (PH).

Methods: Whole exome sequencing for four family members was performed. We included the index patient who manifests both PH and HS, daughter with spherocytosis only, healthy son and healthy wife of index patient. Variants in identified genes were sought in the database Online Mendelian Inheritance in Man to investigate the genotype-phenotype relationship.

Results: No pathogenic variant in any PAH gene could be identified in the index patient. Instead, a missense variant, c.2204C>T p. (Ala735Val), in a gene causative of HS, Solute Carrier Family 4 Member 1 (SLC4A1), was identified in the index patient and his daughter. The variant was absent in the healthy members of the family. It was barely present in controls (0.003%), classified inconclusive by in silico prediction programs, but showed co-segregation with the disease in two family members. No further variant could be identified in the other genes responsible for HS (ANK1, SPTA1, SPTB, EBP42).

Conclusion: The identified variant in the SLC4A1 gene might be causative for HS, hemolytic anemia and cholelithiasis as identified in the index patient and his daughter. All PH patients had undergone splenectomy prior to the onset of the disease. Therefore, PH in this family has been classified as group V, PH due to hematological disorders.

References:

Grants:

Conflict of Interest: None declared.

EP06.010 Hereditary Hemorrhagic Telangiectasia: role of MGP as modifier gene

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Background/Objectives: Hereditary Hemorrhagic Telangiectasia (HHT) is a rare autosomal dominant disease. Genes associated are involved in the TGF-β/BMPs signaling pathway; arteriovenous malformations in lungs (pAVMs) are a clinical sign of HHT. *Matrix Gla Protein* (*MGP*) encodes for an extracellular protein, which inhibits BMP2 and BMP4, regulating ALK1 and VEGF expression. It

also plays a protective role in pAVMs development in HHT-mouse model. The study aims to search for *MGP* variants in HHT patients.

Methods: In 40 pAVMs-HHT patients and 11 non-pAVMs HHT relatives, the whole *MGP* gene was sequenced by Sanger. The variants' allele frequency was compared to GnomAD database controls, using Chi-Squared Test.

Results: Eight variants were observed, one in 5′UTR, three in 3′ UTR, one in the coding region and three in deep intronic regions. They are present in heterozygous and homozygous state in pAVMs patients, while they are present only in heterozygous state in non-pAVMs patients. The haplotype reconstruction on chromosome 12 was possible for 12 families. Chi-Squared Test produced significative *p* values.

Conclusion: These preliminary results, together with evidence from literature, suggest a role of *MGP* in affecting HHT-pAVMs. This can be related to the regulation of HHT-related pathways involving BMP4, ALK1 and VEGF by MGP. Haplotype reconstruction suggests a common pattern for the totality of HHT-patients. The statistically significant differences in allele frequencies between patients and controls encourage the hypothesis of *MGP*-involvement in HHT phenotypic modulation.

References:

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Conflict of Interest: None declared.

EP06.011 Ventricular fibrillation during acute myocardial infarction could be associated with a novel missense variant in the Fibroblastic Growth Factor (EGF) gene

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Background/Objectives: Ventricular fibrillation (VF) during acute myocardial infarction (AMI) is considered the most serious cardiac rhythm disturbance, causing collapse and cardiac arrest. However, the understanding of the genetic contribution to VF is very limited. Variants in genes related to ion homeostasis may increase the risk of developing such cardiac events.

Methods: Rare genetic variants in 244 genes encoded proteins were analysed by NGS in 46 patients with FV during AMI. Visualization of variants was performed using IGV and the bioinformatic analysis of its possible effect was performed with MutationTester, SNAP2, SIFT2, Polyphen and PhD-SNP.

Results: The variant c.1752A>C in EGF gene was identified in a 50 years old male, leading to the aminoacidic substitution p.Lys584Phe.

It encodes a member of the EGF superfamily, that after processing leads to the 53-amino acid EGF peptide. Previous studies have shown that EGF is involved in regulation of various epithelial ion channels that govern Na^+, K^+, Cl^- or Mg^{2+} homeostasis. Its receptor (EGFR) has also a role in maintaining contractile homeostasis under physiologic conditions in the adult heart.

EGF acts as an autocrine/paracrine magnesiotropic hormone that stimulates Mg^{2+} reabsorption in the kidney. Mutations in this gene are associated to a renal reabsorption defect, leading to hypomagnesaemia with normocalciuria and normocalcaemia.

Conclusion: c.1752A>C in EGF gene may have a pathological role in the development of VF during AMI due to a constitutive ionic disbalance.

References:

Grants: Instituto de Salud Carlos III (PI18/01737)-FEDER.

Conflict of Interest: None declared.

EP06.013 Complex arrythmogenic right ventricular cardiomyopathy – case presentation and genetic mutation screening by NGS

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Background/Objectives: Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is characterized by progressive fat or fibrofatty replacement of the right ventricular (RV) myocardium. The cause of ARVD/C is not yet clear, but recent studies suggest it is most often related to genetic mutations. Left ventricular (LV) or biventricular involvement are increasingly identified in ARVD/C patients. The genetic background of cardiomyopathies could shed light on the mechanism of their development, clinical presentations and new treatment options.

Methods: We describe here a patient affected by arrythmogenic right ventricular cardiomyopathy with left ventricular involvement, with the presence of coronary artery anomalies. There were aneurisms of coronary arteries and left circumflex artery (LCx) arises from right coronary sinus. Physiological, imaging and invasive study was performed in details. In addition, genetic analysis by next generation sequencing (NGS), using panel of genes for hereditary cardiomyopathy, was done.

Results: We detected a missense mutation in *MYBPC3* gene (c.1316G>A, p.Gly439Asp), classified as variant of unknown significance. We discuss myosin-related mutations in different cardiomyopathies with dosage-dependent effects of MYBPC3 on myosin that occur across the cardiac cycle.

Conclusion: The association of *MYBPC3* mutation with ARVD/C is worthy to be investigated, since it could have an important impact on the application of new treatment, using specific myosin targeted agents.

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Grants:

Conflict of Interest: None declared.

EP06.014 No effect of CRP rs1800947, TNF-α rs1800629 and IL6 rs1800795 polymorphisms on plasma levels of inflammatory markers after treatment with PCSK9 inhibitors

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Background/Objectives: Inflammation plays a key role in the pathogenesis of atherosclerosis. However, the role of genetic variability on inflammation after treatment with proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors remains to be elucidated. For the first time, we examined the influence of polymorphisms in *CRP*, *TNF-α*, and *IL6* genes on plasma levels of hsCRP, TNF-α, and IL6 at baseline and after treatment with PCSK9 inhibitors.

Methods: A total of 69 patients with stable coronary artery disease after a premature myocardial infarction were included in the study. All patients had extremely elevated lipoprotein(a) levels and received a PCSK9 inhibitor. Genotyping for *CRP* rs1800947, *TNF-α* rs1800629, and *IL6* rs1800795 was performed.

Results: Our results showed no significant association between single nucleotide polymorphisms in *CRP*, *TNF-α*, and *IL6* and plasma levels of hsCRP, TNF-α, and IL6, respectively. Consistent with previous studies, no significant change in levels of inflammatory biomarkers was observed after 6 months of treatment with PCSK9 inhibitors. Moreover, genetic variability in selected genes was not significantly associated with the change in plasma levels of corresponding inflammatory markers.

Conclusion: Genetic variability did not affect plasma levels of inflammatory markers, which could be due to background therapy with statins or extremely elevated lipoprotein(a) levels, because lipoprotein(a) itself contributes to inflammation. Further studies are needed to clarify which factors contribute most to the modulation of inflammation in high-risk patients.

References: Ruscica et al., Atherosclerosis, 2019.

Grants: The study was funded by the Slovenian Research Agency (P1-0170, P3-0308). T.L. was granted a scholarship from the University Foundation of ing. Lenarčič Milan.

Conflict of Interest: None declared.

EP06.015 Circulating microRNAs as biomarkers for pulmonary arterial hypertension

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Background/Objectives: Pulmonary Arterial Hypertension (PAH) is a rare disease where the thickening of the precapillary pulmonary arteries ends up inducing right heart failure. The prognosis of PAH patients depends on multiple factors, being the time of diagnosis a critical one. Currently, diagnosis is complicated and usually delayed until performing right-heart catheterization.

Methods: We perform small RNA sequencing in plasma of idiopathic PAH patients and controls. We used classification models to analyse the potential of the microRNAs, that we found differentially expressed, as PAH predictors. Also, we use miRBase to predict the targets for the dysregulated miRNAs we detected. Finally, we performed functional assays based on qPCR and western blotting to confirm our results.

Results: We were able to find 29 differentially expressed microRNAs and validate 7 of them in a nationwide cohort (let-7a-5p, let-7b-5p, let-7c-5p, let-7f-5p, miR-9-5p, miR-31-5p, miR-3168). In our cohort, we obtained a model with an AUC of 0.738. Also, we identified miR-3168 as a novel upregulated miRNA in PAH patients. We demonstrate that it targets the Bone Morphogenetic Protein Receptor type 2 (BMPR2), as validated at mRNA and protein levels. Preliminary results show that miR-3168 overexpression increases resistance to apoptosis and enhanced angiogenesis.

Conclusion: We found novel downregulated and upregulated microRNAs in idiopathic PAH patients. We were able to develop a 3-microRNA signature for diagnosis and functionally characterized in vitro the effect of miR-3168 as a possible modulator of the disease.

References:

Grants:

Conflict of Interest: None declared.

EP06.016 22q11.2 microdeletion is the most common genomic abnormality in Serbian newborns with critical congenital heart disease and could be rapidly detected by Multiplex ligation probe amplification analysis

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Background/Objectives: Genetic tests may facilitate rapid and effective diagnostics but unfortunately their high costs usually limit their application in all patients (1). We aimed to investigate the utility of rapid, cost effective and high sensitive Multiplex ligation probe amplification analysis (MLPA) for detection copy number variants (CNV) in newborns with critical CHD, admitted to the Neonatal Intensive Care Unit (NICU).

Methods: Study included 100 consecutive newborns admitted to the NICU, University Children's Hospital in Belgrade from August 2014 to September 2019. Patients with viable trisomies (21, 18 and 13) were excluded. All participants were tested by MLPA analysis using SALSA MLPA P250-B2 Di George and SALSA MLPA P311-B1 Congenital Heart Disease probemixes (MRC Holland, The Netherland).

Results: Pathogenic CNVs were identified in ten (10%) patients. Nine of them had 22q11.2 deletion detected by both kits while one patient had 3p25 deletion detected by P311 kit.

Conclusion: Genetic evaluation of all newborns with critical CHD admitted to the NICU by rapid and inexpensive MLPA analysis using combination P250 and P311 SALSA probemixes could contribute to high detection rate of pathogenic variants.

References: 1. Monteiro RA, Freitas ML, Vianna GS et al. (2017) Major contribution of genomic copy number variation in syndromic congenital herat disease: the use of MLPA as the first genetic test. Mol Syndromol 8: 227-23.

Grants: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement number 451-03-68/2020-14/200042) and the Serbian Academy of Sciences and Arts (MIKRONEURO_no. 01-2021).

Conflict of Interest: None declared.

EP06.017 Is the phenotype for ARVC caused by TMEM43 (p.S358L) becoming more severe in women over time?

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Background/Objectives: Autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVC) due to *TMEM43* p.S358L is a sex influenced cause of sudden cardiac death (median age 40 yrs. in men, 67yrs. in women) and heart failure. We have demonstrated that the implantable cardioverter-defibrillator (ICD) significantly improves survival and described the natural history and clinical course using 27 large multiplex families. Anecdotal evidence suggests that women are presenting with an ICD discharge ≥240 beats per minute (previously shown to be a death equivalent) earlier than family history would suggest. We wished to investigate temporal changes in severity using our dataset spanning 100 years.

Methods: We compared 76 affected women (obligate carriers and/or positive for p.S358L TMEM43) in each of two birth cohorts (DOB 1960–1999 and DOB 1920–1959). Using SPSS v 22 we performed Kaplan Meier survival analysis, and cox proportional regression to the end point of death or an ICD firing of ≥240 beats per minute.

Results:

Cohort	N	# Events	Survival time KM		Test of equality KM			Cox PR
			Mean	Std. Error	Log Rank	Breslow	Tarone Ware	
1920-1959	76	32	75.3	2.26	$p = 0.06$	$p = 0.03$	$p = 0.04$	$p = 0.06$
1960-1999	76	10	52.7	1.08				

Conclusion: There is a trend toward earlier presentation in the younger cohort. We will re-analyse, adjusting for life expectancy differences between 1920 and 1999. These results suggest that non- genetic influences may be affecting the younger cohort, thus women may not be as protected as previously thought. Historic family data is powerful as it allows temporal changes to be seen.

References:

Grants:

Conflict of Interest: None declared.

EP06.018 Aortic disease and cardiomyopathy in patient with a novel variant in the DNMT3A gene causing Tatton-Brown-Rahman syndrome

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Background/Objectives: Tatton-Brown-Rahman syndrome (TBRS) is an autosomal dominant overgrowth syndrome caused by heterozygous and usually *de novo* pathogenic variant in *DNMT3A*. This syndrome was first described in 2014, since then an increasingly wider spectrum of neurological, skeletal and cardiological clinical features has been found in adults.

Methods: We present a 34-year-old male who was referred to a cardiologist due to episodes of weakness, dizziness, and a cold sweat. The patient had psychomotor retardation, tall stature, obesity, kyphoscoliosis, coarse facial features, synophrys and corrected pectus excavatum in childhood. Cardiovascular evaluation revealed left ventricular (LV) dilatation with non-compaction, aortic root dilatation, mitral prolapse with moderate regurgitation and short episodes of ventricular tachycardia. During follow-up, aortic dilatation (from 5.0 to 6.0 cm) and mitral valve regurgitation (from moderate to severe) progressed, indicating the need for surgical correction.

Results: Genetic analysis revealed a likely pathogenic heterozygous variant of the *DNMT3A* gene NM_022552.4:c.2324C>A, NP_072046.2:p.(Ser775Tyr). This variant has not been described in literature before. Segregation analysis in the family showed that c.2324C>A variant was inherited from the 58-year-old mother. Additionally, the genetic alteration was identified to the 39-year-old brother of the proband. Both affected relatives have LV dilatation, mild to moderate mitral valve regurgitation. Furthermore, the older brother was diagnosed with aortic root dilatation (4.5 cm).

Conclusion: Clinical examination of the affected individuals in the family showed variable expressivity and incomplete penetrance of specific clinical features of TBRS, including the aortic disease and cardiomyopathy, which might be major complications of the syndrome and require specific therapeutic management.

References:

Grants: None.

Conflict of Interest: None declared.

EP06.019 Exploiting the Whole Exome Sequencing for the identification of new candidate genes associated with Brugada Syndrome

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Background/Objectives: Brugada Syndrome (BS) is an inherited arrhythmogenic disease with risk of sudden cardiac death in young asymptomatic adults. *SCN5A* is the only known causative gene, though 22 genes are associated with BS susceptibility; 70% patients remain genetically undiagnosed. To identify new candidate genes, we performed Whole Exome Sequencing (WES) of 172 patients, both sporadic and familiar cases. To investigate the oligogenic hypothesis, 22 *SCN5A*-positive patients were enrolled too.

Methods: WES was performed on Illumina Platforms (mean coverage: 180X, 97% target region >20X). Reads were analyzed using Dragen Bio IT Platform, coding and splice regions variants (MAF≤0.01%) prioritized and classified according to ACMG guidelines, with support of eVai-EnGenome software. Burden Test extrapolated genes with higher mutation burden, suggesting a possible pathogenic role.

Results: Most prioritized variants were missense (88%) located in new candidate genes belonging to ion channels (25%), sarcomeric (55%), desmosomal (8%) and nuclear proteins (5%), consistent with disease phenotype. In particular, burden test confirmed the role of *SCN5A* and highlighted also possible association of three genes encoding cytoskeletal and channel proteins.

Conclusion: Our data identified new candidate genes which should be further investigated and confirmed in a larger cohort, evaluating also possible copy number variations. Genotype-phenotype correlations will be performed to better stratify patients, taking into account also the putative oligogenic

inheritance of the disease, evaluating the possible role of multiple variants in the clinical phenotype.

References:

Grants: Funded by Italian Ministry of Health (GR-2016-02362316).

Conflict of Interest: None declared.

EP06.020 DNA methylation patterns in NSTEMI and unstable angina pectoris patients

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Background/Objectives: Acute coronary syndrome (ACS) contributes significantly to mortality accounting for nearly one third of all deaths worldwide. ACS can be divided into ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation infarction (NSTEMI) and unstable angina pectoris (uAP). There is a need for accurate diagnostic tools for the early identification and monitoring of CAD, also with respect for the differentiation of NSTEMI and uAP. The role of DNA methylation has attracted attention in the field of CAD diagnostics.

Methods: A genome-wide DNA methylation discovery was performed (Illumina EPIC BeadChips; n = 23 NSTEMI, $n = 25$ uAP, $n = 24$ NCCP). Technical and biological validation on 89 selected methylation marks was done using a qPCR approach based on methylation sensitive restriction enzymes.

Results: A DNA methylation pattern differentiating NSTEMI, uAP and NCCP was identified. 89 single CpGs with $p < 0.05$ and a delta-β of >0.15 were selected for further validation. Validation of selected CpGs confirmed differentiation of NSTEMI and uAP from controls with high accuracy (AUC: 0.83; sensitivity: 0.84; specificity: 0.68). Clear differentiation of NSTEMI and uAP was hampered by biological similarity of theses two groups (AUC-value: 0.71; sensitivity: 0.72; specificity: 0.6).

Conclusion: A unique epigenetic profile is present in the investigated sample cohort. However, the epigenetic profile between NSTEMI and uAP is quite similar and the observed DNA methylation differences between the two groups are low. Nonetheless, we were able to confirm a set of 14 CpGs between NSTEMI/uAP and NCCP, as well as 7 CpGs, which may facilitate the differentiation of NSTEMI vs. uAP.

References:

Grants:

Conflict of Interest: None declared.

EP06.021 Validation of new gene variant classification methods: a field-test in diagnostic cardiogenetics

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Background/Objectives: In the molecular genetic diagnostics of Mendelian disorders, solutions are needed for the major challenge of dealing with the large number of variants of uncertain significance (VUS) identified using next-generation sequencing (NGS). Recently, promising approaches to calculate case excess scores (CE) and etiological fractions (EF) and gnomAD-derived constraint metrics scores have been reported that estimate the likelihood that rare variants in specific genes or regions are pathogenic. Our objective was to study the usability of these scores into diagnostic variant interpretation, using our clinical cardiomyopathy cohort.

Methods: Patients ($N = 2002$) referred for clinical genetic diagnostics underwent NGS testing of 55-61 genes associated with cardiomyopathies. Previously classified likely pathogenic (LP) and pathogenic (P) variants were used to validate the use of data from CE, EF and gnomAD constraint analyses for (re)classification of associated variant types in specific cardiomyopathy subtype-related genes. Next, we applied these constraint data to interpret and (re)classify 1229 variants (identified in 812 patients) previously classified as VUS.

Results: Using constraint scores in variant interpretation and classification, the classifications of (L)P's was corroborated in 94% (354/378) of cases. Moreover, 23 unique VUSs were reclassified to LP, increasing the diagnostic yield with 1.2%. In addition, 106 unique VUSs (5.3% of patients) were prioritized for co-segregation or functional analyses.

Conclusion: Our analysis confirms that the use of constraint metrics data can improve cardiogenetic variant interpretation and classification. We therefore recommend the inclusion of constraint scores in variant interpretation protocols and to also apply these in other cohorts and disorders.

References:

Grants:

Conflict of Interest: None declared.

EP06.022 A case report of a truncating variant altering the extreme C-terminal region of desmoplakin (DSP) suggests its crusial functional role

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Background/Objectives: Homozygous DSP truncating mutations located in the C-terminal region of DSP are known to mainly cause Carvajal syndrome, an autosomal recessive syndromic form of arrhythmogenic cardiomyopathy with an extra-cardiac cutaneous phenotype.

Methods: The index case and her relatives underwent full cardiological assessment. Genetic analysis of the index case was performed using SOPHiA Extended Cardio solution and Sanger sequencing was used to screen members of the family for the presence of the reported mutation.

Results: We describe a female proband with a severely manifesting arrhythmogenic left ventricular cardiomyopathy and a syncopal episode at the age of 10, who was found homozygous for the novel DSP mutation: NM_004415.4:c.8586delC, p.(Ser2863Hisfs*20) at the extreme C-terminal region of the protein, just 8 amino acids upstream the stop codon. She did not have any of the typical dermatological symptoms that characterize Carvajal syndrome. Her brother had died suddenly during exercise and was post mortem found homozygous for the same variant, while their parents were found heterozygous. When interviewed they stated as region of origin the same geographic area of Greece but they were not aware of any common ancestor.

Detailed clinical examination revealed that the mother displayed a mild arrhythmic profile, while the father was asymptomatic.

Conclusion: These observations pinpoint to a significant functional role of the extreme C-terminal tail of the protein.

References:

Grants: GR i CARDIAC NET: Greek National Network of Precision Medicine in Cardiology and the Prevention of Sudden Death in the Young.

Conflict of Interest: None declared.

EP06.026 Molecular genetics profile of inherited cardiomyopathies in Bahrain

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Background/Objectives: Cardiac diseases are a priority for the health system and the cardiomyopathies are known for their impact into morbidity and mortality. This study, the first in Bahrain, aims to investigate the inherited cardiomyopathies molecular genetics profile and identify a phenotype-genotype correlation.

Methods: 58 patients diagnosed with cardiomyopathies were recruited from Cardiac Center, Bahrain Defense Force Hospital. Genomic DNA was extracted from peripheral blood and saliva samples. Gene sequencing and deletion/duplication analysis was performed using a next generation sequencing (NGS) panel of 106 genes linked with inherited cardiomyopathies. The candidate disease-causing variants were verified by Sanger sequencing, MLPA-seq, array CGH. PolyPhen-2 was used to analyze mutation effect and to predict the effect of the variants of unknown significance (VOUS).

Results: Pathogenic variants were identified in 17/58 patients (29.3%). TNNT2, MYBPC3, MYH7, CACNA1C, PTPN11, MYBPC3, MYH7 and FHL1 genes mutations were identified in 14/17 hypertrophic cardiomyopathy. ALMS1 and TTN genes mutations were found in 3/17 patients with dilated cardiomyopathy. Syndromic cardiomyopathies (Alstrom Syndrome, Noonan Syndrome, Emery Dreyfuss dystrophy) were identified in 4/17 patients. The cardiology management was immediately adjusted for 6/17 patients (4/6 for ICD, 2/6 for medication). 131 VOUS in 52/106 genes were identified in 41/58 patients and 89/131 VOUS were assessed by bioinformatics algorithms as possible damaging.

Conclusion: Genetic results provided a certitude causal diagnosis in 29.3% of the patients, establishing a genotype-phenotype correlation, offering a better estimated prognosis, an accurate genetic counselling and providing therapeutic guidance for the treating cardiologists.

References: Invitae Cardiomyopathy Comprehensive panel https://www.invitae.com.

Grants: AGU G-E016-Pi-11/18.

Conflict of Interest: CRISTINA SKRYPNYK Al Jawhara Center, Arabian Gulf University, Research grant G-E016-PI-11/18 PI, Neale Kalis Cardiac Center, Bahrain Defense Force Royal Medical Services Military Hospital, Research grant G-E016-PI-11/18 collaborator, Leena Sulaibeekh Cardiac Center, Bahrain Defense Force Royal Medical Services Military Hospital, Research grant G-E016-PI-11/18 collaborator, Mary Jozeph Lynch Cardiac Center, Bahrain Defense Force Royal Medical Services Military Hospital, Research grant G-E016-PI-11/18 collaborator, Adel Khalifa Cardiac Center, Bahrain Defense Force Royal Medical Services Military Hospital, Research grant G-E016-PI-11/18 collaborator, Noureddine Ben Khalaf Life Sciences Department, Health Biotechnology Program, College of Graduate Studies, Arabian Gulf University, Research grant G-E016-PI-11/18 collaborator, Sfoug AlShammary Regenerative Medicine Center, Arabian Gulf University, Research grant G-E016-PI-11/18 collaborator.

EP06.028 Ryanopathy in two Iranian families with premature coronary artery disease and sudden cardiac death

Maryam Asadnezhad[1], *Sepideh Mehvari*[1], *fatemeh ghodratpour*[1], *Sara Saki*[2], *Kaveh Hosseini*[3], *Saeed Sadeghian*[3], *Reza Malekzadeh*[2], *Hossein Najmabadi*[1], *Kimia Kahrizi*[1]

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Background/Objectives: Coronary artery disease (CAD), assigns 32% of all deaths to itself with an estimated 17.9 million deaths annually. Approximately 50% of all CAD-related deaths are attributed to sudden cardiac death (SCD) and the proportion of all SCDs resulting from CAD are about 80%. Four etiologies exist for SCD, comprising CAD, cardiomyopathies, cardiac channelopathies, and aortopathy. Ryanodine receptors (RyRs), of which, three members are known, are calcium ($Ca2+$) release channels located on the endoplasmic/sarcoplasmic reticulum (ER/SR). RyR2 is the major ($Ca2+$) channel in cardiomyocytes, in which mutations can lead to cardiac arrhythmia and SCD. Due to several evidence on the contribution of rare variants to CAD, we aimed at identifying the potential rare disease-causing variants in Iranian patients with premature CAD and a positive family history for the disease.

Methods: Whole exome sequencing (WES) was performed on the proband of each family followed by Sanger sequencing for confirmation of the results and co-segregation study.

Results: We identified two heterozygous missense variants, p.Arg3260Trp and p.Thr1730Met, in *RYR2* gene (OMIM:*180902) in two unrelated families with history of CAD and SCD. Both variants have been recently reported in ClinVar database with ventricular tachycardia, catecholaminergic polymorphic, 1 (CPVT1) and/or cardiomyopathy, classified as VUS.

Conclusion: There are some forms of CAD with familial segregation consistent with being a single gene disorder. In a cohort of premature CAD Iranian patients, *RYR2* variants have been identified in two families, one suffered from SCD. Identifying asymptomatic individuals prior to disease onset can help to disease prevention and management.

References:

Grants: D/206/1851 to Kimia Kahrizi.

Conflict of Interest: None declared.

EP06.029 Heterozygous ABCG8 variant as the cause of premature coronary artery disease in an Iranian family

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Background/Objectives: Coronary artery disease (CAD) is the major cardiovascular disease with an estimated 17.9 million deaths annually. One of the major pathways with a significant influence on CAD is lipid metabolism. Sitosterolemia 1 [STSL1;MIM:#210250], characterized by elevated cholesterol and plant sterols (xenosterols) in plasma, is associated with premature coronary atherosclerosis that results from mutations in a member of ABC transporters, known as ABCG8. Sterolins, comprised of ABCG8 and ABCG5, form an obligate heterodimer that pumps xenosterols/cholesterol out of enterocytes and hepatocytes back into the intestinal lumen and bile canaliculi. This function is severely affected upon *ABCG5/8* mutations. In our study, we aimed at identifying the potential rare disease-causing variants in Iranian patients who show familial clustering of premature CAD (PCAD).

Methods: Whole-exome sequencing was performed on the proband of each family followed by Sanger sequencing for confirmation of the results and co-segregation analysis.

Results: We identified a likely pathogenic missense variant [c.G562C(p.V188L)] in *ABCG8* gene (MIM:*605460) in an Iranian family affected by PCAD with autosomal dominant pattern of inheritance. There was only one affected offspring in the family with previously angiographically proven PCAD. However, subsequent segregation analysis showed the carrier genotype in four out of five apparently healthy offspring, for whom coronary CT angiography confirmed the genetic results.

Conclusion: Although the rare STSL1 is caused by homozygous/compound heterozygous mutations in *ABCG8* gene, recent research found that *ABCG8* variants are more common than previously assumed. Furthermore, even heterozygous carriers have altered sterol profiles and are at higher risk of cardiovascular disease.

References:

Grants: D/206/1851 to Kimia Kahrizi.

Conflict of Interest: None declared.

EP06.030 Whole Exome Sequencing (WES) in unravelling complex cases of bicuspid aortic valve

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Background/Objectives: Congenital Heart Defects (CHD) are among the most frequently observed malformations and, to date, several genes have been described (both in syndromic and non-syndromic conditions). Here we present the cases of two patients assessed by genetic counseling for bicuspid aortic valve (BAV) and diagnosed through WES.

Methods: Both patients (P1 and P2) have received a deep clinical characterization through cardiological and genetic counselling and their DNA has been analyzed through WES. After standard WES filtering procedures, pathogenic variants within genes likely associated to CHD have been selected.

Results: P1 and P2 present with similar clinical features. In particular, P1, a boy aged 4 y.o., was diagnosed with BAV at birth. He showed later onset of language delay, facial dysmorphisms and short stature, while P2 is an 18 y.o. girl with BAV, cardiomyopathy and arrhythmias, facial dysmorphisms, joint pain and disproportionate short stature. WES analyses highlighted two variants within *TAB2* gene (NM_001292034.3): P1 carries a novel pathogenic truncating variant c.712C>T,p.Gln238 predicted as deleterious by all the in silico predictor tools, while P2 carries a truncating known mutation c.823C>T,p.Gln275* (NM_015093.4). This genotype-phenotype correlation is in agreement with recent literature studies showing the involvement of this gene in syndromic CHD.

Conclusion: Although *TAB2* has been mostly described for non-syndromic CHD, our results, together with recent literature studies, highlighted its role for syndromic CHD in a precise phenotypic spectrum (i.e. developmental delay, facial dysmorphisms, short stature). In conclusion, we believe the importance of a deep evaluation in CHD patients together with a *TAB2* genetic characterization.

References: PMID:2561515;PMID:26493165;PMID: 21128281.

Grants:

Conflict of Interest: None declared.

EP06.031 Genomic diagnosis usefulness in clinical approach and genetic counseling in vascular Ehlers-Danlos Syndrome (vEDS). A case report

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Background/Objectives: Vascular Ehlers-Danlos syndrome (vEDS) is a genetic disease with dominant autosomal inheritance caused by alterations in COL3A1 gen. Clinical criterias are useful tools to identify affected patients in which mortalitity associated with vascular complications is elevated. The aim of this work is to report the case of male patient, 38 years old, with genomic certification of vEDS. Patient is second son from nonconsanguineous parents. He has been studied by Cardiology Unit for thoracoabdominal aneurism that required prothesic replacement. Among his clinical background chronical hypertension, vertebral cerebellar artery dissection, bruising and dissection of right kidney artery has been referred. The anatomo-pathologic analysis post splenectomy reveled compatible features with muscular fibrodysplasia on splenic artery. Genealogy study supported the clinical suspect because his father had thoracic aneurysm corrected by surgery. On the other hand his mother died after recurrent strokes and his brother died at 32 years by sudden death.

Methods: Considering mayor and minor criterias COL3A1 gen was analyzed by multipanel sequencing (NGS) using ILLUMINA platform.

Results: Genetic testing reported one germline pathogenic, missense variant (c.709G>A/p.Gly237Arg) in COL3A1 gen. According Varsome and Clinvar COL3A1 is a gen with very low proportion of benign missense variants.

Conclusion: Genomic diagnosis allowed to identify the pathology and offer guidelines to prevent complications. Cosegregation variant studies discarded vEDS in offsprings. In families identified on the basis of clinical complications, penetrance of the vEDS phenotype appears to be close to 100% in adults with a missense or exon-skipping alteration; the age at which the pathogenic variant becomes penetrant may vary.

References:

Grants:

Conflict of Interest: None declared.

EP06.032 Periodontal pathogens in atherosclerosis. A metagenomic and in-vitro analysis

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Background/Objectives: It has been stablished that periodontitis is a contributing factor for atherosclerosis, being transitory bacteremia one of the mechanisms involved (1). The aim of this study is to describe the bacteria diversity and detect common species between peripheral blood and dental plaque of Colombian patients affected by both diseases.

Methods: A Metagenomic analysis of peripheral blood and dental plaque samples from 12 adults with atherosclerosis was performed. After DNA extraction (QIAamp® DNA Mini-Kit), the sequencing libraries were obtained using the Illumina HiSeq-2500 platform. Bioinformatic analysis was performed in the platform QIIME 2 (Version 2020.11.1).

Results: The metagenomic and bioinformatic analysis of peripheral blood and dental plaque revealed one bacteria specie (*Prevotella melaninogenica*), six genus, eleven families, and five phyla common for both sample types. Some of the genus present in blood are associated to periodontal disease such as *Prevotella*, *Pseudomonas*, and *Streptococcus*. The richness was greater in plaque samples (mean:47. SD = 5.24) than in blood samples (mean: 4.6. SD = 5.8).

Conclusion: The findings of this study confirm the presence of bacteria associated to periodontal disease (2) in peripheral blood of Colombian patients affected by periodontitis and atherosclerosis. This shows a possible contributing role of periodontal pathogens in atherosclerosis probably by mean of transitory bacteremia.

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Conflict of Interest: None declared.

EP06.034 Two novel mutations, two different clinical phenotypes associated with SCN5A; Brugada and Long QT syndromes

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Background/Objectives: SCN5A gene encodes the alpha subunit of myocardial expressed sodium channel protein, Nav1.5. Generally, loss-of-function mutations of SCN5A are expected to exhibit Brugada syndrome phenotype, meanwhile gain-of-function mutations portray Long QT syndrome. Here we present two patients with novel mutations in SCN5A, who display characteristics of different clinical entities, Brugada and Long QT syndromes.

Methods: Patient-1 (aged 13) was led to cardiac stress test that indicated long QT syndrome, then to our genetics clinic for further analysis. Patient-2 (aged 39), prediagnosed with Brugada syndrome in Ajmaline provocation test, was forwarded to genetic evaluation. Next generation sequencing method for target genes of cardiac arrhythmias was performed on patients' genomic DNA. Data were analysed via Ion Reporter Software (ThermoFisher Scientific Inc.) and SEQ (Genomize) programs.

Results: In Patient-1, a heterozygous missense variant, c.1575C>A (p.Ser525Arg), was identified. This variant's position is not conserved, however is a hot-spot region for nucleotide alterations.

In Patient-2, also a heterozygous missense variant, c.2548G>C (p.Val850Leu) was detected which is located in a highly conserved region.

Both alterations weren't found in population studies (gnomAD); were unidentified in disease specific databases (ClinVar, ClinGen, OMIM) and predicted to have damaging effect on protein function, therefore they were classified as likely pathogenic variants.

Conclusion: As nucleotide changes in SCN5A gene might dysregulate the protein's function, in-vitro studies are crucial for further understanding their effect on channel activity in terms of increase/decrease. Novel mutations in SCN5A gene and the clinical phenomena they resemble might elucidate how structural changes affect the phenotypical result.

References: https://doi.org/10.1016/j.jacep.2018.03.006, https://doi.org/10.1038/ncomms1717.

Grants: None.

Conflict of Interest: None declared.

EP06.035 A polygenic risk score improves the prediction of cardiovascular risk associated with obstructive sleep-apnoea

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Background/Objectives: Obstructive sleep apnoea (OSA) is a prevalent disease characterized by repetitive episodes of partial or complete airway obstruction during sleep. It is associated with an increased risk of cardiovascular disease (CVD) and its severity is based on the apnoea-hypopnoea index (AHI). However, AHI is only partially associated with CVD events. Therefore, we sought to assess the potential benefit of adding a coronary artery disease (CAD) polygenic risk score (PRS) to classical predictors of CVD risk in individuals with moderate or severe OSA.

Methods: We applied the CAD PRS developed by Inouye et al. (2018) and classified the OSA status in 1558 participants of the CoLaus-HypnoLaus population-based cohort, following full night polysomnography. We assessed the association between OSA, clinical factors, PRS and CVD events using multivariate Cox proportional hazard regressions.

Results: A total of 442 study participants (67% men; average 64 years old) had moderate or severe OSA (AHI ≥ 15/h). Of those, 77 experienced a CVD event during a mean follow-up of 7 years. A 10% increase in the CAD PRS was significantly associated with CVD events in a model adjusted for all cofounding factors (HR = 1.22, 95% CI (1.02–1.5), $P = 0.03$). Adding the PRS to the clinical risk factors significantly improved CVD event prediction ($P = 0.03$).

Conclusion: A CAD PRS is significantly associated with the occurrence of CVD in individuals with moderate or severe OSA. The addition of a CAD PRS has the potential to improve risk

stratification of OSA patients, thereby enabling more personalized treatment management.

References:

Grants:

Conflict of Interest: None declared.

EP06.036 Development and validation of a new cardiac screening test

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Background/Objectives: Cardiovascular diseases are the leading cause of illness and death worldwide. There are many types of congenital cardiovascular diseases, ranging from simple to complex diseases with severe life-threatening symptoms. Overlapping symptoms can make it challenging to identify the underlying cardiovascular condition. We have developed and validated an NGS-based targeted cardiac screening test for the detection of multiple genetic conditions with complex phenotypes.

Methods: The test was developed using hybrid-capture enrichment followed by next generation sequencing. Custom target capture sequences (TACS) were designed to capture all coding exons and flanking regions of 292 disease associated genes. A blind validation study was performed on samples with unknown variant status and samples that were found to carry a genetic disorder previously identified by an independent lab to determine 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: 79.4–100%) and specificity of 100% (CI: 99.8-100%). The algorithm was designed to detect CNVs at high-resolution with high sensitivity and specificity when applied to single or few exon CNV. All variants were confirmed by an orthogonal method.

Conclusion: We have developed and validated a cardiac screening test of a great diagnostic and prognostic importance. Clear diagnosis can lead to a notable increase in diagnostic success rate, effective treatment, and management for many cardiac genetic defects.

References:

Grants:

Conflict of Interest: Skevi Kyriakou NIPD Genetics, Achilleas Achilleos NIPD Genetics, Michaella Georgiadou NIPD Genetics, Charalambos Loizides NIPD Genetics, Christos Lemesios NIPD Genetics, Michalis Nicolaou NIPD Genetics, Chrisovalando Soteriou NIPD Genetics, Charalambos 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.

EP06.037 The challenge of interpreting GLA VUS: a case of late-onset Fabry disease

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Background/Objectives: Fabry disease is an X-linked recessive inborn metabolic disorder caused by alterations in the *GLA* gene and with heterogeneous phenotypes, ranging from severe early forms diagnosed in childhood to atypical late-onset forms.

We report the case of a 71-year-old woman with hypertrophic cardiomyopathy (HCM) detected at the age of 26, who was finally diagnosed of an atypical form of Fabry disease many years later.

Methods: NGS analysis using virtual panel of 18 genes. Sanger sequencing for segregation studies. Biochemical study by determining α-galactosidase and lyso-GL-3 levels.

Results: We identified the heterozygous variant c.207C>A in the *GLA* gene. It was previously reported in a hemizygous male with late-onset Fabry disease[1]. In our patient, the variant was initially classified as a variant of uncertain significance (VUS) according to the ACMG/AMP guidelines[2]. A segregation and biochemical study were carried out on 3 clinically unaffected relatives (table).

Case	Age	Phenotype	Genotype	α-Galactosidase	lyso-GL-3
Index	71	HCM	+/-	Normal	↑
Daughter	45	-	-/-	Normal	Normal
Son	43	-	-/-	Normal	Normal
Brother	68	Septal hypertrophy secondary to hypertension	-/-	-	Normal

An endomyocardial biopsy showed typical intracelular inclusions, confirming the diagnosis and allowing reclassification of the variant as likely pathogenic. Enzymatic replacement therapy was initiated.

Conclusion: The interpretation of VUS in recessive X-linked diseases as Fabry, poses a diagnostic challenge that requires a multidisciplinary approach and additional individual and family clinical and genetic studies[3].

References: [1]Umeda. Hum Genome Var. 2015;2:15044.
[2]Richards. Genet Med. 2015;17(5):405-24.
[3]Germain. Clin Genet. 2021. https://doi.org/10.1111/cge.14102.

Grants:

Conflict of Interest: None declared.

EP06.038 Massive parallel sequencing in postmortem genetic analyses of sudden unexplained deaths in the young reveals genetic predispositions for cardiac diseases

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Background/Objectives: Cases of sudden cardiac death (SCD) in young and apparently healthy individuals represent a devastating event in affected families. Coronary artery disease accounts for the majority of SCD in the older population whereas cardiomyopathies and arrhythmogenic abnormalities predominate in younger SCD victims (<40 years) with a significant genetic component. Purpose of this study was to define the portion of underlying genetic heart diseases among young Kazakhstani unexplained SCD victims revealed by massive next-generation sequencing (NGS).

Methods: We included 12 forensic cases of unexplained SCD victims aged less than 40 years. DNA was analyzed by NGS panel of 174 candidate genes with known associations to cardiomyopathies, arrhythmias, aortopathies, and more.

Results: We identified within 1 year 12 cases of SCD among 183 forensic cases. The data were evaluated bioinformatically and detected sequence variants were assessed using common databases and applying in silico prediction tools. Evidence for a genetic disposition was found in 9 of 12 (75%) cases, with likely pathogenic effect in 4 and variants of uncertain significance in 7 of SCD cases.

Conclusion: The study provides strong evidence that molecular genetics improves the post mortem diagnosis of fatal genetic heart diseases among SCD victims and should be integrated in forensic and pathological routine practice. NGS has nonetheless brought new challenges to molecular autopsy, especially regarding the clinical interpretation of the large number of variants of unknown significance detected in each individual.

References:

Grants: Study was supported by a program targeted funding from the Ministry Education and Science, Republic of Kazakhstan (BR10965164).

Conflict of Interest: None declared.

EP06.039 Characterization and dating of a novel variant in the MYH7 gene exclusive of the Balearic Islands that is associated to Hypertrophic Cardiomyopathy

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Background/Objectives:.

Methods:.

Results: Hypertrophic cardiomyopathy (HCM) is a genetic disease characterized by increased left ventricle (LV) wall thickness caused by mutations in sarcomeric genes. We performed an observational and genetic study by NGS sequencing of patients with HCM and found a novel variant in the MYH7 gene (NM_000257.4:c.1955G>A) which putatively causes a p.Arg652-Lys missense protein change. This previously non-described variant was found in twelve families with HCM. Out of 8 families that were clinically characterized 64% of carriers developed HCM. The LV hypertrophy was asymmetric septal in 75% of cases, with LV outflow tract obstruction in 28%. The incidence of a composite of serious adverse cardiovascular events (sudden death, aborted sudden death, appropriate implantable cardiac defibrillator discharge, an embolic event, or admission for heart failure) was observed in five (20%) patients. This p.Arg652Lys variant was classified as likely pathogenic (LP) and associated with the development of HCM for the following reasons: 1) It is found in patients with HCM, but not in controls, 2) There is evident segregation with HCM, and 3) It is located in an active site of the protein where a variant in the same amino acid has already been clearly established as pathogenic (p.Arg652Gly). Interestingly, the exclusive presence of the variant in our region could correspond to a founder effect in the Balearic Islands, Spain, which we have further investigated. IBD/coalescent-based allele dating analysis reveals that the origin of this allele is 96 generations away which would correspond to 1900-2400 years ago.

Conclusion:.

References:

Grants:

Conflict of Interest: None declared.

EP06.040 DNA libraries preparation optimization for sequencing of long DNA fragments using Nanopore sequencing for patients with heart disorders

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Background/Objectives: Heart failure (HF) is characterized by the failure of the heart's function and may be a result of cardiomyopathy, which brings to cardiovascular death and disability. We conducted the whole genome sequencing of patients with HF, by using the method of Nanopore sequencing (Oxford Nanopore), which allowed us to read longer DNA fragments. Nanopore long-read sequencing technology greatly expands the capacity of long-range single-molecule DNA-modification detection as well. Sequenced long DNA fragments allowed evaluating new genetic alterations as well as complex structural variants, repetitive regions and DNA-modification associated with HF in Kazakhstani population.

Methods: Genomic DNA was isolated from 68 frozen blood samples of patients with heart failure, and cardiomyopathy by using the Gentra Puregene Blood (QIAGEN). Then, NanoDrop 2000 and Qubit 2.0 assessed samples quantitatively and qualitatively. Optimizing the original fragmentation protocol obtained Long-fragments of gDNA. We used 3 µg gDNA at a concentration of 100 ng/µl using g-TUBE (Covaris) in 49 µl volume. LabChip GX Touch II (PerkinElmer) analyzed fragmentation results. DNA libraries were prepared using Ligation-sequencing kit (SQK-LSK109) and were sequenced on PromethION 48 (Oxford Nanopore) with loading three times after each 24 h.

Results: Attaining whole genome sequencing with approximate coverage of 30× and N50 between 20 and 23kb.

Conclusion: Optimization methods allowed obtaining long DNA fragment reads. Currently, bioinformatics analysis of obtained sequenced data is performing.

References:

Grants: Study was supported by a program targeted funding from the Ministry Education and Science, Republic of Kazakhstan (BR10965164).

Conflict of Interest: None declared.

EP06.041 Evaluation of subclinical myocardial damage in Cornelia de Lange syndrome

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Background/Objectives: Cornelia de Lange Syndrome (CdLS) is a rare multisystemic congenital developmental disorder. Among others, cardiovascular defects represent a significant cause of morbility and mortality in patients with CdLS. The aim of this study is to evaluate a possible cardiac dysfunction in individuals with CdLS without diagnosed congenital heart disease (CHD) using speckle tracking echocardiography.

Methods: This is a case control study including 20 individuals with CdLS without CHD and 20 healthy controls selected of same ages and gender. All individuals with CdLS were submitted to molecular analysis (16 individuals carrying pathogenic variants in NIPBL, two in SMC1A, one in RAD21 and one in HDAC8). Cardiac function was evaluated using conventional echocardiography, tissue doppler imaging (TDI), two-dimensional speckle tracking and biochemical analyses.

Results: The analytical markers of cardiovascular risk and cardiac function showed no alterations. However the left ventricular global longitudinal strain (GLS) was altered (>−15.9%) in 55% of patients. All values obtained by speckle tracking (strain, strain rate and velocity) showed a downward trend correlated with age ($p < 0.05$). Likewise, the ejection fraction, shortening and TAPSE, although preserved, also decreased with age ($p < 0.05$).

Conclusion: The results of this study suggest that patients with CdLS may develop subclinical myocardial dysfunction, which can be detected by speckle tracking even before the appearance of clinical symptoms and the alteration of other echocardiographic or analytical parameters. Taking this into account, cardiological follow up is suggested even in the absence of CHD in individuals with CdLS.

References:

Grants:

Conflict of Interest: None declared.

EP06.042 Novel variants in ALPK3 and GATA3 in a pedigree with primary cardiomyopathy and multiple congenital abnormalities: concurrence of two monogenic diseases in one family

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Background/Objectives: Prominent phenotypic heterogeneity of primary cardiomyopathies makes precise genetic diagnosis difficult, especially in case of presentation of extracardiac phenotypic abnormalities that may indicate either a syndromic cardiomyopathy or an independent disease. We report a case of successful genetic investigation of familial noncompaction cardiomyopathy complicated with diverse dysmorphologic features.

Methods: The proband is 40-year-old man with noncompaction cardiomyopathy, heart failure, arrhythmic complications and short stature. His sister has hypertrophic cardiomyopathy (HCM), myocardial hypertrabeculation, compensated heart failure, sensorineural deafness and congenital abnormalities of genitourinary system. The siblings' father has HCM. Besides, the proband's eldest daughter demonstrates developmental delay. We performed clinical examination and whole-exome sequencing for all available family members (three affected and six unaffected, a total of nine persons). Variants with gnomAD frequency <0.01% were selected for clinical interpretation.

Results: In affected siblings and their father, we found a c.4411-2A>C variant in canonical acceptor splice site in the *ALPK3* gene. Recently, heterozygous truncating variants in *ALPK3* were identified as the cause of adult-onset HCM. Based on sequence properties, we suggest that our finding causes premature truncation of *ALPK3* product. None of unaffected persons harbored the c.4411-2A>C variant. Additionally, in the proband's sister we found a p.Trp329Gly missense in *GATA3*, the gene responsible for the rare syndrome "hypoparathyroidism, sensorineural deafness, and renal dysplasia". Both findings in *ALPK3* and *GATA3* are previously unreported.

Conclusion: The accurate genetic diagnosis is the way to optimize the follow-up scheme and to improve our understanding of complex clinical cases.

References:

Grants: The Ministry of Science and Higher Education of Russia (agreement №075-15-2020-899).

Conflict of Interest: None declared.

EP06.043 Mitomycin C induced genotoxic stress in endothelial cells is associated with inflammatory response

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Background/Objectives: Genotoxic stress triggers endothelial disfunction and atherosclerosis [1], but mechanism of this process is poorly investigated. Inflammation plays the important role in atherogenesis. This study aimed to access the expression of inflammatory markers in endotheliocytes exposed to mitomycin C (MMC).

Methods: Primary human coronary- (HCAEC) and internal thoracic artery endothelial cells (HITAEC) exposed to 500 ng/ml MMC (experimental group) and 0.9% NaCl (control) was used in this research. Gene expression was evaluated by RT-qPCR after 6-h exposure to MMC with MMC followed by 24-h incubation in the mutagen-free cell growth media. The level of cytokine release in endotheliocytes was studied by dot blotting.

Results: We discovered that HCAEC exposed to MMC are characterized by increased mRNA level of *IL-8*, *MCP-1*, *IP-10*; decreased expression of *TIMP-2* and no differences in the expression of *MIF*, *MIP-1b*, *PDGFB* compared to the control. In HITAEC, increased mRNA level of *IL-8*, *IP-10*; decreased expression of *MIF*, *TIMP-2* and no differences in the expression of *MCP-1*, *MIP-1b*, *PDGFB* was shown. At the proteome level MIF, IL-8, MCP-1, IP-10, PDGFB were upregulated both in HCAEC and HITAEC, and there are no differences in MIP-1b release. TIMP-2 was upregulated in HCAEC but not in HITAEC.

Conclusion: Genotoxic stress in endotheliocytes induces by MMC leads to differential inflammatory response that can trigger endothelial dysfunction.

References: Sinitsky M.Y. et al. The gene expression profile in endothelial cells exposed to mitomycin C. Biochemistry (Moscow), Supplement Series B: Biomedical Chemistry, 15(3):255-261, 2021.

Grants: Grant of Russian Science Foundation №27-75-10052, https://rscf.ru/project/21-75-10052/.

Conflict of Interest: None declared.

EP06.044 Non-desmosomal genes in Arrhythmogenic Cardiomyopathy: genetic variants rating

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Background/Objectives: Arrhythmogenic Cardiomyopathy (AC) is an inherited disorder of the myocardium with a highly heterogeneous clinical presentation including life-threatening arrhythmias at risk of sudden death. Genetic testing impacts greatly in reaching AC diagnosis, but gene-disease associations has yet to be determined for the increasing number of genes included in clinical panels.

Methods: Genetic variant re-appraisal was undertaken in most relevant non-desmosomal disease genes, based on current adjudication guidance, identified in 320 unrelated Italian AC patients who did not carry pathogenic/likely pathogenic (P/LP) variants in desmosome-coding genes and reported literature data.

Results: In our cohort, 28 rare genetic variants in non-desmosomal genes were identified in 30 patients, of which 17 *FLNC* (Filamin C*)*, 7 *DES* (Desmin), 2 *TMEM43 (*Transmembrane protein 43), and 2 *CDH2 (*Cadherin-2*)*. No P/LP variants were found in *PLN* (Phospholamban) and *TJP1* (Tight junction protein-1) genes. Gene-based burden analysis, including P/LP variants reported in literature, showed significant enrichment only for *TMEM43* (3.52-fold), *DES* (9.55-fold), *PLN* (117.8-fold) and *FLNC* (93.22-fold). Evolutionary conservation analysis made evident a positive selection pressure (Ka/Ks ratio >1) for *CDH2* and *TJP1*, indicating that missense variants impact less the protein structure. Genotype-phenotype correlation highlighted 71% and 89% of left-dominant AC in *FLNC* and *DES* carriers, respectively.

Conclusion: Genes lacking robust clinical and genetic evidences impact greatly the number of variants-of-unknown-significance detected and should be removed from clinical AC-targeted genetic panels since the findings cannot drive clinical decision-making. About two thirds of non- desmosomal P/LP variants occur in *FLNC* leading to fully-penetrant left-dominant AC.

References:

Grants:

Conflict of Interest: None declared.

EP07 Metabolic and Mitochondrial Disorders

EP07.001 Nuclear DNA changes in patients with suspected mitochondrial disorder

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Background/Objectives: Mitochondrial diseases represent genetically and phenotypically diverse group of disorders, characterized by dysfunctional mitochondria. Pathogenic variants have been identified in more than 350 genes, but at least 40% of patients remain undiagnosed (Stenton, 2020).

Methods: Next-generation sequencing (NGS) methods were applied for 24 patients in a group of 80 unrelated individuals with suspected mitochondrial disease after exclusion of mtDNA mutations by whole mtDNA (Sanger) sequencing. Gene panels for mitochondrial and other neuromuscular disorders were performed for 8 patients, whole exome sequencing was performed for 16 patients.

Results: In the study four patients (16.7%) were found to have pathogenic variants in POLG, SURF1, PNPLA8, RRM2B, and BTD genes. SURF1 gene is involved in the biogenesis of the cytochrome c oxidase complex. POLG and RRM2B genes are involved in mtDNA synthesis, pathogenic variants lead to qualitative or quantitative changes in mtDNA. The protein encoded by PNPLA8 gene is essential for maintaining efficient bioenergetic function through tailoring mitochondrial membrane lipid metabolism. Pathogenic variants in the genes CACNA1A, DDX3X, TPP1, YARS and ANO5, leading to other neuromuscular diseases, were identified in six patients (25.0%). Two patients were diagnosed with two diseases caused by pathogenic variants in nuclear (RRM2B and BTD) or in both mitochondrial (MT-ND4) and nuclear (ANO5) genes.

Conclusion: The application of NGS allowed to confirm rare mitochondrial or neuromuscular disorders emphasizing the importance of clinical genetic-based research in improving the care of these patients. However, the genetic causes of other patients remain unknown and challenging to investigate the molecular mechanisms as well as disease progression.

References:

Grants:

Conflict of Interest: None declared.

EP07.006 Medium chain acyl co-A dehydrogenase (MCAD) deficiency due to an exon 8 duplication in ACADM

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Background/Objectives: Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common disorder of fatty acid β-oxidation, caused by bi-allelic variants in the ACADM gene. Targeted sequencing of ACADM establishes a molecular diagnosis for most- but not all- patients.

We sought to establish the molecular basis of MCAD deficiency (MCADD) in a cohort of patients with a biochemical working diagnosis of MCADD, for whom sequencing of ACADM failed to detect bi-allelic pathogenic variants.

Methods: sequencing of the ACADM gene could not establish a bi-allelic pathogenic variant in fourteen of the 63 newborns detected by the Israeli Newborn Screening (NBS) Program with suspected MCADD. We pursued multiplex ligation-dependent probe amplification (MLPA) of ACADM for these fourteen patients.

Results: The fourteen patients, 22% of all detected MCADD by NBS, were found to harbor a common duplication of exon 8 of the ACADM gene. The exon 8 duplication was in either homozygous form or compound heterozygous with a previously recognized mutation by sequencing. All patients share a North-African Jewish (Tunisian, Moroccan) descent. Exon 8 duplication was not detected in 110 chromosomes of matched ethnic background controls.

Conclusion: A likely pathogenic exon 8 duplication in the ACADM gene leading to MCAD deficiency consists a founder mutation in the North-African Jewish population. Due to the beneficial effect of early diagnosis and intervention towards a positive outcome and better prognosis, we suggest that in patients with a biochemical working diagnosis of MCADD for whom sequencing of ACADM fails to establish a molecular diagnosis, MLPA should be considered.

References:

Grants:

Conflict of Interest: None declared.

EP07.008 A novel heterozygous mutation of CYP17A1 gene in a child with micropenis and isolated 17,20-lyase deficiency

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Background/Objectives: Disorders of sexual development (DSD) are a heterogeneous group of congenital conditions associated with atypical development of chromosomal, gonadal or anatomical sex. These alterations can be associated with numerous hormonal, genetic and often physical alterations. The micropenis consists in the presence of a stretched penile length of less than 2–2.5 SD for age, can represent a clinical sign of various forms of DSDs.

Methods: In the present study we report the case of a 5-year-old child with isolated micropenis, a typical feature of 46,XY DSD, showing low basal and post hCG stimulation testosterone levels. Molecular analysis using a NGS panel of 50 genes involved in DSDs was performed. Then a serum steroid profiling for the child was also determined by Liquid Chromatography coupled to Tandem Mass Spectrometry (LC–MS/MS) analysis.

Results: NGS analysis evidenced the presence of an heterozygous mutation in the CYP17A1 gene. Segregation analysis in the unaffected parents show the presence of the same mutation in the mother of the child, who however presented no features of the disease. Biochemical analysis of the child revealed low levels of testosterone, progesterone and dehydroepiandrostenedione.

Conclusion: These results suggest that in some cases even heterozygous mutations in recessive genes involved in adrenal steroidogenesis can influence the patient's phenotype. The presence of mutation in the mother, who does not have the disease, does not contrast with the results obtained, since low testosterone levels in female subjects do not necessarily correlate with a pathological phenotype.

References: 1. Hiort et al,2014-Nat Rev Endocrinol.

Grants:

Conflict of Interest: None declared.

EP07.009 Ethylmalonic encephalopathy masquerading as meningococcemia

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Background/Objectives: Ethylmalonic encephalopathy (MIM #602473) is a rare autosomal recessive metabolic condition caused by biallelic variants in *ETHE1* (MIM #608451), characterized by global developmental delay, infantile hypotonia, seizures and microvascular damage. The microvascular changes result in a pattern of relapsing spontaneous diffuse petechiae and purpura, positional acrocyanosis and pedal edema, hemorrhagic suffusions of mucous membranes and chronic diarrhea. This case provides a timely reminder to consider rare genetic diagnoses when atypical features of more common conditions are present, with an early referral to ensure prompt biochemical and genomic diagnosis. Here we describe an instructive case in which ethylmalonic encephalopathy masqueraded as meningococcal septicemia and shock.

Methods: Ultra-rapid whole genome testing (time to result 60 hours) and prompt biochemical analysis facilitated accurate diagnosis and counselling with rapid implementation of precision treatment for the metabolic crisis related to this condition.

Results: WGS identified a homozygous frameshift variant in exon 2 of *ETHE1* (MIM #608451), c.131_132del; p.(Glu44Valfs*62).

Conclusion: This case provides a timely reminder to consider rare genetic diagnoses when atypical features of more common conditions are present, with an early referral to ensure prompt biochemical and genomic diagnosis.

References: Nil
Grants: Nil
Conflict of Interest: None declared.

EP07.010 Glutaric aciduria type 1: Variant classification and distribution in the GCDH gene

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Background/Objectives: Glutaric aciduria type I (GA-1) is a rare autosomal-recessive inherited dysfunction of the enzyme glutaryl-CoA dehydrogenase (GCD) catalyzing lysine, hydroxylysine and tryptophan break down. The disease is caused by pathogenic variants in the *GCDH* gene and leads to an irreversible movement disorder as main symptom when untreated. The molecular genetic basis of GA-1 is poorly investigated. Therefore, the main objective of this project was to provide an extensive collection and classification of variants in combination with reported phenotypes in GA-1 in the openly accessible Leiden Open (source) Variation Data base (LOVD). Further investigations on this up-to-date largest GA-1-patient-cohort were conducted and will soon be admitted to publication.

Methods: An intensive literature research was conducted on PubMed. Variants were classified according to adjusted ACMG guidelines.

Results: The preexisting *GCDH*-section of LOVD was complemented by 96 new publications, 342 new patient data and 71 new variants. 227 of the overall 229 listed variants were classified. Finally, more than 220 variants and phenotypes from more than 500 mostly published patients are listed in the LOVD database so far. Almost one third of these variants are classified as VUS and the remaining as (likely) pathogenic. Most variants were missense variants and found towards the middle and 3′ end of the gene localized at functional domains on protein level without clear hot spots.

Conclusion: Internationally uniform criteria for variant classification, such as the ACMG criteria, are important tools. Nevertheless, they aren't easily applicable and need to be adjusted in case of rare diseases.

References: https://databases.lovd.nl/shared/genes/GCDH.
Grants: No.
Conflict of Interest: Isabelle Rinke Voluntary unpaid curator of GCDH-section of Leiden Open (Source) Variation Database (LOVD), Alexandra Tibelius Voluntary unpaid curator of GCDH-section of Leiden Open (Source) Variation Database (LOVD), Christine Fischer: None declared, Anna Jauch: None declared, Katrin Hinderhofer Voluntary unpaid curator of GCDH-section of Leiden Open (Source) Variation Database (LOVD).

EP07.011 Mutation analysis of the PAH gene in phenylketonuria patients from West Ukraine

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Background/Objectives: More than 1280 pathogenic variants have been described in the international database of patients and genotypes causing hyperphenylalaninemia (HPA)/PKU (BIOPKUdb, accessed on 26 August 2021). The occurrence of different mutant alleles varies among ethnic groups and geographic regions worldwide. We report the spectrum and frequency of PAH gene mutations in 160 subjects with PAH deficiency from Western Ukraine.

Methods: DNA was isolated from peripheral blood by treatment with proteinase K with subsequent salting-out protocol and target PAH gene mutations testing was performed by RFLP-PCR. The majority of the patients were diagnosed by newborn screening.

Results: Mutation analysis revealed that 62.5% of all variant alleles carry five mutations. The most prevalent mutation c.1222C>T (p.R408W) was detected on 185 alleles (57.8%) with a very high degree of homozygosity (35%). Mutation p.R408W presented on one or two alleles in 81% patients (129/160). The other 16% were accounted for c.1066-11G>A (8%), c.473G>A (4%), c.1241A>G (2.7%) and c.754C>T (1.2%). Using the direct automated DNA sequencing it has been found 5 different mutations: c.722G>A and c.838G>A in compound heterozygous state, one case each of c.727C>T and c.116_118del, and rare pathogenic variant c.581T>C in homozygous state. The additional testing is required to identify the rest of 37.5% alleles.

Conclusion: The results of study have showed that the most common mutation in PKU patients from West Ukraine is c.1222C>T accounted for 58% of mutated chromosomes. The mutational spectrum is corresponded to that observed for the East European population.

References:
Grants:
Conflict of Interest: None declared.

EP07.013 Novel variants in COQ7 gene cause infantile fatal multysistemic mitochondrial disorder

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Background/Objectives: Coenzyme Q10 is a major electron transporter in mitochondrial chain, thus essential for energy production. Primary COQ10 deficiency is a heterogeneous disease caused by biallelic variants in genes involved in the CoQ10 biosynthesis pathway: 10 "COQ genes" have been identified for being responsible of this disease, including most rarely involved *COQ7*. We report a case of Primary COQ10 deficiency due to *COQ7* gene mutation.

Methods: A multidisciplinary protocol including biochemical and genetic analyses of patient's biological samples was implemented to diagnose a complex metabolic case with suspected mitochondrial dysfunction.

Results: Patient presented a prenatal onset of increased nuchal translucency, cardiac hypertrophy, increased bowel wall echogenicity. At birth, meconium ileus required corrective ileal stenoatresia surgery; in the following months, intestinal resections have been needed due to recurrent intestinal occlusions. Clinical course was complicated by left ventricular non-compaction

cardiomyopathy, ascending aorta dilation, arterial hypertension, renal dysfunction, diffuse skin desquamation, axial hypotonia, neurodevelopmental delay, and growth retardation. Brain MRI showed thalamic abnormalities. Mitochondrial respiratory chain activity revealed an increase in citrate synthase (234.7 nmol/mg/min). Riboflavin and high-dose oral CoQ10 supplementation ameliorated psychomotor development and skin. Patient suddenly died at 16 months. Exome sequencing revealed compound heterozygosis rare variants in COQ7 gene, c.613_617delGCCGGinsCAT (p.Ala205HisfsTer48) and c.403A>G (p.Met135Val), whose pathogenicity was predicted by several bioinformatic tools and confirmed by functional studies.

Conclusion: Our case expands the clinical spectrum of manifestation of primary COQ10 deficiency due to COQ7 gene, and highlights the essential role of multidisciplinary and combined approaches for a timely diagnosis.

References:

Grants:

Conflict of Interest: None declared.

EP07.014 A novel gross deletion in SLC25A15 gene not detected by regular WES analysis

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Background/Objectives: Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (HHHS) is an autosomal recessive disorder of the urea cycle. The main clinical symptoms are hypotonia, developmental delay, mental regression and motor dysfunction. In the acute phase hyperammonemia accompanied by vomiting, ataxia, lethargy and confusion are characteristics of the disease. We detected a Pakistani 3 year-old girl with mental and motor retardation, spastic paraplegia and vomiting. Parents were consanguineous. Urine and plasma amino acids were suggestive of HHHS. We wanted to confirm molecular diagnostic.

Methods: We performed WES analysis using Nextera DNA Exome (Illumina). We also analysed the candidate genes individually in the generated bam files. We also performed PCR analysis and Sanger sequencing of introns 1 and 2 and exon 2 of *SLC25A15* gene.

Results: To identify the genetic defect in our patient, we performed WES but no variants were found in *SLC25A15* gene or in any other urea cycle genes. Due to the HHHS suggestive biochemical and clinical phenotype, we checked *SLC25A15* gene in the bam files and we observed a possible deletion involving exon 2. Oligonucleotides designed to delimitate and characterize the deletion gave rise to the detection of a novel homozygous gross indel. The deletion was c.-69-1221_55+1683delinsAGA, which deleted 3028bp and inserted 3bp instead. Her parents were both carriers for the mutation. This variant classified as pathogenic could be considered the disease causing mutation.

Conclusion: When clinical and biochemical phenotype is very suggestive of a disease, it is crucial to further investigate the candidate genes, even though WES results are negative.

References:

Grants:

Conflict of Interest: None declared.

EP07.015 Adult-onset CblC deficiency: a challenging diagnosis involving different adult clinical specialists

Silvia Kalantari[1], *Brigida Brezzi*[2], *Valeria Bracciamà*[1], *Antonella Barreca*[3], *Paolo Nozza*[4], *Tiziana Vaisitti*[1], *Antonio Amoroso*[1;5], *Silvia Deaglio*[1;5], *Marco Manganaro*[2], *Francesco Porta*[6], *Marco Spada*[6]

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Background/Objectives: Methylmalonic aciduria and homocystinuria, CblC type (OMIM #277400) is the most common disorder of cobalamin intracellular metabolism, an autosomal recessive disease, whose biochemical hallmarks are hyperhomocysteinemia, methylmalonic aciduria and low plasma methionine. Despite being a well-recognized disease for pediatricians, there is scarce awareness of its adult presentation.

Methods: The PubMed database was searched for adult-onset CblC cases. All cases with first symptom at onset ≥18 years old were included.

Results: Available clinical, biochemical and molecular data from 22 reports on cases and case series were collected, resulting in 45 adult-onset CblC cases, including the description of a patient followed in our center. We describe the onset of the disease in adulthood, encompassing neurological, psychiatric, renal, ophthalmic and thromboembolic symptoms. From a molecular point of view adult patients are usually compound heterozygous carriers of a truncating and a non-truncating variant in the MMACHC gene.

Conclusion: Adult onset CblC disease is a rare disorder whose diagnosis can be delayed due to poor awareness regarding its presenting insidious symptoms and biochemical hallmarks. To avoid misdiagnosis, we suggest that adult onset CblC deficiency is acknowledged as a separate entity from pediatric late onset cases. To further aid diagnosis, it is important that genes belonging to the intracellular cobalamin pathway are included within gene panels routinely tested for atypical hemolytic uremic syndrome and chronic kidney disorders.

References: Kalantari et al., Adult-onset CblC deficiency: a challenging diagnosis involving different adult clinical specialists. Orphanet J Rare Dis. 2022 Feb 2;17(1):33. https://doi.org/10.1186/s13023-022-02179-y.

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Conflict of Interest: None declared.

EP07.016 A challenging metabolic acidosis management in a young patient with Trasnsalodase deficiency, T1DM, and pRTA

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Background/Objectives: We report a rare case of a 14 year old girl with Eyaid syndrome - TransAldolase deficiency1,2 (OMIM 606003) based on both clinical and molecular finding of

homozygous pathogenic variant in TALDO1 gene, c.793del p.(Gln265Argfs*56).

She developed type 1 diabetes at around the age of nine and was found to have a baseline non-ionic gap metabolic acidosis that was persistent despite adequate management of her diabetes. Extensive work up for possible renal causes -giving that they are part of her primary syndrome1,3– revealed proximal renal tubular acidosis evident by increased urinary excretion of amino acids and glucose, phosphate and normal renal ultrasound1,2 she also had developmental delay and progressive liver failure resulting in liver cirrhosis, portal hypertension and esophageal varices.

Methods: Case report.

Results: In one ER visit; she presented with one day history of mild abdominal pain, vomiting, diarrhea and lethargy, labs showed metabolic acidosis, her VBG was: pH 6.93, HCO3 3.3, K 3.8, N 136.

Conclusion: Her metabolic acidosis could be related to her underlying pRTA, missed insulin and sodium bicarbonate dosage, the acute illness itself (viral gastroenteritis); Making the diagnosis and management challenging to identify which of them is the major contributor to her acidosis, and what would be the best course of action in regards to when to stop insulin infusion and when to start sodium bicarbonate for which we will highlight in this case report.

References: Clinical, biochemical, and molecular overview of transaldolase deficiency, Clinical utility of anion gap in deciphering acid–base disorders.

Grants:

Conflict of Interest: None declared.

EP07.017 Phenotypic and genotypic spectrum of MTRFR-related disorders

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Background/Objectives: Biallelic loss of function variants in *MTRFR* (previously known as *C12orf65*) have been reported to cause a variable clinical spectrum which includes combined oxidative phosphorylation deficiency 7 and spastic paraplegia 55 (*613541). *MTRFR* is a nuclear gene involved in mitochondrial translation. It contains a functional RF-1 domain with a conserved GGQ motif.

Methods: A retrospective review of all reported patients with biallelic *MTRFR* pathogenic variants was undertaken, focusing on phenotype and possible genotype-phenotype correlations.

Results: 31 patients and 13 pathogenic variants in *MTRFR* are reported in the literature. Clinical presentations were varied but the core triad of optic atrophy, peripheral neuropathy and spastic paraparesis was present in most patients. Other common features included intellectual disability, reduced visual acuity, ophthalmoplegia, nystagmus and ataxia. Ophthalmological abnormalities were the most common initial presentation. Approximately half the patients had normal early milestones, with regression after the first year of life. Variants within the RF-1 domain, and in particular those disturbing the GGQ motif, appeared to produce a more severe phenotype with bulbar dysfunction, respiratory insufficiency and brain MRI abnormalities but this was not the case in all reported patients.

Conclusion: It has been suggested that the length of the truncated protein is inversely proportional to the severity of the clinical phenotype. However, this is not consistent in all reported patients, with three pairs of patients sharing the same variants with divergent phenotype severity. Further work is ongoing recruiting patients with *MTRFR*-related disorders to further understand the natural history of this condition and clarify genotype-phenotype correlations.

References:

Grants:

Conflict of Interest: None declared.

EP07.018 Identifying the molecular basis of Glycogen-storage-disease III in a family with negative exome testing

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Background/Objectives: Glycogen storage disease type III (GSD-III) is characterized by liver, cardiac, and skeletal muscle involvement, and is caused by mutations in the AGL gene alone. We studied a highly consanguineous Palestinian family of two boys affected with GSD-III. WES testing was performed on DNA samples from the two boys and their parents. No pathogenic variants were found, but a homozygous deep intronic variant of uncertain significance (VOUS) AGL:c.1735+130A>G, in intron 13 was identified in both probands. Our objective was to discover the genetic basis for the disease in this family.

Methods: cDNA sequencing and qRT-PCR methods were used to evaluate alternative splicing and gene expression. Long-range PCR and NGS methods were used to identify structural variants.

Results: cDNA sequencing of exons surrounding the VOUS variant, showed no exon skipping in the probands and their parents. But, expression analysis using qRT- PCR showed lack of expression of exons 28-30, at the end of the gene, in the two probands. Long-range PCR of genomic DNA revealed a homozygous deletion of 5.5Kb. This deletion causes a frameshift in the AGL gene with deleterious effect. Their parents were found to be heterozygous.

Conclusion: Traditional Exome sequencing mainly concentrates on the detection of SNVs and small indels. Larger deletions may be missed by these methods. The development of long-read sequencing can help the detection of small CNVs. These methods helped to identify the disease causing variant in this family which will enable the parents to proceed to PGT/pre-natal diagnosis in the future.

References:

Grants:

Conflict of Interest: None declared.

EP07.020 GM1-Gangliosidosis in four Romanian patients: clinical picture and mutation report

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Background/Objectives: GM1 gangliosidosis (GM1) is an autosomal recessive lysosomal storage disease caused by deficiency of acid beta-galactosidase activity, due to mutations in the GLB1 gene. This work reports clinical and molecular characteristics of four Romanian patients with GM1.

Methods: All patients presented type I (infantile) form. A full biochemical and imagistic workout was performed. Molecular diagnosis was confirmed by low enzyme activity of beta-galactosidase measurement in leukocytes, using artificial 4-methylumbeliferyl beta-galactosidase. Mutations were identified by PCR amplification and direct sequencing of the entire coding region, plus exon / intron boundaries of the GLB1 gene, or for the specific exon, only[1].

Results: Four infants, three males and one female, aged between 8 and 13 months, coming from three different families were analyzed. Natural history and presentation were similar: onset in early infancy, coarse facial features, skin infiltration, abdominal distention due to visceromegaly, hydrocele (males), respiratory distress. Developmental regression, seizures, generalized hypotonia, cortical and optic atrophy were hallmarks of this disease. Hypertrophic cardiomyopathy was associated, with variable severity. Fatality was 100%, occuring between 10 and 18 months of age. Beta-galactosidase activity ranged from 4 to 14 % of the inferior limit. A single mutation in homozygous state was identified in all patients: c.176G>A (p.Arg59His).

Conclusion: This is the first report of GM1-gangliosidosis cases from Romania. Similar phenotypic traits were found in all four patients, as they proved homozygous for c.176G>A (p.Arg59His) mutation. Poor outcome was associated with this genetic defect.

References: [1]Silva et al. Hum Mutat. 1999;13 (5):401-409.

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Conflict of Interest: CRISTINA COLDEA: None declared, Dragos Serban: None declared, Felicia Galos: None declared, Eliza Cinteza: None declared, Anna Caciotti For research purpose genetic analysis, pro bono, Gabriel Smarandache: None declared.

EP07.021 Three cases of GLB1-related disorders of the same genotype in patients in Ukraine

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Background/Objectives: *GLB1*-related disorders comprise two phenotypically distinct lysosomal storage disorders: GM1 gangliosidosis and mucopolysaccharidosis type IVB (MPS IVB). GM1 is characterized by severe degenerative lesions of the central nervous system, regression of development, convulsive syndrome, ataxia. While MPS IVB is characterized by lesions of the skeletal system without cognitive lesions: joint contractures, short stature, scoliosis, lesions of the heart valves and retina. Mutations in the gene cause a deficiency of the lysosomal enzyme galactosidase. And the disease is divided purely clinically.

Methods: Herein we present three patients 9–15 years with the same phenotype and genotype.

Results: All children presented the onset of the disease at the age of 3 years with pronunciation disorders and gradual regression of mental development. From the age of 7 they began to develop an atactic syndrome, muscle weakness, stiffness and contractures of the joints, scoliosis, hip dysplasia. At the age of 9 they had seizures. At the age of 8, they were diagnosed with myopia. No patient had hepatolienal syndrome.

Galactosidase activity is increase. Molecular genetic work-up heterozygous c.841C>T (p.His281Tyr) and c.602G>A (p.Arg201His) gene GLB1.

Conclusion: According to the existing phenotype, it is difficult to classify patients as GM1 or MPS IVB. In terms of manifestations, they occupy an intermediate position between these two diseases. Mutation c.841C>T is common in patients with GM1. Mutation c.602G>A is rare. The presence of the same clinical manifestations in these patients by the same genotype gives us additional data on the gene-phenotypic correlation of gene GLB1 damage.

References:

Grants:

Conflict of Interest: None declared.

EP07.022 Molecular diagnosis of MCAD in the Macedonian neonates with elevated medium-chain acylcarnitines identified through MS/MS-based newborn screening

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Background/Objectives: Medium Cchain acyl-CoA dehydrogenase (MCAD) deficiency is an autosomal recessive disorder of fatty acid oxidation, with potential fatal outcome. It is diagnosed by acylcarnitine analysis on newborn screening blood spot cards by tandem mass spectrometry. Early diagnosis of MCAD and presymptomatic treatment can potentially reduce morbidity and mortality.

Methods: A total of 38,578 newborns were screened for inborn errors of metabolism during May 2014 - Jan 2022, using the LC/MS/MS method. Eight newborns showed elevations of medium-chain acylcarnitines with predominance of octanoylcarnitine, and C8/C10 ratio as well. Molecular *ACADM* gene analysis was performed by whole exome sequencing using NovaSeq 6000 (Illumina), and confirmed by Sanger sequencing. Sequencing data were analysed using the bcbio_nextgen bioinformatics pipeline (version 1.2.7; GRCh37 genome).

Results: Molecular analysis of the *ACADM* gene was performed in eight patients with positive metabolic screening for MCAD deficiency. Two different *ACADM* mutations were obtained in a total of 15/16 (93.75%) alleles of the patients. The common c.958A>G mutation (p.Lys329Glu) was detected in 12/16 (75%) alleles, while 3/16 (18.75%) alleles had c.244dupT pathogenic variant (p.Trp81fs). Five of the patients were homozygous for c.985A>G variant, and one was homozygous for c.244dupT. One of the patients was compound heterozygote (c.985A>G/c.244dupT) while another was a heterozygote for c.958A>G without second mutant allele detection.

Conclusion: The sensitivity of medium-chain acylcarnitines as screening markers for early detection of MCAD was confirmed through the molecular analysis of the *ACADM* gene. Early detection and treatment have successfully prevented adverse health outcomes in patients with MCAD.

References:

Grants:

Conflict of Interest: None declared.

EP07.023 A novel homoallelic founder variant of RTN4IP1 in a consanguineous population

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Background/Objectives: *RTN4IP1* encodes a mitochondrial ubiquinol oxydoreductase that interacts with reticulon 4. Such interaction is considered to be vital for reticulon-induced inhibition of neurite growth. Inherited deficiencies in *RTN4IP1* causes mitochondrial deficiency in human and characterized by early-onset recessive optic neuropathy, atrophy, and encephalopathy (1).

Methods: We performed mtDNA sequencing on the collected DNA samples that revealed no pathogenetic variants. We then performed whole exome sequencing (WES) on the index cases and also utilized genome-wide SNP screening to detect shared runs of homozygosity (ROHs) in the families. Sanger sequencing was used to confirm variants' segregation in the families. The age of the mutation calculation was done using published protocols (2).

Results: We identified a missense variant (NM:032730; c.G475T, p.Val159Phe) in *RTN4IP1*. The variant was fully segregated with the phenotype in both families and absent among large ethnically matching controls. The variant was predicted to pathogenic by different classifiers. Amino acid sequence alignment revealed that the mutation site is highly conserved in various species. Immunoblotting experiments revealed a decrease in RTN4IP1 steady-state levels in the index's fibroblasts. Mutation age calculation predicted that the mutation goes back to 56 generations.

Conclusion: Our study expands phenotypic spectrum and pathological outcome of RTN4IP1 deficiency.

References: 1. Angebault, C., et al., Recessive Mutations in RTN4IP1 Cause Isolated and Syndromic Optic Neuropathies. Am J Hum Genet, 2015. **97**(5): p. 754-60.

2. Al-Hassnan, Z.N., et al., ISCA2 mutation causes infantile neurodegenerative mitochondrial disorder. J Med Genet, 2015. **52**(3): p. 186-94.

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Conflict of Interest: None declared.

EP07.024 Identification and classification of a childhood form of hereditary hypophosphatasia

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Background/Objectives: Hypophosphatasia (HPP) is a rare, multisystemic disorder caused by loss-of-function mutations in the ALPL gene that encodes the tissue-nonspecific alkaline phosphatase(ALP) responsible for calcium-, and phosphate mineralization. Our aim was to associate the suspected childhood HPP with the test results and classify the manifestation.

Methods: An 8-year-old child was investigated. Neurological, electrophysiological, radiological, biochemical and genetic tests were performed. Pyridoxal-5′-phosphate(PLP) in blood and phosphoethanolamine(PEA) in blood and urine were measured by HPLC. ALPL gene was analysed by Sanger sequencing and copy number variants were detected by MLPA. Bone manifestation was classified by the Radiographic Global Impression of Change(RGI-C) and Rickets Severity Score(RSS).

Results: Patient had craniosynostosis reconstruction surgery, dental anomalies, generalized muscular atrophy, spastic tetraparesis, significant flexion contarctures, serious somatomental retardation and movement disability, convulsions. Midline and right hemisphere epileptiform signs occurred by EEG. Brain MRI detected periventricular leukomalatia with pons and mesencephalon atrophy. RGI-C and RSS scoring:1/10. Bone age appropriate. Extremely low serum ALP value (-2SD compared to the age-specific average) was detected. PLP in blood and also PEA in blood and urine increased.

Heterozygous missense mutation c.1374C>A(p.Asp458Glu) was detected, which had not been described. The predictive software classifies it as likely pathogenic.

Conclusion: Hypophosphatasia is confirmed by biochemical and genetic level. As to the clinical history and the phenotype, moderate form of childhood hypophosphatasia (HPPC) was diagnosed.

References: 1. OMIM https://www.omim.org/entry/ 241510.
2. Tournis S et al.J ClinMed.2021 Dec; 10(23):5676.
3. Varsome database https://varsome.com.

Grants: This study was supported by KTIA_13_NAP-A-III/6; KTIA_NAP and with FIKP program.

Conflict of Interest: None declared.

EP07.025 Mutations in PPOX gene in Czech patients with variegate porphyria

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Background/Objectives: Variegate porphyria (PV) is an acute hepatic porphyria resulting from partial deficiency of the protoporphyrinogen oxidase (PPO). This enzyme is encoded by the *PPOX* gene and catalyzes the seventh step of the haem biosynthesis pathway. PV is an autosomal dominant disorder with low penetrance. Clinical manifestations may be triggered by many nongenetic factors and include acute neurovisceral attacks and/or cutaneous photosensitivity. Biochemical diagnosis of PV is based on an abnormal faecal porphyrin profile (increased faecal coproporphyrin and protoporphyrin and reversal of the ratio of copro III/I) and characteristic fluorometric plasma scan. A rare variant of PV is caused by biallelic *PPOX* gene mutations and presents with developmental delay, severe skin and neurologic symptoms in early infancy.

Methods: *PPOX* gene was analyzed in 6 probands with clinical and biochemical diagnosis of PV and 2 probands with suspected severe biallelic variant of PV. All exons and adjacent non-coding regions were analyzed by Sanger sequencing.

Results: Pathogenic mutations were confirmed in all probands and subsequent genetic analysis of 22 family members revealed 14 carriers with mutations.

Conclusion: Identification of asymptomatic family members is very important to reduce the risk of PV symptoms by avoiding triggering factors.

References:

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Conflict of Interest: None declared.

EP07.026 Functional RNA analysis of a TAFAZZIN intronic variant in a patient with Barth syndrome

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Background/Objectives: Barth syndrome is a monogenic X-linked recessive disorder characterized by cardiomyopathy, skeletal myopathy, prepubertal growth delay, neutropenia, and 3-methylglutaconic aciduria. It is caused by cardiolipin deficiency and is associated with pathogenic variants in the *TAFAZZIN* (*TAZ*) gene. To date, few patients with a molecular diagnosis have been reported. We describe the characterization of an intronic variant identified by whole-exome sequencing (WES) in a 14-month-old boy who presented with cardiogenic shock.

Methods: Genomic DNA was obtained from skin fibroblasts. We used SpliceAI for in silico splicing predictions. RNA samples from cultured skin fibroblasts and whole blood underwent splicing analysis by Sanger sequencing.

Results: WES analysis identified an unreported variant in hemizygosity in *TAFAZZIN*: c.109+3G>C in the NM_000116.5 transcript (short), corresponding to the variant c.112G>C, p.(Glu38Gln) in the less expressed NM_001303465.2 transcript (long). The variant was inherited from his mother, in whom presumably it appeared *de novo*.

SpliceAI indicated the disruption of exon 1 donor site (DS) of the short transcript and the activation of a cryptic DS in the c.109+34 position. Nevertheless, RNA analysis showed the activation of a different cryptic DS located in the c.82 position, causing the deletion of the last 28 nucleotides of exon 1, and leading to a truncated protein p.(Val28SerfsTer3). The full-length short transcript was not detected, and the expression of the long transcript was significantly increased.

Conclusion: Our findings show that discrepancies between in silico predictions and in vitro results may exist, highlighting the requirement of RNA studies to characterize splicing complex variants.

References:

Grants:

Conflict of Interest: None declared.

EP07.027 Deciphering MALSU1 in a consanguineous family with mitochondrial cardiomyopathy

Laura Holthöfer[1], *Mareike Selig*[2], *Verena Engelhardt*[1], *Dewi Hartwich*[1], *Hanna Müller*[1], *Clemens Sommer*[3], *Torsten Konrad*[4], *Alexandra Marx*[4], *Anne Schänzer*[5], *Matthias Bauer*[6], *Jennifer Winter*[1], *Susann Schweiger*[1]

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Background/Objectives: We describe a consanguineous family with two brothers showing the clinical picture of a mitochondrial cardiomyopathy. The older boy has a history of learning difficulties but was considered as healthy until the age of 17. He then showed signs of a decompensated heart insufficiency and was diagnosed with non-compaction cardiomyopathy (LVNC) and died at the age of 19. The younger brother had been diagnosed with hypertrophic cardiomyopathy (HCM) at the age of 3 years. He also showed learning difficulties. At the age of 16 he showed progressive heart insufficiency. The family´s third child is completely healthy.

Methods:.

Results: A homozygous missense variant in *MALSU1* (*C7orf30*) was detected in genomic DNA of the two patients. Both parents and the healthy brother were heterozygous carriers. *MALSU1* encodes an assembly and stability factor of the large subunit of the mitochondrial ribosomes (mt-LSU), suggesting a critical role in mitochondrial translation for MALSU1. Protein modelling suggested substantial destabilization of MALSU1 through this variant. Western blot analysis confirmed significant reduction of protein expression in cells of the homozygous family members. Comparative seahorse analysis of fibroblasts of the two homozygous and the heterozygous brother showed a decrease in mitochondrial activity in the homozygous samples but not in the heterozygous sample. Ongoing Western blot analysis of mitochontrial translated proteins versus cytosolic synthesized proteins will show if the MALSU1 variant influences mitrochondrial protein synthesis.

Conclusion: Mutations in mito-ribosomal proteins are a common cause of mitochondrial protein synthesis deficiencies. Here, we provide further evidence for MALSU1 to likely play a role in mitochondriopathies.

References:

Grants:

Conflict of Interest: None declared.

EP07.028 The c.1274_1277dupTATC variant causing Tay-Sachs disease in Tunisian family: a case report

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Background/Objectives: Tay-Sachs disease (TSD) (GM2 gangliosidosis I) is an autosomal recessive lysosomal-storage disorder confined to the central nervous system, resulting from deficiency of β-hexosaminidase A.

Methods: Present case is a 3-year-old girl with normal early developmental milestones until 10 months of age when she developed psychomotor regression. She started paroxysmal tonic deviation at the age of 22 months and she subsequently developed myoclonic jerks and an exaggerated startle reaction to sharp noise. Neurological evaluation showed normal head conference,

axial hypotonia and slight spastic tetraplegia. EEG showed interictal bi-temporal spikes, spikes-waves discharges and an episode of increased startle response. Brain MRI showed diffuse white matter and striatum hyperintensity on T2 with swollen appearance and thin corpus callosum. Bilateral cherry-red spots were found with a low level (5%) of HEXA enzyme activity.

Results: The homozygous pathogenic variant in HEXA gene (NM_000520.5):c.1274_1277dupTATC was detected using Human Inherited Disease QIAseq Targeted DNA panel. This variant creates a premature translational stop signal (p.Tyr427Ilefs*5), resulting in a disrupted protein product.

Conclusion: Our patient presented an acute infantile form of TSD supporting the low level of the residual HEXA enzyme activity. The c.1274_1277dupTATC variant has previously reported as the most frequent mutation (80%) in TSD of Ashkenazi Jews (AJ), suggesting the occurrence of a common founder in AJ population or the localization in a hotspot mutation region.

References: 1. https://doi.org/10.1007/s12687-011-0057-x.
2. https://doi.org/10.1007/s00439-003-1072-8.

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Conflict of Interest: None declared.

EP07.029 A clinical presentation of patient with ATP6AP1 -CDG and liver transplantation

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Background/Objectives: Congenital disorder of glycosylation, type IIs (OMIM# 300972) is a rare X-linked recessive complex syndrome characterized by liver dysfunction, recurrent bacterial infections, hypogammaglobulinemia, and defective glycosylation of serum proteins.

Methods: Here we report the 1-years-old male patient of Buryat origin, who presented with hypogammaglobulinemia and liver dysfunction. At the age of 3 months, he was hospitalized with jaundice and hepatosplenomegaly. Laboratory evaluation revealed thrombocytopenia, elevated transaminases, alkaline phosphatase of 4164 IU/L, alpha-fetoprotein of 85602 ME/mL and normal level of gamma-glutamyltransferase. MS/MS analysis of acylcarnitines and amino acids in plasma was normal. At the age of 10 months, the child successfully underwent orthotopic liver transplantation. After transplantation, the use of Tacrolimus led to the development of severe colitis with perforation. It required a change of Tacrolimus to Everolimus.

Results: The whole-exome sequencing identified an ATP6AP1 gene missense variant NM_001183.6:c.938A>G (p.Tyr313Cys) in the hemizygous state, which was previously reported by Jansen et al. in the patient with immunodeficiency type 47.

Conclusion: The previously reported patient demonstrated abnormal N- and O-glycosylation, and as for our patient, isoelectric focusing (IEF) of serum transferrin was performed only after liver transplant and showed a normal IEF pattern. We suggest that a possible explanation for normal glycosylation of transferrin in our patient might be a normalization of the glycosylation profile due to liver transplant, as Mirian et al. reported on the first successful liver transplantation in a patient with congenital disorder of glycosylation, after which, normal glycosylation of transferrin was found.

References:

Grants:

Conflict of Interest: None declared.

EP07.030 Novel mtDNA variants: a key role of muscle biopsy in defining their pathogenicity

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Background/Objectives: Pathogenic variants in mitochondrial DNA (mtDNA) are associated with variable clinical symptoms usually with neuromuscular manifestation. The variability of the symptoms may be affected by heteroplasmy level of the mtDNA variant in tissues. We report on two novel (*MT-ND1*: m.4135>C (p.Tyr277His), *MT-TK*: m.8315A>C) and one extremely rare (*MT-ATP6*: m.8719G>A (p.Gly65*) mtDNA variants identified in 3 patients by mitochondrial genome sequencing. To characterize the pathogenicity of the variants, muscle biopsies were necessary.

Methods:.

Results: Patient 1 is 39-years-old man with progressive loss of visually acuity due to mtDNA variant *MT-ND1*: m.4135>C (p.Tyr277His). In muscle with mutation load 93%, markedly decreased activities of complex (C) I and CI+III were observed and only mildly lower amount of CI holoenzyme. Patient 2 is 39-year-olf woman with fatigue, epilepsia partialis continua, and myoclonus based on variant *MT-TK*: m.8315A>C. A combine deficiency of CI, CIV, and CV was found in her muscle (heteroplasmy 85%). A 55-year-old man with cataract, hearing loss, and leukoencephalopathy (Patient 3) is carrier of a variant *MT-ATP6*: m.8719G>A (p.Gly65*). Separation of muscle (heteroplasmy 70%) mitochondria by native electrophoresis revealed reduced amount of CV (<20% of controls) with accumulated sub-complexes: V^*(F1-part with c-ring), free F1-part and a free c-ring.

Conclusion: We described two novel *(MT-ND1*: m.4135>C (p.Tyr277His), *MT-TK*: m.8315A>C) and one extremely rare (*MT-ATP6*: m.8719G>A (p.Gly65*)) pathogenic mtDNA variants and defined their impact on oxidative phosphorylation complexes. Our data underscores the necessity of muscle biopsy especially for characterization of pathogenicity of novel mtDNA variants.

References:

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Conflict of Interest: None declared.

EP07.031 The role of exome sequencing in newborn screening

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Background/Objectives: Public health newborn screening (NBS) programs provide population-scale ascertainment of rare, treatable conditions that require urgent intervention. Tandem mass

spectrometry (MS/MS) is currently used to screen newborns for a panel of rare inborn errors of metabolism (IEMs). The NBSeq project evaluated whole-exome sequencing (WES) as an innovative methodology for NBS.

Methods: We obtained archived residual dried blood spots and data for nearly all IEM cases from the 4.5 million infants born in California between mid-2005 and 2013 and from some infants who screened positive by MS/MS, but were unaffected upon follow-up testing.

Results: WES had an overall sensitivity of 88% and specificity of 98.4%, compared to 99.0% and 99.8%, respectively for MS/MS, although effectiveness varied among individual IEMs.

Conclusion: WES alone was insufficiently sensitive or specific to be a primary screen for most NBS IEMs. However, as a secondary test for infants with abnormal MS/MS screens, WES could reduce false-positive results, facilitate timely case resolution and in some instances even suggest more appropriate or specific diagnosis than that initially obtained. This study represents the largest, to date, sequencing effort of an entire population of IEM-affected cases, allowing unbiased assessment of current capabilities of WES as a tool for population screening.

References: Adhikari AN et al. 2020. The role of exome sequencing in newborn screening for inborn errors of metabolism. *Nature Medicine 26:*1392-1397. PMID:32778825. https://doi.org/10.1038/s41591-020-0966-5.

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EP07.032 Circulating transcripts in maternal blood for non-invasive prediction of gestational diabetes mellitus

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Background/Objectives: Gestational diabetes mellitus (GDM) is the most prevalent pregnancy disease, which can lead to serious adverse pregnancy outcomes. Early intervention in pregnant women at high risk of GDM can reduce the incidence. Plasma cell-free RNA (cfRNA) comes from various tissues and cells, it can provide a valid marker for risk prediction of GDM.

Methods: We included 54 GDM and 57 matched healthy control pregnant women between 14 and 19 weeks of gestation. Transcripts profiling of plasma cfRNA was performed in whole samples. Then, we divided them into two sets, predictive models were constructed by using multiple machine-learning algorithms in the training set and tested the performance in the validation set.

Results: The altered mRNAs were enriched in acute phase response, leukocyte migration, bone marrow leukocyte activation and response to bacteria pathways, both of which are associated with insulin resistance. This suggests that cfRNA could reflect the mechanism of GDM. Using lasso and random forest (RF) algorithm, five mRNAs and six lnRNAs were screened from all transcripts based on multiple iterations, then we constructed prediction model with the AUC of 0.89 in validation set. And, the significance of the model was further evaluated by permutation tests. Subsequently, we included 9 clinical features that AUC can up to 0.94. Tetraiodothyronine had a good predictive value.

Conclusion: Plasma cfRNA transcripts can characterize GDM noninvasively, and in combination with clinical features can easily and accurately predict the risk of GDM, thereby reducing the incidence of GDM and improving pregnancy outcomes.

References:

Grants:

Conflict of Interest: None declared.

EP07.033 Expanding the phenotype of MSTO1 related mitochondrial cytopathy and questioning the existence of an autosomal dominant transmission

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Background/Objectives: *MSTO1* is a nuclear gene encoding a mitochondrial distribution and morphology regulator, protein misato homolog 1. The *MSTO1* gene-related disease was first described as a dominant mitochondrial cytopathy. Three siblings and their affected mother were reported to carry the c.22G>A, p.Val8Met substitution and suffer from cognitive and/or psychiatric disorders, distal muscle weakness and normal creatine kinase (CK) level. To our knowledge, this is the only reported dominant *MSTO1* variant. In contrast, recessive were reported in 27 patients from 21 unrelated families. The *MSTO1* recessive phenotype contrasts the dominant one: early-onset proximal muscle weakness, elevated CK level and a variable constellation of symptoms including non-progressive cerebellar atrophy/ataxia, visual

impairment, motor developmental delay, cognitive alteration, corticospinal tract involvement, and skeletal abnormalities.

Methods: Studying a multiplex family presenting with early-onset muscle weakness and adult-onset optic neuropathy,.

Results: we identified two novel *MSTO1* variants: c.65C>A (p.Ala22Glu) predicted as "probably pathogenic" and absent from public databases, and c.220+5G>C which resulted in exon skipping in patient fibroblasts. The phenotype was consistent with typical recessive *MSTO1*-gene related disease, but this is the first report of a severe bilateral optic neuropathy as part of the *MSTO1*-gene related disease phenotype.

Conclusion: Through the subsequent analysis of a cohort of patients with bilateral optic neuropathy, we uncovered that the G>A substitution reported as a dominant *MSTO1* mutation was highly frequent and is most probably located in the *MSTOP2* pseudogene instead of *MSTO1*, questioning the dominant inheritance of the *MSTO1*-gene related disease.

References:

Grants:

Conflict of Interest: None declared.

EP07.034 Genomic risk prediction of type 2 diabetes in a clinical trial setting

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Background/Objectives: Polygenic risk scores (PRS) can capture the aggregate contribution of the physiological effects of known type 2 diabetes (T2D) risk alleles [1]. We tested the predictive performance of PRS for T2D risk in a clinical trial setting.

Methods: We constructed distinct PRS for overall T2D, six pathophysiological pathways in T2D, and diabetic complications (liver dysfunction markers, CAD, and CKD). We estimated the relative risk overall and across PRS percentiles using logistic regression in a post-hoc analysis of 4,872 European T2D patients from AstraZeneca clinical trials, EXSCEL [2] and Cotadutide-T2D-Ph2 [3]. We used 4,987 randomly selected UK Biobank Europeans without diabetes as controls.

Results: The genetic T2D risk for trial participants was 1.98-time higher against controls ($P = 3.72 \times 10^{-189}$). The top quintile of the T2D PRS had an odds ratio (OR) of 6.32 ($P = 2.82 \times 10^{-150}$). Among the T2D PRS subtypes, adiposity showed the highest genetic risk (OR = 1.32, $P = 2.29 \times 10^{-40}$). T2D patients had a 1.37-fold increased risk for CAD ($P = 7.13 \times 10^{-52}$) and 1.19-time higher liver transaminase levels ($P = 1.99 \times 10^{-17}$). T2D patients in the top quintile of the T2D Insulin Secretion PRS showed 1.11-time higher transaminase levels ($P = 2.61 \times 10^{-3}$) against the remaining T2D patients.

Conclusion: Our study demonstrated the predictive performance of PRS for T2D risk in a post-hoc analysis of clinical trials. We will expand on this work by testing the utility of the derived PRS for determining the response to anti-diabetes treatment.

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2. Holman et al. N Engl J Med. 377 (2017): 1228-1239.
3. Ambery et al. Lancet. 391 (2018): 2607-2618.

Grants: None.

Conflict of Interest: Xiao Jiang Xiao Jiang is a current full-time employees of AstraZeneca., Abhishek Nag Abhishek Nag is a current full-time employee of AstraZeneca., Katherine Smith Katherine Smith is a current full-time employee of AstraZeneca., Benjamin Challis Benjamin challis is a current full-time employee of AstraZeneca, Philip Ambery Philip Ambery is a current full-time employee of AstraZeneca., Björn Carlsson Björn Carlsson is a current full-time employee of AstraZeneca., Jan Oscarsson Jan Oscarsson is a current full-time employee of AstraZeneca., Dirk Paul Dirk Paul is a current full-time employee of AstraZeneca.

EP07.035 a shared pattern of altered gene expression in mitochondrial respiratory chain deficient human embryos

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Background/Objectives: Quantitative and qualitative anomalies of the mitochondrial DNA (mtDNA) are reportedly associated with impaired human embryonic development, but the underlying mechanisms remain hitherto unexplained. The goal of the present study was to investigate whether mitochondrial mutations affect gene expression in human blastocyst embryos.

Methods: A total of 42 blastocyst embryos (day-5/6/7) from 27 unrelated couples were collected after a preimplantation genetic testing analysis. Among them, 9 embryos were carrying pathogenic variants in either mtDNA genes or in a nuclear gene encoding a mitochondrial protein (mitochondrial group) and 33 were affected by a non-metabolic genetic disorder (control group). Gene expression profiling was performed on whole blastocyst embryos, by RNA-Sequencing.

Results: Transcriptomic analyses showed a similarly decreased gene expression pattern in embryos from the mitochondrial group, altering a number of differentiation factors and nuclear genes encoding mitochondrial proteins. Expression of oxidative phosphorylation genes was most impacted, cell survival and autophagy were also severely decreased, questioning embryonic viability.

Conclusion: The presence of a pathogenic mitochondrial variant induces changes in gene expression programs of human preimplantation embryos and probably compromises development, cell differentiation and survival of the embryo. While identification of reliable markers of normal embryonic development is urgently needed in assisted reproductive technologies, we suggest considering the under-expressed genes reported here as predictive biomarkers of mitochondrial dysfunction during preimplantation development.

References: None.

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Conflict of Interest: None declared.

EP08 Immunology and Hematopoietic System

EP08.001 Revisiting diagnostic procedures in patients with hereditary red blood cell membranopathies– Description of 9 new genetic variants

Friederike Haeuser[1], *Heidi Rossmann*[1], *Anke Adenaeuer*[1], *Berhnhard Laemmle*[2;3;4], *Claudia Paret*[5], *Karl J. Lackner*[1], *Joerg Faber*[5], *Olaf Beck*[5]

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Background/Objectives: Red blood cell membrane defects are a group of hereditary diseases with highly variable severity of symptoms and caused by a set of different variants of genes encoding for erythrocyte membrane and skeleton proteins. Although the most common hereditary hemolytic disease in Caucasians, hereditary spherocytosis (HS) is underdiagnosed, because mild clinical course is common and only the minority of patients have been genetically characterized so far. We aimed to develop a simple strategy to confirm the suspected diagnosis of HS using an NGS (next generation sequencing)-based approach.

Methods: 12 patients with clinical and laboratory signs of HS and one patient with initially inconclusive laboratory results, were genetically (exome sequencing) and phenotypically (standard laboratory tests) characterized. Family members of the index patients were studied by Sanger sequencing.

Results: In all 13 patients, pathogenic variants fulfilling ACMG-criteria were found, limited to only four genes: ANK1, SLC4A1, SPTA1, and SPTB. Four predescribed and nine new variants were detected, typically for HS or pyropoikilocytosis respectively.

Medical history and standard laboratory tests are usually sufficient to establish the diagnosis of HS. More sophisticated methods such as SDS- and native PAGE or ectacytometry can elucidate unclear cases.

Conclusion: The diagnosis of membranopathies was confirmed in all 13 patients by NGS. Panel sequencing of the five most common genes (incl. EPB42) is nowadays a comparatively rapid and inexpensive method to confirm the diagnosis of HS and related disorders. Time-consuming or low-specificity tests can be avoided, and family members can be easily genotyped.

References:

Grants:

Conflict of Interest: Friederike Haeuser: None declared, Heidi Rossmann: None declared, Anke Adenaeuer: None declared, Berhnhard Laemmle Potential conflict outside the topic of this Abstract: Bernhard Lämmle is chairman of the data safety monitoring committees (DMC) for the Baxalta 281102 study (recombinant ADAMTS13 in congenital TTP), the Shire SHP655-201 study (recombinant ADAMTS13 in acquired TTP) and the TAK-755-3002 study (Phase 3b continuation study of recombinant ADAMTS13 in congenital TTP), now all three run by Takeda; he was a member of the Advisory Board of Ablynx, now part of Sanofi, for the development of caplacizumab for the treatment of autoimmune TTP; he received congress travel support and/or lecture fees from Baxter, Ablynx, Alexion, Siemens, Bayer, Roche, and Sanofi., Claudia Paret: None declared, Karl J. Lackner: None declared, Joerg Faber: None declared, Olaf Beck: None declared.

EP08.002 Dominant negative effect of ETV6 germinal mutations as common pathogenic mechanism

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Background/Objectives: ETV6-related thrombocytopenia (ETV6-RT) is a rare autosomal dominant platelet disorder characterized by an increased risk to develop haematological malignancies. The disease is caused by mutations in *ETV6*, a gene encoding a transcription factor that plays a key role in haematopoiesis. Since ETV6-RT has been discovered only in 2015, many aspects of this disease still remain unknown. For this reason, we studied the pathogenic effect of seven germline missense variants identified in thrombocytopenic patients.

Methods: We performed bioinformatic analysis and gene reporter assays on a target promoter of ETV6 to determine the potential effect of the variants. Western Blot and immunofluorescence assays were used to evaluate the pathogenic mechanism.

Results: Analysing thrombocytopenic families by NGS approach, we identified seven different germline missense variants in *ETV6* gene, including four novel amino acid substitutions.

Consistent with bioinformatic analysis, we demonstrated the pathogenicity of all but two variants, whose protein products are no longer able to migrate into the nucleus, impairing the ETV6 repressive activity. Moreover, we ascertained that the pathogenic mutations act through a dominant negative effect, which retains the WT-mutant dimers of ETV6 in the cytoplasm consequently affecting its function.

Conclusion: We confirmed the pathogenicity of five out of seven variants and evaluated their pathogenic mechanism. Understanding the mechanism by which mutations exert their effect is important to clarify the ETV6 role during megakaryopoiesis, to identify possible therapeutic approaches to correct platelet biogenesis and to prevent the onset of leukaemia in ETV6-RT patients.

References:

Grants:

Conflict of Interest: None declared.

EP08.003 Autoimmune Polyglandular Syndrome Type 1 with Clinical and Genetic Characteristics

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Background/Objectives: Autoimmune polyglandular syndrome type 1 is a rare autoimmune disease characterized by polyendocrinopathy and enteropathies [1,2], resulting from a mutation in the autoimmune regulator (AIRE) gene located on chromosome 21q22.3 [3]. AIRE protein which acts as a transcription factor is responsible for immune system functions such as thymic self-representation and immune self-tolerance. Therefore, defects in

the protein levels cause enhancement of autoreactive T cells and autoimmunity. Autoimmune polyglandular syndrome type 1 progresses majorly with chronic mucocutaneous candidiasis, hypoparathyroidism and adrenocortical failure [4]. We aimed to report a 41-year-old female patient with APS-1 disorder.

Methods: We performed clinical exome sequencing from the patient's DNA which was isolated from peripheral blood leukocytes.

Results: As a result of the analysis, it was detected that the patient had a homozygous c.927 C>G mutation in the AIRE gene which is responsible for APS-1.

Conclusion: It was decided to follow up the patient's clinical course with the light of the literature and genetic results.

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Grants:

Conflict of Interest: None declared.

EP08.004 De novo mutation in the C1 inhibitor gene in the female patient with hereditary angioedema

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Background/Objectives: Hereditary angioedema (HAE) is a rare hereditary disease potentially dangerous for patients due to angioedema of larynx, intestines and other vital organs. However, there are HAE de novo in 25% of cases. We describe the clinical case of the patient with characteristic symptoms of HAE I (registered since 2017) without family history. The symptoms manifested after labor as angioedema and abdominal attacks up to 20 per year (AAS28 – 105 points) and were accompanied by decreased levels of complement component C4 to 0,04 g/l, C1-INH quantitative value to 3,3 mg/dl ($N = 15–35$ mg/dl), C1-INH functional activity to 24,1% ($N = 70–130\%$). The waiting period for a diagnosis was 4.5 years.

Methods: A DNA study of a 27-year-old patient was carried out for the presence of mutations in the SERPING1 gene (C1NH) (NM_000062) with the method of direct Sanger sequencing of all exons and exon-intron junctions.

Results: A new variant c.308_311dup (p.(Gln104Hisfs*30)) in the heterozygous state was found in exon 3. It had not been described in literature and databases (HGMD, ClinVar, LOVD) yet.

Conclusion: SERPING1 mutations heterogeneity is confirmed by discovering new gene variations, including de novo mutations. The identified DNA sequence variant should be considered as probably pathogenic and confirming the HAE diagnosis by a molecular genetic method. Genetic epidemiology demonstrates at least 25% of unrelated HAE cases. It should be taken into consideration during a patient consultation. For all patients with suspected HAE without family history, prompt genetic consultation or molecular genetic testing is necessary.

References:

Grants:

Conflict of Interest: None declared.

EP08.005 Tumor-specific methylation of p53-responsive oncosuppressive microRNA genes in Diffuse Large B-cell Lymphoma

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Background/Objectives: To identify the frequency and specificity of p53-responsive oncosuppressive MIR-34B/C, MIR-34A, MIR-203 and MIR-129-2 genes methylation in Diffuse Large B-cell Lymphoma (DLBCL).

Methods: Methylation status of the genes in tumor tissue ($n = 73$) was studied by methyl-specific PCR and methyl-sensitive analysis of high-resolution melting curves. DNA isolated from lymph node biopsies with reactive polyclonal B-cell proliferation ($n = 11$) was used to control the tumor-specificity of the detected methylation. The quantitative analysis of the combined methylation of the studied genes was carried out with the calculation of the one-sided Fisher exact criterion (p-value) and the frequency of false discoveries (FDR) (q-value) was calculated using the Benjamin-Hochberg procedure.

Results: Aberrant methylation of the promoters of the studied genes can serve as a significant mechanism for reducing the miR-34B/C, miR-34A, miR-203 and miR-129 micro-RNAs expression in the tumor tissue of DLBCL. It is occurs in combination and is tumor-specific. Thus methylation of MIR-129-2, MIR-203, MIR-34A and MIR34B/C in lymphoma samples occurred with a frequency of 67%, 66%, 27% and 62%, respectively, and there was no reactive lymph node tissue. Combined methylation of MIR-203, MIR-129-2 and MIR-34B/C genes ($p < 0.013$, $q < 0.020$), as well as pair of MIR-34B/C and MIR-34A genes ($p = 0.010$, $q = 0.029$) was detected.

Conclusion: Aberrant methylation of oncosuppressive microRNA genes associated with underlying p53 signaling pathways is a potentially useful molecular biomarker in the diagnosis, prognosis of tumors and the development of a strategy for targeted therapy of DLBCL.

References:

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Conflict of Interest: None declared.

EP08.006 Non-coding RNA profile associated with systemic lupus erythematosus activity, relevance of exosomal fraction

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Background/Objectives: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease where the exosomes have a regulating role throughout their cargos, especially non-coding RNA (ncRNA). The aim of this study is to identify a global ncRNA profile in plasma and plasma exosomes, associated to SLE activity.

Methods: Plasma samples were obtained from 96 SLE patients and 25 controls. RNA was extracted from both plasma and plasma-

exosomes (EXO-P). NcRNAs were identified using SmallRNA sequencing analysis. Results were validated in a higher cohort by qPCR.

Results: MicroRNAs (miRNAs) were the biotype with the highest mapped reads in all groups for both biofluids, followed by piRNAs, lncRNA and Y-RNA. Then, it was observed that plasma presented the greatest diversity of differentially expressed ncRNA biotypes, being the miRNA and lncRNA the most representative. Analysing only miRNAs in SLE, it was observed that they are biofluid-specific, being up-regulated in exosomes and down-regulated in plasma, and only 1.2% were common in both fractions. MiR-144-3p and miR-144-5p were the highest up-regulated in EXO-P versus P in all patient groups (3.92 and 3.03, $p < 0.001$, respectively). The ROC curve analysis showed the discriminatory power only of the hsa-miR-144-3p in EXO-P for the presence of SLE (AUC = 0.71, $p < 0.01$).

Conclusion: The results showed a biofluid specificity for the ncRNA profile, being up-regulated in plasma exosomes. MiRNAs are the most representative biotype of ncRNA, and exosomal miR-144-3p could be a potential biomarker of SLE activity.

References:

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Conflict of Interest: None declared.

EP08.007 The molecular genetic and laboratory features of HBB gene mutations and prevalence of beta-thalassemia in Russia: preliminary results

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Background/Objectives: HBB gene encodes the beta-globin that is a subunit of hemoglobin. HBB gene mutations can lead either to the decreased beta-chains synthesis and beta-thalassemia or to the beta-chains conformation changes and hemoglobinopathy. Thalassemia is the most common inherited disease in the world however the diagnostics is still a complicated complex process. Our aim was to characterize the molecular genetic and laboratory features of HBB gene mutations and to evaluate the prevalence of beta-thalassemia in Russia due to a little data about it.

Methods: The first group consisted of 268 patients with supposed inherited anemia in accordance with clinical and laboratory data. Capillary electrophoresis (CE) and Sanger sequencing the HBB gene were performed. Calculation of erythrocyte indexes, CE of hemoglobin and the HBB gene genotyping were carried out consistently to 4918 patients to evaluate the approximate prevalence of beta-thalassemia in Russia.

Results: The first group had 33 electrophoresis positive patients: 69,6% had the elevated HbF, 60,6% – HbA2. There were also detected HbS, Hb Shepherds Bush and the unknown pathological variant. 73% of patients had aberrations with the most frequent HBB:c.25_26delAA. The second group had 11 electrophoresis positive patients out of 32 chosen by indexes. 9 of them had the aberrations in the HBB gene.

Conclusion: It is necessary to evaluate both HbA2 and HbF fractions while screening for beta-thalassemia. The approximate prevalence of beta-thalassemia in Russia is 0,18%. The HBB:c.93-96CT mutation evaluated by NCBI base as mutation of uncertain significance was defined by us as pathogenic due to the detected unknown pathological hemoglobin variant.

References:

Grants:

Conflict of Interest: None declared.

EP08.008 The First report of sextuplicated alpha genes in a heterozygote beta thalassemia revealed by a severe beta thalassemia intermedia phenotype

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Background/Objectives: Co- inheritance of an extra copy of the alpha-globin gene cluster in individuals heterozygous for beta-thalassemia usually results in beta thalassemia intermedia. This study reports two members of an American family having eight alpha-globin genes due to an alpha-cluster segmental triplication combined with a beta+-thalassemia gene variant expressing a severe beta-thalassemia intermedia.

Methods: Standard hematology, HPLC and Sanger sequencing have been performed. FISH analysis, Multiplex Ligation-dependent Probe Amplification, SNP- and fine tiling array analysis of the alpha-globin gene cluster were performed to determine the triplication location and breakpoints.

Results: Two patients became transfusion dependent at age 15 and 10 years respectively. Sequencing the HBB gene has revealed a heterozygote beta+-thalassemia variant (HBB:c.*113A>G) inherited from father. MLPA and array analysis, performed in the probands and the parents, have shown a maternally inherited interstitial triplication of the alpha-globin gene cluster on chromosome 16p13.3 (approx. 900 kb), for which the mother was 25% mosaic. One child inherited eight alpha-genes, without the beta +-thalassemia variant of father. The triplicated segment involves the complete alpha-globin gene cluster and 18 additional protein coding genes. Still the only phenotype expressed is that of a thalassemia intermedia when in association with beta-thalassemia trait.

Conclusion: Our results clearly show that the presence of eight alpha globin genes does not have a discernible phenotype on its own, however, it actively contributes to globin chain unbalance when co-inherited with a beta thalassemia variant.

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Conflict of Interest: None declared.

EP08.009 PADI4 and PADI2 enhance collagen-initiated inflammatory responses

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Background/Objectives: Previously, peptidylarginine deiminase type 4 (PADI4) was identified as a susceptibility gene for Rheumatoid arthritis (RA) by genome-wide association studies. Peptidyl citrulline is a target antigen of anti-citrullinated peptide antibodies, and only PADs (translated protein from PADI genes) can

provide peptidyl citrulline via modification of protein substrates. Also the distribution of PADI4 and PADI2 has overlap in immune cells. The aim of this study was to investigate the relationship between PADI4 and PADI2 in the progression of RA.

Methods: To clarify the physiological function of PADI4 and PADI2 in RA, we used collagen-induced arthritis (CIA), known as a RA model mouse. We generated PADI4Knockout (KO) and PADI2KO mice, and performed CIA. In PADI4KO mice sera, serum anti-type II collagen (CII) IgM, IgG, and inflammatory cytokine levels were also significantly decreased compared with those in wild-type mice sera. We also examined that the clinical disease score of CIA mice and expression levels of Padi genes in PADI2KO CIA mice.

Results: We demonstrated that the clinical disease score of CIA was significantly decreased in PADI4KO mice. Interestingly, PADI2 expression was complementary induced in CD11b+ cells of PADI4KO mice. Gene expression levels of CIA of Padi2 using wild type mice was not observed significant difference between CIA and control mice, however, the clinical disease score was decreased in PADI2KO CIA mice.

Conclusion: It appears that PADI4 and PADI2 related with collagen-initiated inflammatory responses.

References:

Grants:

Conflict of Interest: Akari Suzuki RIKEN.

EP08.010 De novo gain-of-function variations in LYN lead to an early onset systemic autoinflammatory disorder

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Background/Objectives: To identify the molecular basis of a severe systemic autoinflammatory disorder (SAID) and define its main phenotypical features. To functionally assess the sequence variations identified in *LYN*, a gene encoding a non-receptor tyrosine-kinase.

Methods: (i) Targeted next-generation sequencing; (ii) In vitro functional studies of Lyn phosphorylation state and of Lyn-dependent NF-κB activity after expression of recombinant forms of Lyn carrying different sequence variations.

Results: We identified a *de novo LYN* variation (Tyr508His) in a patient presenting since birth with recurrent fever, chronic urticaria, atopic dermatitis, arthralgia, increased inflammatory biomarkers and elevated plasma cytokine levels. We studied the consequences of the Tyr508His variation and of the two *LYN* variations reported so far (Tyr508Phe and Tyr508*), on Lyn phosphorylation state, and showed that all three variations prevent phosphorylation of residue 508 and lead to autophosphorylation of Tyr397. Additionally, these three *LYN* variations activate the NF-κB pathway. These results reflect a gain-of-function (GOF) effect of the variations involving Tyr508 on Lyn activity.

Conclusion: This study, which demonstrates the pathogenicity of the first three *LYN* variations identified in SAID patients, delineates the phenotypic spectrum of a disease entity characterized by an early onset severe inflammatory disease affecting neonates with no family history of SAID. All three variations affect the same tyrosine residue located in the C-terminus of Lyn, thereby underlining the critical role of this residue in the proper regulation of Lyn activity in humans.

References:

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Conflict of Interest: None declared.

EP08.011 Searching for rare genetic variants associated with thrombosis using high-throughput sequencing technology

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Background/Objectives: We are using high-throughput sequencing (HTS) in search for rare genetic variant in thrombophilia cases and this approach we are implementing to clinical practice. We designed HTS panel for genes (*PROS1, PROC, SERPINC1* and *PROCR*) which encode proteins with important role in anticoagulation system. In addition to FV Leiden and FII Prothrombin, mutations in these genes are an important risk factor for thrombosis. In these genes was recently found more than 800 mutations without mutational hot-spots.

Methods: The selection of patients for HTS testing is primarily based on repeated low levels of a given anticoagulant protein by an adequate functional test while excluding possible exogenous causes. After exclusion was indicated 31 unrelated patients. The Ion Torrent PGM platform and the newer Ion Torrent S5 platform were used. Variant annotation is performed according to the variant description in the ClinVar database, based on low population frequencies, prediction software (PolyPhen, SIFT, etc.), PhyloP score, VarSome, etc.

Results: Overall mutation detection rate was 67.7%. Representation of mutations in responsible genes will be presented in a poster.

Conclusion: This is a pilot project in the Czech Republic, which assesses the impact of rare genetic variants on thrombophilic conditions using the HTS methodology. It is an effective adaptation of the HTS methodology to the sequencing platforms used at our department for searching variants in genes encoding anticoagulant protein in clinical practice.

References:

Grants: Supported by MH CZ – DRO (FNOl, 00098892).

Conflict of Interest: None declared.

EP08.012 Host genomic susceptibility to severe respiratory syncytial virus infection

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Background/Objectives: Infection with respiratory syncytial virus (RSV) is mild in most children, still a fraction suffers from severe disease, which might be due to specific variants in the human genome. We here use exome sequencing and bioinformatics analysis to search for genetic variants predisposing previously healthy children to severe clinical presentation of RSV infection.

Methods: We sequenced the exome of 127 previously healthy children who needed hospitalization and oxygen therapy due to RSV infection. We used GATK, VEP, and Annovar for variant calling and annotation. For variant prioritization, we implemented a Bayesian framework based on ACMG/AMP guidelines. Only variants with odds of pathogenicity >80% were included in downstream analysis. We used random walk with restart (RWR) on STRING networks to search for genes with similar function to known disease-associated nodes (called seeds). RSV-associated seeds were extracted from DisGeNET and OpenTargets. To control for the impact of network structure on the RWR output, we used permutation test, keeping genes with FDR <5%.

Results: We identified 28 deleterious variants in genes of interest, including: [a] three *IFIH1* loss-of-function previously shown to decrease interferon response to RNA viruses; [b] a frameshift *NOD2* variant resulting in a lack of synergistic response to RSV stimulation; and [c] a stop-gained *IFI44L* variant known as a risk factor for multisystem inflammatory syndrome in children.

Conclusion: To search for host genetics variants involved in severe RSV infection, we used a combination of ACMG/AMP guidelines and network-based methods. We identified 28 potentially causal variants, many of which in interferon-related genes.

References:

Grants:

Conflict of Interest: None declared.

EP08.013 Methylation of RUNX3 promoter 2 in the whole blood of children with ulcerative colitis

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Background/Objectives: Runt-related transcription factor 3 (RUNX3) regulates Th1/Th2 balance, and therefore the synthesis of cytokines and inflammation. Ulcerative colitis (UC) results from a complex interplay between the environment and immune cells, in which the relationship between gene methylation and disease activity remains unclear. We aimed to analyze *RUNX3* promoter 2 (*P2*) methylation level depending on several clinical factors.

Methods: This cross-sectional study recruited hospitalized children with UC. Clinical characteristics were assessed: age, sex, body mass index (BMI), C-reactive protein (CRP), serum albumin, disease duration, pediatric ulcerative colitis activity index (PUCAI), the Paris classification, and exposure to medications. Whole blood DNA was isolated and *RUNX3 P2* methylation level was measured with methylation-sensitive restriction enzymes (OneStep qMethyl).

Results: Sixty-four children were enrolled, at a mean age of 14.5 ± 2.8 years. Half of them were female (51.6%) and the mean BMI was 18.0 ± 3.2 kg/m^2 (Z-score −0.44 ± 1.14). The mean methylation level of *RUNX3 P2* was 54.1 ± 13.3%. The methylation level of *RUNX3 P2* did not correlate with age, sex, nutritional status, CRP, albumin, PUCAI or the extent of colitis (Paris E1–E4). *RUNX3 P2* methylation did not differ between patients recruited within two months of diagnosis and children who had UC for at least a year. Current or past exposure to biologics, immunosuppressants or steroids was not associated with *RUNX3 P2* methylation.

Conclusion: Methylation of *RUNX3* promoter 2 in whole blood DNA does not seem to associate with clinical characteristics of UC in children.

References: N/A

Grants: Polish National Science Center 2017/25/B/NZ5/02783, awarded to JW.

Conflict of Interest: None declared.

EP08.014 A novel missense variant in RPS19 gene causing Diamond-Blackfan anemia 1

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Background/Objectives: Diamond-Blackfan anemia (DBA) is a rare congenital erythroid aplasia that usually presents in infancy. It is characterized by a normochromic macrocytic anemia, growth retardation, and approximately 30 to 50% patients have congenital malformations, and predisposition to cancer. Haploinsufficiency of gene encoding ribosomal protein S19 (RPS19; MIM 603474) accounts for approximately 25% of DBA patients. These patients (DBA type 1) usually don't have craniofacial abnormalities.

We report a three-month-old girl of a healthy non-consanguineous parents, with clinical features of Diamond-Blackfan anemia: low birth weight, pale skin, sleepiness, severe normocytic anemia, congenital heart defects, but without craniofacial dysmorphism. She responded well to steroids.

Methods: Clinical exome sequencing including genes associated with Diamond-Blackfan anemia was performed in family members using Illumina TruSight One Kit.

Results: In the RPS19 gene, one novel, de novo, missense variant p.Arg56Gly was identified. According ACMG classification is pathogenic.

Conclusion: Already reported alternative variant Arg56Gln is also classified pathogenic according ACMG. We assume that these variants at codon 56 may disrupt/weaken interactions between arginine and 18S rRNA. In order to develop better diagnostics and treatment, it is necessary to resolve the molecular basis of the pathogenic variants, especially missense that are challenging to decipher. The novel reported variant will further contribute to that knowledge.

References:

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Regenerative Medicine and by the EU through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project „Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials", Mijana Kero Scientific Center of Excellence for Reproductive and Regenerative Medicine and by the EU through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project „Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials".

EP08.015 Next-generation sequencing with a 64-gene panel in Slovenian patients with myelofibrosis

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Background/Objectives: Myelofibrosis (MF) is a chronic myeloproliferative neoplasm that may present as primary disease (PMF) or as post-polycythemia vera MF (PPV-MF) or post-essential thrombocythemia MF (PET-MF). Over the last decade, many additional secondary mutations have been found to have a prognostic impact on disease course.

Methods: We performed analysis of 80 MF (PMF, $N = 62$; PET-MF, $N = 11$; PPV-MF, $N = 7$) samples with our custom 64-gene NGS panel. he variants were manually checked in the ClinVar and COSMIC databases to determine if they had been previously reported as pathogenic.

Results: Fifty-two patients were tested positive for the *JAK2* V617F driver mutation, and 5 were positive for *CALR* and 2 for *MPL* driver mutations. Aside from driver mutations an initial total of 190 variants were detected across 75 clinical samples, five patients did not show mutations in any of the gene tested. The majority of cases had mutations in ≥ 1 genes (1 mutation: $N = 20$, 25%, 2 mutations: $N = 23$, 28.8%, 3 mutations: $N = 15$, 18.8%, 4 mutations: $N = 10$, 12.5%, 5 mutations: $N = 5$, 6.3%, 6 mutations: $N = 2$, 2.5%). Ultimately, a total of 40 unique variants (which include known driver mutations) with VAFs above 1% were identified. The most common mutations and those with the worst prognostic impact were found as follows: *TET2* ($N = 37$), *SETBP1* ($N = 17$), *ASXL1* ($N = 9$), *EZH2* ($N = 11$), *IDH1* ($N = 10$), *IDH2* ($N = 7$), *SF3B1* ($N = 6$), *SRSF2* ($N = 2$), *TP53* ($N = 1$), *U2AF1* ($N = 1$) and *FLT3* ($N = 3$).

Conclusions: With the obtained results, we will be able to more appropriately classify patients to prognostic groups and treat them accordingly.

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EP08.016 A small deletion in FAS causes splicing disturbances in a patient with suspected ALPS

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Background/Objectives: Autoimmune lymphoproliferative syndrome (ALPS; MIM#601859) is a disorder of a programmed cell death. The known cause of this syndrome is heterozygous variants in *FAS* (MIM#134637) – Fas Cell Surface Death Receptor. The receptor belongs to the TNF–superfamily receptors and plays a central role in apoptosis. The penetrance of ALPS is variable.

Methods: The proband, 20 years of age female, had autoimmune hemolytic anemia, pancytopenia, and accumulation of double-negative T cells. Exome sequencing was applied to analyse the proband's DNA. The genetic findings were confirmed by Sanger sequencing in the DNA samples of the proband and her healthy mother. This method was also applied to investigate the mRNA structure of the gene *FAS* in the RNA samples of the proband and the mother, extracted from fibroblasts.

Results: A heterozygous deletion NC_000010.11(NM_000043.6):c.16_30+1del which covers a first nucleotide of a donor splice site in *FAS* was identified in the proband's DNA. The variant was inherited from healthy mother. The impact of the splice site variant on mRNA structure was determined by Sanger sequencing which revealed a cryptic splice site formation. In silico, a frameshift and a premature stop codon (NP_000034.1:p.(Thr6Phefs17Ter)) is formed.

Conclusion: The disrupted splicing process and most probably truncated and dysfunctional FAS protein were revealed by the molecular analysis of a small deletion NC_000010.11(NM_000043.6):c.16_30+1del in *FAS*. The disturbed molecular process presumably causes the phenotype of ALPS of the affected individual.

References:

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Conflict of Interest: None declared.

EP08.017 Association of rare genetic variants at the ULBP3 locus with alopecia areata

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Background/Objectives: Alopecia areata (AA) is a common hair loss and autoimmune disorder with an estimated lifetime risk of about 2%. Genome-wide association studies reported 14 susceptibility loci, one of which implicates *ULBP3*, encoding for a ligand of the NKG2D receptor expressed on natural killer cells and a subset of CD8+ T cells. *ULBP3* expression was shown to be highly upregulated in the hair follicle of AA patients. The aim of the current study was to discover whether rare variants in the *ULBP3* locus are associated with genetic susceptibility to develop AA.

Methods: The entire coding and non-coding region around *ULPB3* (10 kb) was sequenced in 1.000 AA patients and 1.000 controls. The results were analyzed by a rare variant burden analysis pipeline, which implements a gene-based scoring system using functional annotations and allele frequency weighting function.

Results: A very restricted number of rare variants were identified in the coding sequence. The analysis of the entire region including the non-coding sequences revealed a significant association of rare *ULBP3* variants (MAF < 0.01) with AA ($p = 0.028$).

Conclusion: Our study shows the contribution of rare genetic variation at the *ULBP3* locus to disease susceptibility in AA. A functional follow-up of some of the identified variants can provide mechanistic insights into the development of AA.

References: Petukhova, L. et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. Nature 466, 113-7 (2010).

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EP08.018 Sickle cell trait - a case report

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Background/Objectives: Sickle cell disease is caused by mutations in the gene encoding the haemoglobin subunit β (*HBB*), and is characterized by chronic haemolytic anaemia, ischemia, severe pain, and necrosis. The disease occurs when *HBB* gene is mutated in both alleles, and at least one of the mutations is c.20A>T p.(Glu7Val) (HbS). Individuals with this mutation alone carry the sickle cell trait and generally are healthy.

We report a case of a couple with a sickle cell trait and normal hemograma (pregnant has also elevated Hb F), and their fetus, that were studied for the variant c.20A>T p.(Glu7Val) in the *HBB* gene.

Methods: Sanger sequencing of the exon 1 of the *HBB* gene and MLPA analysis.

Results: Sanger sequencing revealed the heterozygous mutation c.20A>T p.(Glu7Val) in the father, while both mother and fetus had the c.20A>T p.(Glu7Val) mutation in apparent homozygosity. Those results were confirmed by MLPA: no deletions/duplications on the *HBB* gene were detected in the fetus, that was proven to be homozygous; while the mother has the c.20A>T p.(Glu7Val) mutation in one allele and deletion of the *HBB* and *HBD* genes (known as HPFH-2 Ghanaian) in the other allele.

Conclusion: Heterozygous individuals for the HPFH-2 Ghanaian deletion have normal hematology, with increased levels of Hb F (fetal hemoglobin) and decreased levels of Hb A2, which justifies the phenotype presented by the mother. Homozygous individuals for the *HBB* c.20A>T p.(Glu7Val) variant have sickle cell disease.

References: PMID: 29542687; 23065522; 28356267; 6196781; 2307466; 33238542.

Grants:

Conflict of Interest: None declared.

EP08.019 JAK2 variants associated with congenital erythrocytosis and Polycythemia vera

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Background/Objectives: Erythrocytosis is a condition with an increased erythrocyte mass. The *JAK2* gene is involved in the development of acquired type Polycythemia vera (PV), however its role in development of congenital erythrocytosis (CE) is still poorly understood. The aim of our research was to identify *JAK2* variants associated with different types of erythrocytosis and to analyse their presence in patients suspected with CE.

Methods: Literature and Ensembl database were reviewed for potentially pathogenic *JAK2* variants and their position in the gene. Patients were screened for variants in selected *JAK2* gene regions using targeted NGS and confirmed by Sanger sequencing.

Results: Based on literature mining and bioinformatics analysis, we found several variants causative for PV in exon 12, exon 13, exon 14 and surrounding introns. One variant was found in exon 19 and another in exon 24. While only one variant was reported as causative for CE in exon 19. Sequencing of *JAK2* in cohort of 27 patients suspected with CE did not reveal any of the previously reported causative variants. Nevertheless, we identified two new *JAK2* variants with unknown significance in exon 13 and intron 19 in one family[1,2].

Conclusion: Variants of the *JAK2* gene show considerable potential for development of erythrocytosis based on their location in previously reported regulatory regions. In future, the involvement of selected variants in erythrocytosis development will be validated by functional analysis.

References: 1. Kristan et al., 2021. J Clin Lab Anal, 35:e23715.
2. Kristan et al., 2021. Front Genet, 12:689868.

Grants: ARRS grants L3-9279 and Young Researcher funding.

Conflict of Interest: Monika Banfi: None declared, Aleša Kristan: None declared, Irena Preložnik Zupan: None declared, Andrej Vuga Kemomed, Tanja Kunej: None declared, Nataša Debeljak Leader of the project grant L3-9279..

EP08.020 Chronic idiopathic splenomegaly - a challenging autoimmune lymphoproliferative syndrome

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Background/Objectives: Autoimmune lymphoproliferative syndrome (ALPS) is a primary immune regulatory disorder with a

heterogeneous genetic background frequently confronted with diagnosis challenges. Even today with performant genetic technologies there remain some unclarified situations accepted as ALPS-like cases.

Methods: Case presentation.

Results: We bring to attention a 12 years old girl, without pathological history, who at the age of 9 presented mild splenomegaly, anaemia, and jaundice justifying a diagnosis of congenital microspherocytosis. At the age of 10, she was addressed to our clinic for skin pallor and significant splenomegaly. Laboratory investigations indicated a mild normochromic normocytic anaemia, without microspherocytosis, with important persistent reticulocytosis (>3%), mild inconstant thrombocytopenia, hyperbilirubinemia, very low haptoglobin values, direct positive Coombs test; and significantly high values (>10%) of double negative TCR αβ populations, of vitamin B12 (3757pg/ml) and IL-10 (103pg/ml) were assessed. Bone marrow biopsy revealed hypercellularity. Considering an ALPS, next-generation sequencing was performed revealing in the girl and mother a heterozygous variant of MYH13 gene, with uncertain significance. Meeting the mandatory criteria and 3 secondary accessory criteria, we initially interpreted the case as an ALPS-like syndrome. Later the necessary primary accessory biomolecular criteria were found using the whole genome sequencing which assessed a loss of the number of copies on chromosome 10q23.31, with a heterozygous deletion, including the pathogenic FAS gene, finally confirming the ALPS type I diagnosis.

Conclusion: Initially, miss-interpreted, followed by a presumption of ALPS-like, this case finally reached the right diagnosis proving the importance of a comprehensive exploratory approach.

References: NA

Grants: NA

Conflict of Interest: None declared.

EP08.022 A young adult with vasculitis and stroke at the age of 8: consider adenosine deaminase-2 deficiency

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Background/Objectives: The deficiency of adenosine deaminase-2 (DADA2) is a recently described autoinflammatory and metabolic disease evolving with childhood-onset polyarteritis nodosa, strokes, *livedo racemosa* and immune deficiency, responsive to anti-tumour necrosis factor agents.

Methods: A 26-year male patient with a vasculitis with stroke at the age of 8, presented for headache. Examination revealed *livedo racemosa*, a sluggish speech and splenomegaly. Apart from inflammation (CRP 22 mg/L, normal <6 mg/L) and a selective IgG deficiency (520 mg/dl, normal 700-1600 mg/dl), all immunological tests (including antinuclear, antiphospholipid and anti-neutrophil cytoplasm antibodies, complement fractions and hepatitis B, C and HIV serology) were normal.

Results: A 109 genes assessment (autoinflammatory syndromes panel, Invitae, US) revealed two heterozygous variants of unknown significance (VUS) in ADA2: a c.620T>G(p.Phe270Cys) in exon 4, suggested by predictive algorithms to be disruptive, and c.967G>A (p.Val323Ile), in exon 7, for which the predictive algorithms did not agree (SIFT: deleterious, PolyPhen-2 probably damaging, Align GVGD Class 0).

Conclusion: Nevertheless, the first enzymatic test (Synlab) did not confirm the enzyme deficiency. He was treated with glucocorticoids and azathioprine, with clinical improvement. The patient is being tested further in order to clarify a DADA2 or other type of vasculitis and treat it optimally.

References:

Grants:

Conflict of Interest: None declared.

EP08.023 Two cases of a rare association between ataxia-telangiectasia and juvenile idiopathic arthritis

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Background/Objectives: Ataxia-telangiectasia (AT) is an autosomal recessive condition caused by biallelic pathogenic *ATM* variants, presenting with cerebellar ataxia and telangiectasia, endocrine and immunological features, radiation sensitivity and cancer susceptibility. Immunodeficiency is present in 60-80% of AT patients, most frequently low immunoglobulin levels, lymphopenia, weak vaccine response and common infections. There have been rare reports of AT cases with inflammatory and autoimmune conditions such as thrombocytopenia, vitiligo and cutaneous granulomatosis.

Methods: We present two patients with AT and juvenile idiopathic arthritis (JIA), the most frequent autoimmune rheumatic disease in childhood. Both patients presenting as oligoarthritis originate from the same geographic region.

Results: Exome sequencing found compound heterozygosity in both (patient 1: c.5293C>T; p.Gln1765Ter and c.8305T>C; p.Trp2769Arg and patient 2: c.4383G>A; p. Trp1461Ter and c.6095G>A; p.Arg2032Lys). No significant known gene variants or susceptible HLA alleles associated with JIA were identified.

Conclusion: These two cases provide support of the hypothesis that loss of ATM function leads to immune deregulation and systemic inflammation. It raises questions about the clinical management of patients with dysfunctional DNA repair due to loss of ATM function in terms of systematic screening for autoimmune and inflammatory conditions in patients with AT and rational use of the imaging tools given the radiosensitivity and predisposition to cancer.

References:

Grants:

Conflict of Interest: None declared.

EP09 Intellectual Disability

EP09.001 Ververi-Brady syndrome associated with QRICH1 variants: a report of two new cases in Russia and literature review

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Background/Objectives: Recent advances in sequencing technologies have enabled identification of multiple genes associated with intellectual disability disorders, including QRICH1. The function of QRICH1 is largely unknown but it is likely to play a key role in the unfolded response of endoplasmic reticulum stress. Ververi-Brady syndrome (VBS; MIM:#617982) is a rare developmental disorder, characterized by mild developmental delay, mildly impaired intellectual development and speech delay and mild dysmorphic facial features, and loss of-function variants in QRICH1 were implicated in its etiology.

Methods: We examined 2 patients with epilepsy, developmental and speech delay. The clinical phenotyping, video-electroencephalography, magnetic resonance imaging of the brain were carried out. All patients received informed consent for whole exome sequencing.

Results: We describe two new patients and review the clinical and genetic information on all previously reported VBS cases. Here, we present two unrelated individuals with one missense mutation (c.1711G>A; p. Asp571Asn) and one frameshift mutations (c.1963_1964insT; p.Lys655IlefsTer). These two individuals contributes to the delineation of the VBS phenotype and suggests epilepsy, developmental and speech delay, moderate motor delay, learning difficulties and mildly impaired dysmorphic features.

Conclusion: To date, thirty eight individuals have been reported with QRICH1 mutations in the world. Our new clinical cases presented in Russia also confirms that the phenotype of Ververi-Brady syndrome is relatively nonspecific and includes variable neurodevelopmental features, delayed speech, learning difficulties, epilepsy and mildly impaired dysmorphic features.

References: Kumble S, Levy AM, Punetha J, et al. The clinical and molecular spectrum of QRICH1 associated neurodevelopmental disorder. Hum Mutat. 2021 Dec 2. https://doi.org/10.1002/humu.24308.

Grants: No.

Conflict of Interest: None declared.

EP09.002 Refining the Phenotypic Spectrum of KMT5B-Associated Syndromic Developmental Delay

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Background/Objectives: The role of lysine methyltransferases (KMTs) and demethylases (KDMs) in the regulation of chromatin modification is well established. Recently, heterozygous variants in KMT5B were implicated in individuals with intellectual disability (ID) and/or autism.

Methods: Trio exome sequencing (ES) was performed for three unrelated patients with global developmental delay (GDD) or ID, macrocephaly and additional features. 3D structural models of wild-type (WT) and mutated KMT5B structures were constructed, and intra-structure hydrogen bonds and the distances between atoms were calculated.

Results: Patient A presented at 12 months of age due to profound GDD, and showed macrocephaly, broad forehead, strabismus, short neck and mild hypertelorism. Patient B presented at 2.5 years with GDD and relative macrocephaly, and showed a broad forehead, conical fingers, hypotonia and joint hypermobility. Patient C presented at 35 years due to ID, seizures and overgrowth (weight, height and head circumference >97 centile). Physical examination was notable for occipital protrusion and upsweeping of frontal hair, large hands with long and lean fingers. All three probands had normal Chromosomal Microarray Analysis and normal maternal Fragile X testing. Following ES, each of the probands was found to harbor a distinct de-novo heterozygous disease-causing variant in KMT5B: c.541C>G (p.His181Asp); c.833A>T (p.Asn278Ile); or c.391_394delAAAG (p.Lys131GlufsTer6). Using 3D-computational modeling of the KMT5B protein, structural effects of the two missense variants were demonstrated.

Conclusion: Our findings support the role of de-novo heterozygous variants in KMT5B-associated GDD, and suggest that KMT5B should be considered in the differential diagnosis of neurodevelopmental disorders accompanied by macrocephaly and/or overgrowth.

References:

Grants:

Conflict of Interest: None declared.

EP09.003 Williams-Beuren region triplication syndrome in a 4-year-old girl from Ukraine

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Background/Objectives: Williams-Beuren Duplication Syndrome (7q11.23) is a multisystem developmental pathology with a variety of clinical polymorphisms.

The aim of this study was to diagnose chromosomal changes at the submicroscopic level as a first-line test for patients with intelligence deficits, autism spectrum disorders.

Methods: Chromosomal micromatrix analysis was performed in the laboratory "Vista" (Tai Po Industrial Estable, N.T. Hong Kong).

Results: Parents turned to a geneticist with complaints of delayed physical, intellectual, language development in a 4-year-old daughter to clarify the diagnosis, as well as to determine the prognosis of further childbirth. The girl was born without

congenital anomalies from the 1st pregnancy, premature birth within 36 weeks. At birth: parents age 27 years, body weight - 2620 g, height - 46 cm, APGAR score 7/7. Up to 6 months was diagnosed with hydrocephalus. Began sitting at 8 months, walking - 1 year 3 months. Doesn't talk. Doesn't enter into speech contact, eye contact is relative. Constructive tasks are sometimes performed with the help, emotionally labile. Motor activity is satisfactory.

Siblings of the proband mother's grandfather had mental retardation in anamnesis.

Chromosomal analysis - 46, XX. Chromosomal micromatrix analysis - Duplicate fragment detected of a 1.67 Mb in the region 7q11.23q11.23 (copy number: 3).

Diagnosed Williams-Beuren triplication syndrome (OMIM: 609757) with autosomal dominant inheritance.

In literature only several descriptions of such patients were found.

Conclusion: Due to chromosomal micromatrix analysis diagnosed Williams-Beuren triplication syndrome in the proband and the mother was recommended to perform prenatal diagnosis in the next pregnancy.

References:

Grants: no.

Conflict of Interest: None declared.

EP09.004 Identification of a new frameshift deletion in the PTRHD1 gene in two affected children with juvenile-onset parkinsonism and intellectual disability

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Background/Objectives: Juvenile parkinsonism is a progressive neurodegenerative disease that starts at an early age and is characterized by tremor, muscular stiffness, unbalanced coordination and slowness of movements. While it is known that parkinsonism is associated with cognitive impairment, data on juvenile-onset parkinsonism and intellectual disability are still limited to a few case reports. We present two siblings from a consanguineous Syrian family: a 4-year-old patient with severe speech delay, behavioral problems, gait difficulties and delayed global development, and his less severely affected 14-year-old sister with delayed global development and gait difficulties.

Methods: Morphological examination, fragile X syndrome, Whole Exome Trio analysis, MRT scans and histological staining of skin biopsy were performed.

Results: A Whole Exome Trio analysis revealed a homozygous and likely disease-causing variant c.280delC, p.(Leu94TrpfsTer40) in the PTRHD1 gene of both siblings. This mutation is not listed in public databases but it is located in the functional domain PTH2 of the PTRHD1 gene, known to suppress proteolysis degradation and to be causative for neurodegenerative diseases such as the autosomal recessive juvenile-onset parkinsonism and intellectual disability (Kuipers et al., 2018; Khodadadi et al., 2017).

Conclusion: Given the role of the PTRHD1 gene in parkinsonism and intellectual disability in combination with the molecular and clinical findings of both siblings, we assume this frameshift mutation to be causative for a PTRHD1-associated juvenile-onset parkinsonism and intellectual disability supported by previous results about the function of the PTH2 domain in the PTRHD1 gene. Experimental therapy with Levodopa was successfully started in the 4-year-old patient.

References:

Grants:

Conflict of Interest: None declared.

EP09.005 Child health, parent health, and family functioning in families of children with Down syndrome, congenital heart disease and both conditions from Ecuador, Spain and USA

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Background/Objectives: Down syndrome (DS), the most common chromosomal cause of intellectual disability, can affect the health of the child with DS, parental health and overall family functioning. Approximately 50% of children with DS have congenital heart disease (CHD). The aim of this international collaboration was to compare parental perceptions of child health, parental health (physical and mental) and family functioning in families of children with DS, CHD and both conditions from Ecuador, Spain and USA.

Methods: Parents completed an online survey. Clinicians and group leaders of support groups and foundations shared study information with eligible families.

Results: 560 parents completed the online survey (141 Ecuador, 273 Spain, and 146 USA). One-way analysis of variance F tests were used to compare means for the four variables across the nine groups. While there were significant differences between groups for all four variables, the overall pattern was one of good health and above average family functioning. Generally, health and family functioning were less positive in families of children with both conditions and families from Ecuador.

Conclusion: Findings suggest that despite the ongoing challenges associated with living with a chronic condition, many parents reported good health and above average family functioning. More research is needed to identify families that need extra support, such as families of children with multiple conditions and families from countries known to provide less support to families. Research is also needed to understand how social determinants of health influence child health, parental health and family functioning.

References:

Grants: Dhillon Jordan Shah Innovation Fund in CHD.

Conflict of Interest: None declared.

EP09.006 Case Report: Identification of a de novo nonsense mutation in WASF1 gene in a patient with seizures and developmental delay

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Background/Objectives: De novo heterozygous nonsense or frameshift mutations in the WASF1 gene have been first reported in 5 unrelated patients presenting neurodevelopmental disorder and seizures with absent language. We report on a girl with severe muscular hypotonia and global developmental delay, first observed at the age of 6 months who further developed an epilepsy. A brain MRI did not show any structural abnormalities. No Organ malformations were detected.

Methods: The karyotyping showed a normal female karyotype (46,XX), confirmed by aCGH analysis. Whole exome sequencing detected one pathogenic variant and a variant of uncertain significance in the GBA gene. Furthermore, we could detect a likely pathogenic heterozygous variant in WASF1. Using dried blood spots technique for the evaluation of beta glucocerebrosidase activity the suspicion of Gaucher disease could be ruled out. Therefore, we examined our second candidate, the variant c.1507G>T, p.(Glu503*) in WASF1.

Results: The variant p.(Glu503*) in WASF1 gene predicted to result in a premature stop codon, has not previously been reported. The variant could be excluded in both parents by Sanger sequencing, confirming de novo status of the variant in the patient.

Conclusion: This case report represents the seventh patient with developmental delay and seizure having a pathogenic variant in WASF1. Therefore, this case highlights the utility of exome sequencing for identifying rare genetic causes of developmental delay at a very early age.

References:
1-Ito, Y., et al., De novo truncating mutations in WASF1 cause intellectual disability with seizures. Am. J. Hum. Genet. 2018 Jul 5; 103(1): 144-153.

Grants:

Conflict of Interest: None declared.

EP09.007 A new patient with TLK2-associated Mental Retardation: Further delineation of the clinical and molecular phenotype of a rare Neurodevelopmental Disorder

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Background/Objectives: The TLK2-asscociated Mental Retardation, also referred to as Autosomal Dominant Mental Retardation Type 57 (MRD57, OMIM#618050) is a rare condition characterized by mild to moderate developmental delay, behavioral disorders, and gastrointestinal feeding problems. Certain facial dysmorphic features have been observed frequently. MRD57 is caused by heterozygous mutations in TLK2 (OMIM*608439) and is inherited in an autosomal dominant pattern. Up to now, only about 40 patients have been reported.

Methods: Case report.

Results: Here we present a 9 year and 4 month old boy with MRD57 due to the heterozygous de novo variant c.2034del p.(Glu679Lysfs*9) in *TLK2*. This variant is classified as likely pathogenic (Class 4) according to ACMG guidelines. The patient has a moderate intellectual disability, restlessness, a short attention span, and social-emotional problems. Facial dysmorphisms include telecanthus, prominent nasal bridge, broad nasal tips, upslanting palpebral fissures, and low set ears. These features have been described before in patients with MRD57. Gastrointestinal feedings problems, seen in many other patients with MRD57, have not been observed.

Conclusion: The dymorphisms seen in the boy reported here are overlapping with the patients described before, but a distinct facial gestalt can´t be defined so far. In this report, we present detailed clinical and molecular data of a new patient with MDR57 to delineate further the clinical phenotype of *TLK2*-associated Mental Retardation and to point to this rare intellectual disability syndrome.

References: Reijnders et al. (2018). De Novo and Inherited Loss-of-Function Variants in TLK2: Clinical and Genotype-Phenotype Evaluation of a Distinct Neurodevelopmental Disorder. Am J Hum Genet. 102, 1195-1203..

Grants:

Conflict of Interest: None declared.

EP09.009 How DeepGestalt triggered WES re-analysis and led to the identification of a KANSL1 intragenic deletion causing Koolen-de Vries syndrome

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Background/Objectives: Koolen-De Vries syndrome (OMIM # 610443) is caused either by recurrent heterozygous 500 - 650kb microdeletions at chromosome 17q21.31 including *KANSL1* or by heterozygous intragenic pathogenic variants in KANSL1.

We present a fifteen-year-old girl with global developmental delay and moderate intellectual disability. She has had muscular hypotonia since early childhood. The patient had distinctive facial dysmorphism and her family described her as extremely friendly, but anxious in contact with other children. Chromosomal microarray (CMA) performed at the age of eight years were unremarkable.

Methods: Trio whole exome sequencing (WES) was performed on leukocyte-derived DNA samples. The DeepGestalt algorithm of Face2Gene was used to prioritize syndrome suggestions.

Results: The DeepGestalt algorithm scored remarkably high for Koolen-de Vries syndrome. Nevertheless, Sanger sequencing and MLPA of the *KANSL1* gene gave normal results. We then performed trio WES but again no pathogenic variant was detected in *KANSL1* or any other gene. Only after targeted re-analysis of KANSL1 sequencing data in the Integrative Genomics Viewer we were able to detect a 4708 bp intragenic deletion comprising parts of intron 6 and exon 7 (c.1849-4611_1895del;r.spl). The deletion was verified by qPCR and whole genome sequencing (WGS). It was not detected in the parents; hence, it is highly likely that this variant occurred de novo and is causal for the patient's symptoms.

Conclusion: This case demonstrates the utility of Face2Gene and DeepGestalt in facilitating the diagnosis of genetic syndromes with typical facial dysmorphism and stresses the importance of accurate CNV analysis of WES and WGS data.

References:
Grants:

Conflict of Interest: None declared.

EP09.010 OrphaID: a new platform for rare intellectual disabilities in Orphanet in partnership with ERN-ITHACA

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Background/Objectives: Intellectual disability (ID) is a group of heterogenous disorders affecting up to 3% of worldwide

population and characterized by significant impairment in cognition and behavior which can be associated with other syndromic or dysmorphic features. The heterogeneity of intellectual disabilities and rarity of most forms render the genetic diagnosis challenging in most cases. Hence, stemmed a need for developing a comprehensive list of curated intellectual disability related genes and associated phenotypes.

Methods: Orphanet, which is the largest portal for rare diseases in the world has partnered with the European Reference Network on Rare Congenital Malformations, Autism and Rare Intellectual Disability (ERN-ITHACA) in order to develop a platform (OrphaID) for curated intellectual disability related genes and phenotypes. This list of intellectual disability-related genes is curated in partnership with ERN-ITHACA and SysID/SysNDD (www.sysid.dbmr.unibe.ch).

Results: OrphaID currently contains 728 Orpha-coded ID phenotypes linked to 1385 curated ID genes. This will allow systematic and comprehensive access to and retrieval of ID specific information within Orphanet. The list of OrphaID entries is linked to both internal (e.g. genes information, classification, HPO signs) and external resources (e.g. SysNDD, OMIM). It can be searched or filtered by gene, disease or OMIM number and the results can be downloaded in different file formats (excel, csv and txt).

Conclusion: OrphaID is a valuable tool for all users (patients, researchers and clinicians) with interest in rare intellectual disabilities. The new platform will be publicly available on Orphanet's webpage by March 2022.

References:

Grants:

Conflict of Interest: None declared.

EP09.011 Study of IL6 and TNF genes polymorphisms as a risk factor for the development of early neurological disorders and subsequent consequences in neonates

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Background/Objectives: Intraventricular hemorrhage (IVH) and hypoxic-ischemic encephalopathy (HIE) are the most common types of early neurological disorders which are detectable by neuroimaging in newborns with perinatal asphyxia. They might be reversible, but require adequate and early treatment, otherwise their most serious consequence is cerebral palsy (CP). IVH and HIE are activating immune system and inflammation mediators like interleukin 6 (IL6) and tumor necrosis factor (TNF). Inflamation and neurodegeneration could contribute to development of CP. Aim of this study was to examine association between *IL6* and *TNF* polymorphisms with the development of early neurological disorders and CP in children with perinatal asphyxia.

Methods: Study included 167 patients aged 1 to 16 years with perinatal asphyxia. Clinical evaluation and neuroimaging (ultrasound, magnetic resonance) were performed for all subjects. *IL6* and *TNF* polymorphisms were genotyped using rs1800795 and rs1800629 TaqMan assays.

Results: Frequencies of *IL6* polymorphisms were statistically significantly different between groups with early neurological disorders and without them (43.4% GG, 48.2% GC, 8.4% CC, versus 26.5% GG, 54.2% GC, 19.3% CC, respectively) ($p = 0.027$). GG+GC genotypes were significantly more frequent in patients with early neurological disorders ($p = 0.043$, OR = 0.386, 95%CI 0.150-0.994). There was statistical significant difference in genotype distribution of *TNF* polymorphism in CP onset. AA+GA genotype was more frequent in patients who developed CP ($p = 0.040$, OR = 0.425 95%CI 0.185-0.974).

Conclusion: *IL6* gene polymorphism could be a risk factor for early neurological disorders which require tretment, while *TNF* polymorphism may be predictor for CP.

References:

Grants:

Conflict of Interest: None declared.

EP09.012 CCNK-intellectual developmental disorder with hypertelorism and distinctive facial dysmorphism - the impact of cyclins in neurodevelopment

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Background/Objectives: Cyclin K (*CCNK*) integrates the same cyclin-dependent kinase complex as CDK13, which plays a role in early brain development. Variants in *CCNK* gene are associated with autosomal dominant intellectual developmental disorder with hypertelorism and distinctive facies (*OMIM#618147*). We are aware of at least 4 patients reported in the literature, 3 bearing microdeletions in 14q32.2 involving *CCNK* gene, and 1 with a missense variant, all *de novo*. The condition is characterized by developmental delay (DD) and typical dysmorphism.

Methods: We describe a 3-year-old girl, who is the only child of non-consanguineous parents with no relevant family history. At birth, she was diagnosed with a thin patent ductus arteriosus and pulmonary valve dysplasia with mild stenosis. She evolved with mild DD and mild cerebellar vermis hypoplasia. On physical examination, she presented glabellar hemangioma, high anterior hairline, hypertelorism, flattened nasal root, low-set and posteriorly rotated ears, short columella, thin upper vermilion, narrow jaw and a deep sacrococcygeal pit. ArrayCGH was normal. Sequencing of *CDK13* gene found no relevant variant.

Results: Whole exome sequencing identified a heterozygous missense variant of uncertain significance in *CCNK* gene: c.556T>C, p.(Trp186Arg). De novo origin was confirmed by parental analysis.

Conclusion: Our patient's facial phenotype is consistent with the few cases previously reported in the literature. Similar to the only other case caused by a missense variant, DD is milder compared to deletions, corroborating haploinsufficiency as a pathogenetic mechanism. It overlaps with *CDK13*-ID and shares the same episignature, highlighting their close interaction.

References:

Grants:

Conflict of Interest: None declared.

EP09.013 Pathogenic variants in nucleoporin TPR (translocated promoter region, nuclear basket protein) cause severe intellectual disability in humans

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Background/Objectives: The nuclear pore complex (NPC) is a multi-protein complex that regulates the trafficking of macromolecules between the nucleus and cytoplasm. Genetic variants in components of the NPC have been shown to cause a range of neurological disorders, including intellectual disability and microcephaly. Here we present two siblings who present with a phenotype of microcephaly, ataxia and severe intellectual disability. Whole Genome Sequencing (WGS) identified biallelic variants in TPR and we undertook functional genomics analysis to evaluate pathogenicity.

Methods: WGS and RNAseq were used to search for pathogenic variants, and identified candidate variants in TPR. Functional analysis in patient fibroblasts evaluated pathogenicity of TPR variants. We analysed protein levels by western blot and nuclear pore density and RNA intensity in the nucleus by immunostaining and confocal microscopy.

Results: The variants result in a premature truncation variant (c.6625C>T; p.Arg2209*), and a splice variant (c.2610+5G>A) leading to a 12-amino acid deletion respectively. Functional analyses in patient fibroblasts demonstrate significantly reduced TPR levels by western blot, decreased colocalization of TPR to NPCs, and decreased TPR-containing NPC density. A compensatory increase in total NPC levels was observed, whilst decreased global RNA intensity was apparent in the nucleus.

Conclusion: The discovery of variants that partly disable TPR function provide valuable insight into this protein which is essential for neurodevelopment, and our findings show that TPR variants are the cause of the siblings' neurological disorder.

References: Van Bergen et al. (2021). Human Molecular Genetics; 31 (3): 362–375.

Grants: Royal Children's Hospital Foundation and NHMRC.

Conflict of Interest: None declared.

EP09.014 A novel SMG9 variant enriched in the Finnish population causes intellectual disability

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Background/Objectives: The *SMG9* gene encodes a regulatory subunit of nonsense-mediated mRNA decay (NMD) machinery. We identified five patients from three families with a similar intellectual disability (ID) and an identical homozygous *SMG9* variant.

Methods: Using exome sequencing, a novel *SMG9* variant was identified. RNA sequencing of patients and age- and sex-matched healthy controls was used to assess the effect of the variant.

Results: The homozygous variant *SMG9* (NM_019108.4) c.551T>C p.(Val184Ala) segregated recessively in each family. Characteristic clinical findings in patients were mild to moderate ID, intention tremor, pyramidal signs, dyspraxia, and ocular manifestations. Allele-specific expression analysis did not provide evidence that the nonsense mRNA-induced NMD was affected. Differential gene expression analysis identified a prevalent upregulation of genes in patients, including the genes *SMOX, OSBP2, GPX3*, and *ZNF155*. These findings suggest that normal SMG9 function may be involved in transcriptional regulation without affecting nonsense mRNA-induced NMD.

Conclusion: We demonstrate that the *SMG9* c.551T>C variant causes a neurodevelopmental disorder and impacts gene expression. NMD components have roles beyond aberrant mRNA degradation that are crucial for neurocognitive development.

References: Eur J Hum Genet 2022 https://doi.org/10.1038/s41431-022-01046-5.

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EP09.015 Mutation analysis of NSD1 gene in a cohort of patients with clinical suspicion of Sotos Syndrome: a 18-year single center experience

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Background/Objectives: Sotos Syndrome (SoS) (OMIM #117550) is a rare genetic disorder whose prevalence is estimated to be

1:14,000 live births (1). It is caused by haploinsufficiency of NSD1 (nuclear receptor-bindingSETdomain-containing protein1) gene. Most cases are sporadic with few familial cases reported. Approximately 90% of patients bear heterozygous mutation in NSD1 gene or deletions in the 5q35 region (2).

Affected children exhibit distinctive facial features, learning disability and overgrowth (height and/or head circumference ≥98th percentile) overlapping with few others conditions. No clinical diagnostic consensus criteria are still published for SoS and molecular analysis of the NSD1 gene reduced clinical diagnostic uncertainty.

Methods: Using DHPLC, Sanger sequencing, NGS and MLPA on negative samples, we screened NSD1 gene on 1530 unrelated patients enrolled from 2003 to 2021 at the Human Genetics Laboratory of Gaslini Institute of Genova.

Results: NSD1 variants were identified in 292 patients, including 9 partial gene deletions, 13 microdeletions of the entire NSD1 gene and 115 novel intragenic variants never previously described. Nine patients resulted carriers of a mutation in a different gene from NSD1 out of 239 patients analyzed through NGS.

Our approach allowed molecular confirmation for 292 patients with suspected SoS and the formulation of a differential molecular diagnosis in 9 patients of our cohort.

Conclusion: This study broadens the molecular heterogeneity of NSD1variants in Italy, improving the genotype/phenotype correlation for a more appropriate genetic counseling.

References: 1) Kurotaki N et al., 2002.
2) Tatton-Brown K et al., 2005.

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Conflict of Interest: None declared.

EP09.016 Deep intronic de novo germline EHMT1 variant in a patient with syndromic developmental delay

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Background/Objectives: Heterozygous pathogenic variants in the EHMT1 gene cause Kleefstra syndrome 1 (OMIM # 610253), which is characterized by intellectual disability with severe expressive language delay, distinctive facial features and additional abnormalities, including organ malformations, seizures, and psychiatric disorders.

We present a six-year-old girl with developmental delay with prominent speech involvement and dysmorphic facial features that correlate well with Kleefstra syndrome.

Methods: Trio whole exome sequencing (WES) was performed on leukocyte-derived DNA samples after enrichment with Human Core Exome and RefSeq kits (Twist Bioscience) using a NovaSeq 6000 sequencer (Illumina). Alignment to the reference genome was done using the Burrow Wheeler Aligner software, and variant calling was performed according to the GATK best practice guidelines.

Results: Variant prioritization using phenotype data revealed a heterozygous deep intronic variant (NG_011776.1:c.2712+1866G>A) in EHMT1; no other (likely) pathogenic variant was identified. The variant positon was covered in 90 sequencing reads in the index; however, it was covered by only two and zero reads in the father and mother, respectively. Using Sanger sequencing, the variant was shown to be absent in both parents and confirmed in the girl. Hence, it is highly likely that it occurred de novo in the patient. In silico tools predict that this variant generates a new splice site. To analyze possible splice effects, a transcript-analysis using the patient's cDNA is currently in progress.

Conclusion: Our findings stress the importance of deep intronic variants and highlight this pitfall of WES which can be overcome systematically by e.g. whole genome sequencing.

References:

Grants:

Conflict of Interest: None declared.

EP09.017 Widening of the mutation spectrum in KCNK9 gene for the Birk Barel syndrome

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Background/Objectives: Birk Barel syndrome (BBS) is rarely described imprinting disorder with indistinguishable clinical picture. To date, only 22 molecular diagnosed patients were described in literature, of which 20 patients share the same pathogenic missense variant p.Gly236Arg in maternally expressed KCNK9 gene. Only recently, two new patients with a novel missense mutation p.Ala237Asp and a ring chromosome 8 have expended the genetic cause of this syndrome. Here we report an additional nucleotide variant in KCNK9 gene detected in a 16-year old boy with autism spectrum disorder (ASD).

Methods: WES was performed in a cohort of 148 Slovenian children with suspected ASD. A genotype-phenotype driven analyses was performed to identify a rare variants in 1150 autism associated genes.

Results: Among analysed genes, only one variant was detected in KCNK9 gene, de novo missense c.392G>A mutation, causing the amino-acid exchange p.Arg131His in its protein product. A clinical re-examination of the patient confirmed that the boy had BBS.

Conclusion: In 16-year old boy with intellectual disability, ASD, scoliosis, long face, tented upper lip vermilion, cleft palate, strabismus, and single transverse palmar crease, a clinical diagnosis of BBS was established after determination of de novo missense mutation c.392G>A in KCNK9 gene. This variant was also previously described in one patient from a large study of 2000 patients with suspected genetic disorder but without its clinical characterization and in 5 patients in ClinVar database. Therefore, the role c.392G>A variant as possible new mutational hotspots in KCNK9 gene for BBS is further discussed.

References:

Grants:

Conflict of Interest: None declared.

EP09.018 An inherited loss of function variant in SPTAN1; support of reduced penetrance?

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Background/Objectives: Pathogenic variants in *SPTAN1* are associated with a broad phenotypic spectrum from epileptic encephalopathy and severe developmental delay to mild intellectual disability, some of whom have ataxia and/or spasticity. Most reported variants are missense variants.

Only few loss of function variants in *SPTAN1* are reported – some have familial juvenile onset hereditary motor neuropathy with reduced penetrance, and some have de novo variants with variable neurodevelopmental phenotype. We present a patient with a paternally inherited nonsense mutation in *SPTAN1*, thus adding to the knowledge of the phenotype of *SPTAN1* loss of function variants.

Methods: Whole Exome Sequencing (WES) of patient and parents followed by trio analysis for filtering causal variants. Sanger sequencing for segregation analysis.

Results: The patient was an 11 years old boy with juvenile onset of ataxia, cerebellar atrophy, hypotonia, dysarthria, mild spasticity of the lower extremities, and learning disability.

A heterozygous nonsense variant SPTAN1:c.710G>A p.(Trp237*), predicted to result in nonsense mediated decay, was identified. It was present in the father and paternal grandmother, who both were considered healthy.

Conclusion: The consequences of loss of function variants in *SPTAN1* are not well described, even if hereditary motor neuropathy seems to be a recurrent feature in some families exhibiting reduced penetrance. The phenotype of our patient with an inherited loss of function variant overlaps with that of some patients with missense variants in *SPTAN1*. Our case might add to the knowledge of the phenotypic spectrum of *SPTAN1*-associated disease, and if so, it supports the existence of reduced penetrance for loss of function variants.

References:

Grants:

Conflict of Interest: None declared.

EP09.019 Interactive tools for evaluating attention capacities in patients with severe intellectual disability

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Background/Objectives: Intellectual disability (ID) affects approximately 2% of general population. Within reference center "intellectual disabilities from rare causes", a consultation dedicated to ID adults has been developed since 2005 with different objectives: diagnosis, genetic counseling, treatment, follow-up, phenotypic characterization, natural evolution, research and participation in therapeutic trials. We have been involved in international, multicenter therapeutic trials in Fragile-X syndrome which did not show any benefit on all evaluation criteria used, which leads questions on their relevance… Criteria used are most often behavior scales filled in by caregiver with important subjective interpretation. We realized obvious lack of objective criteria which could be used as primary or secondary endpoints in neurodevelopmental disorders. By definition, a primary endpoint useful to demonstrate efficacy of the treatment studied must validate different conditions and allow calculation number of subjects necessary to obtain statistically significant results.

Methods: We have thus developed and validated in collaboration with PRISME platform (IHU-A-ICM, Brain and Spine Institute), interactive, quantitative tools to assess attention capacities in patients with ID of different degrees (IQ < 70 but often < 50), with no language for some of them, consisting on playful tests on touch screens for a manual response or using eye tracking for patients who have motor disorders that do not allow use of their hands.

Results: We'll present results obtained in cohort of patient with ID of different severity.

Conclusion: Those tools could serve as quantitative attention evaluation criteria in therapeutic trials and as rehabilitation tools for patients.

References:

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Conflict of Interest: None declared.

EP09.020 Genotype-phenotype functional correlation in KCNB1-related epilepsies

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Background/Objectives: Pathogenic variants in the *KCNB1* gene, encoding the voltage-gated K^+ channel α-subunit, are associated with a spectrum of phenotypes ranging from developmental and epileptic encephalopathies (DEE) to mild intellectual disability without epilepsy. KCNB1 exerts an electrical role in neurons and mutations may cause changes in its biophysical properties. We evaluated the functional effect of 6 different variants in *KCNB1* and correlated the results with the electro-clinical phenotype of patients.

Methods: *KCNB1* variants were identified through Next Generation Sequencing. Mutants were expressed in HEK293 cells and membrane currents were evaluated by patch-clamp technique in whole-cell configuration. Patients were deep-phenotyped through clinical charts collected from referring clinicians.

Results: We identified 6 *KCNB1* variants: p.T210M; p.R293C; p.A406V; p.F416L; p.P784L; p.T804A (5 de novo, p.T804A inherited from the affected mother). The T804A mutant showed to stay open at >+50 mV potentials, whereas the R293C mutant showed a different voltage dependence and a modified reversal potential as compared to the wild-type (WT) channel ($p = 9.35e\text{-}6$). These variants were found in 10 patients with focal epilepsy with mild

intellectual disability. The P784L mutant (1 patient with DEE) showed biophysical properties comparable to that of the WT channel. The remaining mutants showed loss of the ion current conduction and were found in 48 patients with DEE.

Conclusion: *KCNB1* mutations may impact patients' phenotypes depending on the functional effect on the channel. Functional data suggested that P784L may not be responsible for the severe phenotype of the patient, confirming that electrophysiological experiments are pivotal in validating disease-causing variants.

References:

Grants:

Conflict of Interest: None declared.

EP09.021 PSMD12 haploinsufficiency is not simply a neurodevelopmental disorder

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Background/Objectives: Syndromic neurodevelopmental disorders (NDDs) are mostly due to de novo variants in coding DNA, disrupting gene function. PSMD12 encodes a subunit of the proteasome and is involved in ubiquitination, thus in cellular homeostasis, brain development and interferon-mediated inflammation. PSMD12 haploinsufficiency is associated to autosomal dominant Stankiewicz-Isidor Syndrome (STIS). Up to now, 45 subjects have been described, including two families.

Methods: Detailed clinical history. X-Fragile test and SNP-array for affected members; familial WES analysis, standard filtering procedures with selection of pathogenic variants. Ongoing analysis of interferon-1 activity.

Results: We report six members (median age 29 years, 3 males) within two unrelated, Italian families with STIS due to a known (p.Arg289*) and a novel (p.Tyr111*) variant in PSMD12. They all had global developmental delay, intellectual disability (1 moderate, 5 mild), dysmorphisms and skeletal anomalies. In family 1, all individuals exhibited a good sociality, while in family 2, siblings showed apathy or irritability. 5/6 subjects developed obesity. Diffuse acne was present in 4/6. Congenital anomalies included interventricular defect (1/6) and cryptorchidism (1/6). Two subjects had strabismus, and one had a hearing loss. One of the subjects had an ovaric teratoma and presented oligodontia. In a single case there was a unilateral, ectopic nail within thumb. None presented specific autoinflammatory disorder.

Conclusion: We described two novel familiar cases of STIS, documenting intrafamilial variability. We expand the phenotype of pre-axial anomalies. We assume to prove an altered interferon signature due to PSMD12 haploinsufficiency.

References:PMIDs: 28132691; 30421579; 34906456; 35080150;.

Grants: NA

Conflict of Interest: None declared.

EP09.022 Potocki-Lupski Mimicking Dubowitz Syndrome: the Importance of Molecular Methodological Approach

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Background/Objectives: Neurodevelopmental disorders are conditions characterized by cognitive impairment, that can be associated with a wide spectrum of signs and symptoms. The etiopathogenesis of these disorders represents a challenge for clinicians and molecular investigations usually play a pivotal role in obtaining the diagnosis.

Methods: We examined a patient presenting with short stature, microcephaly, bitemporal narrowing, mild micrognathia, ear lobe crease, downslanting palpebral fissures, bilateral ptosis, thinning of the lateral third of eyebrows, thin lower lip and flattening of the Cupid's bow. At 4 months, he was referred to a geneticist due to growth retardation, hypospadias, unilateral cryptorchidism, and eczema. These characteristics lead to suspicion of Dubowitz syndrome. Standard karyotype was normal (46, XY). At 33, the man underwent genetic counselling again in order to formulate recurrent risk for his sister's pregnancy. We requested Chromosomal Microarray Analysis (CMA) on the patient's blood sample.

Results: CMA detected a constitutional *de novo* 17p11.2 triplication of 851 Kb (arr[GRCh38] 17p11.2(16,854,250-17,705,309)) encompassing three Morbid genes: *TNFRSF13B*, *FLCN* and partially *RAI1*. The triplication overlaps with the critical region of Smith-Magenis syndrome (17p11.2 microdeletion) and Potocki-Lupski syndrome (17p11.2 microduplication). These two conditions entail cognitive impairment, facial dysmorphisms and other structural anomalies. Intrachromosomal microtriplications are rare copy number variants (CNVs), not always associated with a recognizable phenotype. The patient we described resembled typical features of Potocki-Lupski syndrome.

Conclusion: Chromosomal microtriplications are CNVs of rare occurrence, with complex genotype-phenotype correlations. The present case is the first patient detected with 17p11.2 triplication resembling the features of Potocki-Lupski syndrome.

References:

Grants:

Conflict of Interest: None declared.

EP09.023 A small supernumerary marker chromosome resulting from a balanced maternal translocation and leading to partial trisomy of 13q12-q21.1 and 3p26.3 in a child with syndromic intellectual disability

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Background/Objectives: Small supernumerary marker chromosomes (sSMCs) are rare structural abnormalities in the population; however, they are frequently found in patients with developmental disorders. SMCs are usually observed first by karyotype, and further analysis of their molecular origin is important in clinical practice. We report a case of a sSMC characterized by array-CGH in a 2-year-old boy with neurological disorders with an aim to establish genotype-phenotype correlations. Clinical examination revealed growth retardation, facial dysmorphism, and abnormalities of the extremities. We noted the absence of consanguinity, but rather the occurrence of several spontaneous miscarriages.

Methods: GTG-banding Karyotype carried out on peripheral blood lymphocytes, array-CGH and FISH analysis using Whole Chromosome Painting probes were performed.

Results: Karyotype showed the presence of a homogeneous sSMC. The paternal karyotype was normal, while the maternal karyotype revealed the presence of a balanced (3;13)

translocation. Array-CGH analysis showed a gain of 35Mb and 944Kb in 13q12.11-q21.1 and in 3p26.3, respectively. FISH analysis confirmed the origin of sSCM from chromosomes 3 and 13. Thus, we conclude that the sSMC derived from a 3:1 segregation of a maternal balanced t(3;13)(p26;q12) translocation and resulting in partial trisomy of 13q12.11-q21.1 and 3p26.3 as showed by array-CGH.

Conclusion: Molecular characterization of sSMCs by array-CGH is essential to establish genotype-phenotype correlations leading to an appropriate genetic counseling. Our observation underlines also the importance of analyzing karyotypes of couples with a history of repeated spontaneous miscarriages, in search of a balanced parental chromosomal rearrangement which will be transmitted in an unbalanced manner to the offspring.

References: https://doi.org/10.1159/000106438.

Grants: no.

Conflict of Interest: None declared.

EP09.024 Whole DDX3X gene deletion in a female patient with DDX3X-related Neurodevelopmental Disorder

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Background/Objectives: De novo germline pathogenic variants in *DDX3X* gene account for 1–3% of unexplained intellectual disability (ID) in females (MIM# 300958), with an X-linked pattern of inheritance. Most affected patients are females with *de novo* variants, occasionally some males have been reported with hypomorphic inherited pathogenic variants. The associated phenotype is clinically variable, including ID, behaviour problems, minor dysmorphic features and various others manifestations. *De novo* nonsense, frameshift, splice site or missense mutations have been reported in *DDX3X* gene, suggesting a disease-causing mechanism via haploinsufficiency.

Here we describe for the first time a whole *DDX3X* deletion.

Methods: We evaluated a 17-years-old female patient, characterized by mild-to-moderate ID, long and and hypotonic facies, strabismus, recurrent otitis, primary autoimmune hypothyroidism and precocious puberty. No remarkable alteration on brain MRI or Electroencephalogram were detected.

Karyotipe, aCGH (60K) and subtelomeric MLPA were normal.

The patient underwent WES analysis. Variant interpretation was realized on candidate genes based on HPO prioritization.

Results: A whole *DDX3X* gene deletion was detected (chrX:41193507-41206972, hg19), confirmed by digital PCR. This variant is neither reported in HGMD professional nor in ClinVar. The absence of this gross deletion in the healthy mother is likely indicative of a *de novo* inheritance (analysis on patient`s father was unavailable), consistent with the hypothesis that haploinsufficiency at this locus on the X-chromosome is likely lethal in males.

Conclusion: We report a new case of *DDX3X*-related Neurodevelopmental Disorder expanding the knowledge about phenotypic spectrum and describe for the first time a likely *de novo* whole gene deletion in a female patient.

References:

Grants:

Conflict of Interest: None declared.

EP09.025 An inherited case of 3q29 microdeletion: the unlikely story of a diagnosis

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Background/Objectives: 3q29 microdeletion is a rare autosomal dominant disorder with heterogeneous presentation and variable penetrance of medical, neurodevelopmental and neuropsychiatric phenotypes. Typically, this microdeletion occurs *de novo* and, when inherited, the transmitting parent is affected to some degree. We describe a novel case, initially suspected by conventional karyotyping, inherited from an apparently unaffected mother.

Methods: A 5-year-old boy was referred to our Genetics Department for global developmental delay and behavioral problems. His father had psychiatric pathology, his mother was healthy and a 12-year-old brother had learning difficulties. The karyotype showed a chromosome 3 of atypical conformation. Further studies were performed by *a*CGH (180K-CGX-HD). Segregation was investigated by karyotype and aCGH.

Results: aCGH revealed a ~1.56 Mb microdeletion overlapping recurrent 3q29 syndromic region.

Parental karyotyping showed that the mother also presented an atypical 3qter. aCGH confirmed the 3q29 terminal microdeletion to be maternally inherited. The brother will be studied next.

Conclusion: G-banding karyotype resolution is in the threshold of 5-10Mb. Nevertheless, in this case karyotyping led to the first suggestion of a 3qter abnormality in the proband and in his mother. To our knowledge there are no previous studies reporting such a small deletion suspected in the karyotype.

In the rare inherited cases of 3q29 microdeletion, the overwhelming majority of parents are affected at some level. Penetrance for this deletion is not fully established. However, data from the literature combined with our report of a 3q29 microdeletion carrier unaffected mother, suggests that while penetrance is high, it is likely not 100%.

References:

Grants:

Conflict of Interest: None declared.

EP09.026 Clinical and genetic features of X-linked mental retardation 102 type caused by mutations in the DDX3X gene in adolescent-girl

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Background/Objectives: Mutations in DDX3X are known to cause a characteristic neurodevelopmental disorder in female patients. Analysis of the clinical and genetic features in adolescent-girl with mental retardation due to mutations in DDX3X.

Methods: In this study we performed whole genome sequencing of patient (girl, 10 y.o.) by high-throughput sequencing (NGS) on DNBSEQ-G400, BGISEQ-500 and T7 platforms with

the reading of 75 billion nucleotides, conclusion according to ACMG recommendations.

Results: Girl was born from 2 pregnancy, the 1pregnancy ended intrauterine death of female fetus. In the first year of life, there was a delay in psychomotor and speech development. The physical examination of the patient revealed delayed speech development, elongated faces, high forehead, lagophthalmos, upturned nose, wide filter, 2-4 finger syndactyly, Hallux valgus, scoliosis, high growth (SDS +2.1) and early sexual development (Tanner III).The patient has learning difficulties, is being monitored by a psychiatrist with an autism spectrum disorder. A variant of rs1569238002, not previously described in the literature, was found in a heterozygous state in exon 8 of 19 of the DDX3X gene, leading to a synonymous replacement of p.Gly248Gly.

Conclusion: The results obtained may testify in favor of the existence of a dependence of the severity of the phenotype on the localization and nature of mutations in the gene and determine the relevance of further research aimed at searching for clinical and genetic correlations.

References: Dadali E.L. et al. Clinical and genetic characteristics of X-linked mental retardation 102 type caused by novel mutations in the DDX3X gene. Neur Dis. 2020; 10;75–80.

Grants: None.

Conflict of Interest: None declared.

EP09.027 A recurrent de novo mutation in ZMYND11 associated with global developmental delay genocopy the 10p15.3 deletion syndrome: a case report

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Background/Objectives: A 7-year-old patient initially diagnosed as Silver-Russel syndrome due to mosaicism of chromosome 7 returns to our genetic consultation for diagnostic re-evaluation. Characteristics symptoms are global developmental delay (84%), hypotonia, feeding difficulties, short stature and several facial dysmorphisms (microcephaly, depressed nasal bridge and microretrognathia).

Methods: Karyotype and CGH array were performed. MLPA assay (MS-MLPA Probemix ME032 DUP7-DUP14) was also done to test if there was maternal uniparental disomy of chromosome 7 (DUP7). Clinical exome was sequenced by Human Whole-Genome Sequencing with the Nextera™-DNA-Flex-Library Preparation Kit (Illumina).

Results: Karyotype and CGH array were normal. MLPA assay revealed absence of DUP7. The analysis of the clinical exome showed a heterozygous autosomal dominant missense variant: c.1798C>T p.(Arg600Trp) in *ZMYND11* (NM_006624.5), classified according the ACMG guidelines as pathogenic. Genetic testing of both parents showed it was arisen *de novo*.

Conclusion: Zinc finger MYND-type, expressed in many human tissues, acts as a transcriptional repressor, playing an inhibitory role in the muscle and neuronal differentiation steps. Specifically, the mutated position in this case Arg600 is very conserved and essential for its binding to ligands (Kateb et al.,2013). *ZMYND11* has also been proposed as a candidate gene for 10p15.3 microdeletion syndrome, which shares common clinical features with our patient (DeScipio, 2021). Moreover, other authors have described the same mutation (Cobben,2014), so that it can be considered as definitely pathogenic. Finally, the same SNP has been reported 4 times in Decipher and 8 times in ClinVar thus the variant here reported could be a *hotspot* mutation.

References:

Grants:

Conflict of Interest: None declared.

EP09.028 A novel intragenic deletion in oligophrenin-1 features in a mother, her two sons, and one daughter with intellectual disability, retrocerebellar arachnoid cysts, and neuropsychiatric disorders, but without cerebellar hypoplasia

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Background/Objectives: OPHN1 gene mutations can cause X-linked mental retardation (XLMR), cerebellar hypoplasia, and facial dysmorphism. A few cases of symptomatic females carrying a novel OPHN1 intragenic deletion have been reported. we report a Saudi family in which a mother, one daughter, and two sons were affected by this variant.

Methods: 19-year-old boy presented with attention-deficit/hyperactivity disorder, severe mental retardation, facial dysmorphism, mild cerebral volume loss, and retrocerebellar arachnoid cyst. Whole exome sequencing identified the hemizygous likely pathogenic variant in OPHN1c.1105-13_1109del, consistent with the diagnosis of XLMR with cerebellar hypoplasia and distinctive facial appearance. The family was screened and his mother, sister, and brother were found to be carriers.

Results: The mother had mild mental retardation, depression, and schizoaffective personality disorders. Her sons, 19 and 14 years of age, had attention-deficit/hyperactivity disorder, severe mental retardation, facial dysmorphism, mild cerebral volume loss, and retrocerebellar arachnoid cyst. The 7-year-old daughter shared the same phenotype as her brothers but with mild mental retardation and thinning of corpus collosum. No seizure or cerebellar hypoplasia were noted in any of them.

Conclusion: The phenotype in this family with two severely affected boys and two females with milder forms, was suggestive of XLMR. This report confirms previous findings that women carrying OPHN1 c.1105-13_1109del may be symptomatic and mildly affected by facial dysmorphic features and mild cognitive delay, with or without abnormal findings on brain imaging.

References: l-Owain M, et al. Novel intragenic deletion in OPHN1 in a family causing XLMR with cerebellar hypoplasia and distinctive facial appearance. Clin Genet. 2011;79(4):363-370. https://doi.org/10.1111/j.1399-0004.2010.01462.x.

Grants: None.

Conflict of Interest: None declared.

EP09.029 Kleefstra syndrome does not always occur de novo: A family report

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Background/Objectives: Kleefstra syndrome is an autosomal dominant neurodevelopmental disorder characterized by delayed psychomotor development, intellectual disability and dysmorphic features. In this syndrome, mostly *de novo* variants are identified in *EHMT1* and *KMT2C* genes. Here we present an exceptional family

including a mother and two daughters diagnosed with Kleefstra syndrome 2 associated with a novel missense variant in *KMT2C* gene.

Methods: Two sisters sharing the same mother but different fathers, with the age of 5 and 9, were evaluated due to their mild intellectual disability, speech delay, and articulation problems. The older sibling also had aggressive and self-injurious behavior. Additionally, the intellectual capacity and problem-solving skills of the 40-year-old mother were limited. The mother and daughters had similar facial dysmorphic features, which are compatible with Kleefstra syndrome. Etiological evaluations were studied by chromosomal microarray (CMA) analyses in both sisters and clinical exome sequencing (CES) analysis in the younger sister.

Results: CMA analyses were normal and CES analysis revealed a heterozygous c.584A>G (p.Gln195Arg) variant in *KMT2C* (NM_170606) gene. Sanger sequencing confirmed the variant as heterozygous also in the mother and the elder sister.

Conclusion: *De novo* variants of *EHMT1* and *KMT2C* genes are identified in most patients with Kleefstra syndrome. It is thought that the reproductive fitness of this disease is zero, as in most autosomal dominant neurodevelopmental disorders developing by *de novo* variants. Along with another example of paternal transmission in the literature, this family shows that Kleefstra syndrome 2 with mild intellectual disability may be familial.

References:

Grants:

Conflict of Interest: None declared.

EP09.030 Case report of rare 3q27.1 microdeletion syndrome for patient with dysmorphic features, growth retardation and mild developmental delay

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Background/Objectives: Constitutional rearrangements involving chromosome 3q are very rare and overlapping microdeletions of chromosome 3q26-3q28 have only been reported in ten individuals with severe prenatal and postnatal growth restriction and neurodevelopmental abnormalities.

Methods: The proband is 2-year-old female who was born at 38 weeks of gestation, birth weight 2060 g (-2.88 SD). Severe Intrauterine growth restriction was noticed from the begging of 3rd trimester. She failured to thrive during infancy with normal motor development. At the clinical visit her height was 79 cm (-2.44 SD), weight 8 kg (-4.26 SD). The hormonal assessment was performed and normal levels of IGF-1, TSH, FT4, basal cortisol levels were detected. Dysmorphic facial features were noticed: hyperthelorism, high anterior hairline, frontal bossing, depressed nasal bridge, short nose with anteverted nares, low set ears. Developmental delay was assed by scale of Diagnostic inventory for screening children, 1984 (DISC). Test value was 50-77%, especially language delay with feeding difficulties.

Results: Whole Genome NGS-based Large Copy Number Variation Analysis (*Centogene*, Germany) was performed and 867 kb one copy loss within chromosome region 3q27.1 was detected. The deletion covered 21 genes and 9 of them were OMIM-associated with disease, particularly *DVL3, AP2M1* and *PARL* genes. Frameshift variants in DVL3 gene are associated with autosomal dominant type III Robinow syndrome (MIM#: 616894). *AP2M1* (MIM#: 601024) has role in poor speech, developmental delay, hypotonia and seizures. *PARL* (MIM#: 607858) possibly associated with growth restriction.

Conclusion: We suggest that haploinsufficiency of *DVL3, AP2M1* and *PARL* genes can cause 3q27.1 microdeletion phenotype.

References:

Grants:

Conflict of Interest: None declared.

EP09.031 DNA methylation signature associated with Bohring-Opitz syndrome: A new tool for functional classification of variants in ASXL genes

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Background/Objectives: The additional sex combs-like (ASXL) gene family—encoded by *ASXL1*, *ASXL2*, and *ASXL3*—is crucial for mammalian development. Pathogenic variants in *ASXL* genes are associated with three phenotypically distinct neurodevelopmental syndromes. Our previous work has shown that syndromic conditions caused by pathogenic variants in epigenetic regulatory genes show consistent patterns of genome-wide DNA methylation (DNAm) alterations (DNAm signature) in peripheral blood. Given the role of ASXL1 in chromatin modification, we hypothesized that pathogenic *ASXL1* variants underlying Bohring-Opitz syndrome (BOS) have a unique DNAm signature.

Methods: We profiled whole-blood DNAm for 17 *ASXL1* variants, and 35 sex- and age-matched typically developing individuals, using Illumina's Infinium EPIC array.

Results: We identified 763 differentially methylated CpG sites in individuals with BOS. Differentially methylated sites overlapped 323 unique genes, including *HOXA5* and *HOXB4*, supporting the functional relevance of DNAm signatures. We used a machine-learning classification model based on the BOS DNAm signature to classify variants of uncertain significance in *ASXL1*, as well as pathogenic *ASXL2* and *ASXL3* variants. The DNAm profile of one individual with the *ASXL2* variant was BOS-like, whereas the DNAm profiles of three individuals with *ASXL3* variants were control-like. We also used Horvath's epigenetic clock, which showed acceleration in DNAm age in individuals with pathogenic *ASXL1* variants, and the individual with the pathogenic *ASXL2* variant, but not in individuals with *ASXL3* variants.

Conclusion: These studies enhance our understanding of the epigenetic dysregulation underpinning *ASXL* gene family associated syndromes.

References:

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Conflict of Interest: None declared.

EP09.032 MECP2 duplication syndrome: report of an affected female

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Background/Objectives: *MECP2* duplication syndrome also known as Intellectual developmental disorder, X-linked syndromic,

Lubs type, is a neurodevelopmental disorder characterized by early-onset hypotonia, delayed psychomotor development leading to severe intellectual disability, poor speech development, mild dysmorphic features, seizures, progressive spasticity, recurrent infections, autistic features, feeding difficulty and gastrointestinal manifestations[1,2].

This syndrome has complete penetrance in males. Female carriers may have a mild phenotype, as neuropsychiatric features. Due to the skewing of X inactivation against the duplicated X chromosome, females are rarely affected. However, in rare cases, females have the same severe clinical findings as males[3]. Reviewing the literature, at least 23 affected females were reported[4].

We report a case of a 40 years old female with severe intellectual disability, epilepsy, facial dysmorphism, morbid obesity and mild strabismus. She has two sisters with overlapping phenotype and a female cousin with intellectual disability.

Methods: Array CGH was performed with the Affymetrix Cytoscan 750K. The results were analyzed with ChAS software.

Results: We found a duplication from the Xq28 region of 334 Kb, in the genomic coordinates 153254852-153588530 (GRCh37), involving the *IRAK1, MECP2, OPN1LW, OPN1MW2, OPN1MW, OPN1MW3, TEX28, TKTL1* and *FLNA* protein coding genes.

Array CGH analysis of the other affected female relatives was recommended and it is ongoing.

Conclusion: This is a rare case of an Xq28 duplication detected in a severely affected female.

This case demonstrates the importance of the correlation between genetic findings with the patients' phenotype and the family history.

References: [1]https://omim.org/entry/300260.
[2]https://search.clinicalgenome.org/kb/gene-dosage/region/ISCA-46304.
[3]https://www.ncbi.nlm.nih.gov/books/NBK1284/.
[4] PMID: 27761913.

Grants:

Conflict of Interest: None declared.

EP09.033 A novel KCNK9 missense variant adjacent to the typical amino acid substitution causes Birk-Barel syndrome

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Background/Objectives: A 17-year-old girl presented with intellectual disability, and facial dysmorphisms. At birth, she was severely hypotonic requiring tube feeding, and had retromicrognathia, cleft palate, and bilateral club feet. She developed postnatal microcephaly, delay of developmental milestones, and scoliosis, and has hypotonia with areflexia, spasticity, and contractures of the legs. A muscular biopsy at age 4 years showed unspecified myopathy with an excess of type 1 fibres and atrophy of type 2 fibres.

Methods:.

Results: We report a heterozygous novel, maternally inherited, likely pathogenic variant in the *KCNK9* gene: c.710C> T;p.(Ala237Val).

Pathogenic variants in the *KCNK9* maternal allele cause Birk-Barel syndrome, characterized by congenital central hypotonia, developmental delay, and dysmorphic features[1,2]. Almost all patients carry *KCNK9* p.Gly236Arg[1,2].

One additional patient carries the substitution p.(Ala237Asp)[3]. The novel variant in our patient involves a different substitution at the same position (p.(Ala237Val)), adding strength to the interpretation of both variants as likely pathogenic. In addition to the clear phenotypic match, both patients show selective atrophy of type 2 fibres and predominance of type 1 fibres on muscle biopsy[3], which is similar to neurogenic changes seen in spinal muscular atrophy (SMA). Two patients of the originally described group showed signs of SMA on muscle biopsy[1].

Conclusion: Our patient demonstrates the typical phenotype of Birk-Barel syndrome, likely caused by the novel *KCNK9* variant p.(Ala237Val). This case provides further support that substitutions at both positions p.Gly236 and p.Ala237 are to be considered pathogenic.

References: [1]Barel et al, https://doi.org/10.1016/j.ajhg.2008.07.010.
[2]Graham et al, 10,1002/j.ajmg.a,37740.
[3]Šedivá et al, https://doi.org/10.1016/j.ejmg.2019.01.009.

Grants:

Conflict of Interest: None declared.

EP09.034 Biallelic MAPKAPK5 pathogenic variants are associated with a distinct syndromic neurodevelopmental disorder with craniofacial, digital and neuroradiological abnormalities

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Background/Objectives: MAPK activated protein kinase 5 (MAPKAPK5) is an essential enzyme for diverse cellular processes. Dysregulation of the pathways regulated by MAPKAPK enzymes can lead to the development of variable diseases. Recently, three cases from two families presented with a distinct syndromic neurodevelopmental disorder with two different homozygous frameshift variants in *MAPKAPK5* were reported.

Methods: We evaluated three individuals from three consanguineous south-Asian, middle eastern and north-African families. The study was approved by the institutional ethics committees of University College London (UCL) and additional local ethics committees of the participating centres. Exome sequencing (ES) and Sanger segregation analysis were performed separately at two different laboratories.

Results: In the present study, we identified three types of homozygous *MAPKAPK5* variants, frameshift, nonsense, and missense by using exome sequencing, in three unrelated individuals from three consanguineous families. All affected individuals exhibited a syndromic neurodevelopmental disorder characterized by severe global developmental delay, intellectual disability, distinct dysmorphic features, brachycephaly, digits abnormalities and abnormal brain MRIs (cerebellar hypoplasia, hypomyelination) as well as variable vision and hearing impairment. The additional features are failure to thrive, hypotonia and microcephaly.

Conclusion: In this study, we consolidate the causality of loss of MAPKAPK5 function and further delineate the clinical features

associated with biallelic *MAPKAPK5* variants and expand the molecular and phenotypic spectrum of this new ultra-rare neurodevelopmental syndrome.

References:

Grants: MRC (MR/S01165X/1, MR/S005021/1, G0601943).

Conflict of Interest: None declared.

EP09.035 Chromosomal Microarray Analysis in children with unexplained neurodevelopmental disorder

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Background/Objectives: Neurodevelopmental disorders are a heterogeneous group that includes developmental delay (DD), intellectual disability (ID) and autism spectrum disorder (ASD). Recently chromosomal microarray analysis (CMA) has been recommended as the first-tier genetic test in children presenting with an unexplained neurodevelopmental disorder.

Herein, it was aimed to expand the phenotypic spectrum of copy number variations (CNVs) detected in patients with DD/ID/ASD and evaluate the clinical benefits of CMA.

Methods: The size, number of genes, inheritance, and classification (according to ACMG guidelines) of CNVs detected in 31 patients with DD/ID/ASD were evaluated retrospectively along with phenotypic characteristics of the patients.

Results: A total of 41 CNVs were detected in 31 patients, including 24 deletions and 17 duplications.12/31 of patients had at least one congenital anomaly accompanying DD/ID/ASD. The mean age at which CMA performed was 4.1 years. The size of the largest CNV was 51 megabases, the smallest one was 58 kilobases. 17/41 of CNVs were de novo, 5/41 were inherited in the same manner from one of the parents, and 4/41 CNVs were inherited to a larger extent. 22/41 of CNVs were classified as 'pathogenic', 2/41 as 'likely pathogenic', 16/41 as 'variant of uncertain significance (VUS)' and 1/41 as 'benign'. The most affected chromosomal regions were 22q11.2 (4/41) and 15q11.2 (4/41). 10/41 of CNVs were consistent with a previously described microdeletion/duplication syndrome (2q37,7q31, 15q11.2, 16p11.2, 17p11.2, 17q21.31, 22q11.2).

Conclusion: CMA may reveal underlying molecular defects in children presenting with DD/ID/ASD. Knowing the phenotypic features of CNVs enables better patient management and genetic counseling.

References:

Grants:

Conflict of Interest: None declared.

EP09.036 A novel loss-of-function SEMA3E mutation causes severe intellectual disability and cognitive regression

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Background/Objectives: Intellectual Disability (ID) is a neurological disorder arising from early neurodevelopmental defects characterized by subaverage intellectual and adaptive functioning due to abnormalities of brain structure and function[1]. The underlying genetic and molecular mechanisms are complex, but thought to involve, among others, alterations in genes involved in axon guidance and/or neural circuit formation as demonstrated in the developing mouse brain[2]. Here, by combining exome sequencing with in silico analyses, we identified a patient affected by severe ID and cognitive regression, carrying a novel loss-of-function mutation of the semaphorin 3E (*SEMA3E*) gene, which encodes for a key secreted cue that controls brain development in mouse.

Methods: ad hoc in vitro and in vivo experiments were performed. Protein secretion and binding were assessed by western blot, immunocytochemistry and binding assays, while human SEMA3E expression was analysed by immunohistochemistry.

Results: we found that the identified variant impairs protein secretion and hampers the binding to embryonic mouse neuronal cells and tissues. Further, we revealed SEMA3E expression during human brain development.

Conclusion: overall, our findings demonstrate the pathogenic impact of identified *SEMA3E* variant and provide evidence that clinical neurological features of the patient might be due to a defective SEMA3E signalling in the brain.

References:1 Purugganan O. Intellectual Disabilities. *Pediatrics In Review* 2018; **39**: 299–309. 2 Steele JL, Morrow MM, Sarnat HB et al. Semaphorin-Plexin Signaling: From Axonal Guidance to a New X-Linked Intellectual Disability Syndrome. *Pediatric Neurology* 2022; **126**. https://doi.org/10.1016/j.pediatrneurol.2021.10.008.

Grants: Ministry of Health and CHARGE Syndrome Foundation to A.C.

Conflict of Interest: None declared.

EP09.037 Investigation of serotonin, histamine and diamine oxidase in the improvement of child development in ASD: benefits from improved nutrition and diet

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Background/Objectives: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social impairment, repetitive behavior and impaired communication. Alternations in vital neurotransmitters are frequently found in children with ASD, which can be further complicated in cases with the additive phenotype of histamine intolerance.

As diet and nutrition play an important role in maintaining the proper functions of the brain, our aim was to determine if nutritional improvements can alter the symptoms of ASD and improve general development. We analyzed the changes in the levels of serotonin, histamine and diamine oxidase (DAO) activity together with DAO polymorphisms (rs2052129, rs2268999, rs10156191 and rs1049742).

Methods: A cohort of 117 patients with ASD was tested for histamine, serotonin and DAO activity via ELISA prior to and following an appropriate diet. Genotyping was performed for DAO polymorphisms using Sanger sequencing. Statistical analysis was performed using SPSS v26.0.0.0. and Pearson Chi Square Test.

Results: We observed statistically significant changes in all measured parameters including an improvement in the

development of the patients following the assigned diet. Furthermore, we observed a correlation between DAO genotypes and enzymatic activity.

Conclusion: Our study discovered a positive effect of an individualized, clinically-based diet approach on the general condition and development of children with ASD. Further clinical studies are required to validate the correlation between DAO genotype and enzymatic activity.

References:

Grants:

Conflict of Interest: None declared.

EP09.038 Neuroanatomical studies identify VPS13B as an important regulator of brain architecture and hippocampal formation

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Background/Objectives: Vacuolar Protein Sorting 13 homolog B (VPS13B) is a highly conserved protein through evolution, but its function is not well understood yet. Human mutations in *VPS13B* cause Cohen Syndrome, a rare recessive developmental disease characterized by intellectual disability, acquired microcephaly and hypotonia.

Methods: Using a knockout mouse model approach, we set out to identify the role of VPS13B in the brain and animal behavior.

Results: We first showed that homozygous mutant mice of *Vps13b* are sub-viable, half dying during the first week of life. The survivor homozygous mutant mice exhibit growth delay and microcephaly (-27% for weight, and -20% for microcephaly in adult male mice). Using systematic neuroanatomical studies at multiple ages (embryonic E18.5 and postnatal 5, 7, 18 and 33 weeks), we then found severe neuroanatomical changes appearing within the first weeks after birth in homozygous mutant mice when compared to controls. The hippocampal formation is the most affected region with a reduction of 34% of the size of the dentate gyrus. In addition, we performed a battery of behavioral tests and showed hyperactivity, hypotonia and altered memory but enhanced sociability and resilience to anxiety and depression in *Vps13b* homozygous mutant mice. It is noteworthy that heterozygous mutant mice did not show any apparent phenotype.

Conclusion: Together, these findings indicate a highly specific role of VPS13B in the regulation of brain structure and an association with previously unreported features of Cohen Syndrome.

References: Grants: Jérôme Lejeune Foundation and ANR JCJC Young Team, to BY.

Conflict of Interest: None declared.

EP09.039 Two novel variants in POLA1 and TBC1D8B identified in a Japanese patient with failure to thrive, mild intellectual delay, skin pigmentation and renal failure

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Background/Objectives: Whole-exome sequencing (WES) analysis has revealed two pathogenic variants in some patients of rare diseases. Here we report a 23-year-old Japanese patient who was made dual diagnosis by the WES analysis.

Methods: The proband was born to non-consanguineous parents at 41 weeks with birth weight of 2,687g (-0.76 SD), height of 48 cm (-0.48 SD) and head circumstance of 34.0 cm. (+0.5 SD). Failure to thrive was clearly observed at 1 year of age (weight; -2.4 SD, height; -1.4 SD, head circumstance; -2.2 SD). He shows mild intellectual disability and skin pigmentation. Immune dysregulation was not pointed out in his medical history. Proteinuria was observed from 8- years-old and pathologically diagnosed with membranoproliferative glomerulonephritis. He reached renal failure by 23-year-old. After obtaining written informed consent, trio-based WES analysis.

Results: WES analysis revealed two novel pathogenic variants in *TBC1D8B* (NM_017752:c.1711T>C:p.[Tyr571His]) and *POLA1* (c.3082G>A:p.[Gly1028Arg]), on X chromosome in the patient and his mother.

Conclusion: His glomerulonephritis may be caused by the variant in *TBC1D8B* that is known to causative gene for nephrotic syndrome, type 20. *POLA1* is known to causative gene for two allelic disorders with different molecular pathogenesis. One is X-linked reticulate pigmentary disorder, which is characterized by reticular pigmentation abnormalities of the skin and immune dysregulation. Another is van Esch-O'Driscoll syndrome (VEODS), characterized by severe growth retardation, microcephaly and hypogonadism without pigmentation abnormalities. The patient shows both VEODS and skin pigmentation phenotypes. We concluded that he is an intermediate type of the two disorders.

References: AJHG 104, 957-967, 2019.
AJHG 104, 348–355, 2019.

Grants:

Conflict of Interest: None declared.

EP09.040 Genetic diagnosis in Bulgarian autistic spectrum disorder patients

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Background/Objectives: Methods for discovering point variants by using next generation sequencing (NGS), include analysis of the genome, exome, diagnostic panels and single genes. Methods for discovering deletions and duplications include array-CGH and NGS with low coverage which allows for fast scanning of the genome for copy number variations (CNVs). The percentage of genetically confirmed cases with autism spectrum disorder (ASD) is not very high, no matter of the available technologies for diagnostic purposes.

The aim of the study was to screen 12 ASD patients with different diagnostic approaches.

Methods: NGS with low coverage and WES were performed for all 12 patients. Segregation analysis by Sanger sequencing was performed in the families.

Results: Two de novo pathogenic CNVs were detected: duplication on the short arm of chromosome 8 and deletion on the long arm of chromosome 20. In addition, 2 de novo pathogenic

missense variants were found: DDX3X gene (de novo X-linked) - c.857C>A, p.Ala286Asp and HIVEP2 gene (de novo autosomal dominant) - c.7310A>C; p.Asp2437Ala. Two more patients turned out to be double heterozygous: SPATA5 gene c.554G>A, p.Gly185Glu (maternal origin) and c.1831C>T, p.Pro611Ser (paternal origin); UNC80 gene - c.29A>G, p.Gln10Arg (maternal origin) and c.2338A>G, p.Ser780Gly (paternal origin). Altogether 6 point mutations were detected in 4 ASD patients.

Conclusion: Altogether, 50% of our patients with ASD were genetically diagnosed. When the genetic target is clear, Sanger sequencing or MLPA analysis is applicable. If the genetic target is unclear, WES and whole genome analysis for CNVs are of first choice.

References:

Grants:

Conflict of Interest: None declared.

EP09.041 A novel variant in BRWD3 gene in four related male patients with intellectual disability

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Background/Objectives: BRWD3 gene encodes a chromatin modifying protein, acting in the epigenetic regulation of the nervous system development. Intellectual developmental disorder X-linked type 93 (OMIM#300659) is caused by pathogenic variants in BRWD3 and was reported in 36 patients (35 males and 1 female). The clinical picture includes developmental delay (DD)/ intellectual disability (ID), macrocephaly, and facial dysmorphisms (long face, broad forehead, prominent ears, pointed chin).

Methods: We report a 18-year-old male, first child of non-consanguineous parents, with family history of ID (three maternal uncles) and learning difficulties (mother and two maternal half-sisters). Pregnancy, delivery and growth were normal. He has moderate ID, behavioural issues, and unilateral cryptorchidism. Physical examination revealed relative macrocephaly, dysmorphic features (long face, bulbous nose, prognathism), and bilateral clinodactyly of the 5th finger. Previous investigation included metabolic studies, head MRI, karyotype, array-CGH and molecular study for fragile X syndrome (PCR), all normal.

Results: Whole-exome sequencing with copy number variations analysis identified a novel hemizygous variant in BRWD3 gene: c.331+5G>C. Cascade genetic testing revealed maternal inheritance and segregation with the phenotype. Genetic testing of unaffected family members is still ongoing.

Conclusion: The reported variant is predicted to disrupt the highly conserved donor splice site, is absent in control population databases and co-segregates with the phenotype, which is suggestive of causality. This case illustrates that segregation analysis is a powerful tool in variant classification. Further analysis of X-chromosome inactivation pattern in a carrier female may help establish variant pathogenicity. Molecular diagnosis allows proper clinical follow-up and genetic counselling.

References:

Grants:

Conflict of Interest: None declared.

EP09.042 Genetic study of intellectual disability in children with Duchenne muscular dystrophy

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Background/Objectives: Duchenne muscular dystrophy (DMD) is a severe dystrophinopathy linked to mutations of the dystrophin gene. The disease mainly affects the muscles; however, intellectual disability (ID) is present in approximately one third of DMD cases due to dystrophin isoforms' expression in the central nervous system.

We aim to analyze the mutational profile and the dystrophin isoforms in patients with DMD associated with ID.

Methods: Nineteen DMD cases associated with ID are included. DMD was genetically confirmed by MLPA, and we explored the relationship between ID and affected isoforms.

Results: Family history of ID is found in 15.79% of our patients. The age of onset of the disorders is 3.2. Gait disturbances and increased CPK are constant in all our patients. 79% of patients carry deletions between exons 45 and 55. The Dp427 isoform is absent in all patients. Dp140pc is absent in 58% of cases, against 32% for the Dp140utr isoform, while Dp71 is absent in 5% of cases.

Conclusion: Molecular diagnosis helps predict the association of DMD with ID and even have an idea of the severity of ID. This is crucial for adequate care, aiming for a better quality of life of patients.

References: 1. Sequence and Structure Characteristics of 22 Deletion Breakpoints in Intron 44 of the DMD Gene Based on Long-Read Sequencing.Geng C et al. Front Genet. 2021.

2. Timing and localization of human dystrophin isoform expression provide insights into the cognitive phenotype of Duchenne muscular dystrophy. Natatlie et al.Sci Rep. 2017.

Grants: The authors received no financial support for the research.

Conflict of Interest: None declared.

EP09.043 Inherited Maternal Duplication at 15q11.2-q13.1: A new case, detected by Whole Exome Sequencing (WES)

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Background/Objectives: 15q Duplication Syndrome (Dup15q) is caused by one extra copy of the 15q11.2-q13.1 Prader–Willi/ Angelman critical region, subjected to imprinting. Genes like *UBE3A, GABRA5, GABRB3, GABRG3* and *SNRNP* are crucial for development and synaptogenesis and normally present differential monoallelic expression. In maternal Dup15q, inherited in 15% of all cases, allelic balance is disrupted and patients present hypotonia and psychomotor delays, intellectual disability, autism spectrum disorder (ASD), and epilepsy. An interstitial Dup15q was revealed by ExomeDepth during Whole Exome (WES) analysis and is described in the context of intrafamilial clinical heterogeneity, ambiguous presentations, and challenging differential diagnosis.

Methods: A 4-year-old female with low-level 45,X(7)/46,XX(66) mosaicism was referred for developmental delay. From the family history her 10-year-old brother had speech delay and autistic behavior and their mother was reported as socially distant. Following clinical evaluation, pre-test counselling and signed consent, WES was performed with xGen Exome Research v2 kit (Integrated DNA Technologies) on Illumina NextSeq-500 system. Bioinformatic analysis on VarSome Clinical platform, included CNV detection by ExomeDepth. Variant classification followed ACMG and ClinGen recommendations. Mehylation Specific -MLPA (MRC ME028-C1) was applied for further investigation.

Results: A 5.2 Mb heterozygous pathogenic duplication of 15q11.2-q13.1 was detected by ExomeDepth. Subsequent MS-MLPA allowed confirmation and characterization of parental origin, detecting the same maternally-derived duplication (15:23. 060.849-28.277.167/breakpoints BP1-BP3) in both the affected proband and her mother.

Conclusion: ExomeDepth algorithm enables detection of Copy Number Variants and may be used for the elucidation of complex neurodevelopmental disorders like Dup15q syndrome.

References: PMID: 23633446, 22942019.

Grants: None.

Conflict of Interest: None declared.

EP09.044 The divergent pattern of inheritance in siblings with novel homozygous DMN1 gene variant

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Background/Objectives: We present two affected siblings (a brother and a sister) with developmental delay, poor scholastic performance, difficulty to remember, recurrent seizures attacks, abnormal muscle movements, increased deep tendon reflexes, and mild spasticity. EEG showed epileptiform activity. 3-generation pedigree revealed consanguineous parents.

Methods: Whole-exome sequencing (WES) analysis quadro-test was done for both affected siblings plus their phenotypically healthy parents using the Illumina platform at Igenomix laboratory, Dubai.

Results: A novel homozygous missense variant in exon 1 of the *DNM1* gene [NM_004408.4:c.107T>G, p.(Val36Gly)] was identified. This variant has interestingly been detected in a homozygous state in both affected siblings, while it was found to be heterozygous in both phenotypically normal parents.

Conclusion: Although to date the DNM1 gene has only been associated with an autosomal dominant disorder, i.e., developmental and epileptic encephalopathy-31 (DEE31); the above results clearly show autosomal recessive (AR) inheritance. These results also consistent, though different variants; with Goekhan et al. (2021) that showed biallelic variants in individuals with developmental delay and epilepsy [1]. Our case also clearly shows the importance of testing multiple family members at the same time as in this family where WES-quadro was done; in order to reach a definitive diagnosis in a precise manner. Genetic testing of other siblings is on-going.

References: Gökhan Yigit, Ruth Sheffer, Muhannad Daana, Yun Li, Emrah Kaygusuz, et al. Loss-of-function variants in *DNM1* cause a specific form of developmental and epileptic encephalopathy only in biallelic state. J Med Genet. 2021 Jun 25;jmedgenet-2021-107769.

Grants: No grants.

Conflict of Interest: None declared.

EP09.046 Diagnostic yield of exome trio analysis to identify the genetic etiology in 804 previously undiagnosed cases

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Background/Objectives: Exome trio analysis is an effective strategy to identify potentially causal variants, along with their inheritance pattern, on rare genetic disorders. This approach has entered the medical practice as an effective diagnostic test transforming the molecular diagnosis and clinical management of undiagnosed genetic diseases.

Methods: We performed exome sequencing using SureSelectXT Human All Exon V6 technology (Agilent Technologies) and Comprehensive Exome Panel technology (Twist Bioscience). Sequencing reads were analyzed using DRAGEN software. Trio annotation and prioritization was performed with an in-house pipeline that allowed the detection of candidate SNVs and CNVs, based on genetic and phenotypic priorization.

Results: We present the analysis of 804 trios referred to our lab since 2018. Patients were mainly children with neurodevelopmental disorder (92%). The genetic etiology was potentially elucidated in 344 probands, achieving a 43% genetic diagnostic rate. Among these patients, 176 harbored de novo variants, 66 hemizygous maternally inherited variants, 45 in compound heterozygous variants, 27 newly homozygous variants and 30 variants inherited from parents. In two cases, clinically relevant variants detected were pathogenic CNVs.

Conclusion: In our cohort exome trio analysis provides a diagnostic yield of 43% in patients whom traditional molecular diagnostics strategies were uninformative. This analysis also allows CNV screening, but we only detected two CNVs due to CGH array was previously performed in the vast majority of cases. The implementation of exome trio analysis as a first-tier diagnostic approach will provide a higher diagnostic yield and a cost-efficient option particularly specially in patients with neurodevelopmental disorder.

References:

Grants:

Conflict of Interest: None declared.

EP09.049 PHF8-related X-linked phenotype and genotype - review of literature

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Background/Objectives: X-linked intellectual disability, Siderius type (MIM #300263) is a genetic disorder caused by pathogenic loss-of-function variants in the PHF8 gene, with X-linked recessive inheritance pattern. The main clinical features reported include the association of cognitive impairment, cleft lip and/or palate and facial dysmorphisms. Up to date, 16 variants submitted in ClinVar

database were classified as pathogenic or likely pathogenic and 44 as uncertain significance. Regarding phenotypic descriptions other than intellectual disability, nine patients from five families have been published.

Methods: We summarize the clinical features and variants of the patients previously reported in the literature and compare them with an additional patient from our clinic.

Results: We report a 4-year-old male patient with mild global developmental delay, dysmorphic facial features, brain encephaloclastic lesions and symptomatic focal epilepsy. There is a maternal family history of epilepsy. Clinical exome analysis revealed a maternally inherited truncating variant in the PHF8 gene. Unlike those previously reported, there is no personal or family history of cleft lip/palate.

Conclusion: Features like autism spectrum disorder, brain encephaloclastic lesions and epilepsy could be part of the phenotypic spectrum of Siderius type X-linked intellectual disability. There is a high number of uncertain significance variants in the PHF8 gene published in public databases, therefore further phenotype characterization of these patients could be helpful to interpret such variants and provide an accurate diagnosis, that can be beneficial for surveillance, prognosis assessment and familial genetic counselling.

References:

Grants:

Conflict of Interest: None declared.

EP09.050 Case study: deep phenotyping of a case with paternally inherited 16p11.2 duplication and 22q11.2 duplication

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Background/Objectives: Some recurrent copy number variants (CNVs), such as 16p11.2dup and 22q11.2dup, are associated with significant risk for neurodevelopmental disorders (NDD), although associated phenotypes are heterogeneous and familial transmission rates are high. We report for the first time co-occurrence of these NDD susceptibility CNVs in one patient.

Methods: Using standardized instruments, clinical and neurodevelopmental features were assessed in a 9-year-old boy, carrying a paternally inherited 16p11.2dup ([hg19](29,652,360-30,195,608)x3) and 22q11.21dup ([hg19](18,765,102-21,808,998) x3).

Results: This boy is the first and only child of unrelated parents. He was born at 36 weeks PMA following a pregnancy complicated by pre-eclampsia and IUGR (birth weight:1.9kg;length:42.5cm). At age 2, he was referred to the genetics clinic due to nutritional problems, failure to thrive, strabismus, congenital defects such as cryptorchidism and ankyloglossia, and developmental delay. At age 9, he attends special education, received the diagnosis of DCD. Longitudinal follow-up shows a relative stable cognitive trajectory within the borderline range (IQ76), whereas language skills show a growing into deficit trajectory. His father experienced nutritional problems in infancy and attended vocational education. His mother had learning problems, and only completed primary school. Compared to an in-house reference set of unrelated index patients with either 22q11.2dup or 16p11.2dup, scores for IQ, language and behaviour in this boy were within the same range.

Conclusion: No clear additive effect of the co-occurrence of 22q11.2dup and 16p11.2dup was observed on neurodevelopmental outcome of this boy. Systematic familial phenotyping and genotyping is required to explain intra- and interfamilial phenotypic variability of these CNVs.

References:

Grants: NIMH (U01MH119759).

Conflict of Interest: None declared.

EP09.051 Identification through aCGH of CNVs involved in the genetic etiology of intellectual disability - data from a small Romanian cohort

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Background/Objectives: According to WHO Europe, intellectual disability (ID) is a significantly reduced ability to understand new or complex information, to learn and apply new skills (impaired intelligence), and to cope independently (impaired social functioning). There are various causes which could lead to intellectual disability, including genetic abnormalities such as CNVs (copy number variations). The association between CNVs and patients suffering from ID has been acknowledged through multiple studies. The aim of our study is to present the results of aCGH (array comparative genomic hybridization) testing from a small Romanian cohort of patients with intellectual disability, most of which were syndromic.

Methods: Purified genomic DNA from peripheral blood was examined for copy number variations (CNVs) using Agilent Cytogenomics 4x180K/8x60K or OGT Cytosure 8x60K ISCA design oligonucleotide microarrays. Copy number data was analyzed with Agilent Cytogenomics and OGT Cytosure Interpret software, respectively.

Results: From a total of 366 patients with syndromic ID tested through aCGH, 76 presented one CNV and 6 presented two CNVs each, resulting in a diagnostic yield of 22.4%. The group of patients was clinically heterogeneous, as were the identified microdeletions and microduplications.

Conclusion: This study demonstrated the importance of CNVs testing through aCGH in the management of patients with unexplained ID. These results could contribute to the existing data and knowledge regarding the utility of aCGH in the diagnosis of ID.

References:

Grants:

Conflict of Interest: None declared.

EP09.052 Experience to integrate exome sequencing and reanalysis into clinical practice in a tertiary hospital of the public healthcare system

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Background/Objectives: The introduction of exome sequencing (ES) into clinical practice has increased the rate of molecular diagnosis in patients affected with rare disorders. The integration of genomics into the public healthcare system requires a multidisciplinary approach, as well as the implementation of strategies for systematic reanalysis.

Methods: We report our strategy and results of implementing singleton ES in our current diagnostic practice in a cohort of 1111 patients with a suspicion of a genetic condition, evaluated from June 2017 to November 2021 in a specialized genetics consultation with a team including clinical geneticists, genetic counselors, laboratory geneticists, bioinformaticians and other specialists. We also report the results of introducing systematic reanalysis of unsolved cases, combined with a translational research approach and the use of collaborative platforms such as GeneMatcher.

Results: Our overall diagnostic yield was 29% (333/1111) in the first analysis. In a second step, we reanalysed 298 cases. We obtained 20% (60/298) additional diagnoses: 53 through the usual diagnostic process (23 candidate genes/HPOs, 21 improved pipelines, 6 new publications, 2 initially misclassified, and 1 copy-number variant), and 7 through translational research by international data sharing. Our final diagnostic yield was 35% (34% due to a traditional diagnostic approach, and 1% through an additional research strategy).

Conclusion: Our diagnostic yield was similar to that previously described in the literature. We show that the periodic reanalysis of ES allows additional diagnosis in approximately 20% of patients and that it can be integrated into the diagnostic routine.

References:

Grants:

Conflict of Interest: None declared.

EP09.053 KBG syndrome in the Portuguese population: clinical and molecular characterization of 41 patients

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Background/Objectives: KBG syndrome (MIM#148050) is an autosomal dominant syndromic developmental delay/intellectual disability (DD/ID) disorder caused by haploinsufficiency variants in *ANKRD11* gene, characterized by a typical *gestalt*, macrodontia and short stature. It is presumed to be a frequent underdiagnosed aetiology of syndromic DD/ID. We present the clinical and molecular characterization of 41 Portuguese patients with KBG.

Methods: Retrospective review of clinical and molecular data from KBG patients diagnosed in Portuguese medical genetics centres. The study was approved by the Ethics Committee of the Lisbon Academic Medical Centre.

Results: We collected data from 41 patients (21 females, 20 males). All patients had DD/ID and learning difficulties. The typical *gestalt* was recognized in 73%, and in 11% Cornelia de Lange syndrome was first considered. Common findings were 5th finger clino/brachydactyly (86%), macrodontia (79%), attention deficit hyperactivity disorder (ADHD) (64%) and hearing loss (53%). Short stature (<3rd centile) was observed in 38%. 25 different *ANKRD11* sequence variants were identified: 19 frameshift [pathogenic (P)/likely pathogenic (LP)]; 4 missense [2 (P/LP), 2 uncertain (VUS)]; and 1 affecting splicing (P). Four unrelated patients had large deletions involving *ANKRD11* gene, including one case without the typical KBG phenotype. Variants were found in all but two patients, who both had a high KBG Face2Gene score.

Conclusion: This data is consistent with previous literature. KBG syndrome has a distinctive *gestalt* which is easier to recognize later in childhood when macrodontia becomes evident. DD/ID is usually mild; behaviour disorders, particularly ADHD, are common. Target gene sequencing should confirm most clinical diagnoses.

References:

Grants:

Conflict of Interest: None declared.

EP09.054 Beyond craniosynostosis: can TCF12 gene be responsible for a wider clinical spectrum?

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Background/Objectives: *TCF12* gene encodes transcription factor 12 (*tcf12*), a member of the basic helix-loop-helix (bHLH), and plays a key role in neurogenesis, mesoderm formation, and cranial suture development. *TCF12* haploinsufficiency results in coronal craniosynostosis type 3 (CRS3, #615314) and hypogonadotropic hypogonadism 26 (#619718). Several cases of CRS3 presented with other comorbidities such neurodevelopmental impairment, dysmorphims and other congenital anomalies.

Methods: We report a new patient with a likely pathogenic variant in *TCF12* gene aiming to further expand its clinical spectrum.

Results: A 30-year-old female was first referred to our outpatient genetic department at 16-years-old with a central nervous system malformation (focal cortical dysplasia with leftside frontal polymicrogyria), nystagmus, right hemiparesis, epilepsy, hearing loss and intellectual disability. At our observation, she showed low anterior hairline, thin upper lip, and a prominent chin. Extensive investigation was performed with normal karyotype, FRAXA, metabolic investigation, microarray analysis. Exome sequencing identify a *de novo* heterozygous variant in *TCF12* (NM_207036.1):c.1453C>T (p.(Arg485*)), classified as likely pathogenic, previously described as a cause of CRS3.

Conclusion: Although, our patient does not have a craniosynostosis, this result can potentially explain the phenotype and be clinically relevant. We propose that *TCF12* could be responsible for

other craniofacial abnormalities and neurodevelopmental disorders. It is important to note that genetic research suggests that mutations in regulatory elements of genes can cause developmental defects. Therefore, more investigation is necessary to understand the role of *tcf12* as transcriptional regulation factor in other tissues besides cranial suture development, and its impact in additional developmental defects.

References:
Grants:
Conflict of Interest: None declared.

EP09.056 Exploring the phenotypic spectrum of CHD4 gene variants: Case report and literature review

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Background/Objectives: CHD4 is a chromatin remodeler involved in epigenetic regulation of gene transcription. Pathogenic variants in *CHD4* gene are associated with Sifrim-Hitz-Weiss syndrome (OMIM#617159), a well described chromatinopathy characterized by global developmental delay, mild to moderate intellectual disability, brain anomalies, congenital heart defects, and dysmorphic features. Additional abnormalities include hypogonadism, skeletal and limb anomalies, hearing impairment, and ophthalmic abnormalities. Recently, missense variants in *CHD4* were identified in patients with a milder phenotype consisting of epilepsy with sinus arrhythmia.

Methods: We report a case of a novel missense variant in *CHD4* that presented with an intermediate phenotype, aiming to contribute to the clinical and genotypic spectrum characterization.

Results: A 9-year-old boy was referred for developmental delay/mild intellectual disability and epilepsy evaluation. He was the oldest son of a non-consanguineous healthy couple. Prenatal and neonatal history were unremarkable. At our observation, he had no dysmorphisms nor congenital anomalies. FRAXA and microarray were normal. A WES-based gene panel identified a missense variant in the *CHD4* gene, NM_001273:c.2660G>A (p.Arg887Gln). The variant was not previously described in literature, nor in gnomAD population. It occurs at a position that is conserved across species, in the same codon as a known pathogenic variant, and in silico analysis predicts a probably deleterious effect in protein. Finally, familial studies showed a *de novo* origin.

Conclusion: This report contributes to the expansion of the phenotypic spectrum of CHD4-related disorders. It highlights it can be a less obvious diagnosis since there is a wide phenotypic heterogeneity, without known genotypic-phenotypic correlations.

References:
Grants:
Conflict of Interest: None declared.

EP09.057 Heterozygous HMGB1 loss-of-function variants are associated with developmental delay and microcephaly

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Background/Objectives: The 13q12.3 microdeletion syndrome is a rare cause of syndromic intellectual disability for which two candidate genes were previously proposed (KATNAL1 and HMGB1). However, to date, no single point mutation or deletion of either of these genes has been described in patients.

Methods: Thanks to exome sequencing and international data sharing, we report here 6 patients with de novo loss of function ($n = 5$), or missense ($n = 1$) variations in the HMGB1 gene.

Results: The clinical signs found in these patients are consistent with 13q12.3 microdeletion, namely intellectual disability, language delay, microcephaly, dysmorphic features, obesity and atopic dermatitis. In silico data in line with intolerance to loss of function of the gene and previous in vitro studies showing its importance in neurodevelopment support the involvement of these variations in the neurodevelopmental phenotype of the patients. The role of the HMGB1 gene in the inflammatory process could explain cutaneous signs such as atopic dermatitis. Finally, the predicted interaction between HMGB1 and other genes involved in neurodevelopmental disorders (SMARCA4, SMARCA2, SMARCD1) raises the hypothesis of a common epigenetic signature.

Conclusion: Thus, as for many microdeletion syndromes, the search for the genetic basis and the genes responsible for the pathology has been greatly facilitated by high-throughput exome and genome sequencing. Indeed, this cohort allows us to propose HMGB1 as a major gene of the 13q12.3 microdeletion syndrome and as a new gene for intellectual disability. Finally, this study shows once again the importance of international data sharing in the context of rare diseases.

References:
Grants:
Conflict of Interest: None declared.

EP10 Neurogenetic and Psychiatric Disorders

EP10.001 Pipelines to investigate both small and large variants from a small amyotrophic lateral sclerosis family

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Background/Objectives: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease. To date, 90% of ALS cases still have an unknown genetic cause. Most research has focused on small nucleotide level variants only. This project extends ALS gene discovery to consider large structural variation (SVs) by using bioinformatic pipelines to identify both small and large genetic alterations in a small ALS family with limited power for traditional gene discovery methods.

Methods: Whole genome sequencing (WGS) was performed on two affected individuals from a family with ALS history (MQ52). To identify candidate small nucleotide variants, shared variant analysis was carried out to filter for nonsynonymous and indel variants absent/extremely rare from public controls. To identify SVs, we have developed a comprehensive pipeline that calls SVs from WGS data using multiple tools such as Manta, Lumpy and MetaSV. The Duphold tool is then used to filter SVs based on quality scores, followed by annotation using Reciprocal Overlap Annotator and AnnotSV to generate a list of shared or overlapping SVs between the two affected individuals.

Results: Initial analysis of family MQ52 identified 14 small variants and 80 novel/rare SVs, of which 44 SVs lie in coding regions. This SV pipeline is undergoing refinement and resultant SVs will be validated using established PCR techniques.

Conclusion: Our gene discovery pipelines can successfully identify both small and large variants that potentially cause disease within small ALS families. Given that SVs have been largely understudied in ALS, discovery of ALS-linked SVs could help to elucidate the missing genetic architecture of ALS.

References:

Grants:

Conflict of Interest: None declared.

EP10.002 Clinical spectrum and genetic characteristics of four patients with autism spectrum disorders and 15q11-q13 duplication syndrome

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Background/Objectives: Chromosomal copy number variants (CNV) are the most common genetic lesion found in 5–10% of all patients with autism spectrum disorder (ASD). The 15q11.1-13.1 duplication syndrome characterized by ASD, hypotonia, intellectual disability, motor delays and epilepsy. Disease frequency in the population is 1: 30,000 to 1: 60,000. As many as 1–3% of all ASD cases may be the result of duplications of the 15q11.2-q13 region. The duplication described as supernumerary isocentric chromosome in 80% of cases and as an interstitial duplication in 20% of cases.

Methods: We described the clinical picture in 4 children with 15q11-q13 duplication syndrome. Conventional chromosomal analyses at 550 G-band resolution were performed on all 4 of our patients. We used MLPA P245 Microdeletion Syndromes for screening of the most common microdeletion syndromes and MLPA P036 Subtelomeres Mix 1 for screening of subtelomeric deletions/duplications in 3 patients. Array CGH was performed in one case - OGT 4x44k format oligonucleotide microarray with targeted CN resolution of 1 probe every 52kb and backbone CN resolution of 1 probe every 81kb. The slides were scanned on a GenePix 4100A two-colour fluorescent scanner (Axon Instruments, Union City, CA, U.S.A.). The arrays were analyzed by CytoSure Interpret Software.

Results: The chromosomal analysis yielded a supernumerary marker chromosome in 3 children. MLPA analysis demonstrated the presence of chromosome 15 (15q11-q13) duplication in 3 patients. By array CGH we determined arr15q11.2q13.1(22, 765,658-29,030,488)x3.

Conclusion: Our results strongly support the implication of 15q11-13 rearrangements as a predisposing factor for autism.

References:

Grants:

Conflict of Interest: None declared.

EP10.003 Perceptions of causal attribution and attitudes to genetic testing among people with schizophrenia and their first-degree relatives: A qualitative study

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Background/Objectives: Rapid advances in the genetics of psychiatric disorders mean that diagnostic and predictive genetic testing for schizophrenia risk may one day be a reality. This study examined how causal attributions for schizophrenia contribute to interest in a hypothetical genetic test.

Methods: People with schizophrenia and first-degree relatives of people with schizophrenia were recruited through a schizophrenia research bank and mental health organisation. Semi-structured telephone interviews were conducted with 13 individuals with schizophrenia and 8 first-degree relatives. Transcripts were subjected to a qualitative analysis using the thematic analysis framework.

Results: Five themes were developed: (i) "It is like a cocktail", with most participants aware that both genetic and environmental factors contributed to causation, and many mentioning the positive impact of genetic causal explanations; (ii) "Knowledge is power" (i.e., in favour of genetic testing); (iii) Genetic testing provides opportunities for early intervention and avoiding triggers, with participants citing a wide range of perceived benefits of genetic testing but few risks; (iv) Views on reproductive genetic testing for schizophrenia risk with a few participants viewing it as "playing God" but not necessarily being against it; and (v) "It snowballs", whereby participants' understanding of genetics was sophisticated with most believing that multiple rather than single genes contributed to schizophrenia.

Conclusion: Many individuals had a sound understanding of the role of genetic testing if it were to become available, with

evidence of insight into the role of multiple genes and the contribution of other risk factors that may interact with any inherited genetic risk.

References:

Grants:

Conflict of Interest: None declared.

EP10.004 Re-analysis of exome sequencing data of undiagnosed epilepsy cases

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Background/Objectives: The rapidly changing landscape of epilepsy genetics makes it essential to establish new strategies for genetic testing that aim to increase the diagnostic yield. Salinas et al. (2021) have demonstrated the importance of periodic re-interpretation and re-analysis of genetic data to achieve this. Re-analysis of exome data of previously unsolved developmental and epileptic encephalopathy cases was shown to further increase the diagnostic yield by ~15%. The objective of our study is to evaluate the diagnostic potential of exome sequence re-analysis in our cohort of undiagnosed epilepsy cases.

Methods: DNA extracted from blood was enriched using Agilent SureSelect Clinical Research Exome V2 (CRE V2) or Nonacus ExomeCG and sequenced on Illumina NextSeq 500 or NovaSeq. Secondary and tertiary analysis of DNA sequences and review of SNVs and CNVs was undertaken using the Congenica clinical decision platform. Re-analysis was performed 6 - 48 months after initial interpretation, using 1) an updated curated epilepsy gene panel, and 2) gene agnostic prioritisation using Congenica AI.

Results: Through original exome sequencing analysis, a diagnosis was achieved in 34/129 patients (26%). Of 59 cases assessed for CNVs, 2 had pathogenic variants (3.4%). Re-analysis was performed on 95 unsolved cases. Updated figures will presented.

Conclusion: This study illustrates the diagnostic utility of re-analysing exome sequencing data in previously unsolved epilepsy cases.

References: Salinas et al. (2021) Clinical next generation sequencing in developmental and epileptic encephalopathies: Diagnostic relevance of data re-analysis and variants re-interpretation'. Eur. J. Med. Genet. 64(12):104363.

Grants:

Conflict of Interest: None declared.

EP10.005 A novel homozygous nonsense CCDC186 variant that causes neurodevelopmental delay, microcephaly, and seizures. New syndrome?

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Background/Objectives: CCDC186 is hypothesized to play an important role in the biogenesis of dense-core vesicles in neurons and endocrine cells.

Methods: Here, we report a 2 years female Syrian patient who has microcephaly, hypotonia, neurodevelopmental delay, and seizures. She is the 3rd child of a consanguineous couple. There was no significant dysmorphic finding in our patient. No abnormality was detected as a result of routine chromosome analysis and microarray analysis. Exome sequencing was planned in order to identify the genetic etiology that may be responsible for the clinical findings.

Results: Exome sequencing identified a homozygous c.535C>T; p.(Arg179Ter) loss-of-function variant in CCDC186(NM_018017.4).

Conclusion: Any disease phenotype related to the CCDC186 has not been identified in OMIM. There are only two patients with CCDC186 biallelic variants have been reported in the literature so far. Neurodevelopmental delay and some additional anomalies were described in both patients. In addition to all these data, the fact that our patient had clinical findings similar to the other 2 cases in the literature supports that the CCDC186 gene is responsible for a new autosomal recessive neurodevelopmental disease.

References:

Grants:

Conflict of Interest: None declared.

EP10.006 Target next-generation sequencing as a comprehensive test for genetics epilepsy

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Background/Objectives: Next-generation sequencing (NGS) has contributed to the identification of many monogenic epilepsy syndromes and is favouring earlier diagnosis in a subset of paediatric patients with epilepsy. The cumulative information emerging from NGS studies is rapidly changing our comprehension of the epileptic disorders. The aim of this study was to identify new variants, potentially pathogenic, in candidate genes. We performed NGS on 72 epileptic patients.

Methods: We selected 38 epilepsy causative genes for the targeted sequencing panel and performed the analysis using Ion Torrent Proton Sequencer. Raw data were analysed to select the potential pathogenic variants using bioinformatics tools (Mutation Taster, PolyPhen-2). All candidate variants were validated in Sanger sequencing.

Results: The pathogenic significance variants observed were 26: 11 variants were identified in genes encoding ion channels (KCNA2, KCNQ2, KCNQ3, KCNT1, SCN1A, SCN1B, and SCN2A). Other variants were found in genes that encodes for proteins involved in different cell functions: receptor subunits (CHRNA2, CHRNA4, GRIN1, GABRD), enzymatic activity (POMGNT1, PNKP), transmembrane proteins (PRRT2), nucleotide exchange factor (ARHGEF9) and others (RELN, CDKL5, FLNA, DCX).

Conclusion: Our findings provide several potential insights to understand the complex molecular mechanism underlying the epilepsy disorder.

References: J. Symonds, A. McTague, "Epilepsy and developmental disorders: Next generation sequencing in the clinic" Eur J Paediatr Neurol. 2021.

Grants: PRIN Project "Genetic Epileptic channelopathies as disease models for drug discovery toward personalized treatment: an integrated bench-to-bedside and backward approach" - CUP B64I19001030001 – funded by MIUR identification code 2017ALCR7C - Sector LS5 line C – in the following topic: "Epilepsy Genetics".

Conflict of Interest: None declared.

EP10.007 A novel splicing mutation in ANO10 is responsible for Spinocerebellar ataxia, autosomal recessive 10 (SCAR10) in a large kindred in Northern Israel

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Background/Objectives: The hereditary cerebellar ataxias are a heterogeneous group of conditions that is commonly classified according to their mode of inheritance. At least 20 different forms of autosomal recessive cerebellar ataxias (ARCAs) are known to date. Mutations in ANO10 causing slowly progressive spastic ataxia have so far been identified in several families from different ethnicities.

Methods: Five affected individuals from a large Arab-Christian family living in north Israel presented with progressive ataxia, variable degree of pyramidal signs and learning disabilities were studied. Using whole genome homozygosity mapping and targeted gene Sanger sequencing we identify the genetic cause of the disease.

Results: a novel homozygous variant c.139+1G>T was detected at the first nucleotide of the consensus donor splice site of exon 2 in the ANO10 gene, which is fully segregated in the family. This change leads to a frame shift and to a formation of stop codon after 17 amino acids p.G47Efs*9.

Conclusion: To date, at least 26 different pathogenic variants of ANO10 have been reported. These variants are spread throughout the gene and associated with variable phenotypes, with no clear genotype-phenotype correlation. Moreover, we report phenotypic heterogeneity within the same family that strongly suggests the existence of additional environmental and/or genetic factors which modify the phenotype induced by ANO10 variants. Our finding supports the assumption that there is a relationship between ANO10 coded protein TMEM16K, which is important for brain tissue development, and learning disabilities.

References: None.

Grants: None.

Conflict of Interest: None declared.

EP10.008 The diagnostic yield of genetic testing for middle-aged and elderly patients with neurological conditions

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Background/Objectives: The use of genetic testing for neurological disorders is rapidly expanding in clinical practice. However, its yield in middle-aged and elderly population is still unclear.

Methods: We evaluated the diagnostic yield of genetic workup for patients aged 50 years and above, who referred for a specialized neurogenetics clinic within a tertiary medical center in Israel, during the period of 2017-2020.

Results: The study included 156 consecutive Jewish Israeli patients (61% males). All had neurological disorder, without a previous molecular diagnosis. The mean age of first genetic counselling at the clinic was 61.1±7.4 years (range: 50-85), and the mean age of disease onset was 47.1±15.9 years (range: infancy to 75). 43.8% were of Ashkenazi origin, and 41.7% had a positive relevant family history. The main indications for referral were neuromuscular disorders, cerebrovascular disorder/white matter hyperintensities, movement disorders and cognitive impairment/dementia. In total, 60.9% of the patients performed genetic testing, including one or more of the following: tests for specific mutations, gene panel, whole-exome sequencing (WES) or chromosomal microarray (CMA). Costs were covered by public health services, or by patients themselves. Overall, 21.7% of patients received a molecular diagnosis (30.0% of the patients who performed any genetic testing). The highest yield was for neuromuscular disorders (37.9%), and substantially smaller for other indications.

Conclusion: Our experience, in the setting of a specialized neurogenetics clinic, demonstrates that genetic workup for neurological disease among individuals aged ≥ 50 years is beneficial mainly for neuromuscular disorders, with relatively high diagnostic yield.

References:

Grants:

Conflict of Interest: None declared.

EP10.009 Functional genomics and transcriptomics further characterise and potentially improve diagnostic yield of hereditary ataxias

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Background/Objectives: Up to 80% of hereditary ataxia (HA) patients remain molecularly undiagnosed even following high-depth whole genome sequencing. We aimed to leverage detailed multi-omics data to further characterise the genetic architecture of HA and to increase the diagnostic yield of patients.

Methods: We generated 284 genic features without a priori assumption, capturing information about a gene's structure; genetic variation; tissue-specific, cell-type-specific and temporally-relevant expression and protein products. Using modified PanelApp age-of-onset information, we categorised 318 HA-associated genes as childhood-onset, adult-onset and those overlapping both. We then compared these individual genomic features across gene categories and collectively through unsupervised machine learning.

Results: By comparing these genic properties, we demonstrated: (i) an unexpectedly high short tandem repeat density within childhood-onset genes suggesting that we may be missing pathogenic repeat expansions in this cohort; (ii) cell-type-specific expression differentiates childhood- and adult-onset ataxias with CNS glial-specific expression in childhood-onset and Purkinje cell-specific expression in overlap-onset genes; (iii) significant similarities in annotation across the groups using unsupervised analysis suggesting adult- and childhood-onset patients should be screened using a common gene set. We tested the latter hypothesis within the 100,000 Genomes Project by assessing the burden of potentially pathogenic variants among childhood-onset genes in adult-onset HA patients and vice versa. This demonstrated a significantly higher burden of rare, potentially

pathogenic variants in certain childhood-onset HA genes among adult-onset HA patients.

Conclusion: Our analysis highlights genic features of importance to investigate in an unsolved cohort and suggests that a broader testing strategy for HA could increase diagnostic yield.

References:

Grants:

Conflict of Interest: None declared.

EP10.011 Burden of functional variants in epilepsy patients using a deep learning approach

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Background/Objectives: Epilepsy is a neurological disease with a strong genetic component. Nevertheless, classical methods, like GWAS, are not as effective at detecting new causal loci as in other diseases. We used a deep learning approach to predict the tissue specific functional effect of variants in epilepsy patients.

Methods: Whole genome sequence data are available for 493 epilepsy patients and 201 controls. We computed the predicted expression change of variants using the deep learning algorithm ExPecto[1]. We used python's statsmodels to conduct logistic regressions by comparing patients and controls functional variants' burden among different groups of genes such as known epilepsy genes and genes intolerant to loss-of-function variants.

Results: Variants were filtered based on an expression change threshold. It corresponds to the value at which directionality prediction becomes perfect when compared to known eQTLs from GTEx. Using only variants that passed the threshold, we performed a logistic regression to compute the functional variants' burden in known epilepsy genes. Preliminary results show no difference between groups. Nevertheless, future regressions will focus on single genes and the magnitude of the expression change.

Conclusion: Deep learning algorithms are incredibly powerful tools to predict variant functional effects. Those methods are especially useful in neurology since brain tissues can only be acquired post-mortem. ExPecto is an important asset in our study of epilepsy, and it has the potential to be so for many other illnesses.

References: 1. Zhou, J. et al. 2018. PMCID: PMC6094955.

Grants: IVADO.

Conflict of Interest: None declared.

EP10.012 18-year-old patient with progressive respiratory insufficiency due to two variants in GLDN

Britta Hanker[1], *Inga Nagel*[2], *Guido Stichtenoth*[3], *Malte Spielmann*[1;2], *Irina Hüning*[1]

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Background/Objectives: *GLDN* codes for gliomedin which is required for the formation of the nodes of Ranvier and the development of the peripheral nervous system. Biallelic variants in GLDN cause lethal congenital contracture syndrome 11. Wambach et al. (2017) reported that biallelic variants in *GLDN* are not necessarily lethal in the neonatal period.

Methods: We report on an eighteen-year-old male patient, born at 33 weeks of gestation with normal body measurements. He presented with hypotonia and arthrogryposis. Cerebral MRI and testing for metabolic diseases gave normal results. Karyotyping and *SNM1* gene analysis returned normal results. He showed mild motor development delay, learned to walk after 2 years of age and developed progressive thoracolumbar kyphoscoliosis with consequent spondylodesis at 10 years of age. Due to diaphragmatic hypomotility, he suffered respiratory insufficiency requiring non-invasive home ventilation since the age of 16.

Results: At the age of 17, exome sequencing revealed two heterozygous variants in *GLDN* (NM_181789.4: c.82G>C, p.(Ala28-Pro) and c.1178G>A, p.(Arg393Lys)). The variant p.(Arg393Lys) was maternally inherited. DNA of the father was not available for molecular analysis.

Conclusion: Systematic review of the literature showed 16 patients with pathogenic variants in *GLDN* reported so far. Only six of them survived the first months of life (Wambach et al., 2017), the oldest being 17 years old. The phenotype of our patient is consistent with the literature data. We assume that the variants found are compound heterozygous. Long-term outcomes for patients with *GLDN* variants are still unknown. Here we present the oldest known patient with "non-lethal" congenital contracture syndrome.

References:

Grants:

Conflict of Interest: Britta Hanker: None declared, Inga Nagel: None declared, Guido Stichtenoth: None declared, Malte Spielmann Illumina.

Novartis, Irina Hüning: None declared.

EP10.014 Validity of candidate gene association studies in restless legs syndrome

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Background/Objectives: To date, 14 candidate gene case-control association studies have been published for restless legs syndrome (RLS) in populations of European ancestry. The reported associations have not been validated by replication efforts so far. Therefore, we decided to check them in a large genome-wide association study (GWAS) dataset for RLS.

Methods: Candidate gene studies were extracted from PubMed. GWAS data was available from the International-EU-RLS-GENE-Consortium[1] which included 17,220 individuals of European ancestry.[2] Single variant association tests were performed with SNPTEST software. Study power was calculated with the genpwr package in R.

Results: Across all identified studies, five different variants located in the genes *ADH1B, GABRR3, HMOX1, MAOA*, and *VDR* were reported as significantly associated with RLS. We did not replicate these associations in our dataset and no other variants located in these genes reached genome-wide significance either. Power calculations showed that our study had sufficient power (close to or 100%) to detect the reported associations of these

variants, even when accounting for biased effect size estimates due to winner's curse.

Conclusion: Our results indicate that the reported associations were rather false-positive than true-positive associations to RLS. Moreover, our observations are clearly in favour of performing future association studies as large-scale GWAS rather than small-scale studies.

References: 1.International-EU-RLS-GENE-Consortium: https://www.helmholtz-munich.de/en/ing/rls-gene-consortium/about/index.html.

2.Schormair B, Zhao C, Bell S, et al. 2017, Lancet Neurol. https://doi.org/10.1016/S1474-4422(17)30327-7.

I.

Grants:

Conflict of Interest: Barbara Schormair European patent office - WO2021185936A1, Chen Zhao European patent office - WO2021185936A1, Aaro Salminen: None declared, Konrad Oexle European patent office - WO2021185936A1, Juliane Winkelmann European patent office - WO2021185936A1.

EP10.015 LOSS OF SERYL tRNA SYNTHETASE (SARS1) CAUSES COMPLEX SPASTIC PARAPLEGIA AND CELLULAR SENESCENCE

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Background/Objectives: Germline mutations in aminoacyl-tRNA synthetase genes are associated with diverse neurological diseases. Recently, patients affected with microcephaly, intellectual disability and ataxia harbouring biallelic variants in the seryl-tRNA synthetase 1 gene encoded by *SARS1* were reported.

Methods: We used exome sequencing to identify the causal variant in a patient affected by complex spastic paraplegia with ataxia, intellectual disability, and seizures, but without microcephaly. Functional testing using patient's fibroblasts and *S. cerevisiae* strains was performed to examine this variant's pathogenicity.

Results: A *de novo* splice site deletion in SARS1 was identified in our patient, resulting in a 5-amino acid in-frame insertion near its active site. Complementation assays in *S. cerevisiae* and serylation assays in both yeast strains and patient fibroblasts proved a loss-of-function, dominant negative effect. Fibroblasts showed an abnormal cell shape, arrested division, and increased beta-galactosidase staining along with a senescence-associated secretory phenotype (raised IL-6, p21, p16 and p53 levels).

Conclusion: We refine the phenotypic spectrum and modes of inheritance of a newly described, ultrarare neurodevelopmental disorder, while unveiling the role of SARS1 as a regulator of cell growth, division and senescence.

References:

Grants: URD-Cat [SLT002/16/00174] Generalitat de Catalunya, CIBERER [ACCI14-759], ASL-HSP France, Hesperia Foundation, Instituto de Salud Carlos III (FIS PI20/00758), [Sara Borrell, CD19/00221], 'La Marató de TV3' Foundation (202006-30). French National Programme Investissement d'Avenir administered by the "Agence National de la Recherche" (ANR), "MitoCross" Laboratory of Excellence (Labex) (ANR-10-IDEX-0002-02), the University of Strasbourg and CNRS.

Conflict of Interest: None declared.

EP10.017 Genetic and phenotypic associations of serum dicarbonyl metabolites in restless legs syndrome

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Background/Objectives: Altered levels of dicarbonyls such as methylglyoxal (MG) have been linked to several diseases. Little is known about the impact of genetic variation on dicarbonyl metabolism. Genome-wide association studies of the sensorimotor disorder restless legs syndrome (RLS) have identified significant association signals in and close to *GLO1*, which encodes the key MG-detoxifying enzyme, glyoxalase-1 (GLO1). Therefore, we decided to study dicarbonyl levels and their genetic basis in RLS patients and controls.

Methods: Three dicarbonyls (MG, GO, 3-DG) were measured in serum of 246 RLS patients and 482 controls from a population-based cohort (KORA) using liquid-chromatography-mass-spectrometry. Phenotypic information included demographic data, laboratory parameters, and comorbidities. Samples were genotyped on the Axiom® array. Association analyses were done using regression models, MDS and bidirectional stepwise RDA analysis.

Results: Validating our dataset, we replicated known associations of the dicarbonyls to clinical phenotypes, including cardiovascular disease, diabetes, age, obesity, impaired liver, and renal function. These were in accordance with significant associations to objective disease parameters such as waist-to-hip ratio and glomerular filtration rate amongst others. As a novel finding, MG levels were significantly lower in RLS cases compared to controls across all age and sex groups ($p = 4 \times 10^{-23}$). GWAS on dicarbonyl levels revealed only signals below the genomewide significance-threshold, likely due to the limited sample size.

Conclusion: Our study provides first evidence for a role of dicarbonyls in RLS, indicating potential new treatment options. However, our study was underpowered to detect genetic effects. We are currently conducting a replication study to confirm the results and to increase study power.

References:

Grants:

Conflict of Interest: Philip Harrer: None declared, Julica Folberth: None declared, Chen Zhao European patent office - WO2021185936A1, Barbara Schormair European patent office - WO2021185936A1. Erik Tilch: None declared, Christian Gieger: None declared, Annette Peters: None declared, Konrad Oexle European patent office - WO2021185936A1, Markus Schwaninger: None declared, Juliane Winkelmann European patent office - WO2021185936A1.

EP10.018 Mutational screening of Greek patients with axonal Charcot-Marie-Tooth disease using targeted Next-Generation Sequencing

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Background/Objectives: Axonal forms of Charcot-Marie-Tooth disease (CMT) are classified as CMT2, dominant intermediate CMT (DI-CMT), hereditary sensory and autonomic neuropathy (HSAN) and distal hereditary motor neuropathy (dHMN). They are caused by mutations in over 20 genes. The aim of this study was to decipher the genetic landscape of axonal CMT in the Greek population.

Methods: Forty three index patients with CMT2, DI-CMT, HSAN or dHMN, negative for mutations causing CMT1A and CMTX, were screened by an NGS custom gene panel (IoN Torrent) that covers 24 of the most commonly mutated genes in cases of axonal CMT. The study was carried out in the Neurogenetics Unit of the 1st Department of Neurology, NKUA, Eginitio Hospital.

Results: Overall, we identified 9 causative heterozygous mutations corresponding to 9 index cases, representing 20.9% of the cohort. Those were 3 mutations in MPZ (c.103G>T p.Asp35Tyr, c. 449-1G>A, c.186C>G p.Ile62Met),in dHMN, CMT2 and HSAN patients respectively, 3 in MFN2 (c.1070A>C p.Lys357Thr, c.281G>A p.Arg94Gln, c.310C>T p.Arg104Trp), all in CMT2 patients, 1 in BSCL2 (c.263A>Gp.Asn152Ser), in a dHMN patient, 1 in GDAP1 (c.715C>T p.Leu239Phe), in a CMT2 patient, and 1 in DNM2 (c.1072G>A p.Gly358Arg),in a DI-CMT patient. All mutations were found in heterozygosity in accordance with dominant inheritance.

Conclusion: A 24-gene NGS panel designed to screen a Greek CMT2 cohort had a diagnostic yield of 20.9%, in accordance with results of similar studies in other European populations.

References:

Grants: This study was partly supported by a grant from Genesis Pharma (Grant/Award Number: 13044, special account for research grants, NKUA).

Conflict of Interest: None declared.

EP10.019 Screening of the FMR1 premutation in Greek patients with movement disorders

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Background/Objectives: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset, X-linked, neurodegenerative disorder that affects premutation carriers of the *FMR1* gene. Clinical features include gait ataxia and action tremor, while some patients demonstrate parkinsonism, cognitive deficits and peripheral neuropathy. Consequently, FXTAS is often misdiagnosed as spinocerebellar ataxia (SCA) or Parkinson's disease (PD). Herein, we sought to investigate the frequency, genotypic and phenotypic profile of FXTAS in two cohorts of Greek patients with movement disorders, one with late-onset cerebellar ataxia and the other with PD.

Methods: In total, 92 index patients with late-onset cerebellar ataxia (negative for SCA1, 2, 3, 6, 7 repeat expansions) and 171 with PD (negative for p.A53T in *SNCA*) were selected. All cases had no male-to male transmission. Genetic screening for the *FMR1* premutation was performed using fluorescent polymerase chain reaction (PCR) followed by capillary gel electrophoresis.

Results: The *FMR1* premutation was detected in 2 ataxia patients (2.2%), slightly above the range reported by multiple studies (0-1.9%) and 1 PD patient (0.6%), in line with previous studies (<1%). Both FXTAS patients from the ataxia cohort had neuropathy along with cerebellar ataxia, while one patient had mild parkinsonism and cognitive impairment and the other pyramidal signs. The FXTAS patient from the PD cohort had typical PD.

Conclusion: We conclude that, in the Greek population, the *FMR1* premutation is rare in PD, but should be considered in SCA panel-negative hereditary ataxia cases with supportive clinical features. Our study highlights the importance of genetic testing in the differential diagnosis and early management of FXTAS.

References:

Grants: None.

Conflict of Interest: None declared.

EP10.023 Variants inferring high risk for frontotemporal dementia (FTD) in Bulgarian patients

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Background/Objectives: Frontotemporal dementia (FTD) is a heterogeneous disorder with changes in behavior, language, executive control, and often motor symptoms. FTD is difficult to be diagnosed due to the lack of recognition and the overlap with other psychiatric disorders.

Our study aims to reveal the causative genes, and genetic risk variants in a well selected group of Bulgarian patients with FTD using whole exome sequencing approach.

Methods: Genomic DNA was isolated from peripheral blood samples of 140 patients, diagnosed with FTD. WES was performed on two pooled samples. Sequence reads were aligned to the reference genome (GRCh37/hg19), vcf files were generated and annotated with wANNOVAR. The interpretation of the variants was made according to their clinical significance (Varsome and ClinVar database) and frequency (gnomeAD Exomes).

Results: We identified nine variants related to FTD: *PSEN1* rs63751316 (MAF = 0.015) and rs63751287 (MAF = 0.0044); *SQSTM1* rs181263868 (MAF = 0.0044), rs761423892 (MAP = 0.0086), rs535932454 (MAF = 0.0026), *GRN* rs63750116 (MAF = 0.0052), rs774128685 (MAF = 0.0065), *TREM2* rs145080901 (MAF = 0.0025), rs147564421 (MAF = 0.0024). Variant rs63751287 is pathogenic, while the rest are variants of uncertain significance (VUS).

Conclusion: Due to geographical and ethnic variability, the prevalence of the causative genes or high risk variants for FTD may be different. We identified several genetic variations conferring risk for FTD in patients of Bulgarian origin. Their combination with a variety of environmental exposures may result in increased susceptibility to FTD.

References:

Grants: KP-06-N33/5 from 13.12.2019 - National Science Fund of Bulgaria.

Conflict of Interest: None declared.

EP10.024 El-Hattab-Alkuraya syndrome caused by biallelic WDR45B pathogenic variants: further delineation of the phenotype and genotype

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Background/Objectives: Homozygous pathogenic variants in *WDR45B* were first identified in six subjects from with global development delay, refractory seizures, spastic quadriplegia, and brain malformations. Since the initial report in 2018, no further cases have been described. In this report, we present 12 additional individuals from seven unrelated families.

Methods: Retrospective chart review of 12 individuals from seven families was conducted. The molecular diagnosis of *WDR45B*-related disorder, El-Hattab-Alkuraya syndrome, was made by clinical or research exome sequencing.

Results: Six different variants in *WDR45B* were identified, including five novels. Microcephaly and global developmental delay were observed in all subjects, and seizures and spastic quadriplegia in most. Common findings on brain imaging include cerebral atrophy, *ex-vacuo* ventricular dilatation, brainstem atrophy, and symmetric under-opercularization.

Conclusion: El-Hattab-Alkuraya syndrome is characterized by early onset cerebral atrophy resulting in microcephaly, developmental delay, spastic quadriplegia, and seizures. The phenotype appears to be more severe among individuals with loss-of-function variants suggesting a potential genotype-phenotype correlation in this disorder. A brain imaging pattern emerges which is consistent among individuals with loss-of-function variants and could potentially alert the neuroradiologists or clinician to consider *WDR45B*-related El-Hattab-Alkuraya syndrome.

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Grants: 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), NHGRI Baylor College of Medicine Genomics Research Elucidates Genetics of Rare (BCM-GREGoR; U01 HG011758).

Conflict of Interest: Mohammed Almannai: None declared, Dana Marafi: None declared, Ghada Abdelsalam: None declared, Maha Zaki: None declared, Ruizhi Duan: None declared, Daniel Calame: None declared, Isabella Herman: None declared, Felix Levesque: None declared, Hasna Elbendary: None declared, Ibhrahim Hegazy: None declared, Wendy Chung paid consultant for Regeneron Genetics Center., Haluk Kavus: None declared, Kolsoum Saeidi: None declared, Reza Maroofian: None declared, Aqeela Alhashim: None declared, Ali Al-Otaibi: None declared, Asma Al Madhi: None declared, Hager Aboalseood: None declared, Ali Alasmari: None declared, Henry Houlden: None declared, Joseph Gleeson: None declared, Jill Hunter: None declared, Jennifer Posey: None declared, James Lupski 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), NHGRI Baylor College of Medicine Genomics Research Elucidates Genetics of Rare (BCM-GREGoR; U01 HG011758)., has stock ownership in 23andMe, paid consultant for Regeneron Genetics Center.

Scientific Advisory Board (SAB) of Baylor Genetics, Ayman El-Hattab: None declared.

EP10.025 Genetic susceptibility to telomere shortening through the rs2293607 polymorphism is associated with a greater risk of alcohol use disorder

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Background/Objectives: Alcohol use disorder (AUD) is associated with shortened telomere length (TL), and single nucleotide polymorphisms (SNPs) in the telomerase complex may modulate

telomere length (TL). This study was conducted to examine the relationship of TL to AUD and the role of SNPs in TERC and TERT for this association.

Methods: A total of 308 male patients with AUD and 255 sex-matched healthy controls were included. TL was measured in 99 patients and 99 controls paired by age and smoking status and all individuals were genotyped for allelic discrimination of TERC SNPs rs2293607, rs12696304, and rs16847897 and TERT SNPs rs2735940, rs2736100, and rs2736098. Univariate and multivariable logistic regression analyses were performed. A receiver operating characteristic (ROC) curve was used to analyse differences in TL.

Results: The mean TL was shorter in patients with AUD than in controls. The area under the ROC curve was 0.70 ($P < 0.001$). The GG genotype of TERC rs2293607 was more common among AUD patients than among controls (9.8% vs. 5.1%; $P = 0.038$). No difference was found for the other SNPs. Carriers of the GG genotype of rs2293607 had shorter telomeres than did allele A carriers.

Conclusion: Patients with AUD had shorter telomeres. Genetic susceptibility to telomere shortening through the rs2293607 polymorphism is associated with a greater risk of AUD, although mechanisms are not yet established.

References:

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Conflict of Interest: None declared.

EP10.026 Molecular diagnosis of Kufor-Rakeb Syndrome in a 36-year-old patient

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Background/Objectives: Patient involuntarily hospitalized due to psychotic episodes with hallucinations and behavioral alteration. Personal history: normal psychomotor development, cannabis and alcohol consumption. Since 32 years old she showed a gait disturbance and a decline in cognitive functions. The neurological evaluation showed mild parkinsonian signs, moderate pyramidalism and supranuclear gaze palsy. MRI revealed severe cerebral atrophy for her age, suspecting an underlying genetic cause.

Methods: Whole exome sequencing was performed (xGen Exome Panel v2.0 kit). 15 genes were subsequently analyzed related to early-onset hereditary Parkinson's disease.

Results: Two heterozygous variants in the ATP13A2 gene (NM_022089.3) were detected: c.917G>A, p.(Trp306Ter) and the deletion of exon 1 (ExomeDepth tool). Both variants have not been described to date and were classified as pathogenic variants. Biallelic loss-of-function variants are known as a pathogenic mechanism associated with Kufor-Rakeb syndrome and complex hereditary spastic paraparesis 78. In order to confirm the deletion, we designed a long range PCR (GoTaq, Promega) from the last coding exon of the upstream gen (SDHB) to exon 2 of ATP13A2 gene. The amplification was sequenced by NGS (SureSelect, Agilent) and allowed to confirm a 8.8kb deletion fully involving the entire coding region of exon 1 (chr1:17336716-173344506; hg19).

Conclusion: The patient is heterozygous for two pathogenic variants in the ATP13A2 gene that confirm the diagnosis of Kufor-Rakeb Syndrome with a autosomal recessive inheritance. Family studies (unaffected father and sister) are currently in progress.

References:

Grants:

Conflict of Interest: None declared.

EP10.027 A novel SACS variant identified in a patient with autosomal recessive spastic ataxia of Charlevoix-Saguenay

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Background/Objectives: Spastic ataxia of Charlevoix–Saguenay is a rare progressive neurodegenerative disease caused by mutations in the *SACS* gene. Clinical phenotype is characterized by onset in the first decade with a triad of cerebellar ataxia, peripheral neuropathy, and pyramidal tract signs, with a significant disability on disease progression in adulthood. Additionally, other symptoms have been reported, such as deafness and generalized seizures.

Methods: The proband was identified by next-generation sequencing performed in 14 patients with early onset ataxia between 2019 and 2021.

Results: A now 33-year-old female presented with unstable gait and frequent falls when first walking at 12 months. In the first decade, she was diagnosed with early-onset ataxia, polyneuropathy and mixed hearing loss. Since age of 26 she was requiring two canes for walking, which has been disturbed by spasticity in lower limbs. Neurological examination revealed distal weakness of legs (particularly in feet dorsiflexion), decreased vibration sense, absence of tendon reflexes and loss of balance and coordination accompanied by slurred speech. Intragenic deletion NM_014363.6:c.12851_12854del (NP_055178.3:p.(Glu4284Alafs*23), rs786204628) and novel nonsense variant NM_014363.6:c.2764C>T (NP_055178.3:p.(Gln922*)) in the *SACS* gene, inherited from her father and mother, respectively was identified using NGS analysis and targeted multigene panel. Based on in silico analysis, identified variant is classified as pathogenic, and truncates SACS protein in highly conservative DNA sequence.

Conclusion: The identification of novel loss-of-function variant in described patient results the complex disease phenotype.

References:

Grants:

Conflict of Interest: None declared.

EP10.028 Trajectory of Neuroligin/Neurexin dysregulation associates with the establishment of an ASD-like phenotype in Tuberous Sclerosis

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Background/Objectives: Tuberous sclerosis (TS) is an autosomal dominant disorder caused by heterozygous mutations in either *Tsc1* or *Tsc2*, that both negatively regulate mTOR (mammalian target of rapamycin). At the synapse, mTOR is a key enzyme controlling the local synthesis of proteins. Its dysfunction leads to mis-regulation of cell growth and proliferation. The sequential appearance of TAND-symptoms in children with TS suggests a dynamic rather than a static process in disease development.

Methods: In a heterozygous *Tsc2* knock-out model we have longitudinally analyzed behavior at different time points after birth and have matched this with molecular signatures throughout brain development, to carefully characterize the cascade of cellular processes leading into a disease phenotype. To elucidate the molecular causes underlying the behavioral deficits, comparative proteome analysis of cortical homogenate and synaptosomes at different time points from early to late postnatal stages was carried out using serial Western blot and mass spectrometry analysis.

Results: We found that, similar to patients, TAND symptoms develop stepwise in a time-dependent manner. We show that Tsc2 is reduced only at very early stages and is fully compensated at later time points when behavior aberrations occur, suggesting that the behavior phenotype develops independent of the primary defect. Furthermore, we found that the formation of behavior aberrations correlates with a window of Neuroligin and Neurexin mis-expression in cortical but not hippocampal tissue.

Conclusion: Together our data suggests substantial homeostatic dynamics of gene expression underlying the TS phenotype and a correlation of ASD-like disease symptoms with cortical dysregulation of Neuroligins and Neurexin.

References:

Grants:

Conflict of Interest: None declared.

EP10.029 Genetic characterization of patients with bipolar disorder and controls for the generation of induced pluripotent stem cells

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Background/Objectives: Bipolar disorder (BD) is a psychiatric disorder characterized by recurrent episodes of (hypo-)mania and depression. Recent genome-wide association studies support a strong polygenic influence, but little is known about its functional effects in neurons. In this study, we performed genetic characterization of patients and controls for subsequent analyses of the contribution of polygenic factors to neurodevelopmental processes in induced pluripotent stem cell (iPSC)-derived neurons.

Methods: Patients with BD type 1 (BD1) and controls from the FOR2107 cohort (https://for2107.de/) were selected for iPSC generation according to the following criteria: male sex, availability of PBMCs and genome-wide genotype data, high polygenic risk score (PRS) for BD1 in patients and low PRS in controls (Stahl et al., 2019; PRS-CS), and no copy number variants significantly associated with BD or schizophrenia (cnvPartition). 10 patients (highest PRS) and 10 controls (lowest PRS) were whole-genome sequenced, and individuals with rare variants identified in previous BD sequencing studies (MAF < 1%, gnomAD, non-neuro dataset; PHRED-scaled CADD score>20; not present in patients and controls) were excluded.

Results: From 16 BD1 patients and 55 controls with PBMCs and genotype data, one patient was excluded due to a microdeletion on chromosome 2, and three BD1 patients (highest PRS) and three controls (lowest PRS) with no rare variants implicated in BD were selected for iPSC generation.

Conclusion: We describe a systematic procedure to characterize patients and controls at the genetic level prior to iPSC generation, leading to the exclusion of rare variants that might have influenced subsequent analyses.

References: Stahl et al., 2019, PMID:31043756.

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SC310030L_182731.
BR1337/4-1.

Conflict of Interest: None declared.

EP10.030 Associative analysis of 27 genetic variants (SNPs) with the variability in total cognitive scores defined by the MoCA (Montreal Cognitive Assessment) test in older people

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Background/Objectives: Cognitive decline with age is an important social and medical problem that leads to a significant reduction the quality of life of the elderly. Major cognitive function genes, as well as their relationship with neuropsychiatric diseases, have not been identified.The aim of this work was the association analysis of 27 SNPs with the variability in cognitive function, defined by the MoCA test, in the elderly.

Methods: The study was carried out on a population sample of the 241 elderly Russian individuals (64 men and 177 women). The mean age was 72.5±0.4 years (from 57 to 88 years). On the basis of the selected 27 markers was formed multiplexed SNPs panel Genotyping by MALDI-TOF mass spectrometry on Sequenom MassArray platform was performed. The relationship between the studied polymorphic variants and the MoCA scores were analyzed by using the nonparametric Kruskal-Wallis test.

Results: Four SNPs associated with the total MoCA test score were identified. These were polymorphic variants: 1) rs11191580 in the NT5C2 gene ($p = 0.037$); 2) rs1635 (NKAPL, $p = 0.042$). 3) rs17693963 (MHC, $p = 0.042$) and 4) rs2075650 (TOMM40, $p = 06034$). Previously, the first three genes were found to be associated with schizophrenia according to GWAS*. For the TOMM40 gene, GWAS also revealed an association with Alzheimer's disease.

Conclusion: The data obtained during the implementation of the project expand understanding of the neuropsychiatric diseases inheritance, as well as the biological processes underlying the decline in cognitive abilities in the elderly.

References: *https://www.ebi.ac.uk/gwas/.

Grants: The reported study was funded by RFBR, project number 20-015-00397.

Conflict of Interest: None declared.

EP10.032 Candidate regulatory variants in SNARE complex genes and their involvement in migraine susceptibility

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Background/Objectives: Migraine is a disabling and multifactorial neurological disease, remaining unexplained most of its heritability and susceptibility. Some migraine risk loci have been shown to reside in non-coding regions, which may alter gene expression and epigenetic regulation (1,2). Our aim was to select the best SNP candidates to study cis-regulation of genes previously associated with migraine susceptibility in the Portuguese population: SYN1, SNAP25, VAMP2, STXBP1, STXBP5, SYN2, UNC13B, GABRA3, GABRQ, and STX1A (3,4,5).

Methods: From a set of 22 tagSNPs within the candidate genes, we prioritized variants based on an integrative evaluation of their potential functional impact and regulatory features, by using Variant Effect Prediction, SNPinfo, SNPnexus, and HaploReg tools. We selected variants with at least 3 scores predicting deleteriousness (CADD, FunSeq2, DANN, ReMM, FATHMM, GWAVA, and RegulomeBD) and analysed regulatory features, DNA accessibility and histone modifications.

Results: Seven SNPs met our criteria, priority being given to the three SNPs located in major regulatory regions: rs6951030 (STX1A; promotor), rs2327264 (SNAP25; enhancer) and rs1150 (VAMP2; 3'UTR). Interestingly, both variants in SNAP25 and VAMP2 genes are in CTCF binding sites. We proceeded with functional validation of the regulatory effect of selected SNPs through luciferase reporter gene expression assays with migraineurs' DNAs.

Conclusion: In silico analyses suggested possible alterations in gene regulation of SNARE complex proteins implicated in exocytotic neurotransmitter release in migraine pathophysiology.

References: 1-Neurogenetics.2020;21:149–157. 2-BMC Medical Genetics.2010;11(1):103. 3-Headache.2020;60(10):2152-2165. 4-PLoS ONE.2013;8(9):e74087. 5-Arch Neurol.2010;67(4):422-427.

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Conflict of Interest: None declared.

EP10.034 The advantage of high throughput sequencing in the diagnosis of tuberous sclerosis. Clinical case

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Background/Objectives: Tuberous sclerosis refers to phakomatoses, determined by mutations in the TSC1 and TSC2 genes. Mutations in the TSC1 gene are clinically manifested by a milder course, with TSC2 mutations, the symptoms are more severe and not amenable to therapy. Identification of the mutation in a patient is important.

Methods: Targeted high-throughput sequencing, Sanger sequencing.

Results: The 4-year-old boy with symptomatic epilepsy, frequent polymorphic seizures that first appeared at the 16th week of life. The child from the second pregnancy proceeded against the background of termination of pregnancy in the 1st trimester. Objectively: the skin of the proband's buttocks and torso has multiple dense white matte and depigmented spots that were present from birth. MRI of the brain: cortical and subcortical focal changes in the brain is characteristic of Tuberous sclerosis. The family history of mother and father is aggravated by oncopathology. High-throughput targeted sequencing of clinically important genes has been recommended. The proband was found to have a de novo mutation of the TSC2 gene c.1869del(p.Asp624Thrfs*74). The diagnosis «Tuberous sclerosis» was confirmed. In addition, the patient and his mother were found to be carriers of the pathological mutation of the SGSH c.220C>T(p.Arg74Cys) gene responsible for the development of type IIIa mucopolysaccharidosis.

Conclusion: Using modern sequencing methods, a mutant gene was identified in the patient. In the proband and his mother, a pathological mutation of the SGSH gene was found in the heterozygous state. The information obtained is important for the prognosis of the child's life and for medical genetic counseling of the family.

References:

Grants:

Conflict of Interest: None declared.

EP10.036 The other side of ACOX1 variation and a case of Mitchell syndrome

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Background/Objectives: Homozygous loss-of-function variants in ACOX1 cause peroxisomal acyl-CoA oxidase deficiency. Here we describe the case of a child with a recurrent heterozygous gain-of-function variant, recently proven to cause Mitchell syndrome.

Methods: A 5-years old boy was admitted following an acutely presenting and rapidly progressive ataxia. He was born to unrelated parents, after an uneventful pregnancy and had a normal early development. Around the age of 2 he was diagnosed with mild hearing loss which subsequently progressed to severe/profound hearing loss around the age of 5 years of age. Clinical examination on admission showed ataxia, lower limb spasticity, slurred speech, and developmental regression. Ophthalmological examination revealed corneal erosions, hypermetropia and astigmatism. The MRI showed atypical changes of the dentate nucleus and excluded a brain tumour. The metabolic investigations showed slightly increased lactate level in blood and cerebrospinal fluid, and normal very long chain fatty acids.

Results: The array CGH and mitochondrial sequencing were normal. With parents' consent, we carried out rapid trio exome sequencing which identified a *de novo*, heterozygous missense variant in *ACOX1*. This variant had been reported in three other

patients with Mitchell syndrome, a newly described neurodegenerative condition with a different molecular mechanism and response to antioxidant therapy than *ACOX1* deficiency.

Conclusion: The association of hearing loss, rapidly progressive ataxia, eye abnormalities including corneal lesions with normal metabolic tests in a chid with previously normal development should suggest testing for Mitchell syndrome. Early diagnosis can orient management and genetic counselling.

References: Chung HL, et al., Neuron. 2020 May 20;106(4):589-606.e6.

Grants:

Conflict of Interest: None declared.

EP10.037 Two novel variants in a transmembrane domain of GRID2 confirm the phenotype of GRID2-related dominant non-progressive congenital ataxia

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Background/Objectives: The *GRID2* gene codes for the delta-2 subtype of the glutamate ionotropic receptors (GluD2), the ion channel responsible for rapid excitatory synaptic transmission in the central nervous system. Autosomal recessive ataxia due to loss of function have been described, while a gain of function is suspected in dominant, progressive adult ataxia (inherited missense) or congenital ataxia (CA) (*de novo* missense, two patients reported).

Objective: to describe the phenotype and genotype of two new patients with congenital ataxia related to dominant pathogenic variants in a transmembrane domain of GRID2.

Methods: Using a NGS panel of CA genes including *GRID2*, we identified two novel heterozygous variants in two CA patients.

Results: The variants (p.Ala653Asp and p.Gly841Arg) affect the transmembrane domain of the ion channel, like the two dominant mutations previously published. One was *de novo*, and the second was a somatic mosaic in the asymptomatic mother.

The phenotype combines hypotonia and cerebellar ataxia in children who make progress, and on MRI, global cerebellar atrophy or a more severe pontocerebellar hypoplasia. The cognitive phenotype of patient 1 appears more severe than that of patient 2, whose marked motor involvement contrasts with better preserved cognitive functions, as described in our 2 previously published patients.

Conclusion: These 2 new patients confirm the nonprogressive CA phenotype with cerebellar atrophy or pontocerebellar hypoplasia, associated with de novo dominant mutations, in the transmembrane domain of GRID2.

Reassuring genetic counselling for a *de novo* mutation must be tempered by the risk of parental, germ-line or somatic mosaic, as observed in family 2.

References:

Grants:

Conflict of Interest: None declared.

EP10.039 Childhood onset chorea: an overview of genetic etiologies in a series of 85 patients

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Background/Objectives: Chorea is a hyperkinetic movement disorder (MD), the third most frequent MD in children, after tics and dystonia. It is characterized by random, continuous and brief involuntary movements, and may be acquired or genetic. Autosomal dominant NKX2-1 related chorea is the most frequent cause in children, in which the MD may be associated with hypothyroidism or pulmonary disorders. More recently, other genes have been identified (ADCY5, GNAO1, etc.).

Objectives: To assess the genetic epidemiology of childhood onset chorea.

Methods: We reviewed the clinical history of 85 patients initially referred for *NKX2-1* analysis (2011-2019). When early onset chorea was confirmed, negative *NKX2-1* patients were analysed using a NGS panel (104 MD genes), and then exome or genome sequencing.

Results: 75 patients were included. 34 were related to *NKX2-1* anomalies. Panel analysis allowed the identification of causal variants in 45% non-*NKX2-1* patients (16/38), and exome/genome sequencing in 4. *ATM, ADCY5* and *GNAO1* were the most frequent genes after *NKX2-1*. A diagnosis of glutaric aciduria was made in a 70-year-old patient emphasing the need to carefully look at the MRI. Chorea was a milder phenotype or onset presentation of known pathologies related to *KMT2B, GNB1* (mosaicism), *PDE10A* for example.

Conclusion: We identified a genetic cause in 72% patients of the series, *NKX2-1* being the major gene (45%). NGS panel was a performant tool, allowing also diagnosis of mosaicism and deletions, simpler than exome analysis. Etiological diagnosis of chorea is important for genetic counseling, etiological treatment if available, and management of possible associations like cancer (*NKX2-1*, *ATM*).

References:

Grants:

Conflict of Interest: None declared.

EP10.040 Rare pathogenic variants in whole exome sequencing data of Bulgarian Alzheimer's disease patients

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Background/Objectives: Alzheimer's disease (AD) is a complex disorder of still unclear genetic etiology involving rare and common variants. Taking into consideration the genetic heterogeneity of AD, in the present study we have focused on rare pathogenic variants found in exomes of affected individuals.

Methods: The screening of rare pathogenic variants was performed on whole exome sequencing data for a DNA pool sample of 66 Bulgarian AD patients. Reads were aligned to the reference genome (GRCh37/hg19) and variants were annotated using wANNOVAR.

Results: Five of the detected variants are rare (with minor allele frequency-MAF < 0.001 or not reported in gnomAD) and are classified as pathogenic, likely pathogenic or with uncertain significance in ClinVar or Varsome. Variants rs63750053, rs28936380 and rs104894002 are located in AD-related genes (*PSEN1, PSEN2* and *TREM2*, respectively). They have the following MAFs in our sample 0.0065, 0.0035 and 0.0160, respectively. The remaining two variants are in genes affecting AD susceptibility: rs376113829 in *CLU* (clusterin) and rs781466248 in*CR1* (complement receptor type 1) gene. They are found with MAF of 0.0047 and 0.0031 in our sample. All variants are not found among Bulgarians in gnomAD, with the exception of rs63750053 which is not reported. The higher prevalence of these variants in patients compared to the reference population points to their role in the development of AD.

Conclusion: The better understanding of rare genetic variants in AD can better clarify the disease etiology, leading to introduction of novel blood biomarkers for prevention and early diagnosis.

References:

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Conflict of Interest: None declared.

EP10.041 Analysis Copy Number Variations in 25 Mexican Families with Mesial Temporal Lobe Epilepsy using high-density SNP array

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Background/Objectives: Mesial temporal lobe epilepsy (MTLE) is the most frequent type of focal epilepsy, representing between 40-60% of all types of epilepsy. Studies of familial mesial temporal lobe epilepsy (FMTLE) have shown a wide phenotypic and genetic heterogeneity. Recent studies on different types of epilepsy have shown copy number variations (CNVs) that included genes involved in the epileptogenesis in 1-10% of cases. In this research we focus on Mexican families with MTLE, we analyzed pattern of inheritance and CNV associated to disease.

Methods: We identified families with a diagnosis of MTLE in the epilepsy clinic of the Hospital General de México "Dr. Eduardo Liceaga" in Mexico City. The genealogy was constructed to determine the pattern of inheritance, for the identification of CNVs we used the Cytoscan-HD array and the analysis of the CNVs was carried out with the ChAS v3.1 Software from Thermo Fisher Scientific®. Associations and differences between inheritance patterns and CNV were determined by statistical analysis.

Results: A total of 25 families with 61 members affected with MTLE were included. We observed a 17 families with autosomal dominant (AD), and 8 with autosomal recessive (AR) inheritance patterns. Chromosome microarray analysis revealed mosaic duplication in 1p36.33, segregated in 5 affected members of two families with AD inheritance pattern. arr[GRCh37]1p36.33(849470_3586255)x2[2.25].

Conclusion: In summary, we found that FMTLE had genetic heterogeneity, with a mosaic 1p36.33 duplication being a possible new mechanism associated with the etiology of FMTLE.

References: Candace T and Heather C, Genome medicine, 7(1):91, 2015.

Grants: Recursos federales DI/15/403/03/10, CONACyT: 574048.

Conflict of Interest: None declared.

EP10.042 Rett and Rett-like presentation: Underlying genetic factors among 18 children from a tertiary care centre in India

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Background/Objectives: Rett Syndrome (RS) is a neurodevelopmental disorder, predominantly affecting girls. This condition is caused by mutations in MECP2 gene and has an X linked dominant inheritance. It primarily presents as neuroregression, autistic features and acquired microcephaly. Recent studies have identified mutations in a set of genes presenting with an overlapping phenotype to Rett syndrome.

Methods: We present a series of 18 children diagnosed as having Rett syndrome or variant Rett syndrome. Ethical approval was procured from our Institutional Ethics Committee. Appropriate consenting was done for all the recruited cases. The underlying causative pathogenic variants were identified by Clinical / Whole Exome Sequencing. In-depth analysis was also performed to highlight the genotype- phenotype correlation.

Results: Next Generation Sequencing yielded pathogenic variants in MECP2 gene in 11 patients, lQSEC2 gene in two, and in FOXG1, CDKL5, SHANK3, HNRNPH2 and ATP6V0A1 in one case each, leading to phenotypic presentations ranging from typical Rett syndrome to Phelan-McDermid Syndrome and a congenital variant of Rett syndrome. While 14 mutations have been reported in literature, we report four novel mutations – two in MECP2, and one each in CDKL5 and HNRNPH2 genes. Out of the 8 cases with epilepsy, 5 cases were of atypical Rett. Often, the variant forms of Rett, portray a comparatively more severe condition as indicated in our cases.

Conclusion: Screening of a broader gene panel is essential to ensure accurate diagnosis in clinical presentations of Rett-like phenotype. The novel pathogenic variants we reported also add to the existing literature.

References:

Grants: NIMH/PROJ/GAU/00580/2018-19.

Conflict of Interest: CHETAN GHATI NIMHANS Intramural Grant No. NIMH/PROJ/GAU/00580/2018-19, Sanivarapu Lakshmi Sravanti: None declared, ananthapadmanabha kotambail: None declared, GAUTHAM ARUNACHAL UDUPI: None declared, shruthy sreedharan: None declared, ramya sukrutha: None declared, hansashree padmanabha: None declared, Ravindranadh Mundlamuri: None declared, Raghavendra K: None declared, saraswati nashi: None declared, rajendra k m: None declared.

EP10.043 STR pathogenic expansions are the underestimated cause of neurogenetic disorders

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Background/Objectives: Short tandem repeat (STR) sequences are DNA regions of tandemly repeated nucleotide motifs of 2–6 bases and are highly polymorphic with very high mutation rates. STR expansions in pathogenic range have been proven to be the causal genetic event for various diseases, but mainly neurogenetic.

Our experience have shown that about 70 % of patients with neurological disease remain with an unrecognized causal genetic aberration even after WES or WGS analysis of single nucleotide variants and short insertions and deletions.

Methods: STR expansion analysis was performed on 173 WES samples of patients without genetic diagnosis. The analysis was done by ExpansionHunter (1) on known set of 42 STR locus sites associated with neurological disease, that were already published in literature. The method was successfully tested by identifying STR expansions in 3 WES and 3 WGS samples which were previously laboratory confirmed.

Results: From 173 WES samples, we identified 5 patients with STR expansions in intermediate or pathogenic range in genes CACNA1A, PHOX2B, PRMD12 and ZIC2. ExpansionHunter pinpointed additional 31 samples with STR expansions in pathogenic range in ARX gene. However, manual analysis of ARX locus showed very poor read coverage of this region in WES samples and therefore we do not considered these findings as valid results.

Conclusion: Our preliminary results show that we could increase diagnostic rate of neurological diseases from WES by STR analysis by 2.89 %.

References: 1 – Dolzhenko 2019.

Grants: Supported by the MHCR AZV NU20-04-00279..

Conflict of Interest: None declared.

EP10.044 Genetic markers of CCDC60, DCHS2, LSM1 and LOC105373605 demonstrate association with schizophrenia in Russian population

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Background/Objectives: Data on the common links in the pathogenesis of psychiatric and neurological disorders including schizophrenia, Alzheimer's disease, bipolar disorder, Parkinson's disease are accumulating. Thus, statistical evidence has shown that schizophrenia and Alzheimer's disease have a specific molecular background. Schizophrenia is a complex disease with genetic, environmental and lifestyle factors. GWAS has provided massive information to the public databases. The aim of this study was to analyze associations of 45 SNPs reported in GWAS with schizophrenia in Russian population of Siberian region.

Methods: 45 SNP markers that showed repeated association with schizophrenia and Alzheimer's disease in the GWAS were genotyped by MALDI-TOF mass-spectrometry using MassARRAY Analyzer 4 (Agena Bioscience) in Russian patients with schizophrenia (N = 350) and in healthy control group (N = 650). Allele-specific ORs and associated p values were calculated.

Results: We identified four genetic loci that were significantly associated with schizophrenia risk for the Russian population of Siberian region: rs11064768 at CCDC60 gene, rs1466662 at DCHS2 gene, rs16887244 at LSM1 gene and rs7561528 at LOC105373605. These genetic markers were previously reported in GWAS and associated with schizophrenia (rs11064768 CCDC60 gene, rs16887244 at LSM1 gene) and Alzheimer's disease (rs1466662 at DCHS2 gene, rs7561528 at LOC105373605).

Conclusion: Genetic markers of CCDC60, DCHS2, LSM1 and LOC105373605 are associated with schizophrenia but their role in pathogenesis of the disease needs to further study. Our findings also demonstrate that genetic variability in schizophrenia and Alzheimer's disease has overlapping genetic background.

References:

Grants: This work was supported by the Russian Foundation for Basic Research (project 20-015-00397).

Conflict of Interest: None declared.

EP10.045 Early Infantile Epileptic Encephalopathy related to NECAP1; clinical delineation of the disease and review

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Background/Objectives: Epileptic encephalopathy (EE) refers to a heterogeneous group of epilepsy syndromes characterized by seizures as well as encephalopathies, leading to cognitive and behavioral disturbances. These conditions vary in their age of onset, their severity as well as their electroencephalographic patterns. While genetic factors are involved in approximately 40% of all epilepsy cases, they contribute to 80% of infantile epileptic encephalopathies (EIEE) with around 125 genes previously linked to this disease. Here we report the first homozygous missense mutation in the *NECAP1* gene and we achieved a better clinical characterization of the *NECAP1*-linked disease.

Methods: Whole exome sequencing (WES) was performed in a 9-month-old Lebanese girl presenting with EIEE.

Results: WES enabled the detection of a homozygous missense mutation in the *NECAP1* gene (NM_015509.3: p.Glu8Lys) in the proband.

Conclusion: Here we report the first homozygous missense mutation in the *NECAP1* gene in a 9-month-old girl presenting with EIEE. Our findings allow a better characterization of the *NECAP1*-linked disease and enable to broaden its clinical spectrum by including, in addition to EIEE, severe generalized hypotonia, poor feeding, developmental delay, severe microcephaly, delayed myelination, abnormalities of the corpus callosum, and eye abnormalities.

References: NA

Grants: NA

Conflict of Interest: None declared.

EP10.047 Targeted Next Generation Sequencing (NGS) identified novel mutations in rare neurodevelopmental disorders

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Background/Objectives: Neurodevelopmental disorders (NDDs) are genetically heterogeneous, leading to disruptive cognition, impaired adaptive behavior, and interrupted psychomotor and communication skills. Examples of NDDs include attention deficits/hyperactivity disorder (ADHD), microcephaly, autism spectrum disorder (ASD), learning disabilities, intellectual disability (ID), mental retardation, schizophrenia, conduct disorders, and impairments in speech and hearing. Autosomal recessive primary microcephaly (MCPH) is characterized by a significant reduction in head circumference (-3 to -5SD) with normal architecture of brain. The objective of the study was to identify novel variants through gene panel via Next Generation Sequencing in the individuals affected with MCPH.

Methods: Blood samples of the affected families were collected from various regions of Sothern Punjab, Pakistan. DNA was extracted by performing the Salting-out method of DNA extraction. Mutational analysis was done by performing gene panel sequencing for 86 candidate genes including 28 genes for MCPH in 7 families.

Results: A novel homozygous splice site variant (NM_018136.4; c.442-18T>G) was identified in intron 2 of the *ASPM* gene leading to splicing errors. A homozygous variant (NM_018136.4; c.9492T>G) of the *ASPM* gene leading to protein truncation (p.Tyr3164*) was also identified in one family. In another family, we identified a novel nonsense variant (p.Arg260*) in the *CDK5RAP2* gene. Furthermore, 3 families in which potential disease variants in panel genes were not identified are subjected to whole-exome sequencing.

Conclusion: Our study enhances the mutation spectrum of MCPH and identified novel families with the potential to identify new causative gene/s responsible for MCPH phenotype.

References:

Grants: Nil.

Conflict of Interest: None declared.

EP10.049 A novel DEAF1 gene variant in patient with suspected Vulto-van Silfhout-de Vries syndrome

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Background/Objectives: Vulto-van Silfhout-de Vries syndrome (VSVS; OMIM# 615828) is a rare hereditary disease associated with impaired intellectual development and speech, delayed psychomotor development and behavioral anomalies, including autistic behavioral traits and poor eye contact. To date, 27 patients with VSVS have been reported in the world literature [1].

Methods: We describe a clinical case of a man with autism spectrum disorder and serious malabsorption syndrome, who underwent various genetic tests during his life with non-informative results. At the age of 23 patient underwent whole genome sequencing (WGS) in gastroenterological hospital of GBUZ Moscow Clinical Scientific Center named after Loginov MHD.

Results: We found a heterozygous variant chr11:687913G>A (c.662C> T, p.S221L) in ex 4 of the *DEAF1* gene (NM_001293634.1), that was confirmed by sanger sequencing. A segregation analysis showed wild type of the *DEAF1* gene in patient's mother, father and sister, a "de novo" character of mutation was established. This variant had not been previously described in the gnomAD, 1000G, ClinVar, ExAC, Human Genome Mutation and Human Genome Variation databases. According to the American College of Medical Genetics criteria (PM1, PM2, PM5, PP3, PP4) this missense variant was considered as a pathogenic.

Conclusion: WGS made it possible to identify a new causative genetic variant in the *DEAF1* gene and establish a final clinical VSVS diagnosis. This is highly important for genetic counseling and determining the prognosis of the disease.

References: https://doi.org/10.1016/j.ajhg.2014.03.013.

Grants:

Conflict of Interest: None declared.

EP10.050 Posterior lissencephaly caused by domain specific missense-variants in CEP85L

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Background/Objectives: Lissencephaly is a neurodevelopmental disorder (NDD) characterized by the absence of normal cerebral convolutions with abnormalities of cortical thickness. It can affect the whole brain but also be region specific.

Methods: We report a 39-year-old man with pronounced lissencephaly (parietal, occipital and temporal gyri malformations). We conducted exome sequencing and segregation analysis by RT-PCR. We reviewed all described cases to perform computational variant analyses and standardize the phenotypic description (HPO terms).

Results: We identified the heterozygous *CEP85L* missense variant NM_001178035:c.191C>T, p.(Ser64Phe). Allele specific RT-PCR confirmed the variant on the paternal allele. As the father was healthy, a *de novo* occurrence on the paternal allele seems likely

but cannot be further discerned. A damaging effect is predicted for the affected amino acid substitution by multiple *in-silico* tools. All described variants fall into an unstructured, but highly conserved N-terminal protein domain. Considering this clustering and the low protein wide conservation scores (gnomAD pLI: 0, Z-score: 0.4) a currently unknown functionally critical domain in this region seems likely.

Review of described individuals (n = 19) showed variable NDD in 100% with global developmental delay in 42%, speech or motor delay in 37% and 16%, respectively, and intellectual disability in 32%. Abnormal cortical gyration and lissencephaly were present in 84% and 47% had subcortical band heterotopia. Most individuals had seizures (95%).

Conclusion: After the recent initial descriptions of a *CEP85L* associated disorder, the identification of the variant in the individual presented here further supports the implication of the *CEP85L* gene with posterior cortex lissencephaly with associated clinical characeristics.

References:

Grants:

Conflict of Interest: None declared.

EP10.051 Investigation of variants causing hot water epilepsy by next generation sequencing approach in Turkish population

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Background/Objectives: Epilepsy is a neurological condition characterized by recurrent epileptic seizures that may start at any time in a life-time. Hot Water Epilepsy (HWE) is a rare subtype of partial epilepsy. The contact with hot water especially during bathing may cause seizures in HWE. This study aims to reveal the genetic background of HWE by using whole exome sequencing (WES) data.

Methods: The WES and SNP array data of 10 HWE patients generated as a part of a collaborative study had been analysed using our in house analysis pipelines.

Results: In WES analysis, we have detected novel variants in *SCN9A* in two unrelated individuals. In our case series, we also had other novel variants in epilepsy related genes including *SCN2A*, *SLC1A2*, *KCNB1*, and *RELN*. SNP analysis revealed overlapping deletions in a subgroup of our patients.

Conclusion: Most of the variants identified within the scope of the study were considered compatible because they belonged to the channel genes families and the epilepsies were associated with channelopathy. The analysis of the patient group is also ongoing for new genes and copy number variations.

References:

Grants: We thank the Turkish Academy of Sciences for the 2019 Distinguished Young Scientist Award to SAUI. OFD is a national graduate fellow of the TUBITAK BIDEB 2210/A program. WES and SNP data have been produced in the context of the Epi25 consortium.

Conflict of Interest: None declared.

EP10.052 Genetic and clinical analysis of 17 patients with polymicrogyria

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Background/Objectives: Polymicrogyria is defined as an excessive number of abnormally small cerebral gyri and is one of the most frequent types of malformations of cortical development. Aetiology can be genetic or environmental. Here we present genetic and clinical analysis of 17 patients with polymicrogyria detected by brain MRI 1.5 T.

Methods: Clinical phenotyping was performed using hospital medical records. Genetic analysis included chromosomal microarray for detection of copy number variants (CNVs) and clinical exome sequencing (CES) for detection of single nucleotide variants (SNVs) and small insertions and deletions (indels).

Results: Five patients have isolated polymicrogyria, 12 patients have other associated brain malformations and three patients have additional congenital anomaly. Five pathogenic CNVs were detected: 22q11.2 microdeletion in three patients, 22q11.2 microduplication in one patient, and *MECP2* duplication in one patient. CES revealed novel likely pathogenic variants in two patients: heterozygous SNV in *AKT3* gene (NM_005465.7:c.233A>G, p.Gln78Arg) and homozygous indel in *CEP135* gene (NM_025009.4:c.2968_2969delAT, p.Ile990TyrfsTer11). Six variants of uncertain significance were detected: one CNV in one patient, and five SNVs in three patients. In two patients, with no genetic findings, cytomegalovirus was diagnosed as a cause of polymicrogyria.

Conclusion: Our findings illustrate the aetiologic and clinical heterogeneity of polymicrogyria. Despite extensive diagnostic work-up, definitive molecular diagnosis often remains unknown. Further research is needed, with aim of finding additional genes involved in development of polymicrogyria.

References:

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Conflict of Interest: None declared.

EP10.053 NARS1-related epilepsy in a Bulgarian patient

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Background/Objectives: Epilepsy is a heterogenic neurological disorder which is characterized by epileptic seizures, atypical movements, intellectual and motor disability and behavioural problems. It could be monogenic or multifactorial and more than 500 genes have been associated with this condition. Here we report a patient with epilepsy and microcephaly caused by mutations in the NARS1 gene.

Methods: A patient with epilepsy, microcephaly and global developmental delay was referred for genetic diagnostics. Whole exome sequencing (WES) and Sanger sequencing for segregation analysis in the family were performed.

Results: Two heterozygous missense variants (c.676G>C, p.Val226Leu and c.986G>A, p.Arg329Gln) were detected in the NARS1 gene. The variants segregate in the family: the variant c.676G>C is with maternal origin, while the variant c.986G>A is with paternal origin. These variants have not been described

before in association with human disorders. Based on that we classified them as variants with uncertain significance. Recently reported pathogenic variants (frameshift, nonsense, missense, etc.) in the NARS1 gene have been associated with neurodevelopmental disorder with microcephaly, impaired language, and gait abnormalities (OMIM: 108410), which corresponds to the phenotype, observed in our patient.

Conclusion: The results from the molecular-genetic analysis confirm the necessity of genetic verification of epilepsy in patients. The affected families can benefit adequate genetic counselling and prenatal diagnostics. Novel genetic data can enrich the spectrum of epilepsy related genetic variants and can explain the genetic ethology of the disease.

References:

Grants:

Conflict of Interest: None declared.

EP10.054 Two candidate genes with biallelic variants associated with a neurodevelopmental disorder in a consanguineous family from Turkey

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Background/Objectives: Neurodevelopmental disorders (NDDs) refer to central nervous system dysfunction throughout the developmental stages that resulted in impaired learning, motor function, behaviour, language, or non-verbal communication. NDDs comprise various heterogeneous disorders such as developmental delay, intellectual disability, autism spectrum disorders, and epilepsies. Herein, we present a family with NDD having two different biallelic gene variants to delineate associated phenotypes.

Methods: A family from Turkey with first-cousin parents, two affected boys with NDD, and three unaffected siblings were recruited to this study after their signed consent. SNP-based homozygosity mapping was conducted in five children to define linkage intervals and whole exome sequencing (WES) was performed in the affected sib-pair.

Results: Two pathogenic biallelic variants in FOLR1 and TENM2 genes were detected in affected siblings. Sanger sequencing confirmed homozygous segregation of candidate variants within the family.

Conclusion: FOLR1 encodes folate alpha receptor and has been associated with neurodegeneration due to cerebral folate transport deficiency (MIM: 613068). TENM2 has role in the regulation of synaptic connections in the brain and has been suggested as a potential epilepsy-associated gene previously. We suggest that two pathogenic gene variants contribute to NDD phenotype. Additionally, combined analysis of SNP-based whole-genome genotyping and WES is a powerful and accurate tool to genetically diagnose clinically heterogeneous diseases from consanguineous families.

References:

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Conflict of Interest: None declared.

EP10.056 Diagnosis of Williams Syndrome by Whole Exome Sequencing (WES)

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Background/Objectives: Williams-Beuren syndrome (WBS) is a rare genetic disorder (1:8000 births) characterized by intellectual disability, unique personality characteristics, growth delay, distinctive facial features, and cardiovascular abnormalities. WBS is caused by heterozygous large deletions (Copy Number Variant/CNV) of 1.5 to 1.8 Mb on chromosome 7q11.23 encompassing up to 28 genes including the critical *ELN, GTF2I, GTF2IRD1, DNAJC30* and *BAZ1B* genes. The gold standards for CNV detection are array-comparative genomic hybridization (array-CGH) and target specific Multiple Ligation Probe Amplification (MLPA) or Fluorescent in Situ Hybridization. Detecting CNVs with Whole Exome Sequencing (WES) applied for single or oligo-nucleotide variants, is still challenging and requires ongoing computational advances.

Methods: A 3-month-old male was referred with hypotonia, global developmental delay, staring episodes and microsomia. Following clinical evaluation, pre-test counselling and signed consent, WES was implemented using xGen Exome Research v2 kit (Integrated DNA Technologies) for library preparation and NextSeq-500 system (Illumina) for sequencing. Bioinformatic analysis was performed on VarSome Clinical platform, including CNV detection by ExomeDepth. Variants evaluated using the VarSome database were categorized according to ACMG and ClinGen recommendations.

Results: A 1.5 Mb heterozygous pathogenic deletion, encompassing the critical WBS region and genes on chromosome 7q11.23, was detected by ExomeDepth and confirmed by MLPA (MRC P245-A2).

Conclusion: Implementation of ExomeDepth in routine WES analysis can contribute valuable information towards elucidating the etiology of rare genetic diseases, increasing the diagnostic yield and minimizing the need for additional tests.

References: PMID: 20089974, 33610060, 22942019.

Grants: None.

Conflict of Interest: None declared.

EP10.057 Phenotypic expansion of SPATA5 associated neurodevelopmental disorder and natural history of this condition in adulthood

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Background/Objectives: Biallelic variants in *SPATA5* (OMIM *613940) cause a neurodevelopmental disorder with hearing loss, seizures, and brain abnormalities (NEDHSB, OMIM #616577). To date, about 30 patients have been described and most of them were diagnosed in childhood. Frequently reported neuroimaging abnormalities included delayed myelination, thin corpus callosum and (progressive) cerebral atrophy.

Methods: The 52-year-old patient had early developmental delay. Fever-related seizures occurred in infancy. Although gross motor abilities developed slowly and she could walk independently at the age of 4.5 years, she never acquired active speech. Hearing impairment was known since adolescence. The patient developed generalized tonic-clonic seizure at the age of 20 years. Since the age of 40 years the patient lived in a residential care unit. At the age of 51 years balance problems and falls developed. Cerebral MRI was initiated and revealed supratentorial leukoencephalopathy. After intensive clinical reevaluation and comprehensive metabolic testing had been inconclusive, WES was performed.

Results: In *SPATA5* an in-frame-deletion (NM_145207.3: c989_919del, p.(Thr330del)), that had been reported in multiple patients with NEDHSB and a very rare, previously not reported missense variant (NM145207.3:c.1361A>G, p.(Glu454Gly)), predicted to be pathogenic (PolyPhen2, Sift, CADDphred 26,5), was identified. The healthy mother carried only the missense variant, the father was deceased.

Conclusion: Here we present the detailed clinical history and adult phenotype of a 52-year-old German woman affected by NEDHSB. This individual is the oldest published NEDHSB case to date, expands the associated phenotype to leukoencephalopathy and provides information on the condition's natural history.

References:

Grants:

Conflict of Interest: None declared.

EP10.058 Phenotypic expansion in KIF1A-related disorders: A description of pathogenic variants two Turkish patients

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Background/Objectives: KIF1A Associated Neurological Disorders (KAND) comprise an identified group of rare neurodegenerative conditions caused by mutations in KIF1A. KIF1A is a member of the kinesin-3 family of microtubule motor proteins. The phenotypic spectrum of KAND includes microcephaly, neurodevelopmental delay, intellectual disability, progressive spastic paraplegia, peripheral neuropathy, optic nerve atrophy, cerebral and cerebellar atrophy, and seizures.

Methods: In this study, we describe the clinical, neuroradiological and genetic features two Turkish patients harboring heterozygous KIF1A variants detected using whole exome sequencing(WES). Pathogenicity of the identified variants has been evaluated in accordance with the American College of Medical Genetics and Genomics(ACMG) guidelines.

Results: Patients one was a 10-months-old female born at term from non-consanguineous parents. At the age of seven months, she presented global development delay, microcephaly, hypotonia, spasticity, seizures, and an abnormal electroencephalogram. MRI showed a white matter abnormalities. WES analysis revealed a heterozygous missense variant, NM_004321.8:c.296 C>T(p.Thr99Met) in the KIF1A gene. The second patient was a 6-six-years-old female whose parents were non-consanguine, who had psychomotor delay, ataxia and urgency. Her brain MRI showed cerebellar atrophy. WES analysis revealed a heterozygous missense variant, NM_004321.8:c.32 G>A(p.Arg11Gln) in the KIF1A gene.

Conclusion: We describe two patients with pathogenic variants in the KIF1A motor domain. Clinical and genetic findings of the patients described in this cases will contribute to the expansion of the genetic and clinical spectrum of the disease. Variants in KIF1A cause a wide spectrum of neurodevelopmental and neurodegenerative disorders. Better understanding of the phenotypic breadth and the disease subtypes could lead to improvements in diagnosis.

References:

Grants:

Conflict of Interest: None declared.

EP10.059 Detection of rare genetic variants in a group of patients with autism spectrum disorders

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Background/Objectives: Autism spectrum disorders (ASDs) are complex neurodevelopmental diseases characterized by clinical and genetic heterogeneity. A wide spectrum of genetic defects is reported in ASDs, among which rare copy number variants (CNVs) have a well-recognized contribution to ASD pathogenesis. We report the results of array-based genomic comparative hybridization (array-CGH) and triplet primed PCR / MS-MLPA screening for fragile X syndrome, in a group of 200 ASDs patients.

Methods: All patients (50 girls and 150 boys) were diagnosed with ASD. The clinical workup included neurological, psychiatric and psychological evaluations. Array-CGH was performed in all patients using 4x180K platforms (Agilent Technologies). *FMR1* promotor was screened for abnormal methylation (MS-MLPA) and trinucleotide repeat expansion (TP-PCR), for male patients only.

Results: Rare pathogenic or likely pathogenic CNVs were detected in 19 patients, with an increased prevalence of pure deletions in 11 patients, while duplications were present in 7 patients; one patient presented a complex rearrangement (a duplication flanked by two deletions). A number of 54 CNVs were interpreted as VOUS (21 deletions and 33 duplications), including candidate ASDs genes. Three patients presented full mutation on the *FMR1* gene. Thus, the genetic etiology was elucidated in 11% of patients.

Conclusion: Taking into account the striking genetic heterogeneity of ASDs, new patient cohorts with deep phenotyping and molecular characterization supplement the existing data on rare CNVs role in ASDs.

References:

Grants: The research leading to these results has received funding from the EEA Grant 2014-2021, under the project contract No 6/2019.

Conflict of Interest: None declared.

EP10.060 Analysis of putative regulatory variants from whole genome sequencing data of 140 patients affected by neurodegenerative disorders by massively parallel reporter assay

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Background/Objectives: Neurodegenerative diseases are characterized by a progressive neurological impairment. A small number of patients showed a disease family history indicating that genetic factors play a crucial role in disease etiology. NGS has increased the rate of genetic detection, however, a missing heritability was reported which could be explained by variants in non-coding regions. Besides affecting splicing mechanism, disease-causing non-coding variants could operate deregulating gene expression. Massively Parallel Reporter Assay (MPRA) allow to analyze hundreds of thousands of regulatory variants and predict their pathogenic impact.

Methods: We selected rare non-coding variants from WGS data of 140 patients affected by neurodegenerative diseases, obtaining 41 variants annotated as possible regulatory regions, using UCSC-GRCh38/hg38 ENCODE-regulation tracks. A MPRA library array was designed including a total of 60 probes for each variant in both forward and reverse strands, further divided equally into reference, alternative and scrambles and identified by a barcode.

Results: A pilot study was previously conduced with a small oligonucleotide library of few variants, which demonstrated the feasibility of MPRA assay in this context. A total of 2460 probes were cloned in pMPRAvectors upstream of an ORF sequence and transfected into SHSY5Y cells. After RNA isolation and sequencing, mRNA counts and plasmid DNA ratio was performed and the bioinformatics analysis is ongoing.

Conclusion: This technique can be used to analyze the pathogenic role of gene expression regulation variants in neurodegenerative diseases.

References: No references.

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Conflict of Interest: None declared.

EP10.061 Homozygous RNASEH2B Mutation Cause Aicardi–Goutières Syndrome

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Background/Objectives: Aicardi-Goutieres syndrome is a progressive encephalopathy that starts in the neonatal period and causes psychomotor retardation, microcephaly and pyramidal dysfunction. It has a prevalence of 1-5 per 10,000 new live births. In most cases, leukodystrophy, corticosubcortical atrophy, calcifications in the basal ganglia are central nervous system findings. There is mostly autosomal recessive inheritance due to changes in many genes. It also affects the skin and immune system.

Methods: Next Generation Sequencing (NGS) revealed a homozygous variant in the RNASEH2B gene. In the RNASEH2B gene; NM001142279: c.554T>G p.(Val185Gly) missense variant was detected homozygously by clinical exome analysis.

Results: The parents of the female infant with homozygous p.(Val185Gly) variant in the RNASEH2B gene are first-degree cousins. Gene separation was examined by Sanger sequencing and it was confirmed that both parents were heterozygous carriers of the mutation detected.The patient was brought to the hospital at the 10th month with axial hypotonia, hypertonia in the extremities, psychomotor regression and dystonic movements. The patient's brain MRI, which was requested after genetic testing, also revealed calcification in the basal ganglia.

Conclusion: Aicardi-Goutieres type 2 syndrome is a rare entity that should be considered in the presence of neuromotor retardation, hypotonia, and intracranial calcifications; we emphasize the importance of diagnosis in order to both know the prognosis of our patients according to their genetic changes and to offer genetic counseling to their families.

References: PMID: 33981319.
PMID: 18343173.
PMID: 27643693.

Grants:

Conflict of Interest: None declared.

EP10.064 Profitability of founder effect variant in the diagnosis of pyridoxine-dependent epilepsy in south Tunisia

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Background/Objectives: Pyridoxine-dependent epilepsy (PDE, #266100) is a rare autosomal recessive metabolic disorder. It is characterized by refractory neonatal seizures that are responsive to pyridoxine treatment. PDE is caused by pathogenic variants in the *ALDH7A1* gene (#107323). With more than 165 pathogenic variants reported in the literature, the majority are missense variants (49%) localized especially in exon 15 (25%) affecting the catalytic domain of *ALDH7A1*. In South Tunisia, the c.1364T>C (p.Leu455Pro) pathogenic missense variant is known for its founder effect.

The aim of our study is to determine the frequency of this founder variant.

Methods: This is a retrospective study including patients with suspected PDE addressed by neonatology or child neurology departments to the medical genetics department from 2016 to 2021. Sanger targeted sequencing of the exon 15 of the *ALDH7A1* gene (NM_001201377.2) in search of the founder variant was performed.

Results: Among thirty patients belonging to twenty-five unrelated families, seven (belonging to five unrelated families) were genetically diagnosed with PDE due to the founder variant, which represents a frequency of 24% (7/30). All positive PDE patients have a compatible electro-clinical phenotype and were totally ameliorated by the integration of pyridoxine.

Conclusion: The presence of a founder mutation in the *ALDH7A1* gene, which is characterized by great allelic heterogeneity, facilitates the diagnostic strategy for early-onset epilepsy and provides special advantages for treatment and genetic counseling for south Tunisian families. High throughput sequencing can be considered at a second level for the rest of the patients.

References: Tlili and all, 2013, Curtis R. and all, 2019.

Grants:

Conflict of Interest: None declared.

EP10.066 Phosphatidylinositol 4-Kinase 2-Alpha (PI4K2A) deficiency leads to neurodevelopmental disorder associated with innate error in intracellular trafficking

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Background/Objectives: Intracellular trafficking together with various signalling pathways controls complex cellular processes such as macroautophagy or apoptosis as part of metabolic homeostasis and quality control of proteins in post-mitotic tissues, including neurons. An essential regulatory component of these endomembrane organelles is the minor lipid, phosphatidylinositol 4-phosphate (PI4P), generated by four distinct phosphatidylinositol 4-kinase enzymes (PI4K, PI4KB, PI4K2A, PI4K2B) in mammalian cells. Data from animal models and human genetic studies suggest vital roles of the PI4K enzymes for the development and functions of various organs and nervous system. Biallelic PI4KA variants have recently been associated with variable neurodevelopmental disorders, brain malformations, leukodystrophy, and primary immunodeficiency with inflammatory bowel disease.

Methods: Through exome quenching of families with undiagnosed neurodevelopmental disorders and screening of large sequencing datasets coupled with deep-phenotyping and functional studies, we describe the detailed clinical phenotypes of a new disorder.

Results: Here, we describe two unrelated consanguineous families with PI4K2A deficiency, with severe-to-profound global developmental delay, dystonia, neuroimaging abnormalities (corpus callosum dysgenesis/hypoplasia, diffuse white matter volume loss), seizures, immunodeficiency and death in one affected individual in infancy, a clinical presentation akin to what was previously reported in 2 affected brothers with homozygous loss of *PI4K2A*.

Conclusion: Given the central role of PI4K2A in Rab7-associated vesicular trafficking and autophagy, these new cases establish a link between late endosome/lysosome defects and neurodevelopmental abnormalities. Our study extends the spectrum of neurodevelopmental manifestations of PI4K defects to the PI4K2A enzyme and allows comparisons between clinical presentations in patients with *PI4KA* and *PI4K2A* associated disorders.

References: N/A

Grants: N/A

Conflict of Interest: None declared.

EP10.067 Early infantile epileptic encephalopathy type 35: About a case

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Background/Objectives: Early infantile epileptic encephalopathy (EIEE) or Ohtahara syndrome is one of the most severe forms of age-related epileptic encephalopathy. It is a rare syndrome that manifests during the first three months of life with refractory epileptic seizures of various types. It leads to psychomotor retardation and has a fatal outcome.

Through this work, we illustrate the role of medical genetics in the diagnosis of early childhood epileptic encephalopathy, the management, and the development of adequate genetic counseling.

Methods: We report the case of a patient referred to the Genetics Medical Day Hospital of the CHU Mohammed VI in Marrakech for epileptic seizures with neonatal hypotonia.

Results: This is a 1-year-old female infant, consanguineous, without neonatal suffering, who has had tonic spasms since birth. On clinical examination, the patient was hypotonic with psychomotor retardation.

Brain MRI showed cerebral atrophy with widening of subarachnoid spaces.

The molecular study by next generation sequencing (NGS) found a variant likely pathogenic in the homozygous state and this at the ITPA gene in favor of type 35 of the EEP.

Conclusion: EIEE are characterized by great genetic heterogeneity. According to OMIM, 94 genotypes have been described. The main mutations concern the genes: ARX, CDKL5, SLC25A22, KCNQ2, KCNT1, SCN2A and STXBP1.

References:

Grants:

Conflict of Interest: None declared.

EP10.068 Genetic diagnosis of Dravet syndrome using next generation, capillary sequencing and multiplex-ligation dependent probe amplification - Romanian showcase

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Background/Objectives: Dravet syndrome(DS), associated with intractable seizures and developmental delay, is one of the most studied genetic epilepsies. De novo variants in SCN1A gene, which encodes for the voltage-gated Na+ channel alpha subunit Nav1.1, are the most frequent causes of DS.

Methods: We are reporting 36 patients with DS presumptive diagnosis, referred to CRGM-Dolj between 2017-2020. Testing options include probemix P137 SCN1A MRC-Holland multiplex-ligation dependent probe amplification(MLPA) and in-house capillary sequencing on a Thermo Fisher 3730xl DNA Analyzer; and next generation sequencing(NGS) panel Illumina TruSight One on Illumina NextSeq550 IVD. Data analysis uses Coffalyser.Net, Mutation Surveyor, and our bioinformatic pipeline based on nf-core/sarek v2.7.1(GATKv4.1.7.0) and Ensembl VEP v104.3.

Results: MLPA identified a mutation in SCN1A for 1 case. Genetic confirmation was mostly achieved though NGS, identifying SCN1A variants for 7 subjects and, for an additional 8 patients, variants in other genes that could explain the clinical phenotype. Capillary sequencing was offered to identify the de-novo status of the identified variants in the extended family, and was accepted by only a few of the parents. Mosaicism was not evaluated, although we intend to include testing options in the future.

Conclusion: Comprehensive clinical phenotyping is crucial for interpreting results. Genetic postnatal assessment of patients with severe epileptic encephalopathy and developmental delay can be a powerful diagnostic tool for clinicians, with implications in the management and counselling of patients and their families.

References:

Grants: This work is supported by the National Health Programe VI.3.3. and POCU ProGeneRare - SMIS code 108073.

Conflict of Interest: None declared.

EP10.069 Mowat-Wilson syndrome – an ongoing genetic disease

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Background/Objectives: Mowat-Wilson syndrome (MWS) is a rare autosomal dominant neurodevelopmental disorder (about 300 reported cases worldwide) caused by *ZEB2* gene pathogenic variants (heterozygous mutations or intragenic deletions) and microdeletions in the critical chromosomal region 2q22-23. Classicaly, the main features of MWS include neurodevelopmental delay, seizures, distinctive facial features that change with age, musculoskeletal anomalies, congenital ocular and cardiac defects, genitourinary anomalies and Hirschsprung's disease.

Methods: The patient is the second child of a non-consanguineous couple with no significant family history. She is four years old and was born after an uneventful pregnancy. Clinical manifestations, with an onset at 7-8 months of age, progressively include: motor developmental delay, stereotyped behaviours involving the hands, language delay, convergent strabismus, a minimal facial dysmorphism and generalized tonic seizures. MRI examination was performed at the age of 3, revealing a focal signal anomaly in the profound and left frontal subcortical white matter with a possible congenital cause.

Results: The initial genetic tests, MLPA for Rett syndrome and arrayCGH, were negative. The diagnosis of Mowat-Wilson syndrome was confirmed after performing an NGS epilepsy panel which identified a nonsense mutation in the *ZEB2* gene (c.1027 C>T (p.Arg343)).

Conclusion: In the case of this probably underdiagnosed pathology, with an unknown incidence and significantly heterogeneous clinical presentation, genotype-phenotype correlations regarding the c.1027 C>T *ZEB2* gene mutation could in fact contribute to a better understanding of the disease, and to an improved management for patients carrying this pathogenic variant.

References:

Grants:

Conflict of Interest: None declared.

EP10.070 PNPLA6 related cerebellar ataxia: a family report with intra-familial phenotypic variability

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Background/Objectives: Autosomal recessive cerebellar ataxias are a heterogeneous group of rare neurological disorders, with a continuously growing genetic architecture. Biallelic missense mutations in the PNPLA6 gene were described in association with a phenotypic continuum of Boucher-Neuhäuser syndrome, spastic paraplegia 39, Oliver-McFarlane syndrome, Gordon-Holmes syndrome and Laurence-Moon syndrome.

Methods: Whole-exome-sequencing was performed to identify the causal variants. The detected variants were classified according to ACMG recommendations.

Results: Here we report the first Hungarian siblings carrying two novel PNPLA6 missense rare damaging variants (c.830G>A p.R277Q and c.3517C>T p.R1173W, NM_001166114.1), associated with progressive, early-onset cerebellar ataxia. Main clinical features were cerebellar dysarthria, nystagmus, gait and limb ataxia. Intra-familial phenotypic variability was observed regarding upper motor neuron signs, demyelinating polyneuropathy and adult onset hypogonadotropic hypogonadism associated with obesity and metabolic disorders. Brain MRI showed cerebellar atrophy and by one sibling parietal cavernoma. Chorioretinal dystrophy was not present. Whole exome sequencing identified the above-mentioned missense variants, which were previously not described in association of PNPLA6 related syndromes. The very low MAF and some computational predictors supported the deleterious effect. Segregation analysis in the family confirmed the compound heterozygous state in probands.

Conclusion: With this report we present new pathogenic variants of the PNPLA6 gene: the c.830G>A and c.3517C>T. Our observation highlights the phenotypic variability of this disease even within one family.

References:

Grants:

Conflict of Interest: None declared.

EP10.071 Recalibrating behavioral scales using polygenic scores identifies biologically-grounded behavioral dimensions

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Background/Objectives: The Child Behavior Checklist (CBCL) is a widely-used clinical assessment of behavior problems in children. However, the established syndrome subscales were developed without any genetic risk information. Our objective was to derive genetically-informed subscales to identify latent CBCL structure that is more biologically meaningful and heritable.

Methods: We performed sparse canonical correlation analysis with 119 CBCL items (residualized for total score/general psychopathology) and 20 behavior and psychiatric polygenic scores (PGS) in 8,429 children from the SPARK autism and ABCD cohorts and calculated SNP-heritabilities of these canonically-derived subscales.

Results: We identified six canonical subscales:

1. rule following: defiance vs. perfectionism [cognitive PGS].
2. drive: regression vs. aggression [psychosis PGS].
3. reward: hyperfunction vs. hypofunction [depression PGS].
4. attention: inhibited vs. inattentive [ADHD & autism PGS].
5. social communication: withdrawn vs. belligerent [cognitive, risky behavior & extraversion vs. autism & OCD PGS].
6. arousal: lethargic vs. hypervigilant [morningness, physical activity, & OCD PGS].

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Each canonical subscale had distinct CBCL and PGS profiles that incorporated multiple PGS and transcended the syndromic boundaries, with many subscales leveraging items omitted in the syndromic subscales.

Conclusion: Our genetically-informed subscales identified latent behavior phenotypes that explain significant additional heritability beyond general psychopathology and are more aligned with Research Domain Criteria than subscales derived solely from phenotypic-only methods.

References:

Grants:

Conflict of Interest: Taylor R. Thomas: None declared, Jacob J. Michaelson R01DC014489.

EP10.072 Diagnostic yield of whole-exome sequencing in neuroregression in a Colombian cohort

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Background/Objectives: Neuroregression is the loss of acquired functions or the absence of functional progress after apparently normal development. Possible causes of neuroregression include genetic, metabolic, degenerative, infectious, neoplastic, and vascular, but it is commonly perceived as a pivot sign of a genetic disease. Nevertheless, the prevalence of genetic causes has not been clearly established when neuroregression is the main phenotype. We aimed to describe the prevalence of a genetic cause in patients with neuroregression and to identify the diagnostic yield of whole exome sequencing (WES) in this group of patients.

Methods: We performed a descriptive, cross-sectional cohort study in paediatric patients with neuroregression who underwent WES. Phenolyzer (http://phenolyzer.usc.edu)[1] identified 226 genes with a strong association (score 0.0742 to 1) to neurological regression. These genes were evaluated as well as other possible genes related to the patient's phenotype.

Results: Twenty cases were included. The average age was 8.7±5 years-old and the onset of neuroregression was at 23±14.2 months. The main clinical features of neurological regression were language (28.6%), motor functions (14.3%), behavioural disturbances (14.3%), and multiple function impairment (35.7%). A molecular diagnosis (pathogenic/likely pathogenic variants) was achieved in 6/20 patients (30%).

Conclusion: WES' diagnostic yield for neuroregression was 30%. It is considerably superior to the diagnostic yield previously reported for other clinical indications (intellectual disability, autism, epilepsy, etc). Neuroregression should be considered as a strong predictor of a genetic aetiology and therefore WES is indicated in these patients.

References: 1) Yang H, et al. *Nat Methods*. 2015 Sep;12(9):841-3.

Grants:

Conflict of Interest: None declared.

EP10.073 Exploring the Genomic and Phenotypic Architecture of Autonomic Dysfunction in a Rare Disease Cohort

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Background/Objectives: Autonomic nervous system dysfunction or dysautonomia, represents a broad group of disorders that can include many diverse phenotypes affecting single – or multiple – organ systems within an affected individual. Many individuals with dysautonomia have neurocardiac involvement characterized by postural orthostatic tachycardia syndrome (POTS), orthostatic hypotension, and vasovagal syncope (VVS). Autonomic perturbations have multiple known molecular etiologies ranging from aberrant development and adult stem cell maintenance, to defects in ion-channels, metabolism of neurotransmitters, inflammation, and neurodegeneration. Despite these mechanistic insights, many families with dysautonomia remain without a molecular diagnosis and limited efficacious treatments.

Methods: Through the Baylor-Hopkins Center for Mendelian Genomics (BH-CMG) and Baylor College of Medicine Genomic Research to Elucidate the Genetics of Rare (BCM-GREGoR) databases, we have identified 38 unrelated probands and families with exome sequencing (ES) data across a range of clinical dysautonomia phenotypes. We applied a case-similarity clustering approach to guide our rare variant analysis.

Results: This cohort was reported to have individuals with different systems involved including: 60.5% (23/38) cardiovascular, 57.9% (22/38) neurological, 39.5% (15/38) musculoskeletal, 36.8% (14/38) pulmonary, and 26.3% (10/38) gastrointestinal abnormalities.

Conclusion: By leveraging existing knowledge sources and phenotypically-defined subgroups that can be used to elucidate potentially distinct forms of dysautonomia, we are presently

investigating the candidacy of several putative candidate genes and variants identified using this approach. We propose that parallel integration of gene-first and phenotype-first analysis approaches offers a productive method for investigation of human disease traits displaying substantial genotypic and phenotypic heterogeneity.

References:

Grants: 1U01HG011758-01, 5K08HG008986-05, 5T32GM136554-02.

Conflict of Interest: Edgar (Andy) Rivera Munoz: None declared, Angad Jolly: None declared, Haowei Du: None declared, Zeynep Coban Akdemir: None declared, James Lupski JRL has stock ownership in 23andMe, 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., JRL is a paid consultant for Regeneron Pharmaceuticals and Novartis., Jennifer Posey: None declared.

EP10.074 CABIN1, a possible candidate gene involved in neurodevelopmental disorders

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Background/Objectives: Neurodevelopmental disorders (NDD) are a broad spectrum of early onset syndromes affecting the central nervous system (CNS) development. Deciphering the genetic basis of these disorders can provide both clinical and personal utility.

Methods: We applied whole exome sequencing (WES) for a patient with neurological developmental defects; global developmental delay, severe mental retardation, walking difficulties, ataxia, vision and hearing impairment, and speechlessness. Then Sanger sequencing was done for variant confirmation and allele segregation study.

Results: We found a single nucleotide variation; *CABIN1* (NM_012295.4):c.668T>G (p.Ile223Ser) with homozygous genotype in WES data of the Proband. Parents were heterozygous (T/G) and two more affected individuals of the family were homozygous (G/G) for the detected variant. According to the.

ACMG guidelines, the variant is classified with uncertain significance (PM2, and PP3). In silico pathogenicity assessment using MutationTaster, PredictSNP, FATHMM, PROVEAN, and CADD showed this variation could be disease-causing. Furthermore, the variant was not present in our in-house database and public databases including GnomAD and BRAVO.

Conclusion: CABIN1 (Calcineurin binding protein 1, or cain) plays role in mammalian CNS development.

Calcineurin regulates neuronal structure, neurotransmission, and activity-dependent gene expression. Cabin1 could act as a regulator of calcineurin activity in the developing nervous system, given their roles in neuronal differentiation and synaptic refinement. Taken together our preliminary findings and the underlying role of this gene in neuronal development provide clues to introduce *CABIN1* as a candidate gene for NDD pathogenesis.

References:

Grants:

Conflict of Interest: None declared.

EP10.076 Biallelic PRMT7 pathogenic variants are associated with a recognizable syndromic neurodevelopmental disorder with short stature, obesity, craniofacial and digital abnormalities

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Background/Objectives: Protein arginine methyltransferase 7 (PRMT7) is a member of a family of enzymes that catalyse the methylation of arginine residues on several protein substrates. Biallelic pathogenic PRMT7 variants have been previously associated with a syndromic neurodevelopmental disorder characterized by Short Stature, Brachydactyly, Intellectual Developmental Disability and Seizures (SBIDDS syndrome). Although 15 cases from 9 families have been reported until now, there is no comprehensive study describing detailed clinical characteristics of the PRMT7-related disorder.

Methods: We assembled a cohort of 45 patients from 35 different families, gathering clinical information from 30 newly described patients, and reviewing data of 15 affected individuals from literature. Of these, we obtained follow-up data from 12 previously reported cases.

Results: The main clinical characteristics of the PRMT7-related syndrome are short stature, developmental delay/intellectual disability ranging from mild to severe, hypotonia, brachydactyly and distinct facial morphology including bi-frontal narrowing, prominent supraorbital ridges, sparse eyebrows, short nose with full/broad nasal tip, thin upper lip, full and everted lower lip and a prominent or squared-off jaw. Additional findings include seizures, obesity, non-specific MRI abnormalities, eye abnormalities (i.e. strabismus or nystagmus) and hearing loss.

Conclusion: This study further delineates and expands the molecular, phenotypic spectrum and natural history of PRMT7-related syndrome characterised by a neurodevelopmental disorder with short stature, obesity, epilepsy, and distinct craniofacial and digital abnormalities.

References: Poquérusse J, Whitford W, Taylor J, et al. Novel PRMT7 mutation in a rare case of dysmorphism and intellectual disability. J Hum Genet. 2022;67(1):19-26. https://doi.org/10.1038/s10038-021-00955-5.

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Conflict of Interest: None declared.

EP10.077 Multi-phenotype GWAS suggests shared common and rare genetic variation between inflammation and depression

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Background/Objectives: Depression is one of the leading causes of disability and socioeconomic burden affecting 322 million people worldwide. Recent studies suggest depression and inflammation share pathways and genetic background, which we aimed to further elucidate for both common (MAF>5%) and rare (MAF < 1%) variants using multi-phenotype genome-wide association studies (MP-GWAS).

Methods: We analysed the Northern Finland Birth Cohort 1966 data (31-year follow-up). We included individuals (N = 2,673) with genome-wide genotyped data (Haplotype Reference Consortium imputation), depressive symptoms (sum scores of Beck Depression Inventory) and 18 inflammatory markers. Using SCOPA and MARV software, we performed MP-GWAS of common and rare variants as a linear combination of residuals for depressive score and inflammatory markers, while adjusting for sex, BMI and three principal components to control for population structure. We evaluated the associations of depression and inflammation either with individual SNPs (SCOPA, $P < 5x10^{-8}$) or with a proportion of rare alleles within a gene (MARV, $P < 1.67x10^{-6}$). Bayesian Information Criterion was used to define the best fitting phenotype combination at each detected signal.

Results: Seven SNPs at/near *ICAM1*, *ABO*, *MAT2B*, *TENM2*, *ST3GAL4, KIRREL3, MIR3681HG*, showed significant associations in the common variant MP-GWAS, however, only rs5498 at *ICAM1* showed effects with both depression and inflammation ($P < 1.04 \times 10^{-8}$). Rare variant MP-GWAS demonstrated significant associations at 273 genes with rare variation at *PRRC2C* ($p = 1.29x10^{-6}$), *DYNC1I2* ($p = 4.65x10^{-75}$), *SHFM1* ($p = 1.37x10^{-6}$), *KCNK4* ($p = 9.11x10^{-7}$) and *TMEM191C* ($P = 4.18x10^{-7}$) associated with both depression and inflammation.

Conclusion: Our results corroborate shared genetic variation between depressive symptoms and inflammation confirming their shared aetiology.

References:

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Conflict of Interest: None declared.

EP10.078 Relationships between intermediate repeat expansions of TBP and spinocerebellar ataxia type 48 / STUB1: genetic modifier of pure digenic inheritance?

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Background/Objectives: CAG/CAA repeat expansions in TBP>49 are responsible for Spinocerebellar ataxia type 17. The pathogenicity of TBP41-49 intermediate alleles remains controversial. We previously detected for the first time the co-segregation of STUB1 variants causing Spinocerebellar ataxia 48 (SCA48) with intermediate alleles of TBP in two families suggesting an interplay between the two loci. This STUB1/TBP co-segregation was recently confirmed in a study that however proposed a digenic inheritance to explain the full disease penetrance of STUB1 variants in SCA48 families.

Methods: We systematically sequenced the TBP CAG/CAA repeats in 34 probands with STUB1 variants, and search for STUB1 pathogenic variants in 49 probands carriers of intermediate or pathogenic alleles of TBP to precisely delineate the interaction between these two loci.

Results: Recurrent pathogenic or novel STUB1 variants were found in half of the TBP cohort, all carriers of intermediate alleles. TBP41-49 carriers were detected in 26% of STUB1 probands. Clinical comparison of SCA48 patients revealed that the carriage of TBP-intermediate alleles was significantly associated with the risk of developing cognitive impairments ($p = 0.0129$) and with a faster progression of the disease until death ($p = 0.0003$). Statistical analyses revealed that pathogenic effects of TBP-intermediate alleles on STUB1 carriers start from TBP repeats = 40. Importantly, twelve STUB1 probands presenting with the full SCA48 clinical phenotype (ataxia and dementia) had normal TBP37-39 alleles, excluding a systematic digenic inheritance.

Conclusion: Altogether, our work shows that TBP-intermediate alleles act as a disease modifier of SCA48 that increases the severity of the disease with important consequences for genetic counseling of SCA48 patients.

References:

Grants:

Conflict of Interest: None declared.

EP10.080 A Homozygous Variant in CHMP3 is Associated with Complex Hereditary Spastic Paraplegia

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Background/Objectives: Monogenic neurodegenerative diseases are heterogeneous disorders caused mutated genes affecting normal cellular functions including autophagy. Defective autophagy is implicated in hereditary ataxia and spastic paraplegia (HSP), amyotrophic lateral sclerosis and frontal dementia, characterized by intracellular accumulation of un-degraded proteins. We investigated the genetic basis of complex hereditary spastic paraplegia in a consanguineous family of Arab Moslem origin, consistent with autosomal recessive inheritance.

Methods: Genetic investigation included Exome sequencing and Sanger sequencing. The impact of the variant on autophagy was analyzed in primary fibroblasts using electron microscopy, immunofluorescence, and western blot and through ectopic plasmid expression.

Results: Exome sequencing identified the variant c.518C>T, p.(T173I) in the CHMP3 gene, encoding the Vacuolar Protein Sorting 24 (VPS24) protein, a member of ESCRTII autophagy complex, which was recently associated with neurodegenerative disease in Drosophila. Segregation analysis confirmed a homozygous state among five affected individuals of a kindred family. Primary patient's fibroblasts showed significantly reduced levels of VPS24 protein through immunoblot. Electron microscopy disclosed accumulation of autophagosomes and autolysosomes in patient's fibroblasts, which correlated with higher levels of autophagy markers, p62 and LC-II. Ectopic expression of wild type CHMP3-GFP in primary patient fibroblasts successfully reduced the accumulation of p62 particles and autophagosomes compared to GFP transfected patient's cells.

Conclusion: Reduced levels of VPS24 are associated with complex spastic paraplegia phenotype, through aberrant autophagy mechanisms affecting cells of ectodermal origin, probably including neurons in the central and peripheral nervous system.

References: Under review.

Grants: Short term Rappaport Grant 2021-8210..

Conflict of Interest: None declared.

EP11 Neuromuscular Disorders

EP11.003 IGHMBP2-related Disease with Genotype-Phenotype Correlation

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Background/Objectives: The IGHMBP2 gene encodes a well-established protein with ATPase/helicase activity. Mutations in *the IGHMBP2* gene are associated with two distinct phenotypes: Charcot-Marie-Tooth disease type 2S (CMT2S) and distal spinal muscular atrophy 1 (DSMA1).

Methods: We report 3 patients with IGHMBP2 gene mutations. Patient 1 is a 7 mo girl, who presented with stridor, weak cry and proximal muscle weakness from one months. The symptoms progressed into diaphragmatic paralysis requiring intubation. Patients 2 and 3 are 12 and 7 yo brothers, who had congenital bilateral foot drop, walking difficulty, slowly progressive distal limb muscle wasting and weakness with progressive sensory loss. EMG showed slow nerve conductance.

Results: For patient 1 genetic analysis revealed two heterozygous nonsense variants c.127C>T (p.Arg43*) and c.958C>T (p.Arg320*), creating premature translational stop signals in IGHMBP2 gene. For patients 2 and 3 genetic testing showed two novel heterozygous missense variants c.181G>C (p.Gly61Arg) and c.613T>C (p.Ser205Pro) in IGHMBP2 gene.

Conclusion: The current cases confirm the existence of genotype-phenotype correlation in IGHMBP2-related disease, with missense variants producing mild phenotype consistent with CMT2S and nonsence mutations presenting with severe form of DSMA1. Moreover, detection of c.181G>C (p.Gly61Arg) and c.613T>C (p.Ser205Pro) novel variants expands the mutation spectrum of the IGHMBP2 gene. We suggest that IGHMBP2 should be considered in any infant presenting with symptoms consistent with spinal muscular atrophy with diaphragmatic insufficiency.

References: YuanJH, HashiguchiA, YoshimuraA, YaguchiH, TsuzakiK, IkedaA, Wada-IsoeK, AndoM, NakamuraT, HiguchiY, HiramatsuY, OkamotoY, TakashimaH. Clinical diversity caused by novel IGHMBP2 variants. J Hum Genet. 2017 Jun;62(6):599-604.

Grants: No grants.

Conflict of Interest: None declared.

EP11.004 Genetics heterogeneity and novel genes in a cohort of Iranian patients with hereditary spastic paraplegia (HSP)

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Background/Objectives: Hereditary spastic paraplegias (HSPs) comprise a group of heterogeneous inherited-neurological diseases with the common feature of prominent lower-limb spasticity. So far, >85 loci and 72 HSP-causing genes have been identified. Extensive application of whole-exome sequencing (WES) has been resulted in a considerable increase in the rate of novel disease-causing genes discovery. Despite using WES, genetic analysis has failed in finding causative-genes in ~50% of HSP cases. Here, we report the result of genetic analysis of a relatively large cohort of Iranian HSP-families.

Methods: WES was performed on DNAs of 72 Iranian unrelated HSP probands. Data were analyzed and candidate variants amplified and Sanger sequenced in the probands, then screened in the family members to co-segregation analysis. Copy number variations (CNVs) were also analyzed using GermlineCNVCaller.

Results: Totally, disease-causing variants were identified in 39 families (54%). The variants were located in 20 known HSP genes, including SPG11, SPAST, ATL1, SPG7, COQ7, CAPN1, KIF5A, GJC2, ERLIN1, ERLIN2, ENTPD1, CYP7B1, ZFYVE26, GCH1, CYP2U1, MFN2, KIF1B, ALS2, TUBB4A, and C19orf12. Among the remaining families four putative novel candidate genes were identified that involved in axonogenesis, ubiquitination and mitochondrial functions.

Conclusion: The research presented the powers of WES in gene discovery and identification of causative-genes in diseases with high genetic heterogeneity. Identification of novel genes and molecular pathways will lead to better understanding of biological mechanisms and disease pathophysiology.

References: Elsayed LEO, et al. Insights into Clinical, Genetic, and Pathological Aspects of Hereditary Spastic Paraplegias: A Comprehensive Overview. Front Mol Biosci. 2021;8:690899.

Grants: 2592.

Conflict of Interest: None declared.

EP11.005 TTN-related recessive limb-girdle muscular dystrophy in an Estonian family caused by a Finnish founder variant (FINmaj) in compound with a novel splice site variant

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Background/Objectives: The Finnish founder variant (FINmaj) is an 11-bp insertion/deletion in the last exon of the TTN gene. In heterozygous state, this variant causes autosomal dominant tibial muscular dystrophy (TMD, MIM# 600334), a slowly progressive late-onset distal myopathy. In homozygous state and in compound heterozygosity truncating variant, the FINmaj variant causes early onset limb-girdle muscular dystrophy (LGMD R10 titin related, MIM# 608807).

Case report: A 12-year-old male patient presented with walking difficulties, abnormal gait, and muscle weakness. His face appeared myopathic and he had positive Gowers' sign. His serum CK was 1993 U/L and ENMG detected myopathic damage. He started walking at 14m and experienced leg pain after longer walks at 3y.

Methods:.

Results: Trio exome sequencing revealed two heterozygous variants in the TTN gene (NM_001267550.2):c.107780_107790delinsTGAAAGAAAAA,p.(Glu35927_Trp35930delinsValLysGluLys) (FINmaj variant, paternally inherited) and c.64672+2dup,p.? (splice site variant, maternally inherited). Muscle MRI showed early degenerative changes in the tibialis anterior and distal hamstring muscles similar to FINmaj homozygous patients. Muscle biopsy showed myopathic changes with moderate fibrosis, many internal (including central) nuclei, and marked fiber type size disproportion. The 36-year-old father carrying the FINmaj variant presented with mild symptoms of TMD. Family

segregation analysis detected a mildly affected younger sister with the same biallelic variants.

Conclusion: We report the first Estonian family with the FINmaj TTN gene variant. Two siblings are compound heterozygous for the FINmaj and a novel splice site variant causing an LGMD R10 phenotype, while the father is heterozygous for FINmaj causing TMD.

References:

Grants: Estonian Research Council grants PRG471, MOBTP175, PSG774.

Conflict of Interest: None declared.

EP11.006 Optimisation of a cell-based strategy for rapid evaluation of compounds in myotonic dystrophy type I

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Background/Objectives: Gold-standard techniques for the evaluation of drug candidates in myotonic dystrophy type I (DM1) are laborious procedures with poor reproducibility and little consensus on their methodological protocols. The objective of this project is to develop a strategy to characterize patient-derived cultures both at protein and RNA level, for rapid evaluation of new compounds in DM1.

Methods: Myoblots and fibroblots are assays for protein quantification in microplates, based in the In-Cell Western method, that we have adapted to the study of DM1-relevant proteins in myoblast and fibroblast cultures, respectively. Also, we have optimised a digital droplet PCR (ddPCR) protocol for the quantification of the mRNA expression of the genes coding for these same proteins.

Results: Optimisation of myoblots and fibroblots allowed us to accurately quantify different proteins in immortalized differentiated myoblasts cultures and primary human fibroblasts cultures. Statistically significant differences in protein expression among DM1 and CTRL groups were found. We further validated our approach by treating our cultures with several small molecules previously proposed as drug candidates for DM1 and quantified their effects on the expression of these key proteins.

Conclusion: The combination of these techniques allows a highly reproducible and less laborious characterization of DM1 patient-derived cultures suitable for evaluation of potentially therapeutic compounds in DM1.

References:

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Conflict of Interest: None declared.

EP11.007 Retrotransposon insertion as a novel mutational event in spinal muscular atrophy

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Background/Objectives: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder resulting from biallelic alterations of the *SMN1* gene: deletion, gene conversion or, in rare cases, intragenic variations. The disease severity is mainly influenced by the copy number of *SMN2*, a nearly identical gene, which produces only low amounts of full-length (FL) mRNA. Here we describe the first example of retrotransposon insertion as a pathogenic *SMN1* mutational event. The 50-year-old patient is clinically affected by SMA type IV with a diagnostic odyssey spanning nearly 30 years. Despite a mild disease course, he carries a single *SMN2* copy.

Methods: We combined exome sequencing (ES) and RNAseq on lymphoid cell lines to characterize a retrotransposon insertion in the *SMN1* gene and document its consequences on the production of SMN transcripts.

Results: We identified a SVA-F retrotransposon inserted in *SMN1* intron 7 and documented the dramatic decrease of FL transcript production in the patient compared to subjects with the same *SMN1* and *SMN2* copy number. We characterized the mutant FL-SMN1-SVA transcript and showed that it was degraded by nonsense-mediated mRNA decay.

Conclusion: The stability of the SMN-SVA protein may explain the mild course of the disease. Indeed, SMNΔ7 read through product was already shown to restore SMN protein functionality by increasing protein stability. Here, the small quantity of FL-SMN1-SVA transcript may generate a non-negligible quantity of functional protein explaining the less severe course of the disease. This observation exemplifies the role of retrotransposons in human genetic disorders.

References:

Grants:

Conflict of Interest: None declared.

EP11.008 Large-scale screening of SMN1 gene duplications in the Russian population from the side of SMN-carrier screening

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Background/Objectives: Spinal muscular atrophy 5q (SMA 5q) is the second most common autosomal recessive disorder in Caucasians. *SMN1* and *SMN2* (highly-similar) genes play a pivotal role. The great majority of SMA carriers can be identified by the presence of a single *SMN1* exon 7 copy. Some of SMA carriers have two *SMN1* copies on one chromosome and 0 copies on the other (2+0).

Methods: Multiplex ligation-depend probe amplification (SALSA MLPA Probemix P060 (MRC Holland)).

Results: A total of 2073 individuals (4146 chromosomes) without family history of SMA 5q underwent genetic screening by MLPA 55 individuals of them have a single *SMN1* exon 7 copy, representing 0,0133 7 exon *SMN1* deletion allele frequency, and 78 three *SMN1* copies individuals, representing 0,0188 *SMN1* exon 7 duplication allele frequency. Therefore 1 of 2000 (95% CI 1998 to 2002) Russian has the haplotype 2+0. The single *SMN1* exon 7 copy frequency is estimated to be 1:39 (95% CI 37 to 41) for Russian. Proportion of cases where analysis will not identify a true

SMA carrier is 0,0007. Therefore 99,93% of individuals having two *SMN1* copies who have applied for diagnostic is not SMA carries.

SMN1 alleles were investigated by MPLA, designed to detect two polymorphisms (g.27134T> G and/or g.27706_27707delAT). Using our ligation system to 60 *SMN1* duplications samples we identified one silent carrier.

Conclusion: By our data we conclude that population-based screening for SMA 5q silent carriers using these polymorphisms is not appropriate in the Russian Federation.

References: https://www.mrcholland.com/.

Grants: no.

Conflict of Interest: None declared.

EP11.010 Genealogy as a predictor of disease progression in patients with myotonic dystrophy type 1: A demonstration of the power of intersectorial research

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Background/Objectives: Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease caused by an abnormal repetition of CTG nucleotide triplet located on the DMPK gene. The presence and severity of signs and symptoms, as well as their progression, vary not only between but also within the phenotypes. However, there is a lack of knowledge in identifying the predictors of multisystemic impairments associated with DM1. The objective of this study is to assess if genealogical clustering can be used to better understand the severity and progression of impairments in DM1.

Methods: The cohort is composed of 200 patients from Saguenay–Lac-St-Jean who participed in a longitudinal study over 20 years. Our team has collected several clinical measures relevant to the progression of the disease. We have also conducted a deep characterization of CTG repeat length. Additionally, we used the BALSAC population file to reconstruct the genealogies. Genealogical clustering was performed on several generations in order to identify individuals and families with high kinship. Additionally, we used linear regressions to assess correlation between pairwise kinship and CTG repeat lengths.

Results: We report correlations between clinical measures and genealogical clustering. This makes it possible to establish whether certain genealogical groupings present stronger clinical manifestations. We also report correlation between kinship and CTG repeats length to assess if kinship can be used as a predictor for CTG repeats length.

Conclusion: We believe that including genealogy in analyzes will allow our team and others to better understand the progression of the disease in large families.

References:

Grants:

Conflict of Interest: None declared.

EP11.012 Whole genome sequencing as a tool for diagnosing people with genetic neuromuscular disorders

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Background/Objectives: Genetic neuromuscular disorders involve pathologies of muscles, neuromuscular junction, nerves, neurons of the spinal cord, etc, and are responsible both for significant morbidity and early mortality. Modern genetic sequencing technologies allow to identify genetic variants causing the disease and screen couples with an increased risk of having a child with a severe neuromuscular disorder. The aim of the study was to analyze sequencing data of patients with neuromuscular disorders in the medical history.

Methods: DNA was extracted from 52 blood samples of affected children with neuromuscular disorders' referral diagnosis, including congenital myopathies, Duchenne-Becker muscular dystrophy, Ulrich congenital muscular dystrophy, etc. Whole genome sequencing (WGS) was performed using DNBSEQ-G400 and DNBSEQ-T7 platforms. Fastq files were annotated via ASTP, BWA-MEM2, SAMTOOLS, SAMBLASTER, STRELKA2, NGS-BITS, CLINCNV, MANTA, EXPANSIONHUNTER and ENSEMBL-VEP packages. Pathogenic, likely pathogenic variants and variants of unknown significance were included in report followed by Sanger sequencing validation.

Results: Earlier different genetic tests showed no mutations in these 52 cases, so WGS was performed. Genetic variants associated with these diseases were identified in 87% (45/52) of cases. There were 7 considered as pathogenic, 5 as likely pathogenic, and 33 as variants with unknown clinical significance. All mutations were validated by Sanger sequencing.

Conclusion: The WGS results allowed 45 out of 52 patients to be diagnosed and started the necessary treatment, which could significantly improve their quality of life. Nowadays, WGS is the most effective method of identifying genetic variants that are likely to cause orphan diseases.

References:

Grants:

Conflict of Interest: None declared.

EP11.013 The CAPN3 gene mutations among patients with muscular dystrophy from Ukraine and new variants description

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Background/Objectives: Limb Girdle Muscular Disease (LGMD) comprise a group of inherited muscular dystrophy with chronic progressive weakness of hip and shoulder girdles, which caused by mutations in the CAPN3 gene.

Methods: Seventy persons with idiopathic muscular dystrophy from Western Ukraine were included in study (37 females, 33 males). The age range of individuals at the time of genetic testing was <1–72 years (median - 25 years).

Genomic DNA was extracted and analyzed for c.550delA, c.664G>A, c.1465C>T, c.1466G>A, c.1714C>T gene mutation by AS -PCR analysis. Full-gene sequencing and deletion/duplication by NGS was performed for panel of 38 genes, that associated with LGMD.

Results: CAPN3 mutations on both alleles were identified in 12 patients (17,1%). Fourteen mutations were distributed along the entire gene including six pathogenic variants, one – likely pathogenic and seven variants of Uncertain Significance. The most common c.550delA mutation was detected in homozygous (2 patients) and compound heterozygous (3 patients). Three detected CAPN3 variants have not been reported before. The

c.1696G>A variant was identified in homozygous in patient with clinical signs of LGMD. Two variants c.1403A>G and c.1345A>G were detected in the compound heterozygous in patient with destructive changes, necrotic myocytes, nuclear chains and nuclear bags on muscular biopsy.

Conclusion: The most common CAPN3 c.550delA mutation cover 25% identified alleles among patients from Ukraine. Newly reported CAPN3 variants: c.1403A>G, c.1345A>G, c.1696G>A has been considering as a pathogenic and expand the current gene mutation spectrum.

References:
Grants:
Conflict of Interest: None declared.

EP11.014 Is c.1160_1161del AP4B1 a founder effect variant?

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Background/Objectives: Pathogenic variants in the AP4B1 gene, encoding adaptor-related protein complex 4 beta-1 subunit, cause hereditary spastic paraplegia SPG47. Which is characterized by early hypotonia progressing to spastic paraplegia, microcephaly, epilepsy, and central nervous system defects and global developmental delay.

Methods: We compared patient's genotypes (n = 4) obtained by WES using four highly heterozygous STR markers located near the gene. Majority of unrelated Russian patients were homozygous carriers of c.1160_1161del variant, but carried different haplotypes.

Results: DNA	D1S2726	D1S502	*AP4B1* variant	D1S250	D1S189
Kb	111 184	112 508	114 439	115 211	116 693
122_1	1	-5	c.1160_1161del	5	3
122_2	12	6	c.1160_1161del	2	3
201.1_1	9	3	c.1160_1161del	3	1
806.1_1	8	2	c.1160_1161del	3	4
806.1_2	9	3	c.1160_1161del	3	2
342_1	8	2	c.1160_1161del	3	3
342_2	7	1	c.1160_1161del	3	1

Conclusion: In contrast to Polish colleagues [https://doi.org/10.1007/s13353-020-00552-w], we couldn't establish the founder effect of c.1160_1161del mutation as a main reason for variant prevalence. Instead, patient's STR haplotypes were distinct across the subjects. Therefore, this variant can be a "hot spot" mutation, or could have spread as a result the founder effect, but occurred hundred generations ago.

References:
Grants:
Conflict of Interest: None declared.

EP11.015 Gamma-sarcoglycanopathy and Becker/Duchenne muscular dystrophy in the south of Morocco: clinical and molecular diagnosis

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Background/Objectives: Neuromuscular diseases are characterized with a clinical and genetic heterogeneity. Therefore, the identification of the genetic etiology may be difficult sometimes. We report the experience of the medical genetics department of Mohammed VI university hospital, Marrakech in the diagnosis of Gamma-sarcoglycanopathy and Becker/Duchenne muscular dystrophy.

Methods: 132 patients suspected with gamma-sarcoglycanopathy or Becker/Duchenne muscular dystrophy from the South of Morocco, referred to the department of genetics between 2017 and 2021, different clinical data were collected. We perform Genomic DNA extraction from peripheral blood using commercial DNA extraction kit or using salting-out method. For male patients suspected with DMD, we performed multiplex polymerase chain reaction to detect deletions in the dystrophin gene. For patients suspected with gamma-sarcoglycanopathy or without deletion in the *DMD* we perform the screening of the mutation c.525delT of the *SGCG* gene using Sanger sequencing.

Results: From a total of 91 of patient suspected with Becker or Duchenne muscular dystrophy, we confirmed the diagnosis in 54 patients. For patients suspected with gamma-sarcoglycanopathy, we confirmed the diagnosis in 12 patients from a total of 31 and in 5 patients without deletion in the *DMD* gene.

Conclusion: The use of molecular biology techniques is useful in the diagnosis and/or to confirm the clinical diagnosis of neuromuscular diseases.

References:
Grants:
Conflict of Interest: None declared.

EP11.016 RFC1 CANVAS: pathogenic AAGGG repeat expansion frequency - CGC Genetics Unilabs overview

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Background/Objectives: Recently, in 2019, a recessive intronic (AAGGG)n repeat expansion in the *RFC1* gene was identified as the cause of the cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS). The carrier frequency of this mutation in healthy controls was found to be 0.7%, a relatively high frequency, which suggests that CANVAS may represent a considerable fraction of late-onset ataxias.

A retrospective study is presented with a review of our data and determination of the frequency of the biallelic pathogenic expansion detected in a cohort of 18 patients from 2020 to 2022.

Methods: Genetic analysis was based on the approach described by Cortese et al. (2019): Amplification of the repetitive region by fluorescent standard PCR. Additionally, three specific repeat-primed PCRs (RP-PCRs) were performed, targeting the main allelic variants. Using long-range PCR, the presence of the pathogenic AAGGG expansion was confirmed in all patients.

Results: In 18 patients with clinical information suggestive of CANVAS, 11 patients have been found with the biallelic (AAGGG)n expansion, which represents 61% of the total cases. Two of the cases were ascertained as carriers (monoallelic AAGGG expansion).

Conclusion: The search for a cause of progressive imbalance in adult and elderly patients has been difficult and often unsuccessful. This study shows that a significant proportion of these cases is explained by a common genetic defect: the presence of

the pathogenic AAGGG repeat expansion. Testing the (AAGGG)n expansion in undiagnosed patients with late-onset ataxia is highly recommended, as it allows determining the genetic etiology in 61% of the cases.

References: PMID: 30926972.

Grants:

Conflict of Interest: None declared.

EP11.018 The role of LRSAM1 in Charcot-Marie-Tooth disease

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Background/Objectives: Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy, characterized by peripheral nervous system degeneration. CMT is clinically and genetically heterogeneous, with more than 60 associated genes. Pathogenic variants in *LRSAM1* have been associated with axonal CMT2P. LRSAM1 is a RING finger E3 ubiquitin ligase that participates in a range of cellular functions. LRSAM1 has been implicated in CMT pathways, but its role remains unclear. We have recently investigated the pathogenetic mechanisms that lead to the development of CMT2P. We have reported a dominant *LRSAM1* mutation p.Ala683ProfsX3. We then showed that a decrease of *LRSAM1* affects neuroblastoma cells growth and morphology. Then, we showed that *TSG101*, *UBE2N*, *VPS28*, *EGFR* and *MDM2* levels were significantly decreased in CMT2P patient lymphoblastoid and SH-SY5H knocked down *LRSAM1* cells.

Methods: We have recently performed transcriptomics analyses. We used the knocked down LRSAM1 SH-SY5H cell model and CMT2P patient lymphoblastoid cell lines.

Results: Data analysis resulted in more than 2000 significantly dysregulated mRNA molecules. More than 20 of these dysregulated molecules correspond to already known CMT genes. Using EnrichR (KEGG) and Reactome, pathways analysis revealed more than 100 statistically significant involved pathways. These include the Proteasome, Autophagy, Axon guidance, Ubiquitination, Chaperone protein folding and Cell cycle.

Conclusion: We have generated high-throughput transcriptomic data under validation, which will shed light on the CMT2P mechanisms and pathways. Our results also provide additional knowledge to enable better understanding and further study of CMT and peripheral neuropathy in general.

References:

Grants: The Cyprus Institute of Neurology and Genetics, Cyprus.

Conflict of Interest: None declared.

EP11.019 Detection of plasma circulating unmethylated DNA sequences of oligodendrocyte death by SYBR Green qPCR in the context of multiple sclerosis

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Background/Objectives: Multiple sclerosis (MS) is a degenerative and autoimmune disease in which the cells of the nervous system that produce myelin, the oligodendrocytes, die. During relapsing-remitting MS (RR-MS), oligodendrocyte DNA reaches the systemic circulation due to the disruption of the blood-brain barrier derived from the inflammatory events that occur.

Differences in DNA methylation patterns between different cell types in the body make possible for each cell to show a particular gene expression profile while having the exact genome. Previous studies have shown hypomethylated regions in the *WM1* locus and in the *MBP (Lehmann-Werman et al., 2016)* and *MOG (Olsen et al., 2016)* genes in oligodendrocytes in comparison to other cells and that these fragments are identifiable in circulating DNA (cfDNA) during RR-MS.

The main objective of the present study is to design a non-invasive diagnostic/prognostic tool based on real time PCR (qPCR) that would allow the follow up of RR-MS patients using cfDNA.

Methods: SYBR Green qPCR and amplicon dissociation temperature analysis of bisulfite converted cfDNA from plasma, serum and cerebrospinal fluid of patients were carried out to differentiate unmethylated and methylated *WM1* and *MBP* sequences and to calculate the proportion of each type in the samples.

Results: We were able to differentiate and quantify unmethylated sequences in cfDNA samples from patients and controls.

Conclusion: The described technique allows differentiating methylated from unmethylated sequences and could be used in the search of biomarkers in the context of MS.

References: Lehmann-Werman, R. et al., 2016.
Olsen et al., 2016.

Grants: 2016111129_BIO17/ND/024.

Conflict of Interest: None declared.

EP11.021 Mutational spectrum of genes related to hereditary neuropathies – data from a molecular diagnostics laboratory

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Background/Objectives: Hereditary neuropathies constitute a large and heterogeneous group of diseases with a prevalence of 1:2,500. Here, we characterize the mutational spectrum of genes related with hereditary neuropathies identified either by WES or conventional single-gene approaches.

Methods: We reviewed our clinical database for patients tested at our lab (2016-2021) with pathogenic, likely-pathogenic or variants of unknown clinical significance (VUS) in genes related with hereditary neuropathies (n = 956).

Results: Single nucleotide variants (SNVs) or small insertions/deletions, located in 109 different genes, were identified among a total of 772 patients, either by single-gene approaches (n = 318) or WES (n = 454). Recessive forms comprised 57 patients: 13 compound heterozygotes, 36 homozygotes and 8 hemizygotes. Whereas autosomal dominant diseases comprise 212 patients, mostly familial amyloid polyneuropathy (n = 189 cases). Only one case had a X-linked dominant phenotype.

Large pathogenic deletions were found more frequently (but not exclusively) associated with dominant neuropathies in 12

patients (4 WES-based), while only one carried a VUS. Fourteen patients carried large pathogenic duplications (13 WES-based), while 2 patients had a VUS (WES-based). Compound variants between a SNV and a copy number variant were found in 2 patients in genes associated with a recessive neuropathy. As for entire gene copy number variants, *PMP22* deletions were found in 49 patients, while 106 carried whole-gene duplications.

Conclusion: Diagnostic yield in hereditary neuropathies is relatively high, when compared to other neurological diseases, reaching 80% in CMT1A, but significantly lower in other subtypes. Many pathogenic/likely-pathogenic variants were present in our cohort, particularly CNVs. These variant data have both scientific and clinical impact.

References:

Grants:

Conflict of Interest: None declared.

EP11.022 A synonymous substitution in the VMA21 gene cosegregating with a severe neonatal form of x-linked myopathy with excessive autophagy (XMEA)

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Background/Objectives: We aimed to establish the molecular diagnosis of a pedigree with 4 males affected by a neonatal myopathy leading to severe disability and early death. Muscle biopsy showed the highly specific features of X-linked myopathy with excessive autophagy (XMEA) and Sanger sequencing of the coding region of the *VMA21* gene initially yielded the synonymous variant c.294C>T/p.(Gly98=) as a candidate to explain this phenotype. Several asymptomatic females were confirmed as carriers of this variant.

Methods: Clinical and genealogical information was gathered for 29 invididuals from 4 generations. Family testing was extended to 1 affected male, 1 healthy male and 2 asymtomatic carriers. Amplicon-based NGS analysis covering the complete *VMA21* genomic region (including UTRs) was carried out in the index case. Interpretation of this variant was performed according to current knowledge and guidelines.

Results: This variant is presently absent from disease and population databases. Bioinformatic assessment of functional effect suggested potential alteration of RNA processing, possibly affecting the 3´UTR. Virtually all reported pathogenic changes in *VMA21* (around 12) have an intronic/UTR location and cause RNA and protein reduction. Sequencing the complete genomic region did not reveal any further candidate variants. Genetic study of 11 family members was consistent with cosegregation of this variant with the phenotype.

Conclusion: Clinical course, inheritance pattern and the exceedingly distinctive hallmarks in the muscle pathology strongly support XMEA. Cosegregation, a potential molecular effect consistent with previously reported variants and lack of other alterations within *VMA21* underpin c.294C>T/p.(Gly98=) as the cause of the disease in this family.

References:

Grants:

Conflict of Interest: Patricia Blanco Full time Health in Code, Luisa Arrabal Fernández: None declared, Miguel Ángel Fernández García: None declared, Francesca Perin: None declared.

EP11.027 High frequency of nonsense mutations in Russian patients with Duchenne muscular dystrophy

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Background/Objectives: Duchenne-Becker muscular dystrophy (DMD/DMB) is the most common form of muscular dystrophy, accounting for over 50% of all cases. In this regard, in Russia we carry out a program of selective screening for DMD/DMB, which mainly involves male patients. The main inclusion criteria are an increase in the level of creatine phosphokinase (>2000 U/l) or an established clinical diagnosis.

Methods: At the first stage of screening, patients are scanned for extended deletions and duplications in the *DMD* gene using multiplex ligase-dependent probe amplification (MLPA SALSA P034 and P035 DMD probemix, MRC-Holland). The second stage is the search for small mutations using a custom NGS panel, which includes 31 genes responsible for various forms of limb-girdle muscular dystrophy.

Results: In a screening of 1038 patients with a referral Duchenne/Becker diagnosis, pathogenic variants in the *DMD* gene were found in 750 boys (in 72.3% of cases). Here, we report that when analysing the spectrum of *DMD* gene mutations, the share of nonsense mutations is 19.8% (149 patients out of 750), while this percentage in other multiethnic samples is 10-15% [1]. It is important to note that currently Ataluren (Translarna) is registered for the treatment of patients with nonsense mutations.

Conclusion: The mutation spectrum in *DMD* gene in Russia includes a high percentage of nonsense mutations (19.8%). This is essential for the Ataluren treatment.

References: 1. https://doi.org/10.1002/humu.22758.

Grants:

Conflict of Interest: None declared.

EP11.028 A novel hemizygous DRP2 mutation: clinical, molecular, and histopathological characterization of an ultrarare CMT neuropathy

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Background/Objectives: DRP2 encodes the dystrophin-related protein-2 that functionally interacts with periaxin in the PRX-DRP2-dystroglycan (PDG) complex. The PDG complex supports and maintains Cajal bands, cytoplasmic extensions running along myelinated axons. To date, DRP2 mutations were reported only in three families with X-linked CMT disease. Here we report a novel DRP2 variant and describe clinical and biopsy findings.

Methods: A clinical and electrophysiological characterisation was performed. Optic and electron microscopy study including morphometric analysis was completed on sural nerve biopsy specimens. A NGS panel including 116 genes associated to hereditary neuropathy was analysed.

Results: The patient reported from 66 years of age episodes of acute pain and weakness in the upper limbs evolving in muscle atrophy. Physical examination at 68 years revealed upper and

lower limbs strength deficit, atrophy of the biceps brachial, forearm and interosseous muscles bilaterally, bilateral foot drop, bilateral thermal, tactile and pinprick sensation deficit, painful paraesthesia in the upper limbs, absent osteotendinous reflexes, dyspnea and dysphagia. EMG/ENG analysis showed a predominantly axonal sensorimotor polyneuropathy. Optic and electron microscopy revealed significant reduction of myelinated fibers (5995/mm2), cluster-like aspects of regeneration, axonal atrophy, loss of unmyelinated fibers with numerous collagen pockets. The selected NGS panel detected the novel hemizygous variant c.224A>T (p.E75V) in DRP2 gene.

Conclusion: Our report describes the clinical and pathological features associated to a novel DRP2 mutation. Although myelinated axons are predominantly affected, the histological findings and the morphometric analysis confirm the unexpected involvement of unmyelinated fibers and help to improve our knowledge about DRP2 physiopathology.

References:
Grants: Ingene 2.0.
Conflict of Interest: None declared.

EP12 Multiple Malformation/Anomalies Syndromes

EP12.001 Inpatient epidemiology, healthcare utilization and association with comorbidities of turner syndrome: a national inpatient sample study

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Background/Objectives: To investigate prevalence, healthcare utilization, and comorbidities of patients with TS who were hospitalized in the United States.

Methods: Patients with TS were identified within the Nationwide Inpatient Sample (NIS) database from the year 2017 to 2019 using ICD-9 diagnostic code. This database collected data from over 4000 hospitals across the US. Data on patient and hospital characteristics, morbidities, mortality and expenditures were retrieved. A propensity-matched cohort of non-TS patients from the same database was constructed to serve as comparators.

Results: We identified 9,845 TS patients, corresponding to inpatient prevalence of 10.4 per 100,000 admissions. The most common reason for hospitalization was sepsis (28%). Compared to the comparators, TS patients had higher inpatient mortality (adjusted odds ratio [aOR] 2.16 (95% CI 1.57- 2.96) and various morbidities such as shock (aOR 1.68, 95% CI 1.30- 2.17), ICU admission (aOR 1.31, 95% CI 1.10- 1.55), and multi-organ failure (aOR 1.34, 95% CI 1.17- 1.54). They also had significantly higher odds of several comorbidities such as stroke, myocardial infarction, and autoimmune diseases. After adjusting for confounders, TS patients had longer length of stay (5.1 days vs. 4.5 days, $p < 0.01$) and displayed a mean additional $5,382 ($p < 0.01$) in total hospital costs and a mean additional $20,083 ($p < 0.01$) in total hospitalization charges when compared to non-TS patients.

Conclusion: Hospitalization of patients with TS was associated with a significantly higher morbidity, mortality, and expenditures compared to non-TS patients. Patients with TS have a higher risk of cardiovascular complications and autoimmune diseases.

References:
Grants: Not applicable.
Conflict of Interest: Jirat Chenbhanich University Hospitals/Case Western Reserve University, Patompong Ungprasert Cleveland Clinic, OH, Paul Kroner Mayo Clinic, FL.

EP12.002 Mutational characterization of the CORO2B gene and its relationship to ciliopathies

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Background/Objectives: The CORO2B gene belongs to the family of actin-binding coronin proteins involved in cellular processes such as cell division, cell migration and movement, vesicle trafficking within the cytosol and phagocytosis These processes are often altered in ciliopathies, one of them is Bardet Biedl syndrome (BBS MIM# 209900), a multisystem disease whose main symptoms are retinal degeneration, obesity, polydactyly, mental retardation, cryptorchidism and defects in renal structure and function. This high genetic heterogeneity cannot fully explain the large inter- and intra-familial phenotypic variability when members of the same family carry the same causal variant(s), suggesting that other mechanisms are involved in the development of the phenotype.

Methods: Exome sequencing of two patients with clinical suspicion of BBS, subsequent validation by Sanger sequencing. Functional analyses were composed of confocal microscopy of the CORO2B subcellular localization and quantification of expression levels. Immunoprecipitation and cofactor proteomics are currently undergoing.

Results: We describe two new mutations in the CORO2B gene placing it as a possible candidate to modulate the BBS phenotype. In the present work we functionally characterize a set of 3 mutations (Ala 129 Val, Leu 194 Gln and Pro 318 Leu), two found by sequencing and one more extracted from VarSome.

Conclusion: We identified novel the mutations Ala 129 Val, Leu 194 Gln and Pro 318 Leu in the CORO2B gene in two BBS patients. Functional analyses indicate that Ala 129 Val and Pro 318 Leu could have a pathogenic character.

References:
Grants:
Conflict of Interest: None declared.

EP12.003 A case of structural brain anomalies with impaired intellectual development and craniosynostosis caused by a novel ZIC1 gene variant

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Background/Objectives: Structural brain anomalies with impaired intellectual development and craniosynostosis (BAIDCS) is an extremely rare autosomal dominant condition caused by certain mutations in the ZIC1 (Zic Family Member 1) gene. It encodes a member of the ZIC family of C2H2-type zinc finger proteins which are important during development.

We present a seven-month-old boy, born small for gestational age, with uneventful neonatal period. Due to severe dysmorphic features (turricephalic head shape, facial asymmetry, mild ocular proptosis, hypertelorism, tented upper lip, low set ears, short neck, and broad thumbs) along with psychomotor delay, the child was admitted to our department. Head CT scan revealed hypoplasia of corpus callosum, dilated lateral ventricles and bicoronal craniosynostosis.

Methods: Conventional cytogenetic assay and MLPA for microdeletions, subtelomeric deletions and duplications showed normal results. Subsequently we used a NGS panel for craniosynostosis.

Results: The sequence analysis revealed a heterozygous variant of uncertain significance in ZIC1 - c.1199G>T (p. Gly400Val). This sequence change replaces glycine with valine at codon 400 which disrupts the p. Gly400 amino acid residue in the ZIC1 protein. Variants that disrupt this residue are likely to be disease causing. As far as we know this variant is not present in population databases and has not been reported in the literature in individuals affected with ZIC1-related conditions.

Conclusion: A novel ZIC1 variant causing BAIDCS was discovered in a seven-month-old patient that presented with severe craniofacial dysmorphism and developmental delay.

References: N/A

Grants: N/A

Conflict of Interest: None declared.

EP12.004 Male patient with a novel missense variant in SMC3 causing severe phenotype of Cornelia de Lange syndrome

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Background/Objectives: Cornelia de Lange Syndrome (CdLS) is a developmental disorder caused by different genes that affect the cohesin complex. Among those are SMC3 variants, which are rare and usually associated with mild to moderate clinical phenotypes. Here we report a 12-year-old patient with CdLS and a missense variant in SMC3.

Methods: We acquired a patient's family history and clinical data. Chromosome banding analysis, array-CGH, targeted gene analysis, exome sequencing and sanger sequencing were performed using blood samples. The disease's severity was assessed using a scoring system proposed by (1).

Results: The patient presented with typical facial features of CdLS, short stature and global developmental delay. Furthermore, he has epilepsy, strabismus, tear duct malformation, hirsutism, a complete atrioventricular canal defect, gastroesophageal reflux, inguinal hernias, hypospadias and cryptorchidism. An inherited duplication of chromosome region 5q14.1 was detected using array-CGH, classified as likely benign. Exome sequencing showed a likely pathogenic heterozygous missense mutation in SMC3 (c.3442G>T, p.Ala1148Ser), confirming the clinical diagnosis of CdLS. The disease's severity was classified as severe. A segregation analysis is pending.

Conclusion: Our patient presents with CdLS caused by a novel missense SMC3 variant. Whereas most previous case reports of CdLS with SMC3 variants describe mild to moderate phenotypes, this patient shows a severe phenotype. Our case report emphasizes the genetic heterogeneity and the variability of the phenotype of CdLS.

References: (1) Kline AD, Krantz ID, Sommer A, Kliewer M, Jackson LG, FitzPatrick DR, Levin AV, Selicorni A.; Am J Med Genet A. 143A(12):1287-96 (2007); https://doi.org/10.1002/ajmg.a.31757.

Grants: None.

Conflict of Interest: None declared.

EP12.005 Genotypic and phenotypic characterization of Romanian patients with KMT2D-related disorders

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Background/Objectives: Kabuki Syndrome (KS) is a rare disorder of epigenetic dysregulation due to some defects of histone methylation. The incidence of KS is about 1/32,000 of live births. The clinical features of this syndrome are highly variable, making the diagnosis of Kabuki-like phenotypes difficult and that are often mistaken for other diagnostic problems. Kabuki syndrome follows two specific inheritance patterns: KMT2D-related KS is inherited in an autosomal dominant manner and KDM6A-related KS is inherited in an X-linked manner.

Methods: We report 4 unrelated cases referred to the Genetics Department for clinical diagnosis. The patients presented with physical abnormalities (three of them with high suspicion of Kabuki phenotype), prenatal history of polyhidramnios, other medical conditions, skeletal findings, neurodevelopmental and behavioral problems. Next generation sequencing was performed and identified heterozygous mutations in KMT2D gene: c.5138_5146delinsCCTGC, c.15641G>A, c.12039_12046del, c.7228C>T, also confirmed by Sanger sequencing.

Results: An accurate phenotypical observation of certain dysmorphic features, although subtle, can direct to KS diagnosis. Pathogenic variants in KMT2D and KDM6A genes account for 70% of individuals with clinical diagnosis of Kabuki syndrome. The Kabuki molecular genetic panel (KMT2D, KDM6A) may be appropriate for confirmation of a clinical diagnosis or to establish a diagnosis in an individual with suspected Kabuki syndrome. But in case of complex phenotype with additional unspecific features is indicated a comprehensive genetic approach based on NGS technologies.

Conclusion: Early detection is essential. Genetic testing can provide an accurate diagnosis, which may help guide medical management and surveillance decisions, predict disease progression and outcome, and indicate the recurrence risk.

References:

Grants:

Conflict of Interest: None declared.

EP12.006 The burden of congenital anomalies

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Background/Objectives: Congenital anomalies are rare individually, however according to EUROCAT, about 2.6 percent of newborns in Europe are born with one or more anomalies. Major anomalies increase infant morbidity, being the leading cause of death and hospitalization.

Methods: We present an overview of the economic impact of congenital anomalies.

Results: Over the last few decades, progress in medical care led to increased survival of these patients. They require various medical interventions and often face life-long disability. It has been established that costs related to the treatment of congenital anomalies are disproportionately high. Waitzman, N. J. et al. estimated that total medical costs for patients with spina bifida were 235,839 USD. However, direct non-medical and indirect costs were valued at 399,924 USD, exceeding direct medical costs. Decreased life expectancy, lost work capacity, need for special education, caregiver time cost, and other factors have a more significant economic burden than medical care. Given the scale of the

economic and societal impact, not enough studies have so far researched the overall burden of congenital anomalies. More research should be focused on prevention possibilities to reduce this impact. It is known that many congenital anomalies are linked to genetic causes. Current advances in genetic testing allow better risk assessment and offer various diagnostic options before and during pregnancy. However, further understanding of the genetic role is necessary to improve existing preventive measures.

Conclusion: Total burden of congenital anomalies remains undervalued. Researching the causes and prevention together with timely genetic counselling and testing could significantly reduce the overall cost.

References:

Grants:

Conflict of Interest: None declared.

EP12.007 A case of Cat Eye Syndrome (CES) and mosaic additional dic(15;22) marker chromosome

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Background/Objectives: Cat eye syndrome is chromosomal disorder mainly associated with the bisatellited supernumerary chromosome, inv dup(22). Cat eye syndrome critical region in chromosome 22 is multiplied causing partial trisomy or tetrasomy of region 22q11. Mosaicism is described in 1/3 of cases. CES phenotype varies largely resulting from mild to severe malformations affecting multiple organs – eye (chorioretinal coloboma), anus (atresia), ears (preauricular pit or tag), heart and/or kidney (malformations). Mild to moderate intellectual disability is described.

Methods: We report a case of CES in female newborn with congenital tetralogy of Fallot and one tiny preauricular tag. The phenotype is associated with additional dicentric marker chromosome derived from chromosome 15 and 22 centromeric regions.

Results: First chromosomal microarray analysis revealed a pathogenic copy number gain of chromosome 22 region 22q11.1-q11.21 (2,2Mb) containing of CES causing genes and benign gain of chromosome 15 centromeric region 15q11.1-q11.2 (2,7 Mb). Karyotype from blood showed one to two additional marker chromosomes in mosaic form – 48,XX,+mar1x2[14]/47,XX,+mar1[12]/46,XX[4]. FISH analysis with chromosome 15 centromeric probe showed signal on both markers. Additional FISH analysis with chromosome 14/22 centromeric probe showed an additional signal on both markers.

Conclusion: The patient has one to two extra marker chromosomes, dicentric and bisatellited, containing 15pter-q11.2 and 22pter-q11.21 material. The multiplied region of 22q11.21 is causative for CES. The patient's final karyotype is: mos 48,XX,+dic(15;22)(q11.2;q11.21)x2 dn/47,XX,+dic(15;22)/46,XX.ish dic(15;22)(D15Z4+,D22Z1+). To our knowledge the dicentric marker of chromosomes 15 and 22 associated with CES has not been described before.

References:

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Conflict of Interest: Piret Mertsina: None declared, Piret Ilisson: None declared, Ave Auser: None declared, Kati Kuuse: None declared, Gita Taurina: None declared, Gunta Kalnberza: None declared, Zane Enina: None declared, Agnese Berzina: None declared, Katrin Ounap part-time, Estonian Research Council grant PRG471.

EP12.008 CEDNIK syndrome in a Brazilian patient with compound heterozygous pathogenic variants

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Background/Objectives: CEDNIK (Cerebral Dysgenesis, Neuropathy, Ichthyosis, and Keratoderma) syndrome is a neuro ichthyotic syndrome characterized by a clinical constellation of features including severe developmental delay, microcephaly, and facial dysmorphism with autosomal recessive pattern (Sprecher E et al; 2005). Here, we report a characterization of a Brazilian patient with CEDNIK syndrome harbouring two compound heterozygous variants in the SNAP29 gene.

Methods: Whole-exome sequencing was performed with Illumina platform and revealed two genetic variants affecting SNAP29 gene. To validate the findings, it was performed chromosomal microarray (CytoScan HD, Affymetrix) and Sanger sequencing on peripheral blood DNA from the patient. Parental inheritance of the variants was investigated on peripheral blood.

Results: We identified compound heterozygous mutations in the SNAP29 gene (NP_004773.1:p.Leu119fs and arr[GRCh37] 22q11.21(21033398_21798907)×1). The father was a carrier of the NP_004773.1:p.Leu119fs variant, and in the mother, SNP-array showed an interstitial deletion at 22q11.2, with similar breakpoints to the proband's (arr[GRCh37] 22q11.21(21033398 21798907)×1), indicating a maternal inheritance of the deletion.

Conclusion: This report provides a patient with unprecedented genetic events leading to the CEDNIK phenotype and may contribute to accumulating research regarding this rare clinical condition.

References: Sprecher E et al; (2005); A mutation in SNAP29, coding for a SNARE protein involved in intracellular trafficking, causes a novel neurocutaneous syndrome characterized by cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma. Am J Hum Genet 77: 242-51. https://doi.org/10.1086/432556.

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Conflict of Interest: None declared.

EP12.009 Minimal critical region and genes for a typical presentation of Langer-Giedion Syndrome

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Background/Objectives: Langer-Giedion Syndrome (LGS) is caused by a contiguous gene deletion at 8q23-q24. The syndrome is characterized by multiple exostoses, facial, ectodermal, and skeletal anomalies, and, in some cases, intellectual disability. Patients with LGS have been diagnosed clinically or by routine

cytogenetic techniques, thus hampering the definition of an accurate genotype-phenotype correlation for the syndrome.

Methods: We characterized two unrelated patients with deletions involving chromosome 8q through cytogenomic techniques, assessed the pathogenicity of the genes within our patients' deleted segments, and reviewed other molecularly confirmed cases described in the literature.

Results: We identified deletions in the 8q23-q24 region in both patients with one of them, to the best of our knowledge, being the smallest deletion reported in classic LGS cases. Thus, our findings suggest a 3.2 Mb minimal critical region for a typical presentation of the syndrome, emphasizing the contribution of the TRPS1, RAD21, and EXT1 genes' haploinsufficiency in establishing its most common features, including bone dysplasia, intellectual disability, and exostoses, respectively. Additionally, we identified a possible role for the CSMD3 gene, whose deletion could contribute to other uncommon phenotypic characteristics, such as central nervous system anomalies.

Conclusion: Since studies performing this correlation for LGS patients are still limited, our data contribute to improving the genotype-phenotype characterization for patients with LGS.

References:

Grants: CAPES, FAPESP, Brazil.

Conflict of Interest: Bianca Pereira Favilla: None declared, Bruna Burssed: None declared, Érika Mitie Yamashiro Coelho: None declared, Ana Beatriz Alvarez Perez: None declared, Maria de Fátima de Faria Soares: None declared, Vera Ayres Meloni: None declared, Fernanda Teixeira Bellucco: None declared, Maria Isabel Melaragno FAPESP 2019/21644-0.

EP12.010 A maternal inherited rare case with chromoanagenesis-related complex chromosomal rearrangements and de novo microdeletions

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Background/Objectives: Complex chromosomal rearrangements (CCR) are structural rearrangements involving three or more chromosomal breaks and exchange of genetic materials between at least two chromosomes. We here present a 3 years-old boy exhibiting multiple congenital malformations and developmental delay. Cytogenomic techniques identify the proband has a maternal-inherited CCR with the features of chromoanagenesis, a phenomena of massive and chaotic shattering and restructuring of chromosomes.

Methods: Karyotyping, fluorescent in situ hybridization (FISH), spectral karyotyping (SKY) and microarray were performed to characterize the genomic aberrations by the proband's and parental bloods.

Results: The proband presented with speech delay, psychomotor retardation and facial dysmorphism. Cytogenetic analysis found a highly complex chromosomal rearrangements involving four chromosomes (2, 3, 6 and 11), 5 breakpoints as a result of one deletion, one insertion, and three translocations forming three derivative chromosomes. FISH and SKY identified a chromoanagenesis-related derivative chromosome 11 contained three parts connecting the intact 11q telomere to partial 6q and 3q fragments. Microarray studies further revealed one submicroscopic deletion at 6p12.1 (626 kb) inherited from the mother, and two additional de novo deletions at 6q14.1 (914 kb) and 6q16.1~6q16.3 (2.1 Mb), respectively.

Conclusion: We report one maternal inherited CCR with congenital anomalies and the use of cytogenomic techniques to characterize the genomic alterations. FISH and SKY revealed one derivative chromosome 11 linked 11q telomere with 6q and 3q fragments. Microarray identified two de novo microdeletions at 6q. We suggest that chromoanagenesis could be a possible mechanism involved in the repair and restructuring of these rearrangements.

References:

Grants:

Conflict of Interest: None declared.

EP12.011 Clinical and cytogenetic correlation in a case of tetrasomy 9p syndrome

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Background/Objectives: Tetrasomy 9p is a rare chromosomal disorder, characterized by the presence of a supernumerary chromosome, containing two short arms of chromosome 9. It could be present in all cells or in some of them in a form of mosaicism.

A patient (newborn) was the first child of unrelated healthy parents. During mother's pregnancy, NIPD test indicated duplication of the region 9p24-p13.1, confirmed using amniocentesis. Fetal ultrasound scan showed intrauterine growth retardation and multiple congenital anomalies. Newborn had facial dysmorphia (hypertelorism, cleft lip and palate, microretrognathia, poorly formed and low set ears), short neck, hypoplastic digits and nails, partial syndactyly, widely spaced cranial sutures, partial corpus callosum agenesis, multiple heart defects and underdeveloped genitalia.

Methods: Cytogenetic and FISH analysis were used for samples of peripheral blood and buccal smear.

Results: Karyotype 47,XY,+psu idic(9)(pter→q12::q12→pter) was confirmed in 100% lymphocytes and 71,4% buccal smear cells.

Conclusion: Clinical symptoms of tetrasomy 9p vary from multiple malformations, developmental and intelectual dissorders to normal fenotype and high intelectual level. It is assumed that tetrasomy 9p in fibroblasts leeds to more severe symptoms than the one limited only to lymphocytes. Because of a variety of clinical symptoms, more cases of tetrasomy 9p in different tissues should be described.

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Grants:

Conflict of Interest: None declared.

EP12.012 Case Report: Compound Heterozygous COL3A1 Variants in a Child with Brain Anomalies including Polymicrogyria and Heterotopia

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Background/Objectives: Homozygous or compound heterozygous variants in the COL3A1 gene can cause Polymicrogyria with or without vascular-type Ehlers-Danlos syndrome (PMGEDSV – OMIM #618343), a very rare autosomal recessive disorder with a highly variable phenotype.

Methods: Here we report on a 7-month-old developmentally delayed boy who presented with a broad, high and prominent forehead, widely spaced eyes, a thin upper lip and translucent skin. He was delivered by emergency C-section in the 36th gestation week due to severe fetal tachycardia in CTG. He had persisting supraventricular tachycardia until day 5 which ceased after medication. Echocardiography revealed a small pericardial effusion and a tachycardia-induced cardiomyopathy. Brain MRI performed at the age of 4 months showed extensive bilateral frontal and parietal migration disorder mainly polymicrogyria as well as multiple subcortical heterotopias in the occipital region, a thin corpus callosum and symmetrically dilated ventricles.

Results: Investigations showed that the child carries two pathogenic COL3A1 gene variants (heterozygous variant c.1744G>A; p.Gly582Ser and heterozygous variant c.2338-2A>G; p.?) inherited from either parent.

Conclusion: The child's clinical presentation was in keeping with previous reports of patients with PMGEDSV even though a severe supraventricular tachycardia has not been described previously. Whereas heterozygous variants in the COL3A1 gene may cause the vascular type of Ehlers-Danlos syndrome (EDSVASC – OMIM #130050), either parent displayed only very subtle features with no vascular involvement.

References: OMIM #618343, OMIM #130050.

Grants:

Conflict of Interest: None declared.

EP12.013 Clinical and radiological aspects of 34 Brazilian individuals with oculoauriculofrontonasal syndrome: diagnostic implications

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Background/Objectives: Oculoauriculofrontonasal syndrome (OAFNS) is a rare condition, with unknown aetiology, characterised by the clinical association of oculoauriculovertebral spectrum and frontonasal dysplasia features. Here we report a Brazilian cohort with 34 affected individuals and describe the most frequent clinical findings.

Methods: Individuals with clinical diagnosis of OAFNS from the Hospital for Rehabilitation of Craniofacial Anomalies, University of São Paulo, Brazil, were included. Clinical and genetics aspects were considered.

Results: Thirty-four individuals were included in this study. Sex ratio showed male predilection (22M:12F). No recurrence and consanguinity were noted. The individuals were classified as bilateral (85,3%) and unilateral (14,7%). Main clinical features included facial asymmetry (100%), ocular hypertelorism (100%), rare craniofacial clefts (97,0%), preauricular tags (91,1%), and microtia (85,3%). Tessier cleft number 0 (72,7%), 2 (66,7%), and 7 (54,5%) were the most frequent. Complex craniofacial clefts combinations were noted in some cases. Central nervous system abnormalities were observed in 64,2% (18 out 28), mainly corpus callosum lipoma. The ectopic nasal bone was observed in 8 (40,0%) out 20 individuals evaluated through computed tomography scan. Whole exome sequencing performed in one individual was normal.

Conclusion: The phenotype is heterogeneous and variable, affecting most individuals bilaterally. There is a male predilection. Tessier cleft number 2 is an important finding in this condition. The ectopic nasal bone was frequent in our cohort, confirming this is a hallmark of OAFNS. The absence of recurrence, consanguinity, chromosomal and genetic abnormalities reinforce the hypothesis of a non-traditional inheritance model.

References:

Grants: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES).

Conflict of Interest: None declared.

EP12.014 Novel finding in a patient with 17p13.1 deletion syndrome: a case report

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Background/Objectives: Chromosome 17p13.1 deletion syndrome (OMIM#613776) is a rare contiguous gene syndrome characterized by developmental delay, intellectual disability, absent of speech, and various dysmorphic features. This deletion size ranges from 287kb to 4.4Mb. Here, we report the first patient with 17p13.1 deletion syndrome from Turkey who has thoracic syringomyelia; a new finding that has not been reported before.

Methods: After obtaining informed consent from the family, Chromosomal Microarray Analysis (CMA) was performed using Illumina HumanCytoSNP-12 BeadChip kit, and scanned by Illumina iScan system. Results were interpreted using Illumina BlueFuse Multi software.

Results: An 8-month-old girl was referred to our clinic due to hypotonia, neuromotor developmental delay, sacral dimple, facial dysmorphism, bilateral kidney stones, and thoracic syringomyelia. CMA revealed a 1.1 Mb heterozygous deletion; arr[GRCh37] 17p13.2p13.1 (chr17:6,163,462_7,258,861) x1.

Conclusion: According to the American College of Medical Genetics and Genomics (ACMG) guidelines, CMA is recommended as a first-tier test for patients with intellectual disabilities, autism, and/or congenital anomalies. To the best of our knowledge, 17p13.1 deletion syndrome has been identified in only sixteen patients with various phenotypes, and here we reported the seventeenth patient with 17p13.1 deletion syndrome, presenting with a new finding.

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Grants: None.

Conflict of Interest: None declared.

EP12.015 Severe psychomotor delay and new eye findings in a child with biallelic mutations in LAMA1 gene - expanding clinical spectrum of Poretti-Bolthauser syndrome

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Background/Objectives: *LAMA1* gene (OMIM: LAMININ, ALPHA-1; LAMA1; #150320) encodes for laminin, which is a basement membrane protein and a crucial component of the extracellular matrix. Aldinger et al. (2014) identified homozygous or compound heterozygous variants in the *LAMA1* gene in patients presenting with Poretti-Bolthauser syndrome. The described patients had cerebellar dysplasia and cysts, vermis atrophy, high myopia, variable retinal dystrophy, and eye movement abnormalities. All patients had delayed motor development, and most had speech delay, whereas cognitive function was variable.

Methods: We present a case of a 3.5-year-old child with severe psychomotor developmental delay, hypotonia, intractable epilepsy, cerebral abnormalities, including agenesis of the corpus callosum, polymicrogyria and grey-matter heterotopia, colobomatous optic discs and chorioretinal colobomas. Trio exome sequencing (ES) was carried out.

Results: ES revealed two missense variants in *LAMA1* gene in heterozygous state: paternally inherited NM_005559.3(LAMA1):c.7748C>T, p.(Ala2583Val) and maternally inherited NM_005559.3(LAMA1):c.554A>G, p.(Tyr185Cys). Our patient's cerebral malformation was substantially more prevalent than cerebellar abnormalities described in the literature. She also presented with global developmental delay that has not yet been described. In addition to this, our patient did not present with high myopia but rather hypermetropia and had colobomatous findings in the fundus.

Conclusion: According to our knowledge, this is the first case published with this severe phenotype in a patient with Poretti-Bolthauser syndrome. We would like to reach out to collaborators who have expertise in this syndrome with this severe clinical presentation.

References:

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Conflict of Interest: None declared.

EP12.016 A syndrome of severe intellectual disability, hypotonia, failure to thrive, dysmorphism and thinning of corpus callosum maps to chromosome 7q21.13-q21.3

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Background/Objectives: Seven individuals of consanguineous Bedouin kindred presented at infancy with an autosomal recessive syndrome of severe global developmental delay, positive pyramidal signs, craniofacial dysmorphism - consisted of a triangular face with a prominent forehead and midface hypoplasia, severe failure to thrive with normal birth weights, and over-riding toes, with clinodactyly or syndactyly. Magnetic resonance imaging demonstrated thinning of the corpus callosum and paucity of white matter. Early-onset axial hypotonia evolved with progressive muscle weakness, reduced muscle tone, and hyporeflexia.

Methods: Genome-wide linkage analysis was carried using single nucleotide polymorphism arrays for nineteen family members (bead chips) and the HomozigosityMapper and SuperLink softwares (1, 2). Whole-exome-and-genome sequencing was performed for three individuals (arrow heads) and analysed using the Qiagen Clinical Insight software and our in-house database of ~700 samples.

Results: A single ~4 Mbp disease-associated locus was identified on chromosome 7, between rs6952664 and rs13234589 (maximal LOD score of 5.01). Next generation sequencing analysis identified no non-synonymous pathogenic biallelic variants within this locus. Notably, no variants were found in any of the genes previously associated with cases of Russell-silver, Seckel, or Zellweger syndromes.

Conclusion: Considering the consanguinity of the kindred and the homozygosity locus shared by the affected individuals, recessive heredity is the most likely, although other forms of monogenic heredity, such as genomic imprinting, cannot be entirely ruled out. Following the exclusion of partially resembling syndromes, we now describe a novel autosomal recessive syndrome mapped to a ~4Mbp locus on chromosome 7.

References:

Grants: The Morris Kahn Family Foundation.

Conflict of Interest: None declared.

EP12.017 Clinical or whole exome sequencing for mutation analysis in families with rare genetic syndromes?

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Background/Objectives: During the last two years we have sequenced and analyzed over 150 patients with various clinical features and suspect diagnoses, including those of rare genetic syndromes.

Methods: We performed massive parallel sequencing of gDNA isolated from whole blood on NextSeq550 using panel Clinical Exome Solutions (CES v2) and Whole Exome Solutions (WES v2) by Sophia Genetics. CES v2 covers the coding regions (± 5bp of intronic regions) of 4,490 genes related to rare and inherited diseases. WES v2 covers 19,425 nuclear genes and the full mitochondrial genome. SOPHiA DDM™ platform analyses complex genomic NGS data by detecting, annotating and pre-classifying genomic variants and includes trio analyses approach with variant analysis inheritance mode.

Results: Using molecular genetic analysis, we have identified previously unreported de novo variants in trio analyses in families with rare syndromes, that could not be found by CES v2. In 3-year old boy with psychomotor retardation we reported de novo frameshift heterozygous variant c.3943dupA in SON gene. SON gene is associated with ZTTK syndrome, that is characterized by mental retardation, malformations and developmental delay. A 19-year old woman with suspected Wiedermann-Steiner syndrome was proven to bear de novo frameshift heterozygous variant c.126delC in KMT2A gene, a causative gene in Wiedermann-Steiner syndrome.

Conclusion: Performing WES in trio analyses with no causative variant found by CES demonstrates the role of WES in molecular diagnostics for families with rare syndromes caused by de novo and previously unreported mutations.

References:

Grants:

Conflict of Interest: Helena Paszeková researcher, Lucie Hrušková researcher, Tomáš Píš researcher, Zděnka Vlčková head of genetics, Renáta Michalovská head of laboratory.

EP12.018 Copy number variations identified using whole-genome sequencing as genetic markers for pediatrics patients with developmental defects

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Background/Objectives: Whole-genome sequencing (WGS) has been widely applied in studying the genetic causes, such as copy number variations (CNVs), for both rare and complex diseases. However, its clinical applications are limited due to the high false positive detection rate. Despite many bioinformatics tools have been developed to identify the CNVs for WGS data, a stable and reliable approach for CNV calling in clinical samples is still pending.

Methods: We systematically evaluated several CNV calling methods and investigated the effects of sequencing depth and detection resolution in CNV calling by using synthetic data. We built a pipeline with the best performance by integrating the results from different methods. Then we applied the optimized pipeline to WGS data from 88 pediatric patients with developmental defects to discover potential pathogenic CNVs. Validation experiments were performed to assess the findings.

Results: Our newly constructed pipeline (integration of GATK_gCNV, LUMPY, DELLY, cn.MOPS and CNVKit) gave the highest sensitivity and lowest false discovery rate for 10x-depth sequencing data under 5k resolution. For patient data analysis, we not only found many CNVs that were reported to be associated with developmental defects but also identified several novel pathogenic CNVs and genes that might contribute to the diseases. In addition, some mutated genes were enriched in the pathway of immune response.

Conclusion: We developed a CNV calling pipeline for whole-genome sequencing data of clinical samples. This method helped identify novel pathogenic CNVs for pediatric patients with developmental defects.

References:

Grants: This study was funded by Health and Medical Research Fund (no. 16172331 to C.L.H).

Conflict of Interest: None declared.

EP12.019 Extensive deletion of 22q12 in a patient with bilateral schwannoma, mental retardation, sensorineural hearing loss and epilepsy: case report

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Background/Objectives: In contrast with the well-known and described deletion of the 22q11 chromosome region responsible for DiGeorge syndrome, 22q12 deletions are much rarer. However, this region contains genes responsible for cell cycle control, chromatin modification, transmembrane signaling, cell adhesion and neural development. We present a 25-years old man with cleft palate, sensorineural hearing loss, vestibular dysfunction, epilepsy, mild to moderate mental retardation, divergent strabism, pes equinovarus, platyspondylia, and bilateral Schwannoma.

Methods: NGS testing for cancer susceptibility genes, MLPA analysis and Microarray-based Comparative Genomic Hybridization (array-CGH) was performed.

Results: CNV analysis of the sequencing data discovered a heterozygous loss of all exons of *CHEK2* tumor suppressor gene, as well as heterozygous loss of the neurofibromin 2 (*NF2*) gene. The MLPA analysis confirmed the presence of *NF2* deletion. Finally, array-CGH determined an extensive, 3.8Mb sized, 22q12.1-22q12.3 heterozygous deletion, including the *CHEK2* and *NF2* regions. The interstitial deletion was not detected in either parent, so *de novo* origin of the deletion was presumed.

Conclusion: In this article, we focus on the clinical presentation, individual characteristics of the proband and comparison to previously reported cases with similar large deletions.

References:

Grants: Supported by the Ministry of Health of the Czech Republic (under Grant NU20-08-00137); Masaryk University (grant MUNI/A/1330/2021) and the European Regional Development Fund-Project „A-C-G-T" (under Grant CZ.02.1.01/0.0/0.0/16_026/0008448).

Conflict of Interest: None declared.

EP12.020 Expanding the phenotypic picture of Witteveen-Kolk syndrome: the first Hungarian case

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Background/Objectives: Witteveen–Kolk syndrome (WITKOS) is an extremely rare genetic disorder mainly characterized by mild to moderate intellectual disability, global developmental delay, and dysmorphic facial features, occasionally supplemented with skeletal abnormalities, microcephaly, malformation of the brain, ectodermal symptoms, and ophthalmic abnormalities. Haploinsufficiency of *SIN3A* is responsible for this syndrome. The transcriptional repressor protein encoded by *SIN3A* plays a regulatory role of cortical expansion and maturation. To date, only 18 patients with WITKOS have been reported in the medical literature including 11 patients with mutations in the *SIN3A* and seven patients having microdeletions encompassing *SIN3A*. Hereby, we report the first Hungarian patient with WITKOS presenting pronounced lower limb hypertrophy and high arched feet as additional findings, supplementing the previously described clinical picture.

Methods: Array comparative genomic hybridization (aCGH) was applied for the investigation of a Hungarian boy with intellectual disability, global developmental delay and minor anomalies using Agilent SurePrint G3 Human CGH Microarray 8x60K oligo-array.

Results: aCGH examination of the patient revealed a 1.415 Mb (74419546_75834623) copy number loss of the 15q24.1q24.2 chromosomal region. The affected chromosomal region contains several genes including *SIN3A*, responsible for the development of WITKOS.

Conclusion: We report the first Hungarian patient with WITKOS harbouring a novel 15q24.1q24.2 deletion including *SIN3A* presenting psychomotor delay, minor anomalies supplemented with lower limb hypertrophy and high arched feet, which have not been described so far. This report contributes to the expansion of the phenotypic picture of WITKOS, and highlights the importance

of detailed phenotyping and application of aCGH in the investigation of such patients.

References:

Grants:

Conflict of Interest: None declared.

EP12.021 Axenfeld-Rieger anomaly masking a myopathy Ehlers-Danlos syndrome in a family segregating FOXC1 and COL12A1 mutations

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Background/Objectives: Axenfeld-Rieger syndrome (ARS) is a disorder affecting the anterior segment of the eye and causing systemic malformations, and follows an autosomal-dominant inheritance pattern. Pathogenic variants in COL12A1 cause a rare form of congenital connective tissue/myopathy overlap syndrome subsumed under the classification of "myopathic Ehlers-Danlos syndrome (EDS)". The aim of the present study was to identify the genetic cause of complex phenotype in a pedigree affected by ARS.

Methods: A Bulgarian family presented with an autosomal-dominant inheritance pattern for ARS that affected mother and the two children was recruited. Targeted sequencing of clinical exome of the affected daughter was performed on MiSeq platform of Illumina, followed by Sanger sequencing for segregation analysis. CNVs identified by next-generation sequencing were confirmed by MLPA.

Results: A heterozygous deletion of the only exon of the forkhead box C1, FOXC1, gene was detected and then confirmed by MLPA in all affected family members. A novel missense variant, c.4166C>G (p.Ser1389Cys), that was classified as likely pathogenic according to ACMG criteria was found in the gene coding collagen type XII alpha-1, COL12A1. Both mutations were not present in the healthy father.

Conclusion: Our results demonstrate that a novel mutation in COL12A1 and a known exon 1 deletion of FOXC1 co-segregates with two autosomal-dominant clinically overlapping syndromes – AR anomaly and myopathic EDS, respectively. Therefore, molecular-genetic analysis is of emerging importance for clinically diagnostic and genetic counseling of overlapping complex diseases.

References: OMIM (https://www.omim.org/).

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Conflict of Interest: None declared.

EP12.022 A new case of Perching syndrome detected by WES in a patient with severe failure to thrive, dysmorphic features and LOH of 7p21p14.33

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Background/Objectives: Perching syndrome is a rare autosomal recessive condition characterised by global developmental delay, dysmorphic features, feeding and respiratory difficulties, poor overall growth, axial hypotonia, and joint contractures. We present a newly diagnosed patient with clinical signs of Perching syndrome and a previously unreported homozygous pathogenic variant in *KLHL7* gene.

Methods: The patient was examined as a neonate for polyhydramnios, growth restriction and dysmorphic features. Repeated consultations at the age of 5m, 20m and 4yr8m revealed severe failure to thrive, feeding difficulties and vomiting requiring PEG, developmental delay, frequent infections, febrile convulsions, excessive sweating, episodes of raised temperature, bilateral inguinal hernias, undescended testes, and dilatation of the frontal horns of lateral ventricles. Dysmorphic features included triangular face, hypertelorism, proptosis, wide nasal bridge, arched palate, low set ears, overlapping fingers and toes, single palmar creases.

Results: Karyotype was 46,XY, SNPaCGH revealed LOH in 7p21p14.33. WES detected a homozygous frame shift variant *KLHL7*(NM_001031710.3): c.969del (p.Phe323LeufsTer15) in a highly conserved area of the gene not found in gnomAD.

Conclusion: Our report helps to further delineate the phenotype of patients with biallelic variants in *KLHL7* gene. It also presents previously unreported variant in *KLHL7* gene. Homozygosity of the variant was caused by a loss of heterozygosity of the critical region of chromosome 7 in unrelated parents. The diagnosis was only reached due to the results of WES, which highlights the usefulness of the method in diagnostics of rare syndromes.

References:

Grants: MH CZ – DRO (FNOL, 00098892), BBMRI-CZ: LM2018125.

Conflict of Interest: None declared.

EP12.023 Paternal occupational exposures to endocrine disruptors and the risk of congenital malformations using job-exposure matrix

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Background/Objectives: Prenatal exposure to endocrine-disrupting chemicals adversely affects the development of reproductive organs in the offspring, but also other organs. Our study aims to evaluate the effect of prenatal paternal exposure to endocrine-disrupting chemicals to assess their association with the risk of congenital malformations.

Methods: A case-control study was carried out on 101 congenital malformation cases and 101 controls. Paternal occupational exposure to endocrine disruptors at the workplace was assessed using two job-exposure matrix from exppro.santepubliquefrance. Detailed questionnaires data were available to evaluate the father's children health during in utero, neonatal and postnatal periods as well as to estimate the presence of other risk factors such as any maternal diseases during the pregnancy.

Results: Analysis by occupational title revealed that more case than control fathers were farmers. It revealed that the paternal exposure to pesticides, phthalates, alkylphenolic compounds and other endocrine-disruptors is associated with cardiac and genital birth defects. In addition, exposure to Biphenolic compounds appears to be associated in particular with genital malformations. Odds of having a child with genital congenital malformation was higher if the father was occupationally exposed endocrine-disruptors using Chi-square and Fisher's exact test.

Conclusion: This case-control study identified a significantly increased risk of genital congenital malformations among the offspring of men occupationally exposed to endocrine disruptors at their workplaces.

References: Bonde JPE, Tøttenborg SS, Hougaard KS. Paternal environmental exposure and offspring health. Current Opinion in Endocrine and Metabolic Research. 2019;7:14-20.

Chia SE, Shi LM. Review of recent epidemiological studies on paternal occupations and birth defects. Occup Environ Med. 2002 Mar;59(3):149-55.

Grants:

Conflict of Interest: None declared.

EP12.024 A deficiency of PPP1R7 (SDS22) results in a loss of protein phosphatase-1 and is associated with a human neurodevelopmental disease

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Background/Objectives: PPP1R7 (MIM *602877, alias SDS22) encodes an ancient regulatory subunit of protein phosphatase-1 (PP1) and is implicated in the biogenesis and stabilization of PP1 [1]. It largely consists of leucine-rich repeats (LRRs), which mediate binding to PP1. The C-terminus of PPP1R7, which functions as an LRR-cap, is needed to maintain physiological levels of PPP1R7.

Methods: SNP array, WES trio analysis on peripheral blood gDNA and mRNASeq from muscle biopsy has been performed. Immunoblot and mitotic-progression analyses were performed on fibroblasts from the patient and matched controls.

Results: The patient is an eight-years old girl with developmental delay and hypotonia. She is carrier of a partial isodisomy of chromosome 13 (no pathological mutations have been identified in this region) and a heterozygous de novo p.Trp302* variant in the PPP1R7 gene, predicted to encode a C-terminally nicked variant of PPP1R7. Further studies on the patient's fibroblast revealed that this mutation is associated with a 76% drop in the PPP1R7 protein level. The concentration of PP1 was also decreased by some 45%. However, these fibroblasts did not accumulate measurable DNA damage or mitotic defects.

Conclusion: A heterozygous mutation in PPP1R7 results in the expression of a protein with a nicked C-terminus and is associated with a decreased global level of both PPP1R7 and PP1 proteins. Our data suggest that the patient's phenotype is caused by an imbalance in protein phosphorylation due to a loss of PP1.

References: 1. Cao et al. 2021. PMID: 34028981.

Grants: URDCat (PERIS-2016, SLT/238/2016); FWO G090921N.

Conflict of Interest: None declared.

EP12.025 Identification of novel BICRA variant leading to the newly described Coffin–Siris syndrome 12

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Background/Objectives: Heterozygous variants in *BICRA*, encoding a subunit of the non-canonical BAF complex, have recently been identified in patients with SWI/SNF-related intellectual disability (SSRIDD) – Coffin-Siris syndrome-12. So far, only one article reported SSRIDD associated with pathogenic variants in *BICRA*. We present a patient with pathogenic variant affected by the syndrome.

Methods: The patient underwent exome sequencing and segregation analysis. We compared patient's phenotype with cases reported in original paper.

Results: The proband's phenotype include low birth weight, microcephaly, neuropsychological development delay, stereotypical movements, mumbling, slurred speech, inability to express thoughts, poor sleep, strabismus, astigmatism as well as gastrointestinal symptoms such as, regurgitation, obstipation, and dyspepsia. Also, the patient showed craniofacial dysmorphism and clinodactyly of the 5th finger. Exome sequencing revealed a heterozygous pathogenic variant NM_015711.3:c.535C>T in exon 6 of *BICRA* gene. Identified a de novo c.535C>T transition results premature termination (p.(Gln179Ter)). Additional functional studies of the variant and studies of patient cells were not performed, but it is predicted to result in a loss-of-function and haploinsufficiency. As *BICRA* is highly intolerant to loss-of-function variants, loss of this chromatin remodeling protein leads to a neurodevelopmental disorder.

Conclusion: This report aims at broadening the genotype-phenotype spectrum of *BICRA* gene variations. Phenotype comparison of described patient and previously reported cohort of twelve patients showed that pathogenic variant in *BICRA* is commonly characterized by neurodevelopmental, gastrointestinal, and ophthalmologic symptoms, growth retardation, craniofacial dysmorphism, and absent fifth digit/nail hypoplasia. Our patient presented delayed dental growth and enuresis, which were not previously described.

References: PMID: 33232675.

Grants:

Conflict of Interest: None declared.

EP12.026 Old-fashioned dysmorphology, and the use of adjuvant techniques to reach a diagnosis in the whole exome sequencing era. An unusual ATRX case report

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Background/Objectives: This is a clinical case report, demonstrating the value of careful phenotyping before reaching a molecular diagnosis. It demonstrates how the use of adjuvant techniques facilitated a definite molecular diagnosis after a diagnostic trajectory of 7 years.

Methods: A non-ambulatory severely retarded 9 year old boy, without speech is reported. From the age of 2 years on a diagnosis of ATR-X syndrome, without alpha-thalassemia or HbH inclusions was suspected. Two trio WES investigations failed to identify any coding sequence alteration in ATRX, nor any alternative explanation for the retardation.

Results: X-inactivation studies in the mother using the Humara assay showed 100% skewing of X-inactivation against the AR allele transmitted to her son .Genome wide methylation asssy showed an epigenetic signature characteristic for ATRX syndrome. Targeted WGS for the ATRX region showed a c.[371-1200G>A] deep intronic variant, that was the novo in the mother, with skewed X-inactivation. RNA-seq in the proband revealed the splicing in of

a 94 basepair long cryptic exon, ultimately leading to a frameshift in ~80% of ATRX transcripts. Leading to the following molecular diagnosis ATRX NC_000023.10(NM_000489.6):c.[371-1200G>A];[0] r.[370_371ins371-1208_371-1301].

Conclusion: This case shows that a definite molecular diagnosis in the WES era could only be achieved using the combination of precise phenotyping, X-inactivation studies, genomewide methylation profiling, targeted WGS, segregation of the probable variant and utimateli RNA-seq.

References: Alpha thalassaemia-mental retardation, X linked Richard Gibbons Orphanet J. of rare diseases 2006. Genome-wide DNA methylation analysis for the diagnosis of Mendelian disorders Bekim Sadovic et al. Genet Med 2021.

Grants: None.

Conflict of Interest: None declared.

EP12.027 The impact of 22q11.2 CNVs on human traits in the general population

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Background/Objectives: Copy-number variants (CNVs) in the 22q11.2 region have highly pleiotropic and variable consequences. Although extensive efforts have been made to investigate the 22q11.2 deletion phenotypic effects in clinical cohorts, the impact of 22q11.2 CNVs in the general population remains understudied. We performed a phenome-wide analysis to identify associations of those CNVs with traits previously implicated by their genetic content.

Methods: We retrieved phenotypes linked to the 22q11.2 cytoband in the Human Phenotype Ontology (HPO) and mapped 205 out of 702 to UK Biobank (UKBB) traits using cross-ontology mappings and web-scraping followed by manual curation. We performed association scans between the copy-number state of all SNP-array probes in the region and 134 HPO-implicated traits using linear regression or Fisher's exact test in 405'324 unrelated UKBB participants.

Results: We found seven deletion-associated traits, including decreased height ($\beta = -0.56$ cm, $p = 2.3e-06$) and platelet-count ($\beta = -1.67$ 10^9 cells/L, $p = 4e-08$), reinforcing well-known clinical observations. Fifteen traits were associated with duplications, corroborating phenotypes such as body-mass index ($\beta = 0.34$ kg/m^2, $p = 1.1e-10$) and height ($\beta = -0.2$ cm, $p = 1.43e-06$), but also highlighting new associations such as gastroesophageal reflux ($OR = 2.5$, $p = 7.69e-07$). We have further showed that CNV probes in genes linked to a given HPO term are 15 times more likely ($P < 6e-9$) to show significant association with the corresponding UKBB trait.

Conclusion: Our findings suggest that within the general population, 22q11.2 CNVs affect traits compatible with clinical manifestations seen in the genomic disorders, but also revealed new phenotypes being impacted by the perturbation of dosage of 22q11.2 genes.

References:

Grants: CAPES/FAPESP.

Conflict of Interest: None declared.

EP12.028 Double trouble? – Interpretation of two de novo variants in a case of syndromic developmental delay

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Background/Objectives: A premature infant was presented at the age of 8 months with delayed motor development, cardiovascular abnormalities (bicuspid aortic valve, hypoplastic aortic arch), club feet and unilateral hearing impairment.

Methods: Karyogram, arrayCGH (180k Agilent, design 086332), Trio-Whole Exome Sequencing (WES;Twist Human Core Exome with additional customized probes and NGS with NovaSeq Illumina).

Results: Whereas the performed karyogram and arrayCGH showed no abnormalities, Trio-WES revealed two suspicious *de novo* variants: One which causes a premature stop codon in *KMT2D* and a missense in *BRPF1*. Both variants seem to fit the clinical phenotype of the index. Mutations in the *KMT2D* gene, in particular the pathogenic effect of loss-of-function variants, are well described/characterized as the genetic cause of Kabuki syndrome type 1. Whereas the relevance of the missense variant in the less investigated gene *BRPF1* appears unclear although it can be classified as likely pathogenic variant (class 4).

Conclusion: Here we compare the phenotypically overlap of the index with *KMT2D* and *BRPF1* associated developmental delay and discuss how to interpret and handle two *de novo* variants in a medical report.

References:

Grants:

Conflict of Interest: None declared.

EP12.029 Trichohepatoneurodevelopmental syndrome: a case report

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Background/Objectives: Trichohepatoneurodevelopmental syndrome (THNS), reported by Morimoto et al. (2018), is a complex disorder affecting multiple organ systems. It is characterized by liver dysfunction, hypotonia, global developmental delay, coarse hair, and dysmorphic features. We present the fifth case of THNS in the medical literature.

Methods: Case report.

Results: We report a case of a 32-month-old female of Saudi origin. She presented with multiple dysmorphic features, hyporeflexia, generalized hypotonia, global developmental delay, and high liver enzyme levels. A brain MRI showed mild brain volume loss. An echocardiogram showed a dysplastic pulmonary valve with moderate to severe pulmonary stenosis and moderate pulmonary insufficiency. An abdominal ultrasound revealed

cholelithiasis and grade II right hydronephrosis. Through whole-exome sequencing, a homozygous likely pathogenic variant was identified in the CCDC47 gene (GenBank: NM_020198.2: c.567_570del p. (Glu190Profs*7)). These findings are consistent with the genetic diagnosis of THNS.

Conclusion: This case report will help to determine the characteristics of this complex disorder that exhibits variable clinical presentations.

References:

Morimoto M, Waller-Evans H, Ammous Z, Song X, Strauss KA, Pehlivan D, Gonzaga-Jauregui C, Puffenberger EG, Holst CR, Karaca E, Brigatti KW. Bi-allelic CCDC47 variants cause a disorder characterized by woolly hair, liver dysfunction, dysmorphic features, and global developmental delay. The American Journal of Human Genetics. 2018 Nov 1;103(5):794-807.

Grants:

Not applicable.

Conflict of Interest: None declared.

EP12.030 Imprinting disorders' multilocus screening startegy: Diagnosis contribution in overlapping clinical features

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Background/Objectives: Imprinting disorders (IDs) are a group of congenital diseases caused by molecular changes affecting imprinted chromosomal regions. They are characterised by shared clinical features, affecting growth, development and metabolism [1]. Beckwith-Wiedemann (BWS; MIM #130650) and Silver Russel (SRS; MIM #18860) syndromes are ones of the most frequent and well known IDs. They are usually presenting as respectively overgrowth and growth retardation diseases. Here we present a patient with very suggestive clinical presentation of BWS, who thanks to imprinting disorders' multilocus screening strategy, was surprisingly diagnosed as SRS.

Methods: Clinical study and methylation-specific multiplex-ligation-dependent probe amplification assay (MS-MLPA) using screening for more than 5 imprinting loci at the same time, were performed in a 2 months Tunisian boy displaying suggestive Beckwith-Wiedemann clinical features.

Results: Our patient presented macroglossia, umbilical hernia and facial dysmorphism, fulfilling two major and one minor criteria of BWS [2]. MS-MLPA analysis showed no (epi)genetic defects at the 11p15 locus (GF2/H19 and CDKN1C/KCNQ1OT1). However, it revealed a loss of methylation in the chromosome 7p12.1 involving *GRB10*.

Conclusion: In our Patient, loss of methylation of *GRB10* gene known as a defect related to SRS, is miming a BWS. In fact, besides sharing clinical characteristics, ID can also show overlapping molecular alterations. An imprinting disorders' multilocus screening strategy is strongly required for molecular diagnosis.

References: 1: Congenital imprinting disorders; Eggermann et al. Clinical Epigenetics (2015).

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Grants:

Conflict of Interest: None declared.

EP12.032 Turnpenny-Fry Syndrome: Clinical Report of a 4-year-old female with mutations in the PCGF2 gene

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Background/Objectives: Turnpenny-Fry Syndrome is a very rare congenital disorder, caused by mutations in the PCGF2 gene, first described in 2018. There are 14 cases reported in the literature. We describe here a new case.

Methods: The patient is a 4-year-old female who initially presented with intrauterine growth restriction and dysmorphic facial features when she was 2 months old. She was born at 37 weeks gestation to a 25-year-old G2P2 mother. There was no parental consanguinity. The birth weight was <3th percentile. She was admitted to neonatal intensive care unit due to respiratory distress and malnutrition. The body weight, height, head circumference were <3th percentile at the time of her most recent assessment. Her development was globally delayed. She had dysmorphic features including a broad forehead, frontal bossing, microcephaly, hypotonic facial appearance, sparse scalp hair, periorbital fullness, broad nasal root, malar hypoplasia, low set small dysplastic 'satyr' ears, open mouth posture. No organ anomaly was detected. Brain magnetic resonance imaging revealed bilateral subarachnoid enlargement in the frontal area. Echocardiography examination showed an atrial septal defect. Lumbar lordosis and scoliosis were skeletal features.

Results: The karyotype and Array CGH analysis were reported as normal. Whole-exome sequencing analysis displayed pathogenic heterozygous mutation of c.194 C>T (p.Pro65Leu) in the PCGF2 gene.

Conclusion: Turnpenny-Fry Syndrome phenotype includes facial dysmorphism, intellectual disability, developmental delay and skeletal anomalies. The description of new patients is significant to increase our knowledge of disorders to precisely define molecular characteristics and clinical phenotype.

References: Turnpenny et al. 2018.

Grants: None.

Conflict of Interest: None declared.

EP12.034 A de novo ARID1A variant in a child with Coffin-Siris syndrome and hepatoblastoma

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Background/Objectives: Coffin-Siris syndrome (CSS) is a rare neurodevelopmental and multisystemic disorder with phenotypic variability caused by pathogenic variants in ARID1A, ARID1B, ARID2, DPF2, SMARCA4, SMARCB1, SMARCC2, SMARCE1, SOX11 and SOX4. Malignancy has been reported in 9 cases with CSS phenotype, including 2 cases with ARID1A-CSS: 1 with acute lymphoid leukemia and 1 with hepatoblastoma. We report a child

with CSS phenotype harboring a novel probably pathogenic variant in ARID1A and hepatoblastoma, adding evidence of the cancer predisposition related to this gene.

Methods: A 15-month-old female with global developmental delay, brain malformations, congenital heart disease and hepatoblastoma. Single whole-exome sequencing and parental sanger sequencing of the ARID1A gene in peripheral blood was performed. Liver tumoral genomic profiling was done.

Results: We identified a novel, de novo, heterozygous missense variant c.6182T>C; p.(Leu2061Ser) in the ARID1A gene, classified as probably pathogenic.

Genomic profiling of the liver tumour detected the p.(Leu2016Ser) ARID1A variant, as well as a somatic p.(Gly34Val) CTNNB1 variant, classified as pathogenic.

Conclusion: Is there a role of ARID1A gene in the pathogenesis of hepatoblastoma? ARID1A-CSS should be included among the cancer predisposition syndromes associated with an increased risk of hepatoblastoma. Our patient adds to an 8% incidence of malignancy and 5.6% incidence of hepatoblastoma in ARID1A-CSS. Therefore, we recommend performing tumour screening when suspecting/diagnosing patients with CSS.

References: 1.Tsurusaki, Yoshinori et al. Nature genetics vol. 44,4 376-8. 18 Mar. 2012, https://doi.org/10.1038/ng.2219.

2.Vasko, Ashley et al. Genes vol. 12,6 937. 19 Jun. 2021, https://doi.org/10.3390/genes12060937.

Grants:

Conflict of Interest: None declared.

EP12.035 A unique combination of copy-number and single-nucleotide variants in a patient with developmental delay and congenital malformations

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Background/Objectives: Deafness-infertility syndrome (DIS) is an autosomal recessive disorder caused by large contiguous gene deletions at 15q15.3. Here we present a unique non-contiguous deletion of this region in a patient without hearing loss, referred for genetic testing due to congenital developmental abnormalities.

Methods: The patient is a four-year old male with developmental delay, dysmorphic features, hyperlaxity, congenital vertical talus and congenital dislocation of radial head elbow, born to healthy non-consanguineous parents. He was referred for array-CGH and findings were confirmed by MLPA and refined by Clinical Exome Sequencing (CES).

Results: Array-CGH revealed a 100kb homozygous deletion on 15q15.3 (43,851,548–43,951,301) containing *CATSPER2* and *STRC* genes, causative for Deafness - male infertility syndrome (DIS, OMIM#611102). This finding was not consistent with the reported clinical features, as the child has normal hearing; therefore MLPA and CES analysis were performed and refined the deletion, showing that it was homozygous only for *CATSPER2*, but heterozygous for *STRC*. In addition, CES revealed a novel, *de novo* missense variant NM_0011643317.2(FLNB):c.617A>G p.(Asp206Gly). Hererozygous variants within *FLNB* gene are causative for autosomal dominant Larsen syndrome (LRS) which resembles the patient's phenotype.

Conclusion: This is to the best of our knowledge, the first report of a non- contiguous deletion within DIS region, presented as an incidental finding. Accurate characterization of these deletions is crucial for genetic counseling, while the addition of CES to the diagnostic arsenal is of great value, not only for its single-base resolution, but also for its power to identify and refine copy number variants.

References:

Grants:

Conflict of Interest: None declared.

EP12.036 Two new patients harboring pathogenic variants in SAMD9 contributing to the genotype-phenotype association in MIRAGE syndrome

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Background/Objectives: MIRAGE syndrome (Myelodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes, and Enteropathy) is a rare (<1/1,000,000) and severe multisystemic disorder, caused by the presence of de novo gain-of-function variants in the SAMD9 gene. Different compensatory post-zygotic mechanisms have been described such as upstream loss-of-function variants in cis, uniparental disomy of chromosome 7 wild type allele and/or loss of chromosome 7 carrying the pathogenic variant. Our study aims to contribute to the clinical and molecular description of MIRAGE syndrome.

Methods: DNA samples from two patients, a 20-week-old fetus (SAMD9-01) and a 6-month-old infant (SAMD9-02) with intrauterine growth retardation, adrenal hypoplasia, and disorder of sexual differentiation were analysed using Agilent SureSelect V6 exome capture and Illumina Novaseq sequencer.

Results: Clinical exome analysis of both samples identified the presence of two de novo likely pathogenic variants, p.Gly1559Arg and p.Glu646del, in the SAMD9 gene. In patient SAMD9-02, with a 46,XY karyotype at birth, the p.Glu646del variant was detected in mosaic (allelic fraction ~29%) together with a complete monosomy of chr7, also in mosaic (~70-80%). This monosomy is presumably a consequence of the rescue mechanisms described in this pathology.

Conclusion: The description of two new cases of MIRAGE syndrome allows us to expand its age-related phenotypes.

Clinical exome, including both SNVs and CNVs analysis, improves the diagnosis, prognosis and follow-up of MIRAGE syndrome patients' harbouring compensatory mechanisms such as postnatal chromosome 7 monosomy that could lead to myelodysplastic syndrome.

References:

Grants:

Conflict of Interest: None declared.

EP12.037 Pathogenic variants in the MAP3K7 gene: phenotypic overlap in two patients

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Background/Objectives: Heterozygous pathogenic variants in the *MAP3K7* gene (*602614) have been associated with frontometaphyseal dysplasia-2 (FMD2;#617137) and cardiospondylocarpofacial syndrome (CSCF;#157800), two autosomal dominant skeletal dysplasias with many clinical features in common but with no genotype-phenotype correlation reported to date. Less than 20 causal pathogenic variants in *MAP3K7* gene have been described so far. Here, we report two pathogenic variants and describe the clinical phenotypes in two unrelated patients, expanding the number of reported MAP3K7-related cases.

Methods: Patients underwent clinical examination by a specialized syndromologist and were referred to the Clinical Genetic Service for analysis. A WES and Trio-WES from whole peripheral blood was performed in patient 1 and patient 2, respectively. Genes associated with skeletal dysplasias including MAP3K7, were analysed.Segregation analysis was performed in patient 1.

Results: The clinical features and the pathogenic variants found in the MAP3K7 (NM_145331.2) gene in each patient are detailed in table 1..

Table 1: Clinical and molecular characteristics of patients

Patient	1	2
Gender	Female	Female
Age	10	14
Common clinical features	Short stature Skeletal dysplasia Scoliosis Hearing loss Micrognathia	
Specific clinical features	Ptosis Mesomelia Brachydactyly Clinodactyly of the 5th finger Widely spaced toes Broad hallux	Valvular dysplasia
Variant	c.149T>A (p.(Val50Asp))	c.572G>A (p.(Gly191Glu))
GnomAD	0%	0%
ClinVar(ID)	Not reported	985515
Origin	De novo	De novo
Diagnostic	**FMD2**	**CSCF**

Conclusion: The overlapping features of MAP3K7-related disorders together with the lack of a clear genotype-fenotype correlation, makes diagnosis very difficult. Thus, is essential to work in multidisciplinary teams in order to better characterize patients and provide adequate genetic counselling.

References:
Grants:
Conflict of Interest: None declared.

EP12.038 Homozygous deletion of the entire ADAT1 gene in a patient with intellectual disability and symptoms similar to adenosine deaminase (ADA) deficiency

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Background/Objectives: The *ADAT1* (OMIM: 604230) gene is a member of ADAT (adenosine deaminases acting on tRNAs) family which encodes a tRNA-specific adenosine deaminase. It is believed that the enzymes of ADAT family are ancestral to present-day adenosine deaminase acting on RNA (ADAR) enzymes. The ADAR family mediates the adenosine to inosine (A-to-I) RNA editing which is a post-transcriptional process. In the OMIM database, pathogenic variants in *ADAT1* gene have not been associated to any human disorder yet.

Methods: Whole exome sequencing (WES) Trio analysis was performed on a 7-year-old male with speech delay, poor academic performance, elevated liver enzymes, periods of coughing and sneezing almost every month, skin itching, papular dots like lesions on the arms and foot deformity.

Results: WES revealed a homozygous deletion of the entire *ADAT1* gene and both parents were heterozygous carriers.

Conclusion: ADA deficiency is a metabolic disorder, which affects lymphocyte development, viability and function. The clinical picture in infants with early-onset ADA-deficient includes failure to thrive and opportunistic infections associated with marked lymphocytopenia and the absence of humoral and cellular immune function. Our study suggests that pathogenic variants in the *ADAT1* gene may cause symptoms similar to ADA deficiency in human.

References:
Grants:
Conflict of Interest: None declared.

EP12.039 Future directions in molecular genetic diagnostics and management in patients with Silver-Russell syndrome features

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Background/Objectives: Silver-Russell syndrome (SRS) is a congenital disorder which is characterized by intrauterine and postnatal growth retardation, a typical facial gestalt, body asymmetry and further less constant features. Though a clinical scoring system has been consented recently, the clinical diagnosis remains a challenge due to the non-specifity of the major clinical features. The clinical heterogeneity is mirrored by the molecular heterogeneity of SRS: Molecular alterations of at least three genomically imprinted regions (##11p15, 7q32, 14q32) are associated with a SRS phenotype, but additionally imprinted and non-imprinted loci have been suggested as SRS (candidate) region as well (e.g. #1q12, 8q12, 16).

Methods: More than 1100 patients had been referred for SRS testing, they were all analysed by multilocus methylation assays to identify clinically relevant alterations at imprinted loci. In a subcohort, array analysis and next generation sequencing have been conducted.

Results: In the 1100 patients, approximately 19% showed changes affecting imprinting loci. Among this group, 9% exhibited unexpected findings. Further analysis to characterize these results and to increase the diagnostic yield revealed pathogenic variants of relevance for the patients and their families.

Conclusion: We will illustrate the broad spectrum of molecular changes associated with SRS features, as well as the molecular overlap with other imprinting disorders and differential clinical diagnoses of SRS. Based on this dataset, modifications of the currently implemented diagnostic algorithm for SRS will be suggested. Finally, the significance of the precise molecular diagnosis for clinical management of the patients as well genetic and reproductive counselling of their families will be discussed.

References:

Grants:

Conflict of Interest: None declared.

EP12.040 Biallelic FZD4 mutations result in syndromic familial exudative vitreoretinopathy resembling Norrie disease

Sarah van der Ende[1], *Benjamin Meyers*[2], *Alex Levin*[3], *Jennifer Huey*[4], *Matthew Pihlblad*[5], *Yoshihiro Yonekawa*[6], *Ian Krantz*[7], *Mario Sasongko*[6], *Emma Bedoukian*[7], *Christopher McMaster*[8], *Johane Robitaille*[9]

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Background/Objectives: Familial exudative vitreoretinopathy-1 (FEVR-1) is a non-syndromic autosomal dominant retinal disorder caused by heterozygous mutations in the frizzled-4 gene (FZD4). We report a case of FEVR with hearing loss and mental developmental delays, associated with a biallelic FZD4 mutation. Previous cases have not reported hearing loss and developmental delay.

Methods: Case report. History, ocular examination, fluorescein angiography (IVFA), cell-based signalling assays, and genetic testing with interpretation were performed in the setting of patient care.

Results: By 4 months old, the patient had stage 5 FEVR bilaterally. Sensorineural hearing problems were first noted at 2 years. The patient had developmental delays in walking and language, and wears hearing aids. IVFA of the parents revealed stage 1 and stage 2 FEVR. Genetic testing revealed two heterozygous variants in the FZD4 gene in the patient. Segregation in each parent confirmed on allele in each. Signalling assays revealed that biallelic mutations have significantly worse signalling activity when compared to the heterozygous mutations.

Conclusion: We present the first report of syndromic FEVR due to a biallelic FZD4 mutation with hearing deficit, developmental delays, and more severe retinal phenotype.

The signalling assays showed the biallelic mutation had a compounded effect of the heterozygous mutations, which corroborates the clinical phenotypes seen in the patient and parents. Homozygous Fzd4 knockouts in mice feature abnormal vascular development of both the inner ear and retina.

References:

Grants: This work was supported by a Canadian Institutes of Health operating grant to Johane Robitaille and Chris McMaster.

Conflict of Interest: None declared.

EP12.041 A novel missense mutation of the KDM6A gene in an Albanian female patient with Kabuki type 2 syndrome

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Background/Objectives: Kabuki syndrome (KS) is a rare congenital disorder characterized by typical facial features, postnatal growth deficiency, intellectual disability, skeletal anomalies and cardiac defects with variable expressivity. It is caused by heterozygous mutations in *KMT2D* or *KDM6A* genes. Pathogenic variants in the KDM6A gene are associated with Kabuki syndrome type 2.

Methods: We describe a case of a 15 year old female Albanian patient. She presented short stature, developmental delay, intellectual disability, hypotonia, facial manifestations like long palpebral fissures, short columella with depressed nasal tip, broad eyebrows, strabismus, fingertip pads, hypertrichosis, as well as complex partial seizures. The seizures were partially responsive to antiepileptic drugs. Motor and sensory nerve conduction were normal and peripheral neuropathies were not observed. Brain magnetic resonance imaging MRI showed extensive signal abnormality present in both periventricular regions and a pineal cyst.

Results: Chromosomal microarray analysis resulted normal. Whole exome sequencing performed on the DNA of the patient identified a heterozygous variant with unknown significance c.573G > C (Gln119His) in KDM6A gene. It is classified as variant of uncertain significance, but the phenotype showed compatibility with clinical features of Kabuki syndrome. Parental carrier testing was requested.

Conclusion: To our best knowledge, this is the first case report of Kabuki syndrome in Albania caused by a mutation in KDM6A. Our findings provide evidence on the possible pathogenicity of this variant, broadening the mutation spectrum of KDM6A gene in Kabuki type 2 syndrome.

References: Khodaeian M. Kabuki Syndrome: Identification of Two Novel Variants in *KMT2D* and *KDM6A*.Molecular syndromology, 2021; 12: 118-126.

Grants: No grants.

Conflict of Interest: None declared.

EP12.042 A homozygous nonsense variant in LAMA1 associated with Poretti-Boltshauser syndrome: a consanguineous case report

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Background/Objectives: A 6-year-old girl diagnosed with Arnold-Chiari malformation comes to our genetic consultation due to a repaired occipital encephalocele and multiple MRI abnormalities including periventricular and ependymary heterotopias, cerebellous cortical dysplasia and corpus callosum hypoplasia. Other symptoms are epilepsy, psychomotor delayed, gynecomastia, precocious puberty, ophthalmologic abnormalities (bilateral optic neuropathy, strabismus, and nystagmus) and several facial dysmorphisms (epicanthus, broad eyebrows and synophrydia. The patient was born of consanguineous parents at 38+5 gestation weeks by cesarean due to a prenatally detected neural tube defect.

Methods: Karyotype and CGH array were performed. Clinical exome was sequenced by Human Whole-Genome Sequencing with the Nextera™ DNA Flex Library Preparation Kit (Illumina).

Results: Karyotype and CGH array were normal. The analysis of the clinical exome showed a homozygous autosomal recessive (AR) nonsense variant c.842G>A (p.Trp281X) in *LAMA1* (NM_005559.4), classified according the ACMG guidelines as pathogenic.

Conclusion: *LAMA1* encodes the Laminin alpha-1 protein required for the basement membranes and thus is critical in early embryonic development (Cai 2021). Homozygous or compound heterozygous mutations in *LAMA1* are associated with Poretti-Boltshauser syndrome (PTBHS) (OMIM *#615960*), a rare AR disorder characterized by a non-progressive cerebellar ataxia (cerebellar dysplasia, cerebellar vermis hypoplasia), delayed motor development and speech delay. The cognitive function can range from normal to intellectually disabled. Additional ophthalmological phenotypes such as high myopia, variable retinal dystrophy, and eye movement abnormalities are also associated with this condition (Aldinger et al, 2014). This condition should not be confused with Joubert syndrome since it has a much more limited phenotype (Powell 2021).

References:

Grants:

Conflict of Interest: None declared.

EP12.043 The clinical case of inverted duplication and deletion of the short arm of chromosome 2: clinical manifestations, features of diagnosis verification

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Background/Objectives: Inverted 2p duplication and deletion is a rarely diagnosed chromosomal aberration. Literature data on the phenotype at inv dup del 2p continue to be supplemented. Modern molecular genetic methods make it possible to identify complex structural changes in chromosome 2 and verify the diagnosis in patients with delayed motor, speech and mental development.

Methods: Clinical-geniological, cytogenetic, molecular-genetic, instrumental.

Results: The clinical case of inverted duplication in a two-year-old girl. Developmental delay was observed from the first months of life. At the time of examination, the child is two years old - below average physical development, walks with support; vocabulary is severely limited, delayed psycho-linguistic development, impaired social communication; body structure is symmetrical, proportional; round head, hypertelorism of the eyes, thin upper lip, short bridle of the upper lip; nipple hypertelorism, thoracolumbar scoliosis, congenital bilateral clubfoot; inferior flaccid paraparesis, signs of disturbance of the vestibular apparatus, atactic syndrome, muscular dystonia; ECG – incomplete blockade of the right branch of the His bundle. Echocardiography – left-right shunt; slight regurgitation on the mitral and tricuspid valves, additional left ventricular chord. NSG – slight asymmetry of the lateral ventricles and dilation of the third ventricle.

Karyotyping result: 46,XX, dup(2)pter→q37.3::q37.3::q35→qter. ish dup(2)WCP2+: female karyotype with inverted duplication of segment 2q35-2q37; the orientation of the duplicated region is inverse with respect to pter and qter. Duplication was confirmed by FISH using a probe to whole chromosome 2 - WCP2.

Conclusion: To verify an inverted duplication on the short arm of chromosome 2, it is necessary to use modern molecular genetic methods.

References:

Grants:

Conflict of Interest: None declared.

EP12.044 Noonan Syndrome (NS)/Rasopathies in 136 NON-PTPN11 patients. Something learned from the "other" genes

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Background/Objectives: NS/rasopathies genotype provides key information related to hypertrophic cardiomyopathy (HCM) and oncogenic risk, factors of interest when using rGH (only indicated for NS).

To describe the genotype findings vs clinical suspicion that may alert about the use of genotypic data to define the indications/limitations of rGH-treatment.

Methods: 549 genotyped patients (486 index,63 affected relatives) from 65 Spanish hospitals (2005-2021). Customized panels (26-29 genes) Nextera libraries, capture-enrichment (IDT probes) on MiSeq platform Illumina(n = 191), Sanger for segregation(n = 80families) and stratified monogenic analysis (n = 294patients). Variant interpreter, SIFT, Polyphen-2, Mutation Taster in silico analysis, databases (ClinVar, NS Euronet, Ensemble,HGMD, gnomAD,TopMed,dbSNP).

Results: 315 PTPN11 and 159 non-PTPN11 patients, 136 with pathogenic alterations (ClinVar). Unexpected genotypic findings based on clinical suspicion: a)Suspected Costello (n = 19) HRAS in only 3,the rest: RAF1(3), RIT1(3), MAP2K1(3), PTPN11(2), SOS1(1) and 4 patients with pathogenic alterations in NS-like genes SHOC2(3) and PPP1CB(1); b)Suspected CFC (n = 48) BRAF(14), MAP2K1(6), MAP2K2(3); the rest PTPN11(2), SOS1(2), SHOC2(1) and PPPCB1(1). Eleven NS patients with BRAF alteration.

HCM was present in 55/147 patients with heart disease data in the preanalytical report. Primarily associated with RAF1(18) but also, RIT1(8), BRAF(8),LZTR1(7),SOS1(6), MAP2K1(2),KRAS(1) and HRAS(1).

Conclusion: NS genotypes (PTPN11, SOS1, RAF1, RIT1, SHOC2) are detected in patients whose symptoms had suggested non-Noonan rasopathy. Patients with Costello suspicion carrying the pathogenic SHOC2 alteration (documented rGH-good-response) are noteworthy.

Only RIT1, NF1 and LZTR1, have largely contributed to the diagnostic yield. The later description and recessive pattern of LZTR1 conditioned a higher number of VUS.

References:

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Conflict of Interest: None declared.

EP12.045 a novel stop codon mutation in Iranian patient with Peutz-Jeghers Syndrome

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Background/Objectives: Peutz-Jeghers syndrome (PJS) is a rare uncommon autosomal dominant disorder Genetic heterogeneity of disease has been recognized in over 500 mutation in STK11. Another novel mutation in STK11 stop codon is seen in a 42-year-old woman with mucocuta neous pigmentation and PJ-type hamartomas .

Methods: Genomic DNA was isolated from blood sample following standard protocols. All nine exons were amplified by PCR reactions through designed primers . Sequencing method is applied for mutation detection.

Results: The released data from the sequencing results showed five alteration in exons 1 to 5. The major mutation in stop codon is reported as converted to the amino acid TRP to TER; The TGG codon is converted to TAG by mutation.

Conclusion: In this study, mutation P239W>STOP C.716G>A is distinguished as the PJS novel alteration in EX5 which act as a stop codon in transcribed mRNA.

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Conflict of Interest: None declared.

EP12.047 Partial 3q tetrasomy: Defining the syndrome, neocentromeres, and an additional case report

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Background/Objectives: A 23-year-old male with pigmentary changes along the lines of Blaschko, multiple malformations, intellectual disability, hearing loss, kidney failure, and tethered cord was referred to the department for evaluation. The resultant genetic diagnosis prompted re-evaluation of the symptomatology associated with the identified genetic syndrome.

Methods: Trio exome sequencing, chromosomal micro-arrays (CMAs), and fluorescence in-situ hybridization (FISH) was used to establish the diagnosis. Literature searches were used to evaluate the associated symptomatology.

Results: Trio exome sequencing and a blood CMA yielded no explanation for the patient's symptoms. CMA on biopsies from dark and light skin, however, indicated mosaicism for an apparent partial 3q duplication. A subsequent karyotype was normal. However, FISH on hundreds of cells showed a few to carry an extra structurally abnormal chromosome (ESAC) consisting of two 3q26.1qter segments arranged head-to-head with neocentromere formation. This thus revealed the chromosomal aberration to instead be a partial 3q tetrasomy, with partial loss of the ESAC upon cell incubation. The aberration was found to be maternally derived and no uniparental disomy of chromosome 3 was seen. Systematically evaluating all aspect of published partial 3q tetrasomy cases revealed a syndrome of pigmentary changes, specific dysmorphology as well as specific developmental characteristics and a phenotypic overlap with the partial 3q trisomy syndrome.

Conclusion: Pigmentary changes along the lines of Blaschko should prompt consideration of conducting genetic testing on skin samples. Potential loss of ESACs upon incubation should be kept in mind. Further, our evaluation of partial 3q tetrasomy suggested specific points for clinical care.

References: None.

Grants: None.

Conflict of Interest: None declared.

EP12.049 HIDEA Syndrome: a new case report highlighting similarities with ROHHAD syndrome

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Background/Objectives: Patients with ROHHAD syndrome present significant resemblance to HIDEA syndrome. This entity is caused by bi-allelic loss of function variants in *P4HTM* gene and encompasses Hypotonia, Intellectual Disabilities and Eye Abnormalities, Hypoventilation and Dysautonomia. We report the first HIDEA syndrome patient identified from our ROHHAD cohort.

Methods: This 21 month-old girl had history of severe respiratory infections requiring intensive care, hypotonia, abnormal eye movements and rapid weight gain. A polysomnography identified a severe central hypoventilation. During her follow-up, a severe psychomotor delay and an absence of language were observed. Prolactinemia was initially increased. Hypothermia was reported at 4 years. Exome sequencing identified a new homozygous truncating *P4HTM* variant.

Results: Our patient encountered diagnosis criteria for ROHHAD including a rapid weight gain, central hypoventilation appearing after 1.5 years of age, hyperprolactinemia suggesting a hypothalamic dysfunction and an autonomic dysfunction manifesting as strabismus and hypothermia. However, she also presented with severe neurodevelopmental delay, which was not a classic feature of ROHHAD syndrome.

HIDEA Syndrome presents similarities with ROHHAD including Hypoventilation, Obesity, and Dysautonomia. No endocrinological data have been reported for HIDEA patients apart from the observation of advanced bone age. Better delineation of both syndromes is needed to investigate eventual involvement of *P4HTM*, a regulator of calcium dynamics and gliotransmission, in ROHHAD.

Conclusion: In case of clinical evidence of ROHHAD in a child with abnormal neurological development or eye abnormalities, we suggest that the *P4HTM* gene should be systematically interrogated in addition to the *PHOX2B* analysis.

References:

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Conflict of Interest: None declared.

EP12.050 De novo splice pathogenic variant in the ODC1 gene causing Bachmann-Bupp syndrome in a French patient

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Background/Objectives: We report an 11-year-old French patient who consulted for mild to moderate delayed psycho-motor acquisition, hypoplasia of the middle part of the corpus callosum and absence of eyelashes. She is the first child of a healthy unrelated couple. Diagnosis was obtained by WES.

Methods: The patient had a sequencing of a panel of Noonan-like syndrome genes which came back negative. She subsequently had a sequencing of an intellectual deficiency genes panel which also returned normal. A solo WES was therefore performed.

Results: Solo WES revealed a splice variant in the *ODC1* gene (NM_002539.3: c.1242-2A>G). This variant is absent in the population data base GnomAD. It has been reported in Clinvar as a pathogenic variant and most gene prediction algorithms classify this variant as damaging. Parental samples were tested and neither maternal nor paternal sample showed the ODC1 variant.

Conclusion: We identified in our patient an ODC1 splice de novo mutation causing Bachmann-Bupp syndrome that was first described in 2018. It is a neurometabolic disorder associated with global developmental delay, ectodermal abnormalities including alopecia, absolute or relative macrocephaly, dysmorphic features and characteristic neuroimaging features. Treatment is possible for patients with ODC1 gene gain of function variants as α-difluoromethylornithine (DFMO) is being tested on patients in the US. The first patient treated showed neurological improvement and significant hair growth.

References: Bupp CP et al. Novel de novo pathogenic variant in the ODC1 gene in a girl with developmental delay, alopecia, and dysmorphic features. Am J Med Genet Part A. 2018; 176A:2548–2553.

Grants:

Conflict of Interest: None declared.

EP12.051 Broadening the phenotypic spectrum of EVEN-PLUS syndrome through identification of HSPA9 pathogenic variants in the original EVE dysplasia family and two siblings with milder facial phenotype

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Background/Objectives: EVEN-PLUS syndrome is a rare autosomal recessive disorder caused by biallelic variants in *HSPA9*. It is characterized by the involvement of the **E**piphyses, **V**ertebrae, **E**ars and **N**ose, **PLUS** associated findings. So far, five individuals presenting with EVEN-PLUS and biallelic variants in *HSPA9* have been published, showing a very distinctive craniofacial phenotype with extremely underdeveloped nose and narrow and triangular nares. We describe two additional siblings showing the distinctive radiological findings of EVEN-PLUS, but milder facial features. We report biallelic variants in *HSPA9* in these individuals, and in the original EVE-dysplasia family. In addition, we review the clinical features of these cases and those described in the literature to refine the phenotype of this disorder, and provide in silico 3D modelling data to support the pathogenicity of the unreported variants.

Methods: WES (TruSeq Exome Kit, Illumina), Sanger sequencing, SWISS-MODEL software, Chymera Software.

Results: Compound heterozygous variants p.Ile124Thr and p.Arg126Trp were identified in both siblings, and a homozygous p.Thr362Ile variant was found in the original EVE-dysplasia family. In silico 3D modelling revealed that p.Ile124Thr and p.Thr362Ile changes alter canonical hydrogen bonding, supporting the impact of these unreported variants on HSPA9 function.

Conclusion: We show the phenotypic variability of EVEN-PLUS syndrome. We suggest that this diagnosis should be considered in individuals with bilateral microtia with or without associated findings such as anorectal anomalies and aplasia cutis. Radiological investigations help to confirm the diagnosis. Further examinations are warranted for early detection of additional anomalies.

References: PMID: 10424819; 26598328; 32869452.

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EP12.052 Identification of a unbalanced de novo complex chromosome rearrangement involving chromosomes 5 and 6: case report

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Background/Objectives: Complex chromosome rearrangements (CCRs) are rare events. Their clinical identification is important since CCR carriers can display various phenotypes. We report the CCR involving chromosomes 5 and 6 which is characterized using a combination of aCGH, G-banding and fluorescence in situ hybridization (FISH).

Methods: Two-year-old boy was referred because of aortic coarctation, perimembranous VSD, nasolacrimal duct narrowing and facial dysmorphia. Molecular karyotyping on proband and his parents was performed on the SurePrint G3 Human CGH 8x60k microarray (Agilent Technologies, Santa Clara, CA, USA). In order to precisely determine the breakpoints involved in the paracentric inversion, conventional karyotyping and FISH analysis were performed.

Results: aCGH revealed an 8,35 Mb deletion at the 5p14.1p13.2 region (5:27788557_36143306, hg19) containing 25 OMIM genes. In addition, an interstitial deletion of 1.9 Mb at the chromosomal region 5p12p11 (5:44208999_46114984, hg19), and terminal deletion of 3.49 Mb at chromosomal region 6q27 (6:165030335_168524228, hg19) were detected. Karyotypic analysis identified the boy as a carrier of a paracentric inversion of chromosome 5, with breakpoints at p14.1 and p15.31 chromosomal regions in all metaphases analysed. Parental microarray analysis and conventional karyotyping were normal.

Conclusion: Precise structural chromosome abnormality characterization is important due to the possibility of gene disruption and/or position effect. As CCRs are rare, it is difficult to fully predict the whole clinical presentation. Therefore, careful regular monitoring of the carrier is of the utmost importance.

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Grants:

Conflict of Interest: None declared.

EP12.053 DEGCAGS syndrome: a novel epigenetic Mendelian disorder?

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Background/Objectives: The DEGCAGS (**De**velopmental Delay with **G**astrointestinal, **Ca**rdiovascular, **G**enitourinary, and **S**keletal Abnormalities) syndrome is caused by biallelic *ZNF699* variants previously delineated in thirteen individuals[1]. ZNF699 is a large nuclear zinc finger protein, suggesting a role in nucleic acid binding and, in Drosophila melanogaster, is required for normal development of ethanol tolerance[2]. Our aim is to further delineate the geno-phenotypic spectrum of DEGCAGS and explore a possible methylation signature.

Methods: We report three new affected individuals with DEGCAGS syndrome and their immunology profile and review the literature for common clinical and molecular characteristics.

Results: We report two novel *ZNF699* variants: (NM_198535.3) c.421_424delGAGT, (p.Glu141Profs*15) and c.339del, p.(Cys113Trpfs11) both predicting non-mediated decay. The clinical presentation is characterized by distinctive facial dysmorphisms (94%) including a pattern of hypopigmentation that includes hair, eyelashes and eyebrows (31%); global developmental delay and intellectual disability (50%) and other malformations. Immunodeficiency with recurrent infections is frequently reported (44%), while in our 3 patients reduced B-lymphocytes ($n = 2$) and combined immunodeficiency in one patient and different changes in Ig-levels were noticed. The clinical characteristics of the individuals affected partially overlap with other disorders with a distinguishable episignature like chromatin remodeling disorders (microcephaly, feeding difficulties, hypertrichosis)[3].

Conclusion: A comprehensive study of all clinical features and immunological characteristics of our 3 new cases broadens the phenotypic spectrum of *ZNF699*-related DEGCAGS disorder to include reduced B-lymphocytes and combined immunodeficiency.

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Grants:

Conflict of Interest: None declared.

EP12.054 Further delineation of the phenotype of Noonan syndrome with loose anagen hair due to de novo missense variants in the PPP1CB gene

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Background/Objectives: Noonan syndrome (NS) is a genetically heterogeneous disorder, characterized by distinct craniofacial anomalies, postnatal growth retardation, congenital cardiac defects, variable neurodevelopmental abnormalities and ectodermal abnormalities. The recurrent missense change (c.4A>G, (p.Ser2Gly)) in *SHOC2* causes a clinically distinct subtype of NS, NS-like disorder with loose anagen hair (NSLH). In 2016, the first four unrelated patients with a NSLH phenotype and missense variants in *PPP1CB* were published. Developmental delay, relative or absolute macrocephaly, brain anomalies and slow growing, sparse hair and/or an unruly hair texture were the most consistent phenotypic findings. To date 22 patients with missense variants in *PPP1CB* have been described.

Methods: In this study, we describe a novel cohort of 23 patients with Noonan-like syndrome with loose anagen hair due to missense variants in PPP1CB, thereby refining the clinical spectrum of NSLH caused by variants in this gene. Second, we quantify and compare the facial syndromic similarities of patients with NSLH, due to PPP1CB or SHOC2 variants, to patients with NS due to pathogenic variants in PTPN11 using DeepGestalt analysis and GestaltMatcher.

Results:.

Conclusion: This study provides additional evidence that the phenotype in individuals carrying the recurrent c.146G>C (p.Pro49Arg) missense change in *PPP1CB* and possibly a few other variants can be classified as NSLH.

References:

Grants:

Conflict of Interest: None declared.

EP12.055 Case report - 1q44 microdeletion and two heterozygous variants of DOLK gene in a patient with multiple congenital anomalies

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Background/Objectives: 1q44 microdeletion syndrome is a rare condition associated with global developmental delay, hypotonia, seizures and craniofacial dysmorphism. Congenital disorders of glycosylation (CDGs) are a group of metabolic disorders caused by mutations in genes necessary for the addition of oligosaccharides to certain proteins or lipids. Congenital disorder of glycosylation type Im (DOLK-CDG) is a rare autosomal recessive CDG caused by mutations in DOLK gene that lead to a variable clinical spectrum.

Methods: We present the case of an 8 months old girl with facial dysmorphism, inverted nipples, hepatosplenomegaly, important axial hypotonia, dilation of bilateral ventricles, failure to thrive and convulsions. Because our patient presented a particular combination of clinical features, a metabolic disease was suspected, with possible additional genomic imbalances. Therefore, genetic testing using WES-CNV and subsequent aCGH was performed for the proband. Peripheral blood karyotyping and FISH were used for testing of proband and her parents.

Results: The WES-CNV and aCGH detected a 1734 kb microdeletion the 1q44 region that includes COX20,HNRNPU,SNORA100,LOC101928068,EFCAB2,KIF26B,SMYD3 genes. Furthermore, two class 3 VUSs (c.143C>T and c.1318C>A) were detected in DOLK gene. The karyotype and targeted FISH analysis for chromosome 1 showed normal results.

Conclusion: The combined use of WES-CNV analysis for testing of complex syndromes is improving our understanding of the pathogenesis of complex diseases. Our case highlights the role of combined molecular genetic testing in the process of differential diagnosis of multiple abnormalities syndromes and the important implications of this approach for genetic counselling and monitoring for a large number of potential health conditions.

References:

Grants:

Conflict of Interest: None declared.

EP12.056 Unexpected sequencing results in critically affected newborns with extremely severe expression of genetic syndromes

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Department of Medical Genetics, Warsaw, Poland; [6]National Institute of Tuberculosis and Lung Diseases, Department of Genetics and Clinical Immunology, Warsaw, Poland; [7]Wroclaw Medical University, Department of Pediatrics and Rare Disorders, Wroclaw, Poland.

Background/Objectives: Genetic disorders are one of the leading reasons of hospitalization in neonatal intensive care units and neonatal mortality. Apart from new genetic diseases caused by mutations in unknown genes, the reason of severe illness in newborns can be also know genetic disorders with unexpectedly severe and unknown thus far phenotype.

Methods: We present clinical evaluation of 11 critically affected newborns suspected of genetic disorder. The molecular analysis has been performed with exome (8 cases) or targeted (2) next generation sequencing.

Results: All patients were hospitalized in neonatal intensive care units, as they were born prematurely and the majority as dystrophic newborns. All but one had congenital defects. Three patients out of 7 that died soon after birth, had known genetic syndromes. Molecular analysis revealed the presence of pathogenic variants in *DDX11*, *KIF3B*, *GLDN*, *NIPBL*, *KMT2A, SCN1A*, *RIT1*, *RAF1*, *PITX2*, *CHD7*, *TP63* or *NAA10* gene.

Conclusion: The interpretation of NGS results for critically affected newborns is a challenge. There is a need for close cooperation between clinical and molecular geneticists. Not only new disorders should be considered, but also known genetic syndromes with extremely severe phenotype should be suspected. Such severe phenotypes has not been described before, as many of these newborn die soon after birth without genetic diagnosis. Exome or targeted sequencing in the critically affected newborns has a great diagnostic rate and impact not only on medical management, but also on genetic counselling for the family.

References:

Grants: The studies were supported from Institute of Mother and Child intramural grant no. OPK-510-18-41.

Conflict of Interest: None declared.

EP12.057 Outcomes in growth hormone (GH)-treated Noonan syndrome (NS) children: impact of PTPN11 mutation status

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Background/Objectives: Mutations in PTPN11 account for ~50% of NS cases. We assessed the impact of PTPN11 mutation status on growth outcomes in children with NS receiving GH therapy (GHT) with Norditropin® (somatropin, Novo Nordisk).

Methods: Pooled data from previously GHT-naïve, prepubertal children with NS receiving GHT for 4 years in the ANSWER Program (NCT01009905; non-interventional, multicentre study in USA) and GHLIQUID-4020 (NCT01927861; 4-year, randomised, double-blind, multicentre trial in Japan) were analysed.

Results: Sixty-nine patients were included (PTPN11-positive, n = 49; PTPN11-negative, n = 20). Mutations in PTPN11-negative patients included RAF1 (n = 3), SOS1 (n = 2), BRAF (n = 1), KRAS (n = 1), RIT1 (n = 1) and SHOC2 (n = 1). Cardiovascular comorbidities were present in 55% at baseline, including atrial septal defect (n = 16[23.2%]); pulmonary valve stenosis (n = 9[13.0%]); pulmonary artery stenosis (n = 7[10.1%]); hypertrophic cardiomyopathy (n = 7[10.1%]). Baseline characteristics were generally similar between groups. Mean(SD) age at GHT start was 6.4(2.5) years in both groups; GH dose was 0.047(0.015) mg/kg/day in PTPN11-positive and 0.054(0.016) mg/kg/day in PTPN11-negative patients. Table shows change from baseline in height SD score; no significant differences between PTPN11 groups were observed.

Conclusion: Four years of GHT resulted in improved growth outcomes in GHT-naïve, prepubertal children with NS, irrespective of PTPN11 status..

Table: Change in height SD score

		PTPN11-positive		PTPN11-negative	
Height SD score change from baseline	**Year**	**n**	**Mean(SD)**	**n**	**Mean(SD)**
General population	1	41	0.742(0.364)	19	0.755(0.407)
	2	38	1.086(0.531)	19	1.201(0.562)
	3	32	1.272(0.685)	19	1.399(0.624)
	4	30	1.258(0.839)	19	1.488(0.743)
NS population	1	40	0.719(0.352)	19	0.711(0.363)
	2	38	1.058(0.565	19	1.161(0.4920
	3	32	1.292(0.695)	19	1.412(0.550)
	4	30	1.370(0.759)	19	1.522(0.651)

References:

Grants:

Conflict of Interest: Alexander Jorge Principal Investigator of independent research supported by BioMarin, Consultant to Novo Nordisk, Alberto Pietropoli Employee of Novo Nordisk, Nicky Kelepouris Employee of Novo Nordisk Inc, Stock/stock options in Pfizer and Novo Nordisk, Reiko Horikawa Research grants from Sandoz and JCR, Consultant/Advisory Board for Novo Nordisk, Pfizer/Opko, Ascendis, Lumos Pharma.

EP12.058 Eighth patient with Li-Campeau sydrome and expansion of the phenotype

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Background/Objectives: Li-Campeau syndrome (LICAS) is an autosomal recessive neurodevelopmental disorder characterized by global developmental delay with intellectual disability, dysmorphic facial features, hypothyroidism, and abnormalities of the cardiac and genital systems. Seizures, short stature, hypotonia, and brain imaging anomalies can also be seen (1).

To the best of our knowledge our case is the eighth patient with LICAS and expands the phenotype with a novel mutation.

Methods: Whole exome sequencing revealed the c.1185+1G>C variant as homozygous in *UBR7* according to the NM_175748.4 transcript. To the best of our knowledge this variant is novel. Parents were both heterozygous. The identified genetic variation was not found in any databases, located in a highly conserved residue and bioinformatic analysis showed this alteration to be pathogenic and disease causing.

Results: We report a 32month old male with intellectual deficiency and developmental delay, congenital hypotyroidism,

hypotonia, epilepsy, patent ductus arteriosus, exotropia, camptodactyly on both hands and cryptorchidism. Camptodactyly was not reported before. This case further confirms the association of *UBR7* mutations with LICAS and expands the phenotype.

Conclusion: *UBR7* gene expression is reported in human embryonic stem cells and early in embryonic development in *C.elegans* (1), thereby affects multiple parts of the body. We report a patient with LICAS who has camptodactyly that is not reported previously. PRG4 is associated with camptodactyly, but its coexpression with *UBR7* is not reported. *UBR7* gene expression may be a new pathway for hand development and future studies are needed.

References: Li et al. Am. J. Hum. Genet. 108: 134-147, 2021.

Grants: None.

Conflict of Interest: None declared.

EP12.059 Three cell line mosaicism involving numerical and structural abnormalities of chromosome 9 in a newborn girl

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Background/Objectives: Occurrence of three clones mosaicism with chromosomal abnormalities of an autosomal chromosome in the same individual is a rare condition. We report on a newborn girl with ventriculomegaly, secundum atrial septal defect, a small ventricular septal defect, tricuspid valve regurgitation, persistent ductus arteriosus, ovarian cysts and dysmorphic features: asymmetrical palpebral fissures, hypertelorism, dysmorphic ears, high palate and excessive hair growth on her back.

The girl has mosaic karyotype including full trisomy 9, normal cells and in trisomy of 9p.

Methods: aCGH analysis using 4x180K array, karyotyping, FISH analysis with DNA probes along the chromosome 9 and QF-PCR sing chromosome 9 specific microsatellite markers, were performed.

Results: Array CGH revealed a gain of the whole short arm of chromosome 9 in approximately 80% of cells and a gain of the whole long arm of chromosome 9 in approximately 20% of cells: arr(9p)x3[0.8],(9q)x3[0.2]. Metaphase analysis demonstrated mosaic karyotype: 47,XX,+der(9)del(9)(q13)[65]/46,XX[35]. FISH and QF-PCR confirmed/identified the third cell line with trisomy of chromosome 9. The karyotype was re-written: 47,XX,+der(9)del(9)(q13)[60]/47,XX,+9[20]/46,XX[20].

Conclusions: Our data demonstrate that the presented girl is a mosaic with three cell lines. Trisomy 9p dominated in 60%, trisomy 9 and normal cell line were observed in approximately 20% each. The exact contribution of each chromosomal abnormality on a girl's phenotype could not be determined because there are similarities between individuals with trisomy 9 mosaicism and those with duplications of 9p. Although aCGH should be the first-tier test for clinical diagnosis of chromosome abnormalities detection of mosaicism requires a multistep diagnosis approach.

Conflict of Interest: None declared.

EP12.060 Partial deletion of GNAO1, supporting haploinsufficiency of this gene

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Background/Objectives: Pathogenic missense variants in *GNAO1* are associated with a spectrum of phenotypes of early onset developmental and epileptic encephalopathy (DEE), developmental delay and movement disorders. *GNAO1* has also been suggested to be haploinsufficient, and in line with this, it has been suggested to be a very likely candidate gene for the phenotype of the 16q12.1q21 deletion syndrome. The most frequent features of this syndrome are intellectual disability, epilepsy, short stature, microcephaly, eye abnormalities, developmental delay, autism spectrum disorder and dysmorphisms.

Methods: Samples were investigated for copy number variations by chromosomal microarray analysis using the CytoScan HD array (TheromoFisher Scientific).

Results: We describe a mother and her adult daughter, who both had learning disability, epileptic seizures in late childhood, reduced force over the thumbs, and urinary incontinence. Furthermore the daughter additionally experience problems of balance and the mother experiences involuntary movements at the age of 50.

A 154 kb interstitial microdeletion in 16q12.2 including exons 3-9 of *GNAO1* as well as exons 5-14 of *AMFR* was identified in both. The deletion was de novo in the mother.

Conclusion: We report the smallest deletion encompassing *GNAO1* to date in a mother and daughter. The patients have a phenotype which overlaps with the phenotype of the 16q12.1q21 deletion syndrome. This supports that haploinsufficiency of the *GNAO1* most likely explains at least some of the features of the 16q12.1q21 deletion syndrome.

References:

Grants:

Conflict of Interest: None declared.

EP12.062 WES (Whole Exome Sequencing) diagnostic revealed the absence of SRY gene in a patient with a 47,XXY karyotype and a female phenotype

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Background/Objectives: 9 years old girl presenting with global development delay, short stature and behaviour disorder was referred by the physician to perform WES analysis. Previous CGH-array showed a normal female result, nevertheless G banding revealed a 47,XXY karyotype. 47,XXY is associated with Klinefelter Syndrome (KS), which presents with a male phenotype.

Additionally, the *SRY* gene, located in the Y chromosome, is involved in male-typical sex development initiating the cascade of steps necessary to form a testis from the undifferentiated gonad.

Methods: WES was 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: No clinical or relevant variants were found through the exhaustive clinical exome analysis. Nevertheless, during one the checkpoints for analysis procedure, absence of the *SRY* gene was detected.

Conclusion: KS occurs in one of every 600 newborn males. The most widespread karyotype individuals with a KS (47,XXY) usually present with a male phenotype due to the additional Y chromosome. As far as our knowledge goes, only a few cases of female phenotype with 47,XXY have been reported. This patient presents an abnormal genetic condition due to two events: a sexual chromosome trisomy plus *SRY* deletion. It is an example of the different phenotypical ranges found in the KS.

References:

Grants:

Conflict of Interest: None declared.

EP12.063 The phenotype of Floating-Harbor syndrome: clinical characterisation of new individuals of Polish origin

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Background/Objectives: Floating-Harbor syndrome (FHS; MIM#136140) is an extremely rare genetic disorder caused by mutations in the SRCAP gene, characterised by a distinctive facial appearance, various skeletal malformations, delayed bone age, expressive and receptive language delays, and mild to moderate intellectual disability. FHS is passed on in an autosomal dominant manner. The diagnosis is established by identification of a heterozygous SRCAP pathogenic variant.

Methods: We report five unrelated individuals of Polish origin presenting features of FHS. Targeted Sanger sequencing for a hot spot mutation in exon 34 and Next Generation Sequencing (NGS) covering the entire coding SRCAP gene sequences was performed in two and three probands, respectively.

Results: We have identified two common, pathogenic variants c.7303C>T (two patients) and c.7330C>T (one patient), both located in exon 34 of SRCAP gene, and associated with typical FHS features. Moreover, in two other patients variants of unknown significance have been identified in exon 12 (c.1525G>A) and intron 14 (c.2130+6A>T) of SRCAP gene.

Conclusion: Our study defines the phenotypic spectrum observed in patients with variants of unknown pathogenicity identified in exon 12 (c.1525G> A) and intron 14 (c.2130 + 6A> T) of SRCAP gene in relation to the phenotype of patients with common mutations in exon 34 of this gene. Clinical description and molecular findings of the patients expand the knowledge of FHS variability.

References:

Grants:

Conflict of Interest: None declared.

EP12.064 Femoral Hypoplasia Unusual Facies Syndrome as a diabetic mother's baby

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Background/Objectives: Femoral Hypoplasia with Unusual Facies syndrome is an infrequent condition of unenlightened genetic etiology. Bilaterally short hypoplastic femora being the most frequently observed feature, patients present characteristic facial abnormalities and variable spinal, humeral and pelvic involvement. Maternal diabetes, drug exposure, viral infections, radiation and oligohydramnios have been associated with this syndrome. Here we present this very rare syndrome with clinical and radiological findings.

Methods: We presented a 6 years old girl born at 35 weeks of gestation from 29-year-old mother with uncontrolled diabetes. There was no parental consanguinity. Physical examination showed; microretrognathia, high palate, short nose, long philtrum, thin upper lip, frontal bossing, left preauricular pit, genital hypoplasia, scar of cleft palate operation. Skeletal findings were right femur deformation, right knee contracture, pes equinovarus, right elbow contracture and bilateral hip dysplasia. The body height was measured below 3th percentile. Her development was globally delayed. X-ray images revealed femoral hypoplasia, dysplastic acetabular roof, right humero-radial synostosis. Brain magnetic resonance imaging revealed partial fusion of C5-C6 vertebrea.

Results: The karyotype analysis, subtelomeric FISH and whole-exome sequencing analysis were reported as normal, concordant with the diseases nature.

Conclusion: Femoral hypoplasia with unusual facies syndrome is very rare disease. The genetic cause of this syndrome is still unknown. It is important to consider the syndrome in a patient with bilateral hip dislocation, facial and radiological findings, particularly if the mother has diabetes or oligohydramnios. In this syndrome prenatal diagnosis is also important, especially in infants of diabetic mothers.

References: https://doi.org/10.1002/ajmg.a.40425, https://doi.org/10.1016/j.rchipe.2015.08.005.

Grants: None.

Conflict of Interest: None declared.

EP12.065 Delineation of the clinical profile of CNOT2-related disorder and review of the phenotype of 12q15 microdeletion syndrome

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Neonatology, Papeete, French Polynesia; [16]Institut Pasteur, Human Genetics and Cognitive Functions, Paris, France; [17]Robert Debré Hospital, Genetics Department, Cytogenetic Unit, Paris, France.

Background/Objectives: CNOT2-related disorder (IDNADFS, OMIM*618608) is a rare neurodevelopment condition characterized by developmental delay, intellectual disability, nasal speech, craniofacial dysmorphism and variable skeletal anomalies. Due to its clinical overlap with chromosome 12q15 microdeletion syndrome, *CNOT2* haploinsufficiency is believed to be a major contributing event of the latter. CNOT2 is a member of the CCR4-NOT complex, which is a master regulator of diverse cellular processes, such as gene expression, RNA deadenylation, and protein ubiquitination during development. To date, less than 20 pathogenic 12q15 microdeletions encompassing *CNOT2* and a truncating gene variant have been reported. Due to the small number of affected subjects described so far, the clinical profile of IDNADFS has not fully delineated yet, and its relationship with the chromosome 12q15 microdeletion syndrome is still debated.

Methods: Six additional subjects carrying *de novo* missense and non-sense *CNOT2* variants, an intragenic deletion, and a 12q15 microdeletion encompassing the gene were enrolled in the study. A systematic assessment of phenotypes of the affected individuals from the present and previously reported cohorts was also carried out.

Results: With the present data, we expand the series of subjects with *CNOT2* haploinsufficiency and, by comparing the clinical features of individuals with 12q15 microdeletion, we delineate better the clinical phenotype characterizing the disorder.

Conclusion: The critical gene for 12q15 microdeletion syndrome is confirmed to be *CNOT2*, by which its haploinsufficiency can be considered the cause of a unique nosological entity, termed as *CNOT2*-associated disorder.

References:

Grants: Italian Ministry of Health (5x1000 2020 and 2021).

Conflict of Interest: None declared.

EP12.067 A novel mutation in a patient with KIDAR syndrome: tenth patient in the literature

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Background/Objectives: Autosomal recessive, keratitis-ichthyosis-deafness syndrome (KIDAR #242150) is an extremely rare phenotype caused by biallelic mutations of adaptor-related protein complex1, beta-1 subunit (*AP1B1*) gene. Failure to thrive, global developmental delay, photophobia, and low plasma ceruloplasmin and copper levels could be accompanied to keratitis, ichthyosis, and sensorineural hearing loss. Due to few patients reported with KIDAR syndrome in the literature, we present this case to contribute clinical and genetic characterization of KIDAR syndrome.

The proband was a 13-year-old boy, born to a consanguineous parents. He had generalized ichthyosis and palmoplantar keratoderma, scarring alopecia, dysplastic alopecia, and photophobia. He also had dental caries, dysplastic nails, sensorineural hearing loss, and intellectual disability. His medical history is remarkable for failure to thrive and developmental delay.

Methods: Whole-exome sequencing analysis was performed using DNA derived from peripheral blood.

Results: A novel homozygous splicing mutation (NM_001127.4: c.1796+1G>T) was detected in *AP1B1* in whole-exome analysis.

Conclusion: Biallelic mutations in the *AP1B1* gene is responsible for the KIDAR syndrome. *AP1B1* gene encodes beta subunit of the adaptor protein1 complex, which has a role in vesicular transport in eukaryotic cells. Although the role of deafness could be explained by the function of AP1B1, epidermal consequences still elusive. Functional studies are needed to contribute pathophysiology of the KIDAR syndrome.

References: Faghihi F, et al. Phenotypic spectrum of autosomal recessive Keratitis-Ichthyosis-Deafness Syndrome (KIDAR) due to mutations in AP1B1. Eur J Med Genet. 2022.

Grants:

Conflict of Interest: None declared.

EP12.068 PERCHING syndrome: extremely rare and complicate clinical diagnosis

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Background/Objectives: PERCHING syndrome is a recently described multisystem disorder caused by homozygous mutation in the *KLHL7* gene and characterized by clinical presentation overlapping both Crisponi and Bohring-Opitz syndromes.

Methods: A detailed clinical evaluation and family history has been collected. High throughput genetics analyses have been carried out: QF-PCR to exclude Edward's syndrome, SNP-array for CNVs and ROHs identification, WES analysis.

Results: We report an 8-month-old boy presenting with failure to thrive affected by PERCHING syndrome. Review of literature and comparison to previously described patients have been performed. In his medical history: inbred parents from a remote village of Macedonia, referred uncomplicated pregnancy, born at term through CS with auxological parameters at 5th centile, neonatal hospitalization for respiratory distress. The examination revealed: weight -6DS, length -5.1DS and OFC -4.3DS. He had dystrophic appearance, low-set and prominent ears, abnormal posture (internal rotation of shoulders with contractures at elbow and clasped hands), camptodactyly and rocker-bottom feet with overlapping toes. Moreover, he had respiratory failure, developmental delay, partial agenesis of the corpus callosum, peripheral hypertonia, atrial septal defect and unilateral inguinoscrotal hernia. QF-PCR excluded Edward's syndrome. SNP-array analysis revealed a ROH at 7p15.3 including *KLHL7* gene corroborating the clinical hypothesis of PERCHING syndrome. The diagnosis has been confirmed through WES analysis revealing the known c.1051C>T p.(Arg351*) homozygous mutation in *KLHL7* (NM_001031710.3).

Conclusion: Although PERCHING syndrome is a rare disorder, it should be taken into account in the differential diagnosis of an infant with BOS-like posture, feeding difficulties, developmental delay and consanguineous parents.

References: PMID: 27392078; 30300710.

Grants: NA.

Conflict of Interest: None declared.

EP12.069 Delineating the expanding phenotype of HERC2-related disorders: The impact of biallelic loss of function versus missense variation

Krista Vincent[1], *Alison Eaton*[2], *Vahid Yassaee*[3], *Mohammad Miryounesi*[3], *Farzad Hashemi-Gorji*[3], *Lauren Rudichuk*[2], *Helly Goez*[2], *Norma Leonard*[2], *Joanna Lazier*[1;2]

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Background/Objectives: HECT And RLD Domain-Containing E3 Ubiquitin Protein Ligase 2, or HERC2, codes an ubiquitin ligase that has an important role in key cellular processes including cell cycle regulation, DNA repair, mitochondrial functions, and spindle formation during mitosis. While HERC2 Neurodevelopmental Disorder in Old Order Amish is a well characterized human disorder involving HERC2, bi-allelic HERC2 loss of function has only been described in three families and results in a more severe neurodevelopmental disorder.

Methods: Herein, we delineate the HERC2 loss of function phenotype by describing three previously unreported patients, and by summarizing the molecular and phenotypic information of all known HERC2 missense variants and biallelic loss of function patients.

Results: Collectively, these twelve individuals present with recurring features that define a syndrome with varying combinations of severe neurodevelopmental delay, structural brain anomalies, seizures, hypotonia, feeding difficulties, hearing and vision issues, and renal anomalies.

Conclusion: This study describes a distinct neurodevelopmental disorder, emphasizing the importance of further characterization of HERC2-related disorders, as well as highlighting the importance of ongoing work into understanding these critical neurodevelopmental pathways.

References: Vincent KM, Eaton A, Yassaee VR, Miryounesi M, Hashemi-Gorji F, Rudichuk L, Goez H, Leonard N, Lazier J. Delineating the expanding phenotype of HERC2-related disorders: The impact of biallelic loss of function versus missense variation. Clin Genet. 2021 Nov;100(5):637-640. https://doi.org/10.1111/cge.14039. Epub 2021 Aug 9. PMID: 34370298.

Grants: Nil.

Conflict of Interest: None declared.

EP12.070 Investigation of copy number variations as possible genetic modifiers in patients with the 22q11.2 deletion syndrome

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Background/Objectives: The 22q11.2 deletion syndrome (22q11.2DS) has a variable phenotypic expression, suggesting the involvement of complex mechanisms such as genetic modifiers. Copy number variations (CNVs) mapped in several genomic regions have been implicated as genetic modifiers. Therefore, evaluating new 22q11.2DS cohorts could indicate new candidates. We aimed to evaluate the inheritance of CNVs and their contribution to phenotype expressivity in duos and trios.

Methods: Eighteen individuals with 22q11.2DS and available genetic material from at least one parent were included. SNP-array was performed using Axiom Precision Medicine Diversity Array (PMDA). CNVs were called using PennCNV and submitted to quality control, one trio was excluded. Similar CNVs were grouped into CNV regions (CNVRs), which were annotated regarding their inheritance, pathogenicity, frequency in the population, and gene content.

Results: In 11 duos and 6 trios, 257 CNVs outside the 22q11.2 region were identified. The *SPTA1*, *SCAPER*, *KANSL1*, and *SMUF1* genes, annotated in different CNVRs, were correlated to patients' phenotypes. *KANSL1* gene has already been identified as a cardiac genetic modifier in the 22q11.2DS. CNVRs containing *SPTA1*, *SCAPER*, and *KANSL1* genes were inherited from healthy parents; *KANSL1* was also annotated in CNVRs *de novo* and with undefined inheritance. The CNVR containing the *SMUF1* gene was inherited from a mother who has 22q11.2DS.

Conclusion: Here we suggest new genes mapped in CNVRs as potential modifiers for the 22q11.2DS. Investigations regarding possible interactions between these genes and the genetic content in the 22q11.2 region may help elucidate their role in phenotypic expressivity.

References:

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Conflict of Interest: None declared.

EP12.071 Genetic studies of pediatric patients with brain malformations – the experience of a Romanian multicentric team

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Background/Objectives: Brain malformations (BMs) represent a major cause of epilepsy and developmental delay / intellectual disability (DD/ID). A wide spectrum of genetic anomalies have been reported. We report on the results of a study focused on diagnosis of genetic etiology of BMs in a Romanian pediatric population.

Methods: 38 patients with BM were selected from the patients referred for DD/ID and/or epileptic seizures. Phenotypic evaluation included a general clinical exam, neurologic, psychiatric, and psychological evaluations. Genetic investigations included classical karyotyping, fluorescence in situ hybridization, chromosomal microarray, and classical and next generation sequencing – whole exome (WES).

Results: Our patients had complex clinical presentations associated with different BMs types. Genomic imbalances were detected in 10 patients, some of them involving syndromic regions - 1q21.1, 1q43q44, 22q11.2. A pathogenic mutation of *DCX* gene was identified in a girl with double cortex and epilepsy; another patient with a complex phenotype has a pathogenic mutation in *PIK3CA* gene and a compound heterozygous mutation in *VPS13D* gene.

Conclusion: BMs are phenotypically and genetically heterogeneous disorders. Our findings might contribute to the delineation of the phenotypic spectrum of rare genetic defects and further highlights the value of genetic studies in the diagnosis algorithm of BMs.

References:

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Conflict of Interest: None declared.

EP12.072 Targeted next-generation sequencing in Bulgarian patients with RASopathies

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Background/Objectives: RASopathies are a clinically defined group of medical genetic syndromes with overlapping phenotypic features caused by germline mutations in genes that encode components or regulators of the Ras/mitogen-activated protein kinase pathway. These disorders include neurofibromatosis type 1 (NF1), Noonan, Leopard, capillary malformation-arteriovenous malformation, Costello, cardio-facio-cutaneous, and Legius syndromes. Therefore, molecular diagnosis is important for genetic counseling and treatment. The aim of the present study was to identify the genetic diagnosis in a group of Bulgarian patients affected by different types of RASopathies.

Methods: In a selected group of 18 patients diagnosed with different forms of RASopathies, we performed targeted sequencing of clinical exome (including 4813 OMIM genes) on MiSeq platform of Illumina. A detailed analysis, followed by Sanger sequencing and segregation analysis when possible, was used to identify pathogenic variants.

Results: Disease-causing mutations were identified in 10 out of 18 patients (55.55%). We found one pathogenic and two variants of unknown significance (VUS) in *NF1* in 3 out of 7 patients with differential diagnosis NF1. Pathogenic variants in the genes *BRAF*, *RAF1* (in two patients), *SOS1* and *PTPN11*, were found in 5/6 patients with suspected Noonan syndrome. In the remaining 4 patients with malformation syndrome but not clinically diagnosed we found pathogenic variants affecting the genes *NF1*, *BRAF* and *PTPN11* and VUS in *KRAS*.

Conclusion: Targeted sequencing of clinical exome allowed detection of disease-causing mutations in over 55% of our cases, a percentage which exceeds previously reported diagnostic yield of 19-36%.

References: none.

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Conflict of Interest: None declared.

EP12.075 Detection rate of 22q11.2 microdeletion using strict diagnostic criteria

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Background/Objectives: 22q11.2 microdeletion, detected in patients with 22q11.2 Deletion Syndrome (22q11.2DS), is the most common microdeletion syndrome in humans. 22q11.2DS has high risk for neurodevelopmental disorders and is associated with more than 180 malformations. Many investigations of the 22q11.2 microdeletion applying different recruitment criteria, revealed detection rate ranging from zero to 34.7%. Here we analyzed the frequency of 22q11.2 microdeletion among children having at least two out of five major characteristics of 22q11.2DS: congenital heart malformations (CHM), facial dysmorphism, immunological problems, palatal clefts and hypocalcemia.

Methods: Children with clinical characteristics of 22q11.2DS were analyzed. Fluorescence in situ hybridization and multiplex ligation-dependent probe amplification analysis were applied for detection of 22q11.2 microdeletion.

Results: 22q11.2 microdeletion was detected in approximately 40% of children. CHM was found in all patients with 22q11.2 microdeletion. Dysmorphic facial features were present in about 45%, immunological problems in 30%, overt cleft palate in about 4% and hypocalcemia in approximately 60% of patients with 22q11.2 microdeletion.

Conclusion: When at least two major features of 22q11.2DS are taking into consideration higher detection rate is obtained compared to one-feature criterion. These criteria could be considered by centers in low-income countries.

References: /.

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Conflict of Interest: None declared.

EP12.076 Early-onset atypical rare disorders: Precision genetic diagnosis aided phenotypic expansion to the rescue!

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Background/Objectives: Rare diseases frequently present with overlapping clinical features and ambiguous genetic findings can affect accurate diagnosis and thereby the clinical management. Here we report two paediatric cases who presented with failure to thrive in addition to other discernible indications. The first child is a two-year-old girl with a hypopigmented skin patch, chronic napkin rash, grey hair, hepatomegaly, liver failure, and coagulopathy. The second child is a six-month-old girl with hypotonia, recurrent fever, seizures, normal brain MRI, and developmental delay.

Methods: Whole-exome sequencing (WES) analysis was done for the index patients using Illumina platform at Igenomix laboratory, Dubai.

Results: WES revealed a homozygous pathogenic variant in the *CFTR* gene [NM_000492.4:c.580-1G>T; p.(?)] in the 2-year-old child; and a novel homozygous pathogenic variant in the *NTRK1* gene [NM_001012331.2:c.1624delG; p.(Glu542fs)] in the infant. Bi-allelic pathogenic *CFTR* and *NTRK1* gene variations cause cystic fibrosis and congenital insensitivity to pain with anhidrosis (CIPA), respectively.

Conclusion: Hair depigmentation and dermatitis are very rare presentations of cystic fibrosis in early childhood and a retrospective chloride sweat test confirmed that the initial clinical features observed in the first patient fits the atypical presentation of cystic fibrosis.

In the infant, a retrospective phenotypic evaluation revealed loss of pain sensation which confirmed the diagnosis of NTRK1-

CIPA. Recurrent episodic fevers, usually the first clinical sign of NTRK1-CIPA.

Our findings extend the prospect of deep-seated genotype-phenotype correlation utilizing WES in early diagnosis of complex rare genetic disorders, and in turn aid in efficient disease management. Parental and further segregation studies are on-going.

References:

Grants:

Conflict of Interest: None declared.

EP12.077 Kaufman oculocerebrofacial syndrome revisited in view of a new case and novel UBE3B variant

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Background/Objectives: Kaufman Oculocerebrofacial Syndrome (KOS, Blepharophimosis-Ptosis-Intellectual Disability Syndrome(BPIDS), MIM#244450) is an ultra-rare autosomal recessively inherited condition characterized by severe intellectual disability and distinctive craniofacial features, blepharophimosis, ptosis, upslanted palpebral fissures, dysplastic ears with preauricular tags. KOS is caused by biallelic pathogenic variations in *UBE3B* (Ubiquitin protein ligase E3B). Thirty-three patients have been reported with 36 distinct variations in HGMD Professional.

Methods: We here report on a patient first consulted at 10 weeks due to dysmorphic features, laryngomalacia, severe feeding difficulties and history of intensive care hospitalization for 37 days at newborn period. Growth parameters were 53 cm (-2.08 SDS;length), 3720 g (-2.33 SDS;weight), 36.5 cm (-2.28 SDS;OFC). Blepharophimosis, upslanted palpebral fissures, marked telecanthus with epicanthal folds, left epibulbar dermoid, broad/depressed nasal bridge, high palate, micrognathia, overfolded helices, preauricular skin tags, single palmar creases were observed. She had mildly underdeveloped external genitalia, and peripheral hypertonicity. Systemic examination was otherwise normal. Cranial MRI, cardiac ECHO and renal ultrasound showed no abnormalities. At 9 months of age, failure to thrive was persisting with frequent episodes of vomiting, height being 64 cm (-2.66 SDS), weight 5620 g (-3.71 SDS), OFC 41 cm (-2.69 SDS).

Results: Preliminary clinical diagnosis was KOS. WES analysis revealed a novel homozygous likely pathogenic variant in *UBE3B*; c.1261C>T [p.(Gln421X)], and a heterozygous pathogenic variant in *CLCN1*; c.2680C>T [p.(Arg894X)], previously associated with autosomal recessive congenital myotonia.

Conclusion: Neuromuscular specialist consultation, EMG and segregation testing of the parents are pending. We will discuss the findings of new KOS patient in view of all the reported cases.

References:

Grants:

Conflict of Interest: None declared.

EP12.078 Case of Au-Kline syndrome diagnosed during neonatal age with a new missense mutation in HNRNPK gene and history of considerable prenatal findings

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Background/Objectives: Au-Kline syndrome is a multiple malformation syndrome, reported in 2015, characterized by developmental delay, hypotonia, variable intellectual disability, typical facial features, frequently autonomic dysfunction, congenital heart disease, hydronephrosis, palate abnormalities, and oligodontia. Additional complications include craniosynostosis, feeding difficulty, vision issues, osteopenia.

Methods: We report a male newborn that was diagnosed in our clinic during the neonatal period with Au-Kline syndrome. He is the first baby of healthy non-consanguineous parents. During the prenatal period augmented nuchal translucency at 13+4/40weeks and polyhydramnios 32+2/40weeks originated amniocentesis where genetic work up performed (karyotype, molecular karyotype, QF-PCR for chromosomes 13,18,21, X,Y) was normal. At birth, he presented with global hypotonia, excess nuchal skin, wide nasal bridge, overlapping toes, deep plantar creases, patent foramen ovale, tiny patent ductus arteriosus, left aortic arch with aberrant right subclavian artery.

Results: Trio exome sequence analysis revealed a de novo missense mutation c.491G>A(p.Gly164Asp) in HNRNPK gene. The mutation lies at a functional domain, is absent from GenomAD exomes and genomes and 10 prediction software suggest a damaging effect on protein function.

Conclusion: We report the first case of Au Kline syndrome diagnosed during neonatal period. A non-previously reported missense mutation in HNRNPK gene is responsible for the disease. Additionally prenatal findings of augmented TN and polyhydramnios expand the clinical phenotype of the syndrome described before birth.

References: Kline genereviews, https://www.ncbi.nlm.nih.gov/books/NBK540283/, Phenotypic spectrum of Au-Kline syndrome: a report of six new cases and review of the literature PMID: 29904177.

Grants: None.

Conflict of Interest: None declared.

EP12.079 HDR Syndrome: report of a heterogeneous familial presentation

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Background/Objectives: HDR Syndrome is a genotypically heterogeneous autosomal dominant disorder caused by *GATA3* pathogenic variants and characterized by hypoparathyroidism "H"; sensorineural deafness "D"; and renal disease "R". The HDR triad was observed in about 63% of the genetically confirmed suspected patients, with hearing loss and hypoparathyroidism being the most prevalent manifestations, present in 98% and 93% of the cases, respectively. Additional clinical features, like congenital heart disease or intellectual disability, were more rarely reported. Here we describe the genetic characterization of two patients (mother and son) with a heterogenous "HDR" presentation and a strong family history of sensorineural hearing loss and renal disease.

Methods: WES was performed in WBC DNA samples (NovaSeq 6000). In house bioinformatics pipeline and Agilent Alissa Interpret software was used for variant calling and analysis.

Results: The mother, aged 32, had pre-lingual bilateral sensorineural hearing loss, learning difficulties, unilateral renal agenesis with mild chronic renal insufficiency, and hypoparathyroidism. Obesity, hypertriglyceridemia, and mild facial dysmorphic features were also observed. Her 4-year-old son had, in addition to congenital and bilaterally severe sensorineural hearing loss, global developmental delay, a suspected double aortic arch, and normal parathyroid hormone levels. WES study identified the heterozygous loss-of-function pathogenic variant c.708del p.(Ser237-Alafs*29) in *GATA3* gene, already reported associated with classic HDR presentation.

Conclusion: HDR syndrome has a remarkable clinical spectrum, and its familial occurrence is a special opportunity to observe atypical presentations, evidencing the great expressiveness and penetrance variability of each clinical feature in this syndromic condition.

References: PMID 29663634; 17210674; 26282285.

Grants:

Conflict of Interest: None declared.

EP12.080 Severe presentation of acrofacial dysostosis in two unrelated patients

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Background/Objectives: Acrofacial dysostosis (AD) is a group of disorders that are characterized by craniofacial anomalies, limb defects and a variety of other anomalies of other organs and systems. Different mode of inheritance has been reported, suggesting genetic heterogeneity. Severe neonatal forms represent diagnostic and clinical emergencies, frequently of Nager or Rodriguez type of AD.

Methods: We present two unrelated neonates with clinical presentation of severe form of AD. In both families there was no data for similar facial appearance. They were born prematurely on 34/35 gestational week, had low Apgar score and had breathing difficulties that required assistance. Both babies had microretrognathia, cleft palate, antimongoloid slanted palpebraes, deep set eyes, underdeveloped helix and low set ear. Additional finding on the second baby was bilateral athreptic external auditory canal. Arm anomalies include thumb hypoplasia/aplasia in the first child associated with pedes equinovari; the second child had hypoplasia of upper and forearm, associated with short hand and thumb aplasia. Both babies died within the first month.

Results: The novel frameshift mutation of SF3B4 gene (c.749delC;pPro250Leufs*70) leading to nonfunctional protein was found in the first patient verifying the presence of Nager AD. In the second case, the analysis failed to detect any mutation, however the clinical presentation point to the Rodriguez type of AD.

Conclusion: Since only some of the types of AD have clarified molecular defect so far, clinical assessment of anomalies is needed for precise classification. Clinical and molecular heterogeneity is described in lethal forms of AD as well.

References: Drivas TG, Cadieux-Dion M.

Grants: None.

Conflict of Interest: None declared.

EP12.082 Exploring the transgenerational effects of an imprinting disorder - a report of a proband with neuroblastoma and a maternally inherited deletion including EED

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Background/Objectives: Interstitial deletions on chromosome 11q are highly variable in their size and position, and are associated with a range of phenotypes, including developmental delay, non-specific dysmorphism, growth abnormalities, and variable congenital anomalies. While malignancy is not frequently observed in individuals with germline 11q deletions, several cases of neuroblastoma have been reported. Some of these deletions contain *EED*, which has recently been associated with Cohen-Gibson syndrome (CGS), an overgrowth disorder. While neuroblastoma has not yet been described in this condition, it is a well-described feature of several other overgrowth syndromes.

Methods: We present the case of 3 year old boy who presented with global developmental delay, growth parameters at the 97th centile, and dysmorphism, and neuroblastoma.

Results: Microarray revealed an 11Mb deletion at 11q14.1q21 containing *EED*. This deletion was inherited from his mother, who had a similar presentation aside from the neuroblastoma; their features resembled CGS. As *EED* is involved in histone methylation, we are currently pursuing methylation array and RNA-seq on the proband and mother and comparing these to the known epigenetic signatures in CGS. Given the prominent role *EED* plays in H3K27me3 modification in oocytes, we also plan to explore the multigenerational effects of this variant on methylation.

Conclusion: We anticipate that this unique case will contribute to our understanding of 11q deletions in the pathophysiology of neuroblastoma, as well as the multigenerational effects of inherited epigenetic conditions.

References:

Grants:

Conflict of Interest: None declared.

EP12.083 BCORL1 variant in two male fetuses with multiple ultrasonographic abnormalities

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Background/Objectives: Hemizygous mutations in *BCORL1* are associated with Shukla-Vernon syndrome, a rare X-linked recessive neurodevelopmental disorder characterized by developmental delay, intellectual disability, behavioral abnormalities and dysmorphic features. Female carriers are mostly unaffected. Only few cases of Shukla-Vernon syndrome with *BCORL1* pathogenic missense variants haven been reported. Recently, missense variants in *BCORL1* were described in patients with more severe clinical features (e.g. major brain malformations, seizures).

Here, we report a novel hemizygous frameshift alteration c.960dupT, p.(V321Cfs*99) in *BCORL1* in two deceased male

fetuses with multiple ultrasonographic abnormalities (e.g. in brain, heart, kidney, abdomen, oligohydramnios, IUGR) in two consecutive pregnancies of the same phenotypically unaffected mother being heterozygous carrier of a de novo *BCORL1* variant.

This frameshift mutation in the fourth of 13 exons of *BCORL1* leads to a premature stop codon and most likely to a loss of function. In dbSNP this variant is not listed. The probably intolerant of loss-of-function (pLI)-score of 1 might suggest that hemizygous mutations are not compatible with life. The fact that the variant occurred de novo in the mother is indicative for pathogenicity. The loss-of-function might explain the more severe phenotype of the fetuses. Otherwise, hemizygous loss-of-function mutations in *BCORL1* are listed in gnomAD, in *BCORL1 knockout models*, only spermatogenesis was affected and *BCORL1* loss is described to be associated with infertility. In summary, the current data are insufficient to assess whether this variant is associated with the malformations of the two deceased fetuses. Thus, we currently classify the sequence change as a VUS.

Methods:.
Results:.
Conclusion:.
References:
Grants:
Conflict of Interest: None declared.

EP12.084 NF1 Variants and Related Various Phenotypes

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Background/Objectives: Mono-allelic variants of *NF1* (Neurofibromin 1, OMIM *613113) cause various phenotypes such as Neurofibromatosis, type 1, Neurofibromatosis-Noonan Syndrome and Watson Syndrome. Although these phenotypes share the same features as café au lait spots, axillary- inguinal freckling, Lisch nodules, they differ with features such as height, bone deformities, malignancies, cardiac defects and dysmorphic features. We aimed to report the phenotypes of our series with genomic *NF1* pathogenic variants.

Methods: The medical records and the Sanger sequencing and Array CGH results of patients with *NF1*-related phenotypes were recruited retrospectively.

Results: 57 patients (31 female, 26 male) were evaluated, 24 of them were familial. Seven of the 50 *NF1* variants found were novel (c.6876delA, c.5441delAinsCT, c.2671 G>C, c.109_110delGA, c.1241T>C, c.3318 C>G, c.1307C>A) additionally, 2 chromosomal micro-deletions involving *NF1* gene on 17q11.2 were detected. Ten of the patients had Neurofibromatosis-Noonan Syndrome phenotype. All had café au lait spots, 20 of them had central nervous system involvement, 11 had ophthalmologic signs, 17 had short stature, 14 had actual or relative macrocephaly, 10 had mild intellectual disability. 19 had various bone deformities as pectus excavatum, scoliosis, polydactyly and/or segmental hypertrophies of limbs. Three had cardiac defects (two VSD, 1 TOF), three had hypothyroidism, two had cryptorchidism, one had choanal atresia, one had thyroglossal cyst, one had inguinal hernia, and one had degenerative myopia.

Conclusion: *NF1* is one of longest coding genes with high number of pathogenic variants. As an autosomal dominant disorder with variable expressivity pathogenic variants cause various sometimes very rare phenotypes.

References:
Grants:
Conflict of Interest: None declared.

EP12.086 3M syndrome: a diagnosis to consider in patients with prenatal-onset short stature

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Background/Objectives: 3M syndrome (Miller, McKusick and Malvaux) is a rare autosomal recessive disease characterized by severe growth retardation of prenatal onset and postnatal period and particular phenotypic and skeletal characteristics with normal intelligence. Hypogonadism may be present in some males. It is caused by homozygous or compound heterozygous variations in OBSL1, CUL7 and CCDC8 genes.

Methods: We report four patients (two females and two males), two diagnosed during the first year of life and two at 6 and 10 years. Three of them were born from consanguineous parents from Morocco ($n = 2$) and Pakistan. The non-consanguineous was from Spain.

Results: During prenatal period, three cases presented short long bones and one IUGR. Three were delivery by cesarean section and one by eutocic delivery. Three were born at term and one mild premature (34.6w); all birth heights were lower than -2.7 SD with normal head circumference (HC), and birth weights were $p < 1$ in three of them. At diagnosis, height ranged from $p < 3.8$ to $p < 8.8$ and was always smaller than HC (relative macrocephaly). All cases presented anteverted nares, prominent heels and short thorax.

Homozygous variants in OBSL1 ($n = 1$), CCDC8 ($n = 1$) and CUL7 ($n = 1$) were detected in the consanguineous families. The Spanish non-consanguineous family showed compound heterozygous variants in OBSL1.

Conclusion: 3M syndrome presents a recognizable phenotype which should lead to suspicion to confirm by gene studies, being useful for genetic counseling, as well as for possible treatments given that insensitivity to growth hormone and/or IGF-I have been described.

References:
Grants:
Conflict of Interest: None declared.

EP12.087 Recurrent germline variant in ARAF in a neonate with hydrops and congenital chylothorax: a new RASopathy?

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Background/Objectives: RASopathies are a clinically defined group of genetic syndromes caused by germline mutations in genes that encode components of the RAS-MAPK pathway. Although each RASopathy has a unique phenotype, secondary to the common underlying pathway dysregulation, they exhibit

numerous overlapping phenotypic features, such as dysmorphic facial features, cardiac malformations, cutaneous and ocular abnormalities, neurocognitive impairment, lymphatic anomalies and an increased tumour risk. A recurrent gain-of-function variant in the *ARAF* gene, member of the Ras/MAPK pathway, has been associated with lymphatic anomalies. MEK inhibition treatment has been proved to resolve the lymphatic phenotype associated with this recurrent somatic variant and to ameliorate severe lymphatic complications refractory to any other treatments in patients affected by a RASopathy. Here we report the first case presenting with a severe lymphatic disorder and this recurrent variant in a germline manner.

Methods: Rapid trio WES study was performed for the genetic diagnosis.

Results: A de novo recurrent gain-of-function *ARAF* variant (c.640T>C:p.S214P) was identified in a female newborn who was large for gestational age and presented with congenital chylothorax, ascites and anasarca. The child died at 22-days of age due to septic shock.

Conclusion: Here we present the first case affected by a germline variant in the *ARAF* gene, presumably expanding the RASopathy family. We suggest that for patients with *ARAF* defects complicated by a severe lymphatic disorder, inhibition of the RAS-MAPK pathway should be considered as a possible treatment option when conventional treatment has failed.

References: PMID: 31263281; 33219052.

Grants: AES PI 19/01681 Fondo Europeo de Desarrollo Regional.

Conflict of Interest: None declared.

EP12.088 From young to old: Evolutive phenotype of Mandibuloacral dysplasia A. Two novel patients

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Background/Objectives: Mandibuloacral dysplasia with type A lipodystrophy (MADA) is a rare autosomal recessive disorder characterized by craniofacial dysmorphism, type A lipodystrophy, clavicular dysplasia, and acroostelolysis. It is caused by homozygous or compound heterozygous missense mutations in LMNA gene.

Methods: We report two new patients from two families. Patient one is a 5 year old boy and patient 2 an adult woman of 63 years old (the oldest patient reported so far). We aimed to delineate the main characteristics and natural history of the disease.

Results: Both patients presented with typical features of mandibuloacral dysplasia including. The clinical manifestations in both patients raised the suspicion of MADA. LMNA genetic testing identified in patient one an homozygous variant c.1583C>T p.(Thr528Met) and the homozygous variant c.1580G>A (p.Arg527His) in patient 2. The first signs of MADA occur usually in early childhood, like in patient one, and become more evident in the second decade of life. By the sixth decade of life skeletal features were very prominent with development of extensive vertebral osteophytes visible through skin because of severe lipodystrophy and also soft tissue calcifications. It's important to evaluate in these patients the opportunity of therapy with an analogue of human leptin to minimize the impact of lipodystrophy.

Conclusion: he inclusion of new patients with MADA it's essential to delineate the phenotype of this syndrome. By reporting the oldest patient so far we better understand the natural history of this syndrome. Analogues of leptin should be considered to minimize the long term effects of lipodystrophy in these patients.

References:

Grants:

Conflict of Interest: None declared.

EP12.089 NGS findings in girl with onychodystrophia, chylotorax and behavioural disorder

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Background/Objectives: We are presenting a case of girl born in 2015 with yellow triangular nails, chylothorax, recurrent respiratory infections, failure to thrive, mild intellectual deficiency and behavioural disorder. The girl was examined and followed up at our department since her second year of age.

Methods: Clinical examination, standard karyotyping, MLPA for most common microdeletion syndromes, arrayCGH and clinical exome sequencing with NGS tests were performed.

Results: We considered yellow nail syndrome, but any pathogenic variant in *FOXC2* was found. Instead two pathogenic variants in *FZD6* gene were found. So far described variants in this gene are associated with onychodystrophia and chylothorax in some patients. We also found pathogenic variant in *NBEA* gene which is associated with developmental delay.

Conclusion: Clinical exome sequencing plays an important role in diagnostics and can elucidate even complex and combined genetic disorders.

References:

Grants: Supported by MH CZ DRO (FNM 64203, project 6003).

Conflict of Interest: None declared.

EP12.090 Revisiting TOP2B-Related Phenotypes: Turkish family with Three Affecteds in Two Generations

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Background/Objectives: DNA Topoisomerase IIβ (TOP2B) acts on DNA topology during transcription and has a critical role in neural development. Heterozygous pathogenic changes in its encoding gene, *TOP2B* (MIM, *126431), has been linked with three overlapping phenotypes characterized by immunodeficiency, acral and urogenital anomalies: Hoffmann; BILU and Ablepharon-macrostomia-like syndrome. Uptill now only 11 patients and 4 variations have been reported.

Methods: 7 years 3 months old boy, firstborn, was referred to genetic outpatient clinics for urogenital anomalies, glandular epispadias and cryptorchidism. He had distinct facial dysmorphism; maxillary hypoplasia, hypoplastic alae nasi with low-set columella, absent nasolabial sulci, short-flat philtrum, enamel hypoplasia, microstomia, rounded-creased oral commissures, Angle class II malocclusion defect, micrognathia and prominent-dysplastic ears. He showed acral findings of webbed fingers&toes, hypoplastic thumbs with absent flexion lines with no mobility of DIF joint and posteriorly-set halluces.

Results: Whole Exome Sequencing (WES) revealed a heterozygous missense change, p.S483L, in the TOPRIM domain. The mother, in whom the variant was de novo, had the same distinctive facial gestalt, acral findings and microcephaly. Third

pregnancy was complicated with trisomy 18 leading to medical termination. The family didn't opt prenatal testing at 4th gestation and similarly affected male was born. He presented with severe immunodeficiency and failure to thrive, which required regular IVIG treatment.

Conclusion: We herein report on a mother and her two sons, the second and third reported males with a distinct *TOP2B*-phenotype. We will further discuss the genotype-phenotype correlation and possible disease anticipation pattern, lumping together Hoffmann; BILU; Ablepharon-macrostomia-like syndromes.

References:

Grants:

Conflict of Interest: None declared.

EP12.091 A non-linear Beckwith–Wiedemann syndrome diagnosis

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Background/Objectives: Beckwith–Wiedemann syndrome (BWS) is a congenital overgrowth disorder, caused mostly by deregulation of the imprinted 11p15.5 chromosomal region, either by changes in epigenetic modifications or, less frequently, by genetic variants in the imprinted genes of this cluster. Indeed, *CDKN1C* pathogenic variants are implicated in both sporadic and familial forms. Usually there is no developmental delay.

Methods: A 33-week boy with prenatal diagnosis of macrosomia and omphalocele, with normal 11p15.5 imprinting analysis and aCGH developed bilateral intraventricular hemorrhage and refractory multifocal seizures with migrating pattern after birth; craniofacial dysmorphisms (including macroglossia) were noted; an epileptic encephalopathy NGS panel identified a heterozygous VUS in KCNT1, maternally inherited; on follow up a developmental delay with severe bilateral hearing loss and bilateral cataracts were detected; *CDKN1C* was sequenced and identified a probably pathogenic variant (also maternally inherited). Two monozygotic twin sisters, one with a severe phenotype, including facial asymmetry and frontal angioma, global hypotonia, macrocrania, ear pits, macroglossia, and hepatomegaly. Beckwith–Wiedemann syndrome MS-MLPA testing showed loss of methylation for *KvDMR1* imprinting control region (on both sisters); tests on DNA extracted from buccal swab confirm the loss; bisulfite-sequencing confirmed loss of methylation in both sisters' blood samples, aCGH testing, performed due to developmental delay, identified a paternal inherited 5p13.2 microduplication; this CNV partially overlaps the 5p13 Duplication Syndrome (OMIM#613174).

Results:.

Conclusion: We explain two different and rarer causes of BWS combined with other clinical situation and highlight sometimes some clinical complications may mask a true diagnosis, and although rare, some syndromes may come together.

References:

Grants:

Conflict of Interest: None declared.

EP12.092 Research on a pathogenesis of cognitive and neurofunctioning impairments in patients with Noonan syndrome. A role of RAS/MAPK signaling pathway gene disturbances

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Background/Objectives: Noonan syndrome (NS) is one of the most common genetic conditions inherited in autosomal dominant manner with vast heterogeneity in clinical and genetic features. NS is caused by mutations in particular genes of the RAS/MAPK signaling pathway. Patients with NS might have cognitive problems in language skills, memory, attention, executive functioning and decreased overall intelligence level. According to our knowledge this is first study on functional connectivity in NS.

Methods: 28 subjects with Noonan Syndrome and 23 healthy took part in white matter connectivity measurements using diffusion tensor imaging (DTI) and Rs-fMRI data acquisition using a 3 T Siemens PRISMA scanner. A structural T1 MR sequence was obtained as well. Fractional anisotropy (FA) and mean diffusivity probability distributions were calculated. In addition, subjects underwent a complex assessment of cognitive abilities with the use of Stanford-Binet Intelligence Scales.

Results: Reductions of white matter connectivity were revealed by DTI in subjects with NS. Additionally, both hypo- and hyper- connectivity within salience and DMN networks were revealed in subjects with NS. Simultaneously, they demonstrated decreased connectivity within precuneus. In respect of IQ, subjects with NS showed decreased Verbal and Nonverbal IQ compared to healthy controls.

Conclusion: The assessment of the microstructural alterations of white matter as well as rsFC analysis in subjects with Noonan syndrome may shed light on mechanisms responsible for cognitive and neurofunctioning impairments.

References: Cesarini L, et al. Cognitive profile of disorders associated with dysregulation of the RAS/MAPK signaling cascade. *Am J Med Genet A*. 2009;149A(2):140-146.

Grants: Supported from NCN research projects no. 2013/09/B/NZ2/03164.

Conflict of Interest: None declared.

EP13 Cancer Genetics

EP13.001 Whole-exome sequencing in eccrine porocarcinoma indicates promising therapeutic strategies

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Background/Objectives: Malignant sweat gland tumours are rare, with the most common form being Eccrine porocarcinoma (EP). Limited data are available concerning EP biology.

Methods: To investigate the mutational landscape of EP, we performed whole exome sequencing (WES) on 14 formalin-fixed paraffin-embedded samples of matched primary EP and healthy surrounding tissue.

Results: Mutational profiling revealed a high overall median mutation rate. This was attributed to signatures of mutational processes related to ultraviolet (UV) exposure, APOBEC enzyme dysregulation, and defective homologous double strand break repair. All of these processes cause genomic instability and are implicated in carcinogenesis. Recurrent driving somatic alterations were detected in the EP candidate drivers TP53, FAT2, CACNA1S, and KMT2D. The analyses also identified copy number alterations and recurrent gains and losses in several chromosomal regions including that containing BRCA2, as well as deleterious alterations in multiple HRR components. In accordance with this reduced or even a complete loss of BRCA2 protein expression was detected in 50% of the investigated EP tumours.

Conclusion: Our results implicate crucial oncogenic driver pathways, and suggest that defective homologous double strand break repair and the p53 pathway are involved in EP aetiology. Targeting of the p53 axis and PARP inhibition, and/or immunotherapy, may represent promising treatment strategies.

References:

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Conflict of Interest: None declared.

EP13.003 Evaluation of EGFR mutation testing in cf-DNA and tissue from NSCLC patients using Cobas® EGFR mutation test v2

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Background/Objectives: Mutational analysis of circulating cell-free tumor DNA (cf-DNA) extracted from plasma is a minimally invasive approach for targeted therapy of NSCLC patients without adequate tumor material for molecular analysis and for disease monitoring during relapse. EGFR mutation detection in cf-DNA is currently already used in clinical practise.

Methods: 521 blood samples were collected from NSCLC patients and EGFR mutations were detected in cf-DNA using IVD test, Cobas ® EGFR mutation v2. For 128 cases FFPE tissues were available for EGFR comparative analysis using the cobas test.

Results: EGFR mutations were present in 28% (144/521) of the cf-DNA samples. Deletions in exon 19 were encountered most frequently (67%, 97/144), followed by point mutations in exon 21 (25%, 37/144). p.Thr790Met was found concurrently with other mutations in 20% (31/144) of the mutant samples. Primary samples displayed EGFR mutations at a frequency of 10% (26/255) whereas for follow up samples the percentage of mutations reached 45% (118/266). 3/17 tissue samples with invalid FFPE mutation analysis displayed mutations in cf-DNA. The overall concordance of EGFR mutational status between plasma and tissue was 78% and the Specificity 100%, Sensitivity 55%, PPV 100%.

Conclusion: EGFR mutation testing in cf-DNA using cobas IVD test shows significant concordance with analysis of tissue samples and a very high PPV and specificity. However the sensitivity of cf-DNA analysis is not yet optimal.

References: Normanno N et al., Oncotarget. 2016, https://doi.org/10.18632/oncotarget.13915.

Grants: No funding.

Conflict of Interest: None declared.

EP13.004 prognostic value of bile acid transporter SLC10A1 expression in hepatocellular carcinoma

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Background/Objectives: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy that occurs predominantly in patients with underlying chronic liver disease and cirrhosis. Emerging data have implicated that the expression and function of hepatic bile acid uptake transporter NTCP (sodium-taurocholate co-transporting polypeptide) are altered in hepatocellular carcinoma. NTCP, encoded by the SLC10A1 gene (solute carrier family 10 member 1), is a key transport protein involved in the enterohepatic recirculation of bile acids. We aimed to systematically analyze SLC10A1 expression and its prognostic role in HCC using various open databases.

Methods: SLC10A1 expression in HCC was assessed using UALCAN and GEPIA database. The promoter methylation levels were also examined by UALCAN. Correlation between SLC10A1 expression and patient survival was evaluated with OncoLnc. SLC10A1 genetic alterations in HCC were explored using cBioPortal.

Results: SLC10A1 expression is significantly down-regulated in the clinic-pathological characteristics (cancer stages, patient's race, gender, age, weight, tumor grade, nodal metastasis status, and histological subtypes) examined in HCC patients compared to normal counterparts. SLC10A1 promoter methylation level in HCC was higher than that in normal liver tissue. Clinically, low expression of SLC10A1 was correlated with shorter overall survival in HCC patients ($P = 0.0038$). Among HCC cases with SLC10A1 genetic alterations, mutations were the most common type of alteration (data from TCGA Liver Hepatocellular Carcinoma, Pan-Cancer Atlas).

Conclusion: These observations indicate that SLC10A1 may function as a potential tumor suppressor gene. In HCC patients, SLC10A1 expression levels may also serve as a prognostic predictive marker.

References: NA

Grants: NA

Conflict of Interest: None declared.

EP13.005 Germline gene panel testing in 32 ovarian cancer patients from Bulgaria

Zornitsa Kamburova[1;2], *Katya Kovacheva*[1;2], *Savelina Popovska*[2;3], *Diana Dimitrova*[2;4], *Chavdar Tsvetkov*[2;4], *Angel Yordanov*[2;4], *Slavena Nikolova*[1;2]

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Background/Objectives: Ovarian cancer (OC) is not the most common type of cancer, but it is the fourth most common cause of cancer death among women in Bulgaria. This is because the disease typically occurs at a late stage, when the 5-year relative survival rate is only 29% [1].

OC is a multifactorial disease. Rare high penetrant germline mutations in the BRCA1/2 genes increase the lifetime risk of OC and are a major cause of hereditary OC. The evolvement of the genetic testing methods has revealed many other genes that determine susceptibility to OC [2].

We use the panel of cancer predisposition genes analysed by next-generation sequencing (NGS) to increase the knowledge of the genetic etiology of OC in Bulgarian women.

Methods: We tested 32 women with OC (mean age at diagnosis - 54 years) for germline mutations using NGS.

Results: Nine of the women (28%) had a pathogenic mutation - five of the carriers (56%) had a mutation in the BRCA1 gene and the other four had a mutation in the TP53, MSH2, ATM and ERCC3 genes. Patients with pathogenic variants distinct from BRCA1 had no significant differences in clinical features or age at diagnosis.

Conclusion: Germline genetic testing in women with OC could improve their treatment strategy and help relatives of mutation carriers make informed decisions about prophylactic measures.

References: 1.НСИ available on: https://www.nsi.bg/sites/default/files/files/publications/Zdraveopazvane_2019.pdf.

2.Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med. 2017 Feb;14(1):9-32. https://doi.org/10.20892/j.issn.2095-3941.2016.0084. PMID: 28443200; PMCID: PMC5365187.

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Conflict of Interest: None declared.

EP13.007 Detecting disease-causing genetic variants in 48 patients with familial colorectal cancer by using whole exome sequencing

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Background/Objectives: Hereditary genetic mutations causing predisposition to colorectal cancer are accountable for approximately 30% of all cases. However, only a small fraction are high penetrant mutations occurring in DNA mismatch repair, causing Lynch Syndrome. Most of the mutations are low-penetrant variants, contributing to an increased risk of familial colorectal cancer, and often in additional genes and pathways. Aim of this study was to identify such variants.

Methods: Whole exome sequencing was performed on constitutional DNA extracted from blood of 48 patients with suspected familial colorectal cancer. Variant calling was performed to detect single nucleotide, small insertion/deletion and copy number variants. In silico prediction tools and available literature-based evidence were used to investigate for disease association of detected variants.

Results: This study identifies several causative germline variants in genes known for their association with colorectal cancer. Additionally, several variants have been identified in additional genes normally not included in relevant gene panels for colorectal cancer that potentially may be associated with an increased risk for cancer.

Conclusion: Identification of variants in atypical genes that potentially can be associated with familial colorectal cancer indicates a larger genetic spectrum of this disease, not limited only to mismatch repair genes. Whole exome sequencing provides the possibility to explore this larger spectrum. Usage of multiple in silico tools based on different methods and combined through a consensus approach increases the sensitivity of predictions and narrows down a large list of variants to the most significant ones.

References:

Grants: Cancer Institute NSW (Grant-number:12/ECF/2-34); Liaison committee between Helse Midt-Norge RHF and NTNU.

Conflict of Interest: None declared.

EP13.008 STK11 deletion breakpoints characterization by Nanopore long read sequencing with CRISPR/Cas9 or adaptive sampling enrichment

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Background/Objectives: Peutz-Jeghers syndrome (MIM 175200) is an autosomal dominant disease caused by pathogenic variants in the STK11 gene. Only 22 constitutional deletion cases are described. Main implicated mechanisms are Alu sequence-mediated non-allelic homologous recombination (NAHR) (10 cases), non-homologous end joining (NHEJ) (8 cases) and sequence microhomology (MH) (4 cases).

The aim of this work was to characterize mutational mechanisms by breakpoints identification of STK11 deletions in a cohort of 26 patients.

Methods: We used Nanopore long read sequencing with region of interest (ROI) enrichment: (1) real time adaptive sampling: DNA molecules were ejected from the pore if real time-determined sequence was out of ROI; (2) by CRISPR/Cas9: STK11 intron 1 was cut and generated fragments were sequenced from cut sites.

Results: For now, we could characterize 15 deletions: 6 exon 1 (4 NAHR, 2 MH), 1 exons 2-10 (NHEJ), 1 whole gene (MH), 3 exons 2-3 (3 NAHR), 4 intragenic (exons 2-8, exons 4-8, exon 6 and exons 7-8 (MH)).

Conclusion: We took advantage of enrichment to increase ROI sequencing depth to determine mutational mechanisms of STK11 deletions. CRISPR/Cas9 enrichment allows a decreasing ROI sequencing depth starting from cut sites but needs to target a specific known undeleted region. Real-time adaptive sampling allows a homogenous but lower ROI sequencing depth, useful for agnostic analyses.

We showed that Alu-mediated NAHR was one of the major STK11 deletion mechanisms, responsible of half of cases. This could explain the high de novo proportion of deleted patients (16/26).

References: None.

Grants: None.

Conflict of Interest: None declared.

EP13.009 The clinical use of NGS multi-gene panel testing in hereditary cancer analysis

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Background/Objectives: A considerable number of families with pedigrees suggestive of a Mendelian form of Breast Cancer (BC), Ovarian Cancer (OC) or Pancreatic Cancer (PC) have not detectable BRCA1/2 mutations. Multi-gene hereditary cancer panels growth the possibility to identify individuals with cancer predisposing gene variants. Our study was aimed to evaluate the increase in the detection rate of pathogenic mutations when using a multi-gene panel.

Methods: 546 patients analysed in the last 2 years, affected by BC (424), PC (63) or OC (59) entered the study with at least one of the following criteria: (i) positive cancer family background, (ii) early onset and (iii) type of tumour. The patients were tested using a Next-Generation Sequencing (NGS) panel containing 25 genes in addition to BRCA1/2.

Results: Forthy-four out of 546 patients (8%) carried germline pathogenic or likely pathogenic variants (pv/lpv) on BRCA1/2 genes, and 51 out of 546 (9%) patients presents pv or lpv in other susceptibility genes.

Conclusion: Our findings demonstrate the utility of expanded panel testing in patients with suspected hereditary cancer syndromes, since this approach increased the mutation detection rate of 14% in PC, 8% in BC and 5% in OC cases. In absence of multi-gene panel analysis, a considerable percentage of mutations would have been lost.

References: 1: Bono, M et al. ESMO open vol.6,4 (2021).

2: Fountzilas C, Kaklamani VG. Multi-gene panel testing in breast cancer management. Cancer Treat Res.2018;173:121-140.

Grants:

Conflict of Interest: None declared.

EP13.010 A Multigene panel testing reveals high prevalence of germline mutations at prostate cancer

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Background/Objectives: Although many factors are implicated in Prostate Cancer (PrC) development, genetic predisposition represents an important risk factor, correlated with more aggressive disease. Inherited PrC component has been related to Hereditary Breast and Ovarian Cancer Syndrome genes, mainly BRCA2, and moderated predisposition genes such as ATM and CHEK2. This study aimed to evaluate the usefulness of multigene panel testing in PrC patients with positive family history of cancer.

Methods: 64 PrC patients with suspected hereditary origin were screened by On-Demand panel comprising 35 predisposition cancer genes. Next Generation Sequencing (NGS) was performed by ThermoFisher Scientific Technology. Library and template preparations were accomplished using the automated Ion Chef System and then sequenced by Ion S5 with Ion 520 Chip. Finally, results were analysed using Ion Reporter Software.

Results: A total of 7 variants were detected (10,93%), affecting 3 different genes.

Gene	cDNA	Protein	Consequence	Interpretation
ATM	c.802C>T	p.Gln268Ter	Nonsense	Pathogenic/ Likely Pathogenic
	c.1339C>T	p.Arg447Ter	Nonsense	Pathogenic/ Likely Pathogenic
BRCA2	c.3922G>T	p.Glu1308Ter	Nonsense	Pathogenic
	c.793+1G>A	-	Splicing	Pathogenic/ Likely Pathogenic
	c.2808_2811delACAA	p.Lys936fs	Frameshift	Pathogenic
	c.9026_9030delATCAT	p.Tyr3009fs	Frameshift	Pathogenic
CHEK2	c.433C>T	p.Arg145Trp	Missense	Pathogenic/ Likely Pathogenic

Conclusion: This study reinforces the utility of germline testing by NGS panels in those PrC cases with family history. Increasing knowledge about PrC genetic spectrum is essential to develop clinical guidelines which will improve the clinical management of PrC patients and their families, enabling genetic counselling.

References:

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Calendario Benéfico Solidario de Pedrajas de San Esteban.

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EP13.011 Impact of increasing melanoma incidence on germline mutational yield. Five years of (tele)-counseling and gene panel testing within the Italian Melanoma Intergroup

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Background/Objectives: Genetic assessment criteria for hereditary melanoma in Italy are less conservative than in high-incidence countries. However, melanoma incidence keeps increasing, and thus a revision may be needed.

Methods: Since 2016, we prospectively recruited 940 cutaneous melanoma (CM) probands from 25 Italian centers via in-person or tele-genetic counseling, and germline sequenced through a shared panel including CDKN2A, CDK4, BAP1, POT1, ACD, TERF2IP, MITF, ATM. Inclusion criteria: personal/family history of at least 2 cancer events among cutaneous/uveal melanoma, pancreatic cancer (PC), kidney cancer, mesothelioma. We assessed mutation rate (MR) according to familial status, region of origin, presence and type of tumors. We then computed multiple logistic regression with personal/family characteristics as independent predictors of mutational status.

Results: Overall MR was 10.21% (5.74% CDKN2A). Sporadic multiple primary melanoma (spoMPM) with 2 CMs had the lowest overall MR ($p = 0.02$), and >60 was the age category with the lowest CDKN2A MR ($p < 0.01$). ≥3CM cases, spoMPM with ≥3CMs, PC and region were predictive of CDKN2A likely/pathogenic variants (OR = 5.14, 4.97, 3.25 and 4.45, $p < 0.05$), whereas age >60 was a negative predictor (OR = 0.16, $p = 0.014$). In particular, MR was nearly 19% when CMs and PC clustered together.

Conclusion: Our results suggest that a revision of national genetic testing criteria for melanoma should be considered, especially regarding age cut-off and number of CM in absence of familial history. The use of telecounseling in our clinical practice allowed us to have a nationwide picture of the differences between CDKN2A and non-CDKN2A MRs in different regions.

References:

Grants:

Conflict of Interest: None declared.

EP13.012 Analysis of transcripts from alternative PRKAR1B gene promoters in colorectal cancer

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Background/Objectives: The transcriptional regulation of *PRKAR1B* is controlled by alternative promoters, and previous in silico analysis has indicated their differential activity in colon and rectal cancer tissue in comparison to normal gut mucosa. The aim of this study was to investigate *PRKAR1B* promoters and transcripts potentially involved in cancer.

Methods: The sequences of *PRKAR1B* alternative promoters were retrieved from Ensembl database: promoter A 752209 and promoter B 767287 bases upstream from the translation start site. Bioinformatic tools Alggen, AliBaba, CiiiDER, and TFBIND were used to predict binding of transcriptional regulators. Primer extension assay was performed on RNA isolated from malignant colon cell lines using an oligonucleotide probe binding to the sequence at the exon2/exon3 junction common for all *PRKAR1B* transcripts.

Results: Based on analyzed elements, both *PRKAR1B* promoters were found to have atypical structure. According to the prediction, promoter A that encodes transcript PRKAR1B-201 binds several factors involved in cell proliferation, while promoter B that encodes transcript PRKAR1B-203 binds mostly pro-apoptotic factors. In primer extension experiments, a single signal corresponding to the transcript PRKAR1B-212 was observed in malignant cells.

Conclusion: The differential activity of alternative *PRKAR1B* promoters in colorectal cancer can be explained by in silico results, predicting that promoter sequences bind sets of transcriptional regulators with opposing roles. However, experiments point to the transcript unrelated to either of the investigated promoters as potential cancer biomarker and it should be further characterized.

References:

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Conflict of Interest: None declared.

EP13.015 Frequency and spectrum of non-founder clinically actionable BRCA1/2 mutations in Russian patients with breast cancer: a series from one institution

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Background/Objectives: Most common mutations in *BRCA1* (5382insC, 185delAG, 4153delAG, T300G) and *BRCA2* (6174delT) genes account for 6-8% breast cancer (BC) patients of Slavic origin and are major targets for routine testing. But many BC cases with family history remain unsolved. The aim was to estimate the frequency and spectrum of non-founder *BRCA1/2* mutations in a large group of BC patients treated in our clinic.

Methods: A total, 560 women diagnosed with BC, which did not carry any of 5 BRCA1/2 Russian founder mutations, were enrolled. Age ranged from 18 to 83 years, mean age 49,3 years. Target sequencing was performed using multi-gene panel, including all exons of *BRCA1* and *BRCA2* genes.

Results: Clinically actionable non-founder genetic variants in coding regions of *BRCA1/2* genes were found in 35 from 560 (6,3%) patients. *BRCA2* mutations prevailed (22 cases or 62%), while *BRCA1* mutations explained 13 cases (38%). The 28/35 (80%) of mutations were frameshift deletions/insertions leading to premature stop codon, 6/35 (17%) mutations were missense and 1/35 (3%) mutation was from splice site. The majority of mutations was unique in this study, but previously described as pathogenic variants in BC patients from different populations. The recurrent mutations were BRCA2 c.7879A>T (rs80359014) in 3/35 (8,5%), BRCA2 c.9253del (rs80359752) in 2/35 (5,7%) and BRCA1 c.1303_1309del (rs886039941) in 2/35 (5,7%) BC cases.

Conclusion: The contribution of founder and non-founder mutations to the disease was compatible in our study population. The analysis of all *BRCA1/2* coding regions may double the number of identified *BRCA1/2* cases in Russian BC patients.

References:

Grants:

Conflict of Interest: None declared.

EP13.016 Germinal mutations in POLE and POLD1 genes

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Background/Objectives: Germline mutations in the exonuclease domain of POLE and POLD1 repair polymerases affect their proofreading capacity and have been described to confer a high risk for multiple colorectal adenomas and carcinoma. Other tumours were also associated with the syndrome: breast, brain, ovaries, pancreas, stomach uterus and skin.

Methods: An NGS multi-gene hereditary cancer panel called CZECANCA was used according to SeqCap or KAPA HyperCap Workflow (Roche) for the analysis of 3000 adult patients indicated by clinical geneticist for suspected hereditary cancer predisposition: 49% breast and/or ovarian cancers patients; 6.5% polyposis, non-polyposis colorectal or endometrial cancer; 21% with other type of solid cancers; remaining 23.5% were healthy individuals with high risk family history of hereditary cancer syndromes.

Results: Germinal deleterious (FS, N) mutations in the POLE gene have been detected so far only in breast cancer patients in 3 families without family history of CRC. Several potentially significant missense variants were detected in highly conserved amino acids of the exonuclease domains of POLE and POLD1 genes, but even in these cases, breast cancer was predominant.

Conclusion: Cancer patients with POLE/POLD1 mutations were described to respond well to immune checkpoint inhibition. Could

this be a potential pathway for such therapy in breast cancer patients with a germline proofreading mutation?.

References: Soukupova et al, PLoS One 2018; PMID: 29649263. Briggs et al., J Pathol. 2013 Jun; PMID: 23447401.

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Conflict of Interest: Eva Machackova Masaryk Memorial Cancer Institute, Ministry of Health of the Czech Republic: collaborator in AZV project NU20-03-00285, Ministry of Health of the Czech Republic MH CZ – DRO (MMCI, 00209805), Petra Vasickova Masaryk Memorial Cancer Institute, Jana Hazova Masaryk Memorial Cancer Institute, Adela Misove Masaryk Memorial Cancer Institute, Lenka Foretova Masaryk Memorial Cancer Institute.

EP13.017 Hypoxia-induced FAM13A modulates growth and metastatic potential of non-small cell lung cancer cells via cell cycle arrest and actin cytoskeleton disruption

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Background/Objectives: Hypoxia occurrence in non-small cell lung cancer (NSCLC) tumor microenvironment affects cancer progression and metastasis. We previously showed that FAM13A was induced by hypoxia in NSCLC cell lines and tumors. The aim of the present study was to investigate the role of hypoxia-induced FAM13A in NSCLC progression.

Methods: Lentiviral shRNAs were used for stable FAM13A gene silencing in A549 and CORL-105 cell lines. The effect of FAM13A knockdown on cell proliferation (MTS assay, cell tracking VPD450 dye), cell cycle (BrdU assay), migration (wound healing assay), invasion (Boyden chamber assay), apoptosis (APC, 7AAD assays) and cytoskeleton phenotype (F-actin staining) was examined under the conditions of hypoxia (1% O2) and normoxia (21% O2).

Results: Significant reduction in cell proliferation after FAM13A knockdown was observed after hypoxia for both cell lines. The S phase cell cycle arrest in FAM13A depleted A549 cells under hypoxia condition was indicated. The FAM13A inhibition significantly suppressed migration of A549 and CORL-105 cells in both oxygen concentrations. Several disruptions of the cytoskeleton of A549 cells were observed after chronic hypoxia. Cell invasion rates were significantly decreased in A549 FAM13A depleted cells, mostly after hypoxia.

Conclusion: Our findings demonstrated that FAM13A depleted post-hypoxic cells have a decreased cell proliferation ability and metastatic potential, which indicates FAM13A as a potential therapeutic target in NSCLC.

References: https://doi.org/10.3390/ijms22094302.

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EP13.018 Age of breast cancer onset in female BRCA1/2 pathogenic variant carriers is modified by polygenic risk scores

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Background/Objectives: Clinical management of women carrying a germline pathogenic variant (PV) in the *BRCA1/2* genes demands for accurate age-dependent estimators of breast cancer (BC) risks. We assessed the contribution of polygenic risk scores (PRSs) to the occurrence of extreme ages at onset of primary BC, namely, diagnosis before the age of 35 years (early diagnosis, ED) and cancer-free survival until the age of 60 years (late/no diagnosis, LD) in female *BRCA1/2* PV carriers.

Methods: Overall and estrogen receptor (ER) status-specific BC PRSs developed by Kuchenbaecker et al. were computed for 297 female *BRCA1/2* PV carriers. Binomial logistic regression was applied to assess the association of standardized PRSs with either ED or LD under adjustment for selection for patient recruitment criteria for germline testing and localization of *BRCA1/2* PVs in the corresponding BC or ovarian cancer (OC) cluster regions.

Results: For *BRCA1* PV carriers, the standardized overall BC PRS displayed the strongest association with ED (odds ratio (OR) = 1.60; 95% CI: 1.13–2.27, $p < 0.01$). Additionally, statistically significant associations of selection for patient recruitment criteria and localization of PVs outside the OC cluster region were observed. For *BRCA2* PV carriers, the standardized ER-negative BC PRS displayed the strongest association (OR = 2.20, 95% CI: 1.39–3.48, $p < 0.001$).

Conclusion: Our results provide further evidence that PRSs modify age at onset of primary BC in *BRCA1/2* PV carrier and localization of PVs within OC cluster regions should be considered in BC risk predictions.

References:

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EP13.021 A combined visualization of diverse diagnostic methods: Reconstruction of clonal evolution improves understanding of leukemia progression

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Background/Objectives: Patients with myeloid neoplasia are classified due to the WHO system and besides clinical and hematological criteria, cytogenetic and molecular genetic alterations highly impact treatment stratification. In routine diagnostics, a combination of methods is used to decipher different types of genetic variants, i.e. single nucleotide variants (SNVs), insertions/deletions (indels), structural variants (SVs) and copy number variations (CNVs).

Methods: We used a bioinformatic approach to analyze clonal evolution and genetic architecture in patients with myeloid neoplasia based on SNVs, indels, SVs and CNVs. Eight patients were comprehensively analyzed using karyotyping, fluorescence in-situ-hybridization, array-CGH and a custom NGS panel.

Results: Clonal evolution was reconstructed manually, integrating all mutational information (SNVs/indels/SVs/CNVs). Altogether, we differentiate between three cases: 1) The CNV occurred prior to the SNV/indel, but in the same cells. 2) The SNV/indel occurred prior to the CNV, but in the same cells. 3) SNV/indel and CNV exist in parallel, independent of each other. On three samples, we showed that reconstruction of clonal evolution is possible even with data from one time point only. For other samples, providing data on more than one time point, the effect of therapy was estimated.

Conclusion: This bioinformatic approach offers the possibility of analyzing clonal evolution (linear/branching/neutral) and genetic architecture at one or more time points of analysis. The visualization of the results in fishplots contributes to a better understanding of genetic architecture and helps to identify possible targets for the disease (personalized therapy). Furthermore, this model can be used to identify markers in order to assess minimal residual disease.

References:

Grants:

Conflict of Interest: None declared.

EP13.022 Uterine lavage fluid for molecular identification of high grade ovarian cancer

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Background/Objectives: High-grade serous ovarian cancer (HGSOC) is the second deadliest gynaecologic malignancy in the world. HGSOC is diagnosed at an advanced stage due to a lack of specific symptoms and diagnostic tests used for patient screening and monitoring, adding to the low survival of HGSOC patients. Uterine lavage is a promising non-invasive liquid biopsy technique that can be used for gynaecologic cancer molecular analysis. Mutations detected in tumour cell-free DNA from uterine lavage samples could be useful as diagnostic and prognostic molecular biomarkers.

Methods: Mutations in 75 uterine lavage and 47 tissue samples from 78 patients with gynecologic malignancies (40 patients with HGSOC, 22 patients with benign or borderline gynecologic malignancies, and 16 with endometrial cancer or non-serous ovarian cancer) were analyzed using custom *Ion Torrent AmpliSeq*™ On-Demand Panel targeting 10 genes commonly associated with HGSOC and endometrial cancer.

Results: 90 % (29/32) of HGSOC tissue samples had ether TP53 (75 %, 24/32 cases) or BRCA1/2 (53 %, 17/32 cases) mutations, while only one non-serous ovarian cancer case harboured TP53 mutation and all BRCA1/2 mutations in non-HGSOC samples were uncertain significance (VUS). However, in uterine lavage samples, BRCA1/2 or TP53 mutations were detected in 65 % (24/37) HGSOC and only two BRCA1 VUS in non-HGSOC samples.

Conclusion: Our data suggest TP53, BRCA1, and BRCA2 mutation status in uterine lavage samples could be a useful tool for HGSOC diagnosis and prediction.

References:

Grants:

Conflict of Interest: None declared.

EP13.023 Identification of genomic signature as diagnostic blood biomarker for breast cancer and its association with tumor characteristics

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Background/Objectives: Despite the advances in cancer therapies and raising awareness, breast cancer (BC) continues to be one of the most malignant tumors. Unfortunately, the incidence and mortality of BC are still increasing despite all the current advances. Therefore, it is utmost important to detect the disease at early stages.

Methods: Integrated genomic analysis of BC was performed using genome-wide gene expression profiling datasets from both tissue and blood samples from patients with BC that were probed using Affymetrix HGU133 Plus 2.0 array. The diagnostic potential of the identified gene signature is validated using transcriptomic datasets from patients with BC with detailed clinical information.

Results: The integrated analysis revealed a 60-gene signature that are significantly expressed in both tissue and blood of BC patients compared to healthy controls. Hierarchical clustering using 60 genes clearly separated patients from normal controls validating the gene signature's diagnostic potential. The investigation of the 60-geneset score with the BC clinical and pathological features, including age, grade and HER2 indicated that high geneset score was significantly associated with aggressive disease characteristics.

Conclusion: The results suggest that integrated genomic analysis may provide a robust methodology to diagnose patients with BC non-invasively and lead to improved diagnosis and early detection for BC.

References: None.

Grants: None.

Conflict of Interest: None declared.

EP13.024 Molecular genetics of pediatric acute megakaryoblastic leukemia

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Background/Objectives: Pediatric acute megakaryoblastic leukemia (AMKL) is a genetically heterogeneous disease that is represented by Down syndrome (DS) patients with constitutional trisomy 21 (21c) and acquired *GATA1* mutations. In non-DS AMKL various chimeric oncogenes were identified.

Methods: The cohort included 120 AMKL patients diagnosed in 2013-2021 in two national multicenter studies. All patients underwent conventional karyotyping, FISH, fragment analysis, Sanger sequencing of *GATA1* exon 2 and targeted HTS (HMNP, Qiagen).

Results: We analyzed 51 DS patients (2 days to 6.6 y.o, median 1.8, m:f = 1:1) and 69 non-DS patients (1 months to 4.7 y.o., median 3.3, m:f = 2:1).

Assessment of cytogenetic features revealed 49 DS with 21c: 21c solely (37%), within complex karyotype (18%), 21c with trisomy 8 (12%). *GATA1* mutations were found in 90%.

In non-DS *CBFA2T3::GLIS2*, *NUP98::KDM5A*, *RBM15::MKL1* and *KMT2A* rearrangements were identified in 15%, 6%, 9%, and 10% patients, respectively. Patients with complex karyotype comprised 33%, with *GATA1* mutations - 22%.

The landscape of somatic mutations in DS patients included from 1 to 8 additional events. The most frequent mutations were in *JAK/STAT* pathway (37%) and cohesin complex genes (35%), as well as *RAS*-pathway genes (12%) and epigenetic regulators (8%).

Among non-DS patients no coexisting mutations were observed in fusion-positive patients. DS-like patients with acquired *GATA1* mutations carried 1-2 cooperating mutations in *RAS* and *JAK/STAT* pathways.

Two years OS in AMKL patients was 66%, *CBFA2T3::GLIS2* correlates with poor prognosis (OS = 37%), while complex karyotype OS = 85%.

Conclusion: Pediatric AMKL is a heterogeneous disease comprised of distinct genetically subsets with varying outcomes.

References:

Grants:

Conflict of Interest: None declared.

EP13.025 The burden of microsatellite instability in the blood of constitutional mismatch repair deficiency syndrome is associated with patient genotype but not age of tumour onset

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Background/Objectives: Constitutional mismatch repair deficiency (CMMRD) is a very rare recessive childhood cancer syndrome caused by germline variants in mismatch repair (MMR) genes. Microsatellite instability (MSI) in non-neoplastic tissues is a CMMRD diagnostic hallmark[1]. Investigations of associations between constitutional MSI-burden and a patient's genotype and phenotype have been limited by methodology and cohort size. We explored these associations by quantifying blood-MSI in a relatively large series of CMMRD patients, using novel MSI markers selected for instability in blood rather than tumours.

Methods: Three CMMRD, one Lynch syndrome, and two control bloods were genome sequenced to >120x depth. Microsatellite amplicon sequencing[1] was used to analyse a pilot cohort of eight CMMRD and 38 control bloods, a blinded cohort of 56 CMMRD and 43 control bloods, and 80 reference control bloods.

Results: Thirty-two novel mononucleotide repeats were selected for their instability in blood using genome sequence and pilot amplicon sequence data. MSI analysis of the blinded cohort, plus 80 reference controls for MSI scoring[1], gave complete discrimination between groups, with a larger MSI score separation than with tumour-derived markers. Blood-MSI scores were reproducible ($R = 0.994$, $p < 10^{-15}$). Lower scores were associated with MSH6 variants ($p = 0.001$) and VUS ($p = 0.0007$), but did not correlate with age at first tumour ($p = 0.287$) or age at sampling ($p = 0.100$).

Conclusion: Constitutional MSI-burden is associated with CMMRD patients' genotypes, but no phenotype association was found. The new blood-MSI markers enhance microsatellite sequencing as a diagnostic assay for CMMRD.

References: 1 Gallon et al. 2019 https://doi.org/10.1002/humu.23721.

Grants:

Cancer Research UK C569/A24991.

Conflict of Interest: Richard Gallon Cancer Research UK C569/A24991, Inventor on patent filing covering assay markers, Christine Hayes: None declared, Rachel Phelps: None declared, Laurence Brugières: None declared, Chrystelle Colas: None declared, Martine Muleris: None declared, Katharina Wimmer: None declared, John Burn Cancer Research UK C569/A24991, Inventor on patent filing covering assay markers, Michael S. Jackson Cancer Research UK C569/A24991, Inventor on patent filing covering assay markers, Mauro Santibanez Koref Cancer Research UK C569/A24991, Inventor on patent filing covering assay markers.

EP13.026 Beyond BRCA: mutational landscape of a cohort of 186 patients with hereditary breast and ovarian cancer

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Background/Objectives: Pathogenic Variants (PV) are only identified in approximately 10% of patients with Hereditary Breast and Ovarian Cancer (HBOC) syndrome, mostly in *BRCA1* or *BRCA2*. The purpose of this study was to evaluate the diagnostic rate in a cohort of 186 individuals admitted to the Istituto di Genetica Medica of Udine for genetic testing, using of a custom multigene panel coupled to a dual variant assessments based on ACMG/CanVIG-UK classification and AI-based structural prediction.

Methods: NGS sequencing was performed on an Ion Torrent platform using an Ion Ampliseq custom panel of 18 genes. The structures generated by the AlphaFold algorithm were used as structural models and the score in the obtained position specific score matrix (PSSM) was taken as a bona-fide indication of pathogenicity.

Results: The majority of pathogenic and probably pathogenic mutations were found in non-BRCA genes (61.90%). VUS were subdivided into five subclasses, each of which is associated with a probability of being reclassified as pathogenic. Molecular modeling of 12 VUS highlighted that the most deleterious values were assigned to mutations capable of disrupting a disulfide bridge or modifying intra-domain contacts.

Conclusion: We achieved an overall yield of positive results of 11.3%.The genes with the highest prevalence of positive results were *ATM* (9.8% of all PV), *BRCA1* (13.7% of all PV), *BRCA2* (11.8% of all PV), *CHEK2* (13.7% of all PV). Our data demonstrate that integration of ACMG and AI-based structural prediction allows better evaluation of variants that affect the inner structure of the mutated protein.

References:

Grants:

Conflict of Interest: None declared.

EP13.027 Transcriptomic profile of the metabolic status in endometrial cancer

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Background/Objectives: Aberrant metabolism has been identified as a main driver of cancer. Here we establish a transcriptomic profile of metabolism-related pathways in endometrial cancer (EC) using a novel method, NanoString nCounter Technology, to comprehensively characterize the EC metabolic status.

Methods: 57 ECs and 30 normal endometrial specimens were studied using the NanoString Metabolic Panel (Seattle, Washington, USA), further validated by qRT-PCR (Roche, Basel, Switzerland). To examine the functions of the identified differentially expressed genes (DEGs), Gene Ontology (GO) and Gene Set Enrichment Analysis (GSEA) were performed. Statistical analyses were by GraphPad PRISM and Weka software.

Results: 11 DEGs in EC were identified (Fig. 1), all implicated in cancer metabolism. GO showed their direct association with 'central carbon metabolism in cancer'. GSEA identified 'MTORC1 signaling', 'glycolysis', and 'hypoxia' pathways as the most significantly associated with EC. In order to distinguish ECs from controls, the logistic regression-based diagnostic classifier incorporating two genes: *CXCL9* and *HAAO* demonstrated a higher diagnostic value (AUC = 0.82) than the highest AUC found for any separate DEG (for *SLC7A5*, AUC = 0.67). Drug repurposing applied to the metabolism-related transcriptomics in EC revealed 10 new candidate drug compounds.

Conclusion: Central carbon metabolism in cancer, including MTORC1 signaling, glycolysis, and hypoxia, creates the principal metabolic axis in EC.

References: Not included.

Grants: Data were generated by the Centre for Artificial Intelligence of the Medical University of Białystok, Poland. Supported by the Polish Ministry of Education and Science within the project 'Excellence Initiative-Research University'.

Conflict of Interest: None declared.

EP13.028 Highly sensitive Liquid Biopsy Duplex Seq enables molecular diagnosis of a 10 year old child with clinically confirmed overgrowth syndrome in plasma

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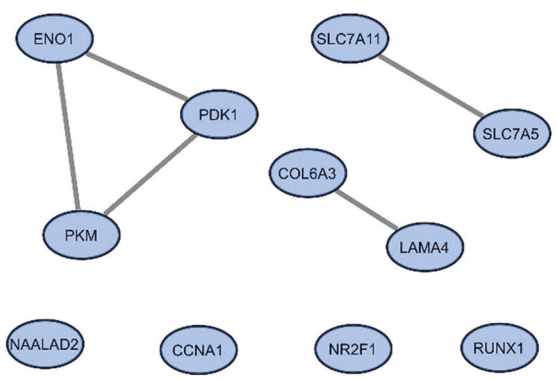
Background/Objectives: Liquid Biopsy is a promising approach for identification a patient´s mutational profile in plasma, but accurate detection of variants at low frequencies is challenging. We established Liquid Biopsy Duplex Sequencing, enabling highly sensitive analysis of variants associated with overgrowth syndrome and tumor disease. Here we present its application in clinical use for elucidating a molecularly unsolved case.

Methods: Somatic mutation profiles of 33 genes were obtained from plasma samples using NGS Duplex Sequencing.

Results: Analytical validation of the Liquid Biopsy Duplex Sequencing panel showed 98% sensitivity and 90% precision for variants with 0.5% allele frequency in a total of 33 genes.

The Liquid Biopsy panel was applied for molecular diagnosis of a 10 year old child with a clinically diagnosed asymmetric overgrowth syndrome including arteriovenous malformations limited to one side of the body. Somatic *KRAS* c.35G>A, p.(Gly12Asp) variant was detected in plasma with 1% variant allele frequency.



Gene Symbol	Gene Name
SLC7A11	*Solute carrier family 7 member 11*
SLC7A5	*Solute carrier family 7 member 5*
RUNX1	*Runt-related transcription factor 1*
LAMA4	*Laminin subunit alpha 4*
COL6A3	*Collagen type VI alpha 3 chain*
PDK1	*Pyruvate dehydrogenase kinase 1*
CCNA1	*Cyclin A1*
ENO1	*Enolase 1*
PKM	*Pyruvate kinase M1/2*
NR2F1	*Nuclear receptor subfamily 2 group F member 1*
NAALAD2	*N-acetylated alpha-linked acidic dipeptidase 2*

Fig. 1 The DEGs' network rendering using the STRING database.

Notably, the variant was not detected in a parallel analysis of a skin biopsy sample.

The *KRAS* c.35G>A, p.(Gly12Asp) variant leads to constitutive overactivation and increased signal transduction into downstream pathways and is associated with overgrowth including various types of congenital nevi and vascular malformations (so-called mosaic RASopathies). Consequently, detection of *KRAS* c.35G>A, p.(Gly12Asp) variant in plasma could molecularly explain the clinically observed overgrowth syndrome.

Conclusion: Liquid Biopsy Duplex Sequencing pushes the boundaries for detection of low frequency variants in plasma. Our broad Duplex Sequencing panel enables highly sensitive screening of all therapy relevant variants for overgrowth syndrome.

References: none.

Grants: none.

Conflict of Interest: None declared.

EP13.029 Insights into somatic mutations associated with oral leukoplakias

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Background/Objectives: Oral leukoplakias (OL) are clinically recognizable precancerous conditions caused due to tobacco and/or areca nut chewing. Up to 40% of the OL cases progress to cancer within one year after diagnosis, whereas remaining continue to have an increased risk. Till date, there are no genome-scale studies that defined OL-specific mutations at hyperplastic stage, representing the early stages of precancerous conditions to understand the molecular events associated with increased cancer susceptibility.

Methods: In this study, we sequenced the exomes of five OLs and paired peripheral blood monocytes. In addition, we sequenced the transcriptomes of three pairs of affected and surrounding normal tissues to identify transcripts carrying OL-specific mutations.

Results: The pair-wise analysis revealed the affected genes carrying mutation signatures associated with age, tobacco chewing, defective mismatch repair, and DNA double-strand break repair. Transcriptome comparisons with the exome data indicated the expression of 54% of genes with pathogenic mutations participating in pathways such as Focal adhesion and ECM-receptor interactions. This study also yielded 111 novel pathogenic mutations in genes participating in ubiquitin mediated proteolysis and base-excision repair. A comparison of the driver genes with mutations and copy number alterations suggested a possible lack of evidence of increased driver gene mutation burden in progressive over the non-progressive forms.

Conclusion: In summary, this first report on molecular profiling of hyperplastic leukoplakias makes a beginning towards understanding the early molecular events leading to oral carcinogenesis.

References:

Grants: This work was supported by funds from BITS-Pilani under the Centre for Human Disease Research program.

Conflict of Interest: None declared.

EP13.030 Expression levels of isoform I and II of NF1 gene in tissue of patients affected by neurofibromatosis type 1

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Background/Objectives: Neurofibromatosis type 1 is a multisystemic disease with high risk of tumor development. NF1 gene encodes for different isoforms. Type I isoform is more expressed in neurons while type II in glial cells and peripheral nerves. Several studies suggested that alterations in isoform II / I ratio can be associated with the development of malignancies [1]. The aim of the project is to investigate an association between the expression levels of NF1 isoforms and the development and progression of cancer.

Methods: qReal-time PCR was used to determine expression levels of NF1 isoforms in NF1-related and in sporadic tumors. Statistical analysis was performed using t-test.

Results: Isoform type I was more expressed in sporadic glioma while isoform type II was prevalent in NF1-related glioma. The isoform II / I ratio showed a predominance of type I isoform in sporadic low grade glioma, while neurofibromas and NF1-related high/ low grade glioma showed a predominance of type II.

Conclusion: Our study highlights an increase of expression of isoform II levels in NF1-related tumors thus suggesting that an alteration of the alternative splicing in favor of the expression of isoform II could play a role in the progression of cancer and be used as a potential tumor marker.

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Grants:

Conflict of Interest: None declared.

EP13.031 Novel HOXB13 variant identified in Galician prostate cancer patients

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Background/Objectives: Recent studies have associated *HOXB13* gene with a significantly increased risk of hereditary prostate cancer, specifically with the variant HOXB13 (NM_006361.6): c.251G>A; p.(Gly84Glu). A high frequency of this variant was identified in Nordic populations, suggesting a founder effect (1). Founder mutations have been previously identified in Galician population (2,3). Here we aimed to study *HOXB13* gene in Galician prostate cancer patients.

Methods: We studied the *HOXB13* gene in 200 Galician patients diagnosed with high-risk prostate cancer using next-generation sequencing technologies. Variant detection, annotation and classification was performed through the use of GATK best practices workflows, ANNOVAR and ACMG guidelines respectively.

Results: A novel *HOXB13* variant was identified in three unrelated patients: HOXB13 (NM_006361.5): c.853T>C; p.(*285Glnext*32). Two of the three patients have reported a family history of prostate cancer, one of these two had an early onset disease. This variant has not been described in population-based databases before.

The variant HOXB13 (NM_006361.6): c.251G>A; p.(Gly84Glu) was found in one patient.

Conclusion: We identified *HOXB13* mutations in 2% of the high-risk prostate cancer patients from Galicia. Segregation data for the novel HOXB13: c.853T>C; p.(*285Glnext*32) variant will shed light on its implication in prostate cancer development.

References: 1. Xu et al,2013. *Human Genetics*. PMID:23064873.
2. Vega et al,2001. *Human Mutation*. PMID:11385711.
3. Fachal et al,2012. *PLOS ONE*. PMID:22511925.

Grants: PI19/01424,INT20/00071 granted by Spanish Instituto de Salud Carlos III (ISCIII), an initiative of the Spanish Ministry of Economy and Innovation partially supported by European Regional Development FEDER Funds, and through the Autonomous Government of Galicia (Consolidation and structuring program: IN607B).

Conflict of Interest: None declared.

EP13.032 Germline genetic variants that affect Wnt signaling as potential cause of serrated polyposis

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Background/Objectives: Extensive efforts have been made to elucidate the inherited factors that predispose to serrated/hyperplastic polyposis (SP), a heterogeneous disease associated with significant personal and familial CRC risk. We aimed to identify additional inherited risk factors by performing exome sequencing in 44 non-related SP cases.

Methods: We selected rare, predicted damaging variants affecting genes involved in DNA repair, TGF-β and Wnt pathways, and performed pathway- and gene-based burden tests. TOP-FOP reporter assays were carried out to test the functional effect on Wnt signalling of the identified candidate genes.

Results: Pathway-based burden analysis comparing the frequency of damaging and predicted pathogenic variants in cases vs. controls identified significant differences only in Wnt pathway components (50%, 44/88 SP patients; 36.12%, 42,692/118,190 controls; $p < 2.2x10-16$). We identified (predicted) damaging variants in 34 Wnt-related genes. The pathway-based analysis performed in 1,006 familial/eoCRC patients not selected for polyposis showed no enrichment of Wnt-related (predicted) damaging variants in nonpolyposis CRC patients compared to controls. A gene-based burden analysis revealed that 11 Wnt-related genes harboured more germline (predicted) damaging variants in SP cases than in controls. TOP/FOP dual luciferase assays were performed to assess the functional inhibitory effect on Wnt signalling of the candidate genes, and the reversion of this inhibition in the presence of the identified variants. This effect was confirmed, in addition to RNF43 (known SP predisposing gene), for 6 additional Wnt-related genes, all of them negative regulators of Wnt signalling.

Conclusion: Germline mutations in negative regulators of Wnt are associated with serrated polyposis predisposition.

References:

Grants:

Conflict of Interest: None declared.

EP13.033 miR-135b expression as a predictor of poor survival in tongue cancer patients

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Background/Objectives: Oral cavity cancer is one of the most aggressive and leading causes of death among cancers of head and neck region. The tongue is the most common location of cancer inside oral cavity. There is a strong need to identify potential prognostic biomarkers and micro RNAs (miRNAs) have promising characteristics of good candidates. miR-135b is up-regulated in numerous cancers and thus considered as an oncomir. This study aimed to investigate the prognostic role of miR-135b in patients with tongue cancer.

Methods: Tumor tissues from 25 patients with tongue cancer were obtained for RNA isolation. After cDNA synthesis, the expression of miR-135b was estimated by TaqMan technology using qRT-PCR. Relative expression of miR-135b, normalized to RNU6B, was calculated and reported as $2^{-\Delta Ct}$. The expression of miR-135b was dichotomized as high or low according to the median. Association of miR-135b expression and survival was analyzed by Kaplan-Meier curves.

Results: Increased expression of miR-135b was observed in 14 (56%) tongue cancer patients. These patients had significantly shorter overall survival compared to patients with low miR-135b expression ($p = 0.043$, log-rank test). The mean overall survival was 19.6 months in patients with highly expressed miR-135b versus 32.2 months in the miR-135b low expressed group. None of the clinicopathological features were associated with miR-135b expression.

Conclusion: Highly expressed miR-135b might be considered as a potential biomarker of poor overall survival in patients with tongue cancer.

References: /.

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Conflict of Interest: Goran Stojkovic University Clinical Center of Serbia, Clinic for Otorhinolaryngology and Maxillofacial Surgery, Belgrade, Serbia.

Faculty of Medicine, University of Belgrade, Belgrade, Serbia, Katarina Zeljic Faculty of Biology - University of Belgrade, Belgrade, Serbia, Ministry of Education, Science and Technological Development of the Republic of Serbia, number: 451-03-68/2022-14/200178.

EP13.034 Differential gene expression analysis of tongue and floor of the mouth tumors: development of a predictive model

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Background/Objectives: This study aimed to perform differential gene expression analysis of tongue and floor of the mouth carcinomas and identify key deregulated genes associated with carcinogenesis and disease evolution.

Methods: RNA-Seq data from 213 patients located in the tongue or floor of mouth was retrieved from The Cancer Genome Atlas (TCGA). The genes that were differentially expressed between the two anatomical locations were determined by applying linear regression to each gene using the *limma* package. From these genes, those with $p < 0.001$ were kept for further analysis by logistic regression.

Results: After pre-processing, 121 genes were identified as differentially expressed between the two anatomical locations, with $p < 0.001$. A logistic regression model with capability to differentiate the tumors between the two anatomical location was built, encompassing 17 genes differentially expressed: *ASPA, CDKN2A, DNPEP, FMO2, GPD1, GTPBP3, HOXB8, LOC340508, MGC45800, SLC19A1, SLC3A2, SNX15, SORCS1, TAC4, ZNF788, PUF60* and *CPSF1*. This model showed an overall accuracy of 89.7%, with the null model's accuracy being 71.4%. The area under the ROC curve was 0.957. All the 17 genes except for *GPD1* are significant ($p < 0.05$) for the distinction between the two anatomical locations.

Conclusion: Our results proved the presence of different gene expression signatures according to tumors location, which will help in the identification of key biological pathways and a better understanding of the specific molecular mechanisms of carcinogenesis and disease evolution in the tongue and floor of the mouth and ultimately in the pursuit of precision medicine for these patients.

References:

Grants:

Conflict of Interest: None declared.

EP13.035 Exploring the expression profiles of TRAIL apoptotic pathway in breast cancer as potential prognostic biomarkers

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Background/Objectives: Currently research efforts focus on the development of new therapeutic approaches for breast cancer patients who are ineligible/unresponsive to the existing treatments. Therapies targeting TRAIL apoptotic pathway, which is selectively activated in cancer cells, constitute an emerging field for clinical research[1]. However, there is an unmet need for suitable predictive biomarkers for patient stratification, in order to maximize the efficiency of TRAIL-targeting therapeutic molecules.

Methods: 94 breast cancer tissues were examined for relative mRNA levels of TRAIL pathway genes (*TRAIL, DR4, DR5, DcR1, DcR2, BCL2, BCL-XL, MCL1*) by RT-PCR/ΔΔCt method. Activation of caspases 3/8 was evaluated by IHC in a subset of cases. Statistical analysis was performed to determine clinicopathological associations.

Results: A variation of expression levels among the examined genes was observed; most of the cases (31-64%) displayed reduced mRNA levels while elevated levels were present in 8-16% of the cases. Multiple mRNA co-expression patterns emerged, with BCL-XL/MCL1 demonstrating the strongest linear correlation ($R = 0{,}723$, $p < 0{,}001$). Clinicopathological characteristics (age, pPrognostic stage, molecular subtype) displayed statistical correlations with relative mRNA levels of various examined genes. Notably, DR4/5, DcR1/2, cFLIP, BCL2, MCL1 elevated mRNA levels were statistically correlated with pPrognostic Stage I ($p < 0{,}05$).

Conclusion: Our results delineate deregulation of TRAIL pathway in breast cancer, indicating complex regulation mechanisms and different mRNA expression profiles of the examined genes. The correlation of relative mRNA levels with clinicopathological parameters supports their importance as potential predictive biomarkers for TRAIL targeting therapies.

References: Dianat-Moghadam H. *et al.Pharmacol. Res.* (2020).https://doi.org/10.1016/j.phrs.2020.104716.

Grants: No funding.

Conflict of Interest: None declared.

EP13.036 The database of variants of nucleotide sequence associated with hereditary cancer syndromes

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Background/Objectives: More than 500 thousand new cases of malignant neoplasms are registered annually in the Russian Federation. Among them more than 50 thousand cases are hereditary. Early diagnosis will allow detecting tumors at the initial stage, including those in mutation carriers, to provide preventive measures and early treatment, as well as to use advanced highly effective targeted drugs.

Methods: The study provided 1860 samples obtained from cancer patients, including those with breast cancer, ovarian cancer, colorectal cancer, small bowel cancer, gastric cancer, pancreatic cancer, thyroid cancer, renal cell cancer, melanoma, etc. DNA libraries were prepared using a custom KAPA Hyper probe panel (Roche) included probes for targeted enrichment of the coding sequences of 44 genes. NGS was performed on the MiSeq platform (Illumina).

Results: 152 pathogenic variants of the nucleotide sequence were detected in 278 patients in the *BRCA1, BRCA2, PALB2, MLH1, EPCAM, MUTYH, TP53, ATM, BARD1, MSH6, NBN* genes. Also 1118 variants of the nucleotide sequence with unknown clinical significance were found. A local database of genetic variants was created, also containing clinical data, 6252 variants of the nucleotide sequence have been added to the database.

Conclusion: As the database is updated with new sequencing data, further statistical and clinical profile of the detected genetic variants will be performed. The accumulated data can be used both to analyze the epidemiology of oncological diseases and to devise tests that are relevant for the Russian population.

References:

Grants: The study was done with a support of the state assignment №№ 388-00102-20-01/388-00154-21-00.

Conflict of Interest: None declared.

EP13.037 Germline genetic alterations associated with multiple primary malignant neoplasms

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Background/Objectives: The proportion of multiple primary malignant neoplasms (MPMN) in cancer is 8-9%. The reasons for the development of subsequent tumors during the life are unfavorable environmental factors, the occurrence of chemo- or radiation therapy during the first tumor treatment, as well as hereditary predisposition.

Methods: The study provides results of 118 patients diagnosed with MPMN, including breast cancer, ovarian cancer, fallopian tube cancer, colorectal cancer, small bowel cancer, gastric cancer, pancreatic cancer, thyroid cancer, salivary gland cancer, renal cell cancer, melanoma, basalioma, Hodgkin's lymphoma, Ewing's sarcoma, etc. DNA was extracted from peripheral blood lymphocytes. DNA libraries were prepared using a custom KAPA Hyper probe panel (Roche). High-throughput DNA sequencing was performed on the MiSeq platform (Illumina).

Results: 32 pathogenic variants of the nucleotide sequence related to the coding regions of the genes *BRCA1, BRCA2, CHEK2, DICER1, EPCAM, MLH1, MSH6, MUTYH, PALB2, TP53, VHL* were detected in 30 patients. Two patients with MPMN showed a combination of two mutations previously described as pathogenic: c.5503C>T, p.Arg1835Ter, rs41293465 in *BRCA1* gene, c.172_175del, p.Gln60ArgfsTer, rs180177143 in *PALB2* gene; and c.1421_1422dupGT, p.Gln475CysfsTer, rs63750854 in *MSH6* gene, c.7879A>T, p.Ile2627Phe, rs80359014 in *BRCA2* gene, respectively.

Conclusion: Multigene panel testing allows the most complete assessment of the contribution of genetic factors to the development of the second primary tumors. The accumulation of knowledge in this area will help to assess the risk and prevent the development of second tumors, primarily by detecting mutations associated with hereditary cancer syndromes.

References:

Grants: The study was done with a support of the state assignment №№ 388-00102-20-01/388-00154-21-00.

Conflict of Interest: None declared.

EP13.038 First genetic study of constitutional MMR deficiency (CMMRD) syndrome in Tunisia

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Background/Objectives: Patients with bi-allelic germline mutations in mismatch repair (MMR) genes develop constitutional MMR deficiency (CMMRD), a rare but severe variant of Lynch syndrome. This pathology is distinguished by the presence of early-onset colorectal cancers, lymphomas or leukemias, and brain tumors [1].

Most of the mutations associated with CMMRD are located in the *PMS2* gene followed by *MSH6*, and more rarely *MLH1* and *MSH2* [2].

A genetic study on this syndrome is carried out for the first time in a Tunisian patient, in order to confirm his affection by this pathology.

Methods: Next Generation Sequencing (NGS) was performed in one patient suffering from CMMRD syndrome.

Results: For this patient, we identified a missense variant; p.P640T (c.1918 C>A) at a homozygous state detected in exon 17 of the *MLH1* gene. This variant was subsequently confirmed by Sanger sequencing. It's a missense variant that was predicted as highly pathogenic.

Conclusion: Our results confirm that CMMRD is caused by homozygous germline mutation in one of MMR genes. Performing functional studies is our outlook to confirm its pathogenicity.

References: [1] Bruno Buecher and al. CMMRD syndrome (constitutional deficiency of MMR genes): genetic bases and clinical aspects. 2018.

[2] Felton KEA and al. Constitutive deficiency in DNA mismatch repair. Clin Genet. 2007.

Grants:

Conflict of Interest: None declared.

EP13.040 Evaluation of breast cancer awareness and perception among women in Albania

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Background/Objectives: Despite the intensive breast cancer awareness campaign for the importance of early detection, breast cancer remained the leading cause of cancer death among Albanian women. The aim of this study is to understand the knowledge about this disease, the understanding of early symptoms as well as attitudes on genetic and diagnostic testing methods among women.

Methods: The validated questionnaire provided three main groups of questions: regarding the demographic characteristics; concerning knowledge of breast cancer and heredity; concerning their attitude and perception of early diagnostics tools and management. Women from different regions of Albania were interviewed face to face by a trained molecular biologist using the questionnaire. The data pre-processing step and statistical analysis were performed in SPSS 26.

Results: Four hundred and thirty-five women, mean age 34.9, SD 1.3 (age from 20-60 years old), has responded to questionnaire. The percentage of women knowledge about the genes related breast cancer was 49.4. There is a statistically significant relationship between age and gene recognition ($X2\ (4) = 11.89$; $p = 0.018$). About 60% of our participants had university level of education. Their response showed confused and misunderstanding about the symptoms and early detection.

Conclusion: Based on the above data, dissemination of knowledge & achievements to the society and education activities with interest for future young generation, is needed.

References: Cobain EF, Milliron KJ, Merajver SD, Updates on breast cancer genetics: clinical implications of detecting syndromes of inherited increased susceptibility to breast cancer. Semin Oncol 2016;43:528-35. https://doi.org/10.1053/j.seminoncol.2016.10.001 pmid: http://www.ncbi.nlm.nih.gov/pubmed/27899183.

Grants: N/A

Conflict of Interest: None declared.

EP13.042 A rare case of familial cancer predisposition syndrome

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Background/Objectives: Mismatch Repair Cancer Syndrome 3 (MMRCS3; OMIM 619097) is a rare autosomal recessive childhood cancer predisposition syndrome characterised by the development of hematologic malignancies, brain or central nervous system and gastrointestinal cancers, although embryonic tumors and rhabdomyosarcoma have been reported. Non-malignant features, in particular manifestations reminiscent of neurofibromatosis type 1 (NF1; OMIM 162200), mainly café-au-lait spots, as well as pre-malignant and non-malignant lesions, such as adenomas or polyps are frequently present before malignancy development.

Methods: The authors present the case of a 7 years old boy with MMRCS3, diagnosed by MiSeq-NGS technology and Illumina TruSight One Sequencing Panel.

Results: The patient had 3 deceased young brothers with similar hematological malignancy. The physical examination revealed multiple café-au-lait spots and superior vena cava syndrome with collateral circulation, swelling on the anterior thorax and dyspnea. The etiology of the oncologic diagnosis of mediastinal T lymphoblastic lymphoma was a homozygous frameshift variant (c.3261dupC) in *MSH6* gene (OMIM 600678) on chromosome 2p16.3.

Conclusion: The case presented is part of the category of rare familial cancer predisposition syndromes and highlights the importance of the early genetic counceling not only in achieving the correct diagnosis but also in explaining the multitude of symptoms and finding out about the possible comorbidities and prognosis.

References: Bakry D, Aronson M, Durno C et al. Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. Eur J Cancer. 2014;50:987–96.

Grants: None.

Conflict of Interest: None declared.

EP13.044 Casuistry of Haematology Analysis in CGC from 2020 and 2021

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Background/Objectives: In CGC, we receive bone marrow or peripheral blood samples for unstimulated karyotype and/or FISH analysis. This classical approach still represents an important contribution for the diagnosis and classification of haematological diseases, their prognosis, decisions regarding the most adequate therapy and consequent patient follow-up, being mandatory for some diseases.

We present a review of the samples received for haematological analysis in 2020/2021, with a total of 1033 patients with successful analysis. Main indications are suspicion of Multiple Myeloma (MM) and Myelodysplastic Syndrome (MDS).

Methods: The karyotype analysis was performed in metaphases stained with GTG bands from non-stimulated cultures of bone-marrow or peripheral blood.

FISH analysis was performed in nuclei hybridized with Vysis Fish probes, using Metasystem software.

Results: 492 and 541 patients were reviewed from 2020 and 2021, respectively, distributed by MM (91+107), MDS (195+204) and other referrals. An abnormal finding was identified in a total of 339 patients (175+164). From these, 68 in MM and 94 in MDS.

Conclusion: From 1033 patients, an abnormal finding was detected in 33% cases, with 34 % detected in MM and 24% in MDS.

With karyotype alone, only 167 patients (16%) had an abnormal result, but combining the two techniques, 339 had an abnormal finding identified.

In conclusion, despite their limitations, integrating unstimulated karyotyping and FISH analysis remains an added value in the diagnosis of haematological diseases, making an important contribution to a patient's diagnosis and consequent follow-up guidance, continuing to be mandatory in some malignancies.

References: https://doi.org/10.1038/gim.2016.50; https://doi.org/10.1038/s41375-019-0378-z.

Grants:

Conflict of Interest: None declared.

EP13.046 Single center experience in genetic testing of suspected MEN1 and MEN2 syndromes

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Background/Objectives: Multiple endocrine neoplasia type 1 and 2 (MEN1 and MEN2) syndromes are genetic predisposition of various endocrine system tumours like thyroid cancer, pheochromocytoma, parathyroid tumours and others. Early detection of such syndromes allows better management as early screening and interventions are possible.

Methods: We report the experience of Hospital of Lithuania Health Science University Kauno klinikos in genetic testing of suspected MEN1 and MEN2 cases in a time period between 2016 - 2021. Sanger sequencing of *RET* (NM_020975.4) or/and *MEN1* (NM_130799.2) gene coding regions was performed.

Results: 18 patients were referred to geneticist due to medullary thyroid cancer. The mean age at which they developed cancer was 48.8. In our study 3 cases had pathogenic variants in *RET* gene c.1833C>G; c.1900T>C, c.2753T>C and were diagnosed with first cancer at 31,37 and 16 years of age, respectively.

9 cases of pheochromocytoma, with mean age at diagnosis 42.3, had no pathogenic variants in RET gene.

A case of hyperparathyroidism in 14 years old patient showed no pathogenic variants in *RET* and *MEN1* genes.

One female patient was referred to due to early prolactinoma at 30 years of age. Later on she was also diagnosed with

insulinoma and hyperparathyroidism. She had a novel c.446-2A>T variant in *MEN1* gene which on RNA Sanger sequencing showed exon skipping and was classified as pathogenic.

Conclusion: In 5 years we have diagnosed 4 MEN syndrome cases what lead to better understanding of patient secondary tumour risk and the need of family screening.

References:

Grants:

Conflict of Interest: Marius Šukys Hospital of Lithuanian University of Health Sciences, Darius Čereškevičius Hospital of Lithuanian University of Health Sciences, Rimvydas Jonikas Hospital of Lithuanian University of Health Sciences, Zivile Zemeckiene Hospital of Lithuanian University of Health Sciences, Inga Nasvytienė Hospital of Lithuanian University of Health Sciences, Sandra Žėkienė Hospital of Lithuanian University of Health Sciences, Kristina Aleknavičienė Hospital of Lithuanian University of Health Sciences, Rasa Ugenskiene Hospital of Lithuanian University of Health Sciences.

EP13.048 Cancer risk and genotype-phenotype corellation in pediatric patients with Basal cell nevus syndrome (Gorlin syndrome)

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Background/Objectives: Gorlin syndrome is a rare hereditary condition caused by mutations in *PTCH1/2* and *SUFU* genes. Patients with Gorlin syndrome are at high risk for development of benign and malignant tumors. The aim of our study is to describe a correlation between genotype and age of manifestation of different tumor types.

Methods: Five patients with Gorlin syndrome were studied: 1 boy and 4 girl aged 4-17 years. All patients had different tumor types: odontogenic keratocysts (n = 2), medulloblastoma (n = 2), basal cell carcinoma (n = 2), ovarian fibroma (n = 1). Three patients had multiple primary tumors. Next-generation sequencing (NGS) was performed using multigene panel, which included coding regions of 415 cancer-associated genes.

Results: Heterozygous mutations in *PTCH1* or *SUFU* genes were revealed in all patients. A 12-year-old boy with mutation c.3450-1G>A in *PTCH1* was diagnosed with odontogenic keratocysts at age 10 and basal cell carcinoma at age 11. A 12-year-old girl with a *PTCH1* c.2198C>G mutation had a basal cell carcinoma at age 11. A 17-year-old girl with *PTCH1* mutation c.199-1G>C had a neck cyst at age 3 and odontogenic keratocysts at age 17. A 4-year-old girl with *PTCH1* c.3206G>A mutation developed medulloblastoma at 8 months. Her mother was a mutation carrier with ovarian fibroma at age 20. A 13-year-old girl with *SUFU* mutation c.915_916del was diagnosed with medulloblastoma at the age of 1,7 and with bilateral ovarian fibroma at the age of 11.

Conclusion: An understanding of genotype-phenotype corellation in patients with Gorlyn syndrome is important to optimize the management strategy in order to reduce cancer risk.

References:

Grants:

Conflict of Interest: None declared.

EP13.049 Evaluation of angiogenesis in head and neck cancer cell lines treated with bevacizumab and paclitaxel

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Background/Objectives: Cancer stem cells (CSCs) present metastasis, drug resistance, and tumor recurrence in head and neck cancer (HNC). Tumor growth is angiogenesis-dependent and activated by vascular endothelial growth factor (VEGF). Bevacizumab and Paclitaxel have shown induce antiangiogenic activity leading to inhibition of cell growth. The study goals were to evaluate the angiogenesis activity in both CSCs, and non-CSCs from SCC-28 and FADU HNC cells line treated with Bevacizumab and Paclitaxel.

Methods: CSCs and non-CSCs were isolated from SCC-28 and FADU HNC cells line by flow cytometry using Aldehyde dehydrogenase (ALDH1) antibodies, followed by spheres-formation, invasion and migration assay, VEGFA gene, and protein expression. Angiogenesis assay was performed in CSCs and non-CSCs from both HNC cells line treated with Paclitaxel and Bevacizumab separately and in combination by counting branch points (BP).

Results: CSCs from SCC-28 presented a greater formation of spheres than non-CSCs (p = 0,003). CSCs had greater invasiveness p = 0.0192, p = 0.0036 and migration p = 0.0287, p < 0.0001 respectively for SCC-28 and FADU cell lines. The gene expression of VEGFA was higher in CSCs of the two strains p < 0.0001. Protein expression was 1.34 and 1.71 times higher in the CSCs group than in the non-CSCs of SCC-28 and FADU. Bevacizumab had better efficiency in lowering BP when combined with Paclitaxel than the HNC cell line treated using only one drug.

Conclusion: The combination of bevacizumab with paclitaxel shows be more effective as a possible HNC therapy.

References:

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Conflict of Interest: Gabriela Helena Rodrigues-Fleming CAPES(Financial code 001), Bianca Barbério Bogdan Tedeschi CAPES(Financial code 001), Marcia Maria Urbanin Castanhole-Nunes full: Foundation of Faculty of Medicine of São José do Rio Preto -FUNFARME/FAMERP, Juliana Garcia Oliveira-Cucolo: None declared, Marlon Fraga Mattos FAPESP (Process N.2018/24825-2), Ana Paula Simedan Villa CAPES(Financial code 001), Erika Cristina Pavarino: None declared, Eny Maria Goloni-Bertollo FAPESP(Process N. 2018/26166-6.

CNPq(Process N. 310987/2018-0).

EP13.050 Comprehensive genomic analysis of primary triple negative breast cancer

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Background/Objectives: Triple negative breast cancer defined by negative ER/PR/HER2 expression is highly heterogeneous. Chemotherapy in a neoadjuvant setting remains a standard of treatment. Genomic alterations are extensively investigated to

determine the drivers of tumor evolution, new treatment targets, and to identify the ways of resistance to therapies.

Methods: 88 patients with stage II – III triple negative breast cancer were assigned to receive neoadjuvant chemotherapy with paclitaxel (80 mg/m^2) and carboplatin (AUC 1.5-2) 12 cycles weekly followed by 4 cycles of AC (doxorubicin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2) 3-weekly. Hybrid capture-based next-generation sequencing for genomic profiling of DNA from formalin-fixed paraffin-embedded (FFPE) primary tumor tissue obtained prior to the treatment was performed by FoundationOne®CDx.

Results: The median age of 88 patients was 55 years (ranged 32 - 73 years). One or more mutations were found in all cases. The number of detected gene mutations was an average of 5.6 (range 1 - 18 per case). The most frequent somatic mutations were ***TP53* 94.3%** (83 of 88), ***PIK3CA* 18.2%** (16 of 88), ***PTEN* 14.8%** (13 of 88), *RAD21*, *MYC*, *NSD3*, *NF1*, *FGFR2, CCNE1*, *AKT2*, and other genes. Somatic *BRCA1* mutation was found in 16 cases of 88 (18.2%), while somatic *BRCA2* mutation – in 6 cases (6.8%).

Conclusion: Comprehensive genomic profiling of primary triple negative breast cancer identifies potentially actionable mutations in a large set of tumors and might be clinically important for treatment individualization.

References:

Grants: This study was supported by National Cancer Institute and F. Hoffmann – La Roche Ltd.

Conflict of Interest: None declared.

EP13.052 Correlation between HPV DNA testing and cytological examination of cervical samples

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Background/Objectives: Human papilloma virus (HPV) is part of a large family of double stranded DNA viruses, with more than 200 haplotypes and is one of the most common sexually transmitted viruses [1,2]. Despite the fact that there are many screening programs and detection methods still remains one of the most common causes of cervical cancer in the whole world [2]. The aim of this study was to find out possible correlations between a positive HPV DNA test to one of the high-risk (oncogenic) types with cervical lesions detected in a parallel Pap test performed.

Methods: A total of 1430 thin prep samples were collected from females in Northern Greece in order cytological examination (PAP test) and HPV DNA test to be performed. HPV DNA presence examined by real-time PCR.

Results: Out of 1430 cases, 241 (16.85%) were found positive for at least one of the HPV high-risk haplotypes tested. Concerning cytological examination of the above samples, 1201 (83.98%) had a normal Test Pap while 229 revealed at least one pathological finding. Remarkably, 172 samples positive for one of the HPV haplotypes examined had a normal Pap test.

Conclusion: These results show that despite a normal cytological examination, HPV infection occurs emphasizing the importance of HPV DNA test as screening method for prevention of cervical cancer.

References: 1. Zacharis, K., et al. (2018). Human Papilloma Virus (HPV) and Fertilization: A Mini Review. Medicina, 54(4).

2. Okunade, K.S. (2020) 'Human papillomavirus and cervical cancer', Journal of Obstetrics and Gynaecology.

Grants:

Conflict of Interest: None declared.

EP13.053 Association of TCF7L2, CASC8 and secretogranin V polymorphism with the risk of colorectal cancer and diabetes mellitus

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Background/Objectives: The aim of the study was to explore the correlation between rs7903146 of TCF7L2, rs6983267 of CASC8 and rs 16969681 of secretogranin V and association of colorectal cancer (CRC) with diabetes mellitus (DM).

Methods: Real time PCR was used to determine the genotypes of TCF7L2, CASC8 and secretogranin V in 60 patients with CRC and 30 without CRC or DM. We further classified the CRC patients in group of patients with DM and group of patients without DM. Hardy Weinberg equilibrium was determined in the control group for genotypes distribution of every polymorphism. The genotype and allele distribution differences between groups were analyzed by qui-squared test.

Results: The results showed that the TT genotype of rs7903146, rs6983267 and rs16969681 had a significant higher frequencies in case of CRC group than in controls ($P < .05$). T allele frequency had higher frequencies only for rs7903146 of TCF7L2 ($P = .045$). Between patients with CRC were observed significant higher frequencies of TT genotype of rs7903146 and rs16969681 in group of patient with CCR and DM ($P = .015$ and $P = .0019$).

Conclusion: TCF7L2 rs7903146 and survivin V rs16969681 may be a risk factor for association of CCR and DM. Due to study limitation, further researches to verify this conclusion should be conducted.

References:

Grants: TCF7L2 polymorphism as molecular mechanism of association of diabetes mellitus with colorectal cancer, 03/13.10.2020, Ovidius University of Constanta.

Conflict of Interest: None declared.

EP13.056 The immune checkpoint genes expression in gastric cancer

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Background/Objectives: Recently, PD-L1/PD-1 immune checkpoint (IC) inhibitors have become the most promising innovation in gastric cancer (GC) treatment. However, the proportion of patients responding to treatment is not high. There are other ICs in addition to ICs blocked, whose expression in the tumor may be one of the reasons for the low efficiency of PD-L1/PD-1 blockade. The aim was to study the correlation of IC gene expressions and their connection with GC development.

Methods: The paired samples of the gastric tumor and morphologically normal tissue were studied. Two groups were distinguished: non-metastatic and metastatic GC. The expression level of mRNA was measured by RT-PCR.

Results: The expression profiles of the *PD-L1, IDO1, CEACAM1, PVR, TDO2, CD276, GAL9, ADAM17* genes were studied. A significant correlation with *PD-L1* expression was demonstrated by *IDO1, TDO2* and *GAL9* (R = 0,63; 0.55; 0,59 respectively). Statistical data processing using ROC-analysis and Fisher's exact test revealed that the *PD-L1* and *TDO2* expression have a statistically significant association with GC metastasis. In metastatic GC the *PD-L1* and *TDO2* increased expression is more often observed relatively to non-metastatic.

Conclusion: Three ICs - *IDO1, TDO2* and *GAL9* - are co-expressed with *PD-L1* in GC. In metastatic GC the *PD-L1* and *TDO2* expression is increased. *TDO2* gene may be a promising target for immunotherapy in metastatic GC. The data obtained are important for the development of effective immunotherapy and prognostic markers of GC also.

References:

Grants:

Conflict of Interest: None declared.

EP13.057 The GAS5 rs145204276 ins/del polymorphism is associated with increased susceptibility to colorectal cancer

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Background/Objectives: Colorectal cancer (CRC) remains the second leading cause of cancer-related death in Europe. The involvement of long non-coding RNAs (lncRNA) is well-documented in many types of cancer, including CRC. The aim of our study was to investigate three lncRNA polymorphisms (SNPs): GAS5 rs145204276 ins/del, CASC8 rs10505477 A>G and HOTAIR rs7958904 G>C and CRC susceptibility in Eastern Europe (Romanian population), a region in which these SNPs has not previously been studied.

Methods: A total of 156 patients with sporadic CRC and 195 healthy controls were included. DNA was extracted from blood samples and the SNPs were genotyped by allelic discrimination TaqMan PCR assay with specific probes. The associations between the investigated SNPs and CRC risk were assessed by odds ratio (OR) with 95% confidence interval (CI) using logistic regression analysis. Bonferroni correction for multiple testing was used.

Results: A significant association was observed for GAS5 rs145204276 ins/del, in a dominant model the carriers of allele del were at a 2.13 fold elevated risk for CRC (OR 2.13, 95% CI: 1.24-3.63). In the stratified analysis, this association was limited to invasive stages (OR 2.72, 95% CI: 1.05-7.03) and tumour grade G3 (OR 3.98, 95% CI: 1.49-10.59) subgroups. We did not find any correlation for the remaining SNPs and CRC in any genetic model.

Conclusion: This study found that GAS5 rs145204276 ins/del is associated with CRC in Romanian population, mainly with advanced stages and a poorly differentiated subtype.

References:

Grants: The study was supported by the research grant AE 540/2018.

Conflict of Interest: None declared.

EP13.058 Compound heterozygous BRCA2 mutations are associated with vulvar epithelioid angiosarcoma

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Background/Objectives: Vulvar sarcomas are extremely rare and aggressive tumors. Published cases of gynaecological angiosarcomas have been reported in the uterus and ovaries, in adult women.

Here we report a case of vulvar epithelioid angiosarcoma in 9-year old girl with biallelic *BRCA2* mutations (in „trans"-form).The patient presented with tumor and several distinct phenotypic features (microcephaly, short stature, café-au-lait spots, hypopigmented skin lesions). No thumb or radial abnormality was observed. The patient has never been diagnosed with any hematologic problem typical for Fanconi Anemia.

Methods: We have performed NGS using Illumina Platform 76 gene multi-gene panel (*AKT1, APC, ATM, ATP9, AXIN2, BAP1, BARD1, BMPR1A, BRCA1,BRCA2,BRIP1,CDH1, CDKN2A,CHEK2,CTNNA1,-CYP21A2, DIRC3, DICER1, EPCAM, EXO1, FANCC, FH, FLCN, GALNT12, GDNF, GREM1, HNF1A, HNF1B, KIF1B, MAX, MC1R, MEN1, MET, MITF, MLH1,MLH3, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, NF2, PALB2,PIK3CA, PMS1, PMS2, POLE, POLD1, POT1, PRKAR1A, PRSS1, PTCH1, PTCH2, PTEN, RAD51C, RAD51D, RB1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, STK11, SUFU, TGFBR2,TMEM127, TP53, TSC1, TSC2, VHL, WT1, XRCC1, XRCC2)*.

DNA has been extracted from peripheral blood leukocytes.

Thorough mutation segregation analysis has been performed using blood DNA samples from family members.

Results: Mutations identified in *BRCA2* gene in the DNA of our proband:

I. rs80359604 (ClinVar; dbSNP) c.658_659delGT p.Val220Ilefs*4 inherited from paternal site.
II. rs80359598 (ClinVar; dbSNP) c.6486_6489delACAA p.Lys2162Asnfs*5 inherited from maternal site.

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Conclusion: Biallelic (compound heterozygous) mutations in the *BRCA2* gene may lead to vulvar epithelioid angiosarcoma.

References: 1. Weishaupt J. Vaginal epithelioid angiosarcoma: A literature review of a rare entity in an unusual site https://doi.org/10.1016/j.gore.2021.100706.

Grants: none.

Conflict of Interest: None declared.

EP13.059 Bladder cancer – drug-targets screening

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Background/Objectives: Urinary bladder cancer is socially significant healthcare problem with multifocality, frequent recurrence and growing incidence in the economically developed countries. Most of the tumors at presentation are low grade, non-muscle-invasive, but 10 to 20% of patients with high grade bladder tumor die of metastatic urothelial carcinoma.

The aim of our study is to expand the knowledge about mutations in bladder cancer and to screen for possible drug targets for so-called targeted therapy.

Methods: A total of twenty bladder cancer samples staged pTa to pT4 were collected. Written informed consent and a questionnaire on environmental and professional health hazards, family history, recurrence and treatment were obtained from all participants prior to tissue collection. DNA was extracted by standard phenol-chloroform extractionl and stored at -20○C. They were analysed by Infinium OncoArray-500K BeadChip (Illumina), scanned with iScan and analysed by GenomeStudio® Genotyping Module v2.0.

Results: Clinical and pathological characterization of the studied cohort shows the age of the patients was between 50 and 80 years. Around half of them were tobacco users with an average consumption of more than half package cigarettes per day. Only two of them reported professional health hazards. The interpretations of the results of SNP array are in progress.

Conclusion: Will be given after the data interpretation.

References: No.

Grants: BG NSF No KP-06-OPR01/3-2018.

Conflict of Interest: Zora Hammoudeh BG NSF No KP-06-OPR01/3-2018, Boris Mladenov: None declared, Zornitsa Yordanova BG NSF No KP-06-OPR01/3-2018, Olga Antonova BG NSF No KP-06-OPR01/3-2018.

EP13.060 Cost-utility of universal screening for common BRCA1 and BRCA2 variants among Ashkenazi Jewish women: real-life analysis

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Background/Objectives: Identifying carriers of pathogenic BRCA1/BRCA2 variants can reduce cancer morbidity and mortality through surveillance and prevention. Universal testing for founder BRCA1/BRCA2 variants, found in 2.5% of Ashkenazi Jews (AJ), fulfills WHO criteria for disease screening. We analyzed the cost-effectiveness of BRCA1/BRCA2 population screening (PS) in AJ, as an alternative strategy to two existing strategies for identifying unaffected carriers: international family history (IFH)-based guidelines (corresponding to >10% carrier probability) and cascade testing (CT) (≥25% carrier probability, according to familial variant).

Methods: A decision analytic model was performed to estimate screening and treatment costs, quality-adjusted life-years (QALY) gained, and incremental cost-effectiveness ratio (ICER) for PS vs. IFH-based testing and CT in Israel. The analysis was conducted from a payer perspective using a lifetime time-horizon. Analysis was based on "real life" numbers.

Results: The model predicted 5.8 and 21.6 years gained per 1000 women for PS vs. IFH-based and CT strategies, respectively, reductions of 0.3% and 0.1% in BC incidence, and reductions of 0.4% and 0.1% in OC incidence, respectively.

PS strategy was less costly compared with CT (ICERs/QALY -3097 USD). Although PS was more costly than IFH-based testing (ICERs/QALY +18,968 USD), it was still highly cost-effective. PS was more effective than other strategies in all sensitivity analyses.

Conclusion: PS was highly cost-effective, and less costly than CT. Although PS was more costly than IFH, PS was the most effective screening strategy for BC and OC prevention. Founder BRCA variant testing should be available to all AJ women, irrespective of family history.

References:

Grants:

Conflict of Interest: None declared.

EP13.061 Disclosing CDH1 c.1901C>T as a founder pathogenic variant in the Portuguese population

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Background/Objectives: Hereditary diffuse gastric cancer (HDGC) caused by *CDH1* germline pathogenic or likely-pathogenic variants predisposes to early onset diffuse gastric (DGC) and lobular breast cancer (LBC). In Northern Portugal, an unexpectedly high number of early-onset DGC and LBC in apparently unrelated families carrying the same *CDH1* c.1901C>T variant suggested this as a *CDH1*-founder variant. We aimed to demonstrate that c.1901C>T (formerly known as p.A634V) is a bona fide truncating variant inducing cryptic splicing (rather than missense), to calculate the timing of a potential founder effect, and to characterize tumour spectrum and age of onset in carrying families.

Methods: Splicing impact was proven by using 2 carriers' RNA for PCR-cloning sequencing and allelic expression imbalance analysis with SNaPshot. Carriers and non-carriers from four families were haplotyped for 12 polymorphic markers, and the decay of haplotype sharing (DHS) method was used to estimate time to the most common ancestor of c.1901C>T. Clinical information from 58 carriers was used to explore stomach and breast-associated clinical presentations.

Results: The c.1901C>T variant induced cryptic splicing and an out-of-frame 37bp deletion in exon 12, causing premature truncation (p.Ala634ProfsTer7), and RNA mediated decay. A shared common ancestor was estimated at 490 years (95% Confidence Interval 445–10,900). Among 58 carriers, DGC occurred in 11 (18.9%;4M–7F; average age 33±12) and LBC in 6 females (19.4%; average age 50±8).

Conclusion: The c.1901C>T variant was proven as a loss-of-function splice-site variant, and a founder variant in Northern Portugal, predisposing for very early-onset DGC and later onset LBC.

References:

Grants: SFRH/BD/145132/2019; NORTE-01-0145-FEDER-000003(**DOCnet**), POCI-01-0145-FEDER-007274; POCI-01-0145-FEDER-016390 (**CancelStem**); PTDC/BTM-TEC/30164/2017 (**3D ChroMe**); PTDC/BTM-TEC/6706/2020(**LEGOH**).

Conflict of Interest: None declared.

EP13.062 Mitochondrial genome in sporadic breast cancer: A case-control study of Sri Lankan women of Sinhalese ethnicity

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Background/Objectives: Breast cancer (BC) is the most prevalent malignancy in women world-wide. Mitochondrial dysfunction has been implicated in breast cancer, but data from Sri Lanka is limited to one study on the control region variants. Thus, we investigated the possible association of mt-genome with breast cancer in a case-control study, where cases and controls were matched for age, body mass index and menopausal status.

Methods: Mt-genomes, amplified from genomic DNA extracted from peripheral venous blood of 30 pairs were subjected to next generation sequencing. *MT-CYB* and *MT-ND3* genomic regions were subjected to Sanger sequencing in another 30 pairs. Sequencing data were pooled for statistical analysis (McNemar's test and Z score for two population proportions).

Results: A total of 503 variants were identified out of which 86 were missense variants. No variant identified had a significant difference in the prevalence between patients and the controls. Some of the variants reported as risk factors in other populations were polymorphisms with similar prevalence among cases and the controls. Higher rates of mutations/1000bp were seen in the *MT-CYB*, *MT-ATP6* and *MT-ND2* genes. Significantly higher number of missense variants and their unique combinations were seen among premenopausal and obese patients respectively.

Conclusion: Association of mtDNA variants with sporadic BC in premenopausal and obese women is suggested. However, a larger group should be studied to further confirm the findings.

References:

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Conflict of Interest: None declared.

EP13.063 WWP1, negative regulator of the PTEN/PI3K pathway, as candidate gene for colorectal polyposis

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Background/Objectives: Lee et al.[1] identified germline pathogenic variants in *WWP1* affecting the regulation of PTEN–PI3K signaling, in families with *PTEN*-hamartoma-tumor syndrome-like phenotypes and no germline pathogenic variants in *PTEN*. Specifically, carriers of *WWP1* variants had oligopolyposis, mostly with hyperplastic/serrated and adenomatous polyps, and early-onset CRC phenotypes. Our aim was to assess the involvement of *WWP1* in the predisposition to adenomatous and serrated polyposis.

Methods: We performed mutational screening of *WWP1* in 182 adenomatous polyposis patients, with classic and attenuated forms of the disease, and no germline pathogenic variants in known polyposis genes. Sequencing was performed by PCR amplification of pooled DNAs followed by targeted next generation sequencing[2]. Validations were performed by genotyping or sequencing the individual DNA samples contained in the DNA pools. Mutational screening of *WWP1* in 96 serrated polyposis patients is currently ongoing.

Results: In the adenomatous polyposis patients, the sequencing strategy identified 14 rare, predicted damaging variants in *WWP1*, including one of the likely pathogenic variants identified in the original study[1]. Identification of variant carriers and *WWP1* mutational screening in serrated polyposis patients are currently being performed and results will be presented at the meeting, including the description of the clinical phenotypes of mutation carriers.

Conclusion: Although discovered in patients suspected of *PTEN*-hamartoma-tumor syndrome, the phenotypes of the carriers identified to date suggest that *WWP1* is a polyposis and cancer-predisposing gene, in absence of most typical Cowden's and other *PTEN*-associated syndromes' clinical phenotypes.

References: [1]Lee et al. N Engl J Med 2020.
[2]Belhadj. Hum Mutat 2020.

Grants:

Conflict of Interest: None declared.

EP13.064 differentiation of sessile serrated adenoma polyps from hyperplastic polyps by use of mucin genes expression

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Background/Objectives: Lack of reliable diagnosis of colorectal polyps negatively affects clinical care. Significant histological overlap between sessile serrated adenoma polyps (SSA/Ps) and hyperplastic polyps (HPs) is observed, but SSA/Ps contribute up to 30% of all colon cancers and it is necessary to diagnose these polyps as soon as possible.

Altered mucin expression, the major component of mucus has been strongly associated with both benign and malignant pathologies of colon.

Methods: Real-time PCR of mucin panel genes was performed on 90 formalin-fixed, paraffin-embedded serrated polyp and normal colon samples and also immunohistochemical staining of SSA/Ps and hyperplastic polyps plus controls confirmed the protein expression patterns for CD44, MUC17, MUC2, MUC5AC, MUC6 in SSA/Ps and HPs.

Results: Different expression of these genes is observed in the hyperplastic and sessile serrated adenomas/polyps. MUC2, MUC5A and MUC6, are part of a gel-forming group of mucins that have epithelial barrier functions, provide a habitat for gut microbes and

also contain EGF-like motifs (MUC17) predicted to regulate cell growth that these mRNA was increased in SSA/Ps.

Conclusion: Physical interaction of secretory mucin MUC5AC with transmembrane protein CD44 is evident in the progression of SSA/Ps to CRC.

References: 1) RNA Sequencing of Sessile Serrated Colon Polyps Identifies Differentially Expressed Genes and Immunohistochemical Markers/ 2014.

2) Mechanistic and Functional Shades of Mucins and Associated Glycans in Colon Cancer/ 2020.

3) Molecular Biomarkers of Sessile Serrated Adenoma/ 2019.

Grants: Research Code: 1174 from Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflict of Interest: None declared.

EP13.066 The importance of non-coding RNA in precancerous lesions of colon cancer

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Background/Objectives: Colorectal cancer is assumed third in terms of incidence and second in terms of mortality. The progression of the malignancy is followed by proliferation of precancerous lesions, called polyps. There are two distinct pathways, achieving the attraction for researchers. First, the conventional adenoma-carcinoma pathway, which involves in progression of non-advanced tubular adenomas to larger in size or villous lesions with the potential of developing invasive carcinoma. Second, the serrated pathway which is thought to be originated from distinct traditional or sessile serrated polyps that estimated high-risk to become malignant. Non-coding RNAs participate in the gene expression regulation, demonstrate the effective role in previously described pathways. Different profiles of noncoding expression were observed between the precancerous lesions.

Methods: Based on scientific literature reviews and bioinformatics analysis, non-coding panels were introduced to distinguish different pre-cancerous lesions. The ncRNAs were included in the study only if their expression is differentiated between polyps introduced categories.

Results: We designed a ncRNAs panel with circulating lncRNA CCAT1, CRNDE, UCA1 and miR-335, miR-222, and miR-214. To distinguish serrated and non-serrated via expression evaluation.

Conclusion: ncRNAs are reported as the potential marker for the early detection of cancers. Hence, selecting the accurate and sensitive panel is substantial to differentiate high-risk colon lesions characteristics.

References: De Palma,et al."The molecular hallmarks of the serrated pathway in colorectal cancer." (2019).

Galamb,Orsolya, et al."Diagnostic and prognostic potential of tissue and circulating long non-coding RNAs in colorectal tumors." (2019).

Grants: Research Code: 1172 Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflict of Interest: None declared.

EP13.067 An atypical FISH pattern of the BCR::ABL1 fusion gene in a paediatric B-ALL patient

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Background/Objectives: A combination of methodologies is applied for the diagnosis of acute leukaemia and the identification of any potentially amenable subtype. FISH is a robust and widely used technique for the identification of *BCR::ABL1* gene rearrangements. These cases are assigned to a high-risk group and receive targeted therapy with tyrosine-kinase inhibitors in combination with chemotherapy.

Methods: We present the case of a 15-year-old girl diagnosed with B-cell precursor acute lymphoblastic leukaemia with discrepant genetic and molecular tests due to an atypical FISH pattern of the *BCR::ABL1* fusion gene.

Results: The RT-PCR identified the e1a2 (p190) transcript of the *BCR::ABL1* gene in peripheral blood and bone marrow blasts. However, the karyotype only revealed an inv(12), and the fusion was not seen by FISH using three different commercial ABL1 break-apart probes. Due to the discrepancy in the results, we tested a dual-colour-dual-fusion *BCR::ABL1* FISH probe. Use of D-FISH in interphase nuclei and metaphases show an atypical pattern with the fusion gene inserted at 9q34 instead of chromosome 22.

Conclusion: Therefore, it is crucial to select the correct FISH probe to avoid false-negative results in the *BCR::ABL1* identification due to atypical patterns in complex rearrangements. Besides, metaphase FISH is essential for the accurate interpretation of interphase FISH atypical patterns. Our case exemplifies the need of using complementary methodologies to discard any directly targetable leukemic subtype. Discrepancies due to atypical FISH patterns and the metaphase FISH may help clarify the diagnosis.

References: Primo et al, Leukemia (2003), Robinson et a., Leukemia (2005), Zhang et al, BMC Cancer (2019).

Grants:

Conflict of Interest: None declared.

EP13.068 Reclassification of germline variants in cancer susceptibility genes implementing ACMG/AMP guidelines

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Background/Objectives: In 2015, the American College of Medical Genetics and Genomics and the Association of Molecular Pathologists (ACMG/AMP) publicized joint recommendations for clinical interpretation of variants in routine clinical testing and based on scoring criteria place them into one of five classification tiers. Our laboratory implemented ACMG/AMP guidelines in routine setting in 2018.

Methods: Between 2014 and 2021, 5776 patients with different hereditary cancer predispositions were included in the study. Genomic DNA was extracted from blood and NGS sequencing was performed using Illumina's_TruSight_Cancer/Hereditary_Panel. Between 2014 and 2017 variants were classified without specific guidelines (based on information in the literature, disease databases, population frequencies and in silico predictions). After 2017, variants were classified and reclassified according to ACMG/AMP guidelines.

Results: Among 3623 classified variants in cancer predisposition genes, 5.9% (215/3623) variants were reclassified according to ACMG/AMP guidelines. Three variants (0.1%) were reclassified from VUS to likely pathogenic and one variant (0.02%) from VUS to pathogenic. In addition, 203 (5.6%) variants were reclassified from VUS to benign/likely benign, while 8 (0.2%) variants were reclassified from likely pathogenic to VUS. Detailed information about reclassifications are summarized in Table 1..

Table 1 Reclassifications according to ACMG/AMP guidelines.

Variant reclassification	number	percent(%)
from_VUS_to_benign	12	5.6
from_VUS_to_likely_benign	191	88.8
from_likely_pathogenic_to_VUS	5	2.3
from_pathogenic_to__VUS	3	1.4
from_VUS_to_likely_pathogenic	3	1.4
from_likely_pathogenic_to_pathogenic	1	0.5
total	215	

Conclusion: Variant classification guidelines are useful recommendations for variant classification due to multi scoring/evidence criteria and allows the comparable classification of variants between different laboratories.

References:
Grants:
Conflict of Interest: None declared.

EP13.069 The role of PTEN, mLST8 and REDD1 as prognostic factors in advanced stage rectal cancer patients

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Background/Objectives: Local recurrences and metastases are serious problems in treatment of rectal cancer. Preoperative chemoradiotherapy (CRT) has long been accepted as a method to improve survival and lifetime quality of the patients. However, physiologic effects of these therapies largely depend on resistance of cells to radiation, type of CT agents and individual responses. As one of the signaling cascades involved in chemo- or radiation-resistance, the present study focused on several proteins involved in pTEN/mTOR pathway to explore their prognostic significance.

Methods: pTEN, mLST8 and REDD1, were quantified using qRT-PCR method in samples collected from tumor and adjacent healthy tissues. Kaplan-Meier analysis was used to assess expression-survival relation and correlations among all proteins and clinicopathological features were statistically analyzed.

Results: PTEN expression showed strong correlation to tumor response (p:0.003) and lymph node response (p:0.003). Mean survival for high vs. low expression of PTEN profile was 72.82 vs 66.47 months, but not statistically significant. There was a negative correlation between tumor response and REDD1 with a considerable trend toward significance (rs: −0.271; p: 0.079), and it was found to be correlated significantly with metastasis (rs: 0.370; p:0.015). High associations were noted between mLST8/REDD1 (p:0.000) and mLST8/PTEN (p:0.003), confirming their role in the same cascade.

Conclusion: The contentious role of PTEN as a prognostic biomarker in colorectal cancer was advocated, while REDD1 could be suggested as potential candidate. mLST8, a conjunctive element for both mTORC1 and mTORC2 complexes, had no promising effect in survival.

References:
Grants:
Conflict of Interest: None declared.

EP13.070 Constitutional mismatch repair deficiency syndrome (CMMRD): the significance of customized surveillance protocol for Lynch syndrome-related tumors in relatives at risk—a case report

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Background/Objectives: Constitutional mismatch repair deficiency syndrome (CMMRD) is a childhood cancer predisposition syndrome caused by biallelic dysfunction of DNA mismatch repair (MMR) genes, while monoallelic deficiency of MMR genes leads to Lynch syndrome. Even though CMMRD is highly penetrant, Lynch-related tumors may be absent in up to 85% of CMMRD pedigrees. We are presenting our CMMRD family with a literature overview on guidelines for surveillance protocols.

Methods: We present a 12-years-old girl with multiple malignancies: high grade glioma, colorectal adenocarcinoma and mediastinal lymphoma. Her 3-years-old sister died due to pilocytic astrocytoma. Whole exome sequencing was performed in proband, followed by target carrier testing for family members at risk.

Results: Homozygous VUS c.274C>G variant in MSH2 gene and homozygous VUS c.2426_2428del variant in MSH6 gene as well as several blocks of homozygosity were detected in proband. Subsequently, target testing of family members revealed that both MSH2 and MSH6 gene variants were present as heterozygous in both so far healthy parents and three other sisters.

Conclusion: Surveillance for Lynch-related tumors in heterozygous carriers should be customized and MMR gene–specific, especially in CMMRD families that do not meet clinical selection criteria for Lynch syndrome, in order to avoid subjecting carriers at low cancer risk to the invasive procedures, in some cases from an unnecessarily young age.

References: Bakry D, Aronson M, Durno C, et al. (2014). Genetic and clinical determinants of constitutional mismatch repair

deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. Eur J Cancer 50(5):987-96.

Grants: Non applicable.

Conflict of Interest: None declared.

EP13.071 Use of multi-gene NGS panel testing in polyposis/ colorectal cancer patients identify new mutations in non-CRC cancer genes

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Background/Objectives: Hereditary colorectal cancer syndromes are disorders that may predispose patients to developing colorectal cancer (CRC). The current genetic screens analyze classical high penetrance genes associated with these syndromes. However, this approach only explains the genetic predisposition of a few cases. This study aims to elucidate the potential advantages to implementing a multi-gene NGS panel in routine genetic diagnosis.

Methods: A multi-gene NGS panel (Imegen) was used to analyze 50 genes associated with hereditary cancer syndromes in 60 cases. We selected patients with polyposis and a family history of polyposis and/or CRC with no detected mutations in high penetrance genes. Genomic DNA was extracted from peripheral blood following a standard phenol/chloroform procedure.

Results: 6 germline pathogenic or likely pathogenic variants and 4 variants of unknown significance (VUS) were detected in high penetrance genes (*MUTYH* and *APC*, respectively). 2 pathogenic or likely pathogenic mutations and 10 VUS were found in moderate/low penetrance genes (*POLE*, *BMPR1A*, *MSH6*, *MSH2*, *MSH3*, *MLH1*, *MLH3*, and *NTHL1*). Interestingly, 7 VUS were identified in *ATM*. Additionally, another 14 VUS were detected in other non-CRC cancer genes (*BRCA1*, *RET*, *NBN*, *MET*, *SDHA*, and *PIK3CA*).

Conclusion: Our results highlight that multi-gene NGS panels are an appropriate tool for enhancing the genetic testing potential and should be incorporated into diagnostic routine. Further studies are required to find the potential role of *ATM* and other non-CRC cancer genes in the development of CRC and/or polyposis.

References:

Grants: Study funded by Fundación Mutua Madrileña.

Conflict of Interest: None declared.

EP14 Genome Variation and Architecture

EP14.001 Novel genetic variants that associated with Wilson disease in Saudi families

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Background/Objectives: Wilson disease (WD) is an autosomal recessive inherited disorder caused by homozygous or compound heterozygous mutations in ATP7B gene. The ATP7B gene mediates the excretion of copper into bile and delivers copper to the transporting protein ceruloplasmin in blood. The genetic database reported over 782 pathogenic ATP7B variants that cause WD. In the Saudi population, several mutations are identified in ATP7B gene that are associated with WD such as p.Gln1399Arg, p.Ser744Pro and p.Gly1341Ser. We aimed to investigates and characterize the common and specific genetic variants of WD in Saudi Arabia.

Methods: Blood samples were collected from four WD patients comprising three siblings (P1, P2, and P3), and one unrelated case. Blood samples were also obtained from the unaffected parents of the siblings. Genomic DNA was extracted from blood samples using a Gentra Puregene Blood Kit. DNA was sequenced by utilizing massive parallel sequencing via Ion Torrent PGM with the Ion PI™ Hi-Q™ Sequencing 200 Kit.

Results: A novel homozygous frameshift variant (M851X) was identified in the ATP7B locus at position chr13:52524431delA in three affected siblings. The parents were found to be heterozygous carriers for this variant. A homozygous frameshift identified at this location results in a premature stop codon and putative expression of a non-functional truncated ATP7B protein. In the unrelated case, four homozygous missense variants were also identified: Val1140Ala, Thr977Lys, Arg952Lys and Lys832Arg.

Conclusion: This study reported novel WD-associated ATP7B gene variants in the Saudi population.

References: NO.

Grants: NO.

Conflict of Interest: None declared.

EP14.002 Beyond the canonical splice site: retrospective study of non-canonical splice site variants and their pathogenicity

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Background/Objectives: Variants within canonical splice sites (CSS) can affect splicing, leading to inherited diseases. However, pathogenicity of variants neighbouring CSS is underappreciated and understudied. This study evaluated the distribution of reportable (pathogenic/likely pathogenic) non-CSS variants in a large US carrier screening cohort.

Methods: We assessed the distribution of single nucleotide variants within donor and acceptor sites, ±1 to ±10 bases into the intron, and their pathogenicity in >500,000 presumably healthy individuals undergoing HorizonTM carrier screening for up to 274 genes between April 2018 and April 2021. All variants were classified using ACMG/AMP guidelines.

Results: Within this cohort, 2,201 reportable variants were identified (Table 1). As expected, most reportable variants were found in CSS (±1, ±2) compared to non-CSS. Interestingly, the majority of reportable variants in non-CSS regions, were found at +5 donor and -3 acceptor sites (76/154) (Table 1). Of +5 reportable variants, 35/56 were reported as causing exon skipping or intron retention, and 9/56 were not reported in public databases. Of -3 reportable variants, 2/20 were not reported in public databases.

Conclusion: Our data indicates that the +5 and -3 positions harbor a significant proportion of variants disrupting splicing, compared to other non-CSS analyzed. Frequent monitoring of non-CSS variants, especially at +5 and -3, can increase the accuracy and utility of variant classification for inherited diseases..

Table 1. Reportable Variants

	Reportable variants		
CSS (±1, ±2)	2047		
Non-CSS (±3 to ±10)	154	+5 donor	56
		Other donor sites	41
		-3 acceptor	20
		Other acceptor sites	37
Total	2201		

References:
Grants:
Conflict of Interest: Chiao Ling Lo Full-time employee of Natera Inc., Option to own stock in Natera Inc., Trevor Smart Full-time employee of Natera Inc., Option to own stock in Natera Inc., Jocelyn Wang Full-time employee of Natera Inc., Option to own stock in Natera Inc., Bryan Gall Full-time employee of Natera Inc., Option to own stock in Natera Inc., Bridgette Meyers Full-time employee of Natera Inc., Option to own stock in Natera Inc., Ezen Choo Full-time employee of Natera Inc., Option to own stock in Natera Inc., Nina Sanapareddy Full-time employee of Natera Inc., Option to own stock in Natera Inc., Dianne Keen-Kim Full-time employee of Natera Inc., Option to own stock in Natera Inc.

EP14.003 Mutational analysis of NF1 gene and its interactors highlights role of NF1 3' tertile in spinal neurofibromatosis

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Background/Objectives: Spinal Neurofibromatosis (SNF) is a specific form of Neurofibromatosis type1(NF1) characterized by bilateral neurofibromas involving all spinal roots.While the diagnosis of SNF and classical NF1 can be revealed by spinal MRI,the molecular basis of these conditions is unknown.The aim of the study was to verify the presence of variants contributing to development of a specific NF1 form.

Methods: We investigated 99 NF1 sporadic patients and 74 SNF patients by a NGS panel of 286 genes encoding RAS pathway effectors and NF1 interactors,beside *NF1* gene.

Results: We identified 75 *NF1* variants in SNF and 99 in NF1 cohort.We confirmed the higher prevalence of missense (MS) mutations ($p = 0.0001$) in the SNF(30%) as compared to the NF1 cohort(8%) as previously reported (1). 5 SNF patients had double *NF1* mutations,2 of them were demonstrated to be compost heterozygotes.The prevalence of mutations in *NF1* 3' tertile of the gene was significantly higher in SNF than in NF1 ($p = 0,0085$) patients.Interestingly,a higher prevalence of variants in *NF1* 3' tertile interactors with a MAF < 0.05 and uncertain significance was found in SNF patients (7%) than in NF1 patients (1%),together with *NF1* mutations,all but one,in the 5' and middle tertile.

Conclusion: Our data highlight two different mutational patterns of the *NF1* gene for SNF and classical NF1.Beside confirming the prevalence of MS mutations in SNF,the prevalence of mutations in the 3' tertile of *NF1* gene could indicate the presence of functional sites possibly involved in SNF.This hypothesis should be confirmed in a larger cohort.

References: https://doi.org/10.1111/cge.12498.
Grants: RF-2016-02361293ItalianMinistryofHealth..
Conflict of Interest: None declared.

EP14.004 Cytogenetic mapping of aphidicolin-sensitive fragile sites in human induced pluripotent stem cells

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Background/Objectives: Common fragile sites (CFs) are genome markers of replication stress. They can be detected as chromosomal breaks or gaps on metaphase spreads. Currently, replication stress in induced pluripotent stem cells (iPSC) was only studied in S-phase and early stages of mitosis. Cytogenetic mapping of CFs is complicated due to peculiarities of cell cycle regulation, repair and apoptosis conferred by pluripotency. In this study, we performed cytogenetic mapping of aphidicolin-sensitive fragile sites in human iPSC line RCMGi001-A.

Methods: RCMGi001-A cell line was derived by reprogramming of fibroblasts with homozygous CFTR F508del mutation using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Cells were cultured in Essential 8 medium in presence of aphidicolin for 16-22 hours, arrested with nocodazol, followed by standard metaphase spread preparation. Differential staining was performed using Actinomycin D and DAPI.

Results: Totally 300 breaks induced by replication stress were mapped in the human iPSC line. The most frequently recurring breaks were found at bands on chromosomes 2, 6, 9, 10, and 12 that contain long genes some of which are associated with Parkinson's disease, Alzheimer's disease and a number of enzymes and ion channels expressed in brain cells. The identified breaks only partially coincide with the regions of spontaneous recurrent chromosomal aberrations previously reported in human iPSCs.

Conclusion: This research may help to uncover the biomarkers of genetic instability in cultured cells and will help to understand the mechanisms of induction and functional relevance of chromosomal aberrations. It is also relevant in the context of somatic mutagenesis, genotoxicology, malignant transformation.

References:
Grants:
Conflict of Interest: None declared.

EP14.005 Three case reports of patients with rare copy number variations in the recurrent 2q11.1-q11.2 region

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Background/Objectives: Copy number variations (CNVs) in the 2q11.2 region are known as recurrent for more than a decade but there are still a small number of patients reported in the literature. It has not been observed in the control healthy populations, and the data regarding associated phenotypes as well as evidence supporting the pathogenicity of CNVs in this region are limited at this time.

Methods: DNA samples of 656 patients referred to our laboratory for array- comparative genomic hybridization testing were analyzed by oligonucleotide microarrays in format 8x60K. Clinical indications included developmental delay (DD), autism, and birth defects as the most common features. Case series of information were gathered from health records and thorough research of literature and available databases.

Results: In our sample, we identified three unrelated patients (0.46%) with identical 1.24-Mb CNVs (two deletions and one duplication) in the 2q11.1-q11.2 region that encompasses 22 protein-coding genes of whom 6 are disease-associated: CNNM4, LMAN2L, NCAPH, SNRNP200, STARD7, and TMEM127. All three patients have mild to moderate DD with non-specific dysmorphic features, but some of them overlap with those previously reported like microcephaly, midface hypoplasia, hypertelorism, crowded teeth, and scoliosis. One patient inherited deletion from a supposedly healthy father, and for the other two patients, inheritance is unknown. The variants in our cases were classified as unknown significance, likely pathogenic.

Conclusion: This case series could help clarify the clinical significance of the recurrent 2q11.2 region and genotype-phenotype correlation.

References:

Grants:

Conflict of Interest: None declared.

EP14.006 Reverse mosaicism in Fanconi anemia: a natural mechanism of gene therapy

Ilaria Persico[1;2], *Giorgia Fontana*[3], *Michela Faleschini*[3], *Enrico Cappelli*[4], *Fabio Corsolini*[4], *Clara Mosa*[5], *Angela Guarina*[5], *Piero Farruggia*[5], *Anna Savoia*[1;3], *Dufour Carlo*[4], *Roberta Bottega*[3]

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Background/Objectives: Fanconi anemia (FA) is a genetic instability syndrome which fosters additional genetic events, as reversion or compensatory mutations, resulting in a 15-25% incidence of mosaicism [1]. We report a *de novo* variant in *FANCA* able to revert the cellular phenotype in a FA patient.

Methods: Proband underwent a targeted next generation sequencing (NGS). Variant pathogenicity was assessed by RT-PCR, western blot, diepoxybutane (DEB) sensitivity, and minigene assay.

Results: Targeted NGS of proband's peripheral blood DNA reported two pathogenetic mutations in *FANCA*; a large deletion and c.2778+83C>G with a known splicing impact [2]. Nevertheless, no aberrant products in the RT-PCR on RNA from two distinct patient's lymphoblastoid cell lines (LCLs) established in different moments were observed. By resequencing LCLs DNA, we detected the *de novo* c.2778+86insT variant, predicted to compensate 2778+83C>G role. Accordingly, we reported partial maintenance of FANCA synthesis and FA pathway activity, and wild-type phenotype under DEB treatment in LCLs. Minigene assay is underway to evaluate the effect of the single variants and their combination on splicing.

Conclusion: c.2778+86insT appears as a compensatory variant recovering c.2778+83C>G pathogenicity and, thus, providing a natural gene therapy ongoing in the proband.

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Grants: Associazione Italiana Ricerca Anemia Fanconi.

Conflict of Interest: None declared.

EP14.007 uORF-creating mutations in Van der Woude syndrome: why it is important to study 5'UTRs

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Background/Objectives: Van der Woude syndrome (VWS, MIM 119300) is an autosomal dominant cleft lip and/or palate with typical lower lip pits. Most patients carry loss-of-function mutations in *IRF6*. Upstream open reading frame (uORF)-creating mutations have been reported in four VWS patients. Pathogenic uORF-creating mutations are mostly out-of-frame upstream start codons (uAUG) in the 5'UTR. We searched for *IRF6* uORF mutations and assessed the ability to predict the pathogenicity of uORF-creating variations of 5 prediction tools.

Methods: We analyzed *IRF6* UTR and coding regions in 68 VWS probands. By using a set of 44 reference genes, we assessed 5 in silico tools predicting the probability of ATGs to initiate translation: NetStart, ATGpr, TIS Miner, AltORFev, TIS Predictor. We then assessed the potential pathogenicity of all the theoretical uORFs in *IRF6* 5'UTR.

Results: We have identified two novel uORF-creating mutations (c.-141C>T and c.-162C>T), representing 3% (2/68) of the probands. The 7 carriers of the two families had typical VWS signs.

Our in silico analyses revealed a higher accuracy for AI-based tools over those based on Kozak consensus scoring. There are 28 theoretical uAUG-creating SNVs in *IRF6* 5'UTR. With AI-based tools, the six uAUG identified in VWS patients have high translation initiation site scores; 3 to 4 of the theoretical uAUG-creating SNVs had high scores and could correspond to pathogenic mutations. For the dozen of theoretical SNVs with intermediate scores, predicting pathogenicity remains challenging.

Conclusion: As untranslated regions are frequently understudied in NGS strategies, uORF-creating mutations may be underdiagnosed in VWS and in human pathology in general.

References:

Grants:

Conflict of Interest: None declared.

EP14.008 Catalogue for transmission genetics in arabs: analysis of lebanese variant data

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Background/Objectives: The Catalogue of Transmission Genetics in Arabs aims to address the under-representation of Arab data in genetic databases by showcasing the vast amount of Arab genotype-phenotype data available in the literature.

Methods: CTGA is being hosted online since 2005 at cags.org.ae/ctga. It is a SQL-based open-access compendium of bibliographic data on genetic disorders in Arabs, compiled and curated from searches on Medline, Index Medicus, and Google Scholar. In 2019, the data entry process was streamlined through automation while maintaining a thorough manual curation, and the user interface was revamped into a concise tabular database. The structure of CTGA involves interconnected disease, gene, and variant records hosting anonymous data from Arab individuals across 23 Arab countries. CTGA data involves variant annotation using HGVS terms, phenotypic descriptions using Human Phenotype Ontology terms, as well as linked disease, gene, and variant IDs from databases such as OMIM, dbSNP, and ClinVar. A versatile advanced search was implemented where users can pull specific results by combining multiple filters.

Results: CTGA holds a total of 6764 records (checked February 2022). Our most recent major update incorporated bibliographic data of Lebanese subjects up to the year 2020. Among this data, we report on 641 diseases, 676 genes, and 1317 variant records curated from a total of 814 publications- including 172 variants previously unreported in dbSNP and ClinVar, as well as 15 Lebanese-specific disorders.

Conclusion: CTGA is a valuable resource for clinicians, geneticists, and researchers dealing with Arab patients, and is currently open to data submission of published data.

References: PMID: 34680914.

Grants:

Conflict of Interest: Sami Bizzari Researcher, Centre for Arab Genomic Studies, Pratibha Nair Researcher, Centre for Arab Genomic Studies, Sayeeda Hana Researcher, Centre for Arab Genomic Studies, Asha Deepthi Researcher, Centre for Arab Genomic Studies, Mahmoud Al-Ali Director, Centre for Arab Genomic Studies, Stephany El-Hayek Assistant Director, Centre for Arab Genomic Studies.

EP14.009 Patient Series of Multiple Molecular Diagnoses: A Single-Center Experience

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Background/Objectives: In last two decades, the usage of genome-wide diagnostic techniques have become popular and more accessible and the underestimated number of multiple molecular diagnoses (MMDs) has been brought to light. In this study, we aim to contribute to the literature by presenting the phenotypes of the patients with MMD and the genes involved.

Methods: The data of a series of 932 patients referred to our department with a suspicion of an underlying genetic cause were analyzed retrospectively. All patients were examined and underwent a genome-wide diagnostic technique at our clinic, both chromosomal microarray and whole-exome sequencing.

Results: Of the 932 probands, 381 (40.8%) were diagnosed based on mutations in known disease-related genes. Twenty two (2.4% of all cases) had diagnoses that involved two or more genetic background explaining their clinical phenotype. Among all, 49 damaging variants detected, three variants were CNVs. When CNVs were attributed to the single disease gene, 20 variants were in autosomal dominant (AD) and 26 variants were found to be in autosomal recessive (AR) disease genes. The remaining 3 variants were in the genes associated with X-linked diseases. Fifteen patients had two or three molecular diagnoses showing overlapping features.

Conclusion: In the era of genomic medicine, complete identification of molecular etiology and prediction of final phenotype have a vital importance. Studies in which patient series of MMDs that can create a completely new phenotype, especially when given together with the causative genes and final phenotypes, will enlighten the genetic consultations.

References:

Grants:

Conflict of Interest: None declared.

EP14.010 Ring chromosome 20 syndrome: a case report with refractory frontal lobe seizures and learning disabilities

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Background/Objectives: Constitutional ring chromosomes (RCs) result from rare intrachromosomal fusions of unstable telomeres or subtelomere breaks that stabilise by circularising. Among RCs, ring chromosome 20 [r(20)] is one of the less understood. Between mosaic patients with r(20) syndrome reported in the literature, females seem to be the more frequent (64%), with a r(20) that maintain intact subtelomeric sequences with no genomic imbalances. Post-zygotic telomere fusion is thought to be the most probable mechanism for ring formation.

We report a 24 years-old woman, referred with refractory epilepsy, developmental delay and the suspicion of r(20) due to her electro-clinical phenotype.

Methods: High resolution cytogenetic analysis and FISH, performed on metaphases obtained from peripheral blood lymphocytes.

Results: Cytogenetic analysis revealed a mosaic with two cell lines: one cell line has a r(20) (22% of the metaphases) and the other cell line is normal. The r(20) with subtelomeric probes showed a balanced result. Karyotype (ISCN 2020):.

mos 46,XX,r(20)(::p13->q13.3::)[11]/46,XX[39].ish r(20)(20PTEL 18+,20QTEL14+).

Conclusion: The epileptic phenotype of the patient is characterized by intractable focal seizures and non-convulsive status epilepticus (NCSE). The age of onset of seizures, 8 years-old, is inversely correlated with the degree of mosaicism in blood. Most cases are sporadic, however rare familial cases have also been

reported. The phenotype could be related to ring structure, due to a silencing effect to nearby genes (*CHRNA4*, *KCNQ2*) or intrinsic instability with recombination or loss of the ring. The mechanism through which r(20) causes the typical manifestations remains unanswered. Conventional cytogenetic undoubtedly represent the gold standard for this diagnosis.

References:

Grants:

Conflict of Interest: None declared.

EP14.011 Molecular mapping of chromosome breaks revealed two large fragile genes specific to induced pluripotent stem cells

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Background/Objectives: Induced pluripotent stem cells (iPSCs) are a valuable model for embriogenesis of all tissue types and a promising tool for regenerative medicine. Accumulation of chromosomal aberrations during rapid cell proliferation both in vivo and in vitro is presumably caused by replication stress. The most vulnerable to replication stress genomic regions are referred to as common fragile sites (CFSs). CFSs are prone to formation of focal copy number variations, and can be detected as recurrent breaks on metaphase chromosomes under exposure to replication stress.

Methods: The iPSC line RCMGi001-A was obtained through reprogramming of the skin fibroblasts with a homozygous CFTR F508del sing CytoTune™-iPS 2.0 Sendai Reprogramming Kit and characterised earlier. Culture medium was supplemented with aphidicolin, an inhibitor of replicative polymerases, for 18-24h, followed by standard cytogenetic preparation of metaphase spreads. FISH was performed with custom probes obtained by nick-translation labelling of long-range PCR amplicons.

Results: To choose candidate regions for FISH probe targeting, we analysed the location of large genes within fragile cytogenetic bands on chromosomes 2 and 12 using UCSC genome browser. Hybridization of probes directed to flanking regions of candidate genes to aphidicolin-treated metaphases allowed to molecularly define break localization within or outside the gene. This approach allowed us to narrow down the location of two most active fragile sites in iPSC from cytogenetic bands to specific genes.

Conclusion: Our study tests the impact of replication stress in iPSC culture genome instability as well as its possible involvement in somatic mosaicism and age-related disease pathogenesis.

Conflict of Interest: None declared.

EP14.012 Unmasking small and structural variation in the IKBKG gene with short and long read sequencing technologies

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Background/Objectives: Long-read sequencing can simplify identifying genetic variants that would be difficult to assess relying only on traditional short-read technologies. As a proof of concept, we developed data processing pipelines for the two technologies and focused on unmasking genetic variation typically occurring in *IKBKG* gene[1], whose deficiency affects the interferon-γ production leading to a known immunodeficiency, that is confounded by a nearby pseudogene.

Methods: We used paired-end whole-genome sequencing from 14 donors from Spain obtained with PromethION (ONT) and HiSeq4000 (ILMN). For small variants, PEPPER-DeepVariant[2] (ONT) and BWA-GATK[3] (ILMN) pipelines were used. For structural variants (SVs), a combination of reference-based and *de novo* assembly algorithms was used. Resulting SVs were filtered and combined into a callset per technology using SURVIVOR.

Results: We found that the presence of a pseudogene (*IKBKGP*) in the region hindered small variant discovery with short reads, but not with long reads, revealing additional variants in *IKBKGP* across most samples. Two SVs were also detected in the region with long reads, but not with short reads.

Conclusion: We developed in-house pipelines for variant characterization from two sequencing technologies. Long-read technology allowed us to obtain small and structural variants missed by short reads. ONT based *de novo* assemblies could further help to reveal additional genetic variation in complex regions of the genome.

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Conflict of Interest: None declared.

EP14.016 Report of a case with a small terminal deletion of 18q23 region

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Background/Objectives: Clinical features associated to 18q deletion may be higly variable according to the size and to genes involved in the deletion and comprises intellectual disability and various abnormalities and malformations. Individuals with terminal 18q deletion share some clinical features as ocular abnormalities, cleft palate, hearing defect, heart malformations, scoliosis and foot anomalies[1].

Methods: We describe a 2-years-old child admitted to our department for exotropy, hypermetropia and heart murmur. He was born at 40week of pregnancy, after an uneventful pregnancy and delivery. Neuropsychomotor development was referred adequate. Echocardiography detected an aortic subvalvular diaphragm. Dysmorphic features were recorded including: brachycephaly, broad forehead, prominent metopic suture, long eyelashes, epicanthus, low-set ears, fetal pads, clinodactyly of the fifth toe.

Results: Array CGH showed: Arr[GRCh38] 18q23 (76390288_80254946) x1.

A 3MB deletion in 18q23 was demonstrated. Some deletions involving 18q23 have been reported in literature, but most of

them are longer than this rearrangement, that seems to be one of the smallest reported until now.

Conclusion: The phenotype associated with 18q23 deletions comprises hypermetropia, cleft palate, hearing loss, growth abnormality, IgA deficiency and congenital heart disease; intellectual disability is often absent in patients with a very small deletion[2]. The patient here described share with the typical phenotype heart and ocular abnormalities, with normal neurodevelopment. This report is useful to improve correlation genotype-phenotype correlation of 18q terminal deletions.

References: [1]J.D. Cody, Consequences of Chromsome18q Deletions (2015).

[2]Ghidoni PD Growth hormone deficiency associated in the 18q deletion syndrome (1997).

Grants:

Conflict of Interest: None declared.

EP14.017 Aortic coartation, laterocervical appendix and asimmetric crying face associated to 2p11.2 deletion: a case report

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Background/Objectives: Deletion of 2p11.2 region is widely associated to intellectual disability, microcephaly, short stature and parkinsonism in adults. We describe a two months-old child, that showed asimmetric crying face, heart murmur and laterocervical appendix. Family history was negative for cardiac disorders. The laterocervical appendix was described as "left lateral-cervical branchial residue" at otolaryngological consultation and mild neutropenia at hematologic investigation.

Methods: Echocardiography showed coartation of the aorta at the aortic isthmus associated to a maximum gradient of 52 mmHg. Interventricular defect with left-right shunt was also recorded. The patient underwent surgery. CGH-array analysis reported: arr[GRCh38]2p11.2 (87176199_90226253)x1. Deletion of 3Mb was demonstrated.

Results: Deletion of 2p11.2 region larger than that observed in our patient have been reported in the literature and heart disease is not a cardinal feature of these patients. Deletions of a region overlapping with that of our interest have been described in association with a phenotype similar to DiGeorge syndrome (del 22q11.2), including timic hypoplasia and asimmetric crying face; no one of the case reported in this paper presented heart disease. FOXI3 is included in this region and is implicated in cervical development, interacting with TBX1, involved in 22q11.2 deletion syndrome; FOXI3 knockout in mouse models, is associated with heart malformation.

Conclusion: This is the first description of heart involvement in 2p11.2 deletion, thus confirming the association between 2p11.2 deletion and DiGeorge phenotype.

References: "Recurrent microdeletions at chromosome 2p11.2 are associated with thymic hypoplasia and features resembling DiGeorge syndrome"- Bernstock et al.

Grants:

Conflict of Interest: None declared.

EP15 Cytogenetics

EP15.001 Case of a balanced complex chromosomal rearrangement involving chromosome Y, 15, 6 and 3, 4 in an infertile male patient with cryptozoospermia

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Background/Objectives: Complex chromosomal rearrangements (CCRs) are a rare form of balanced translocations and involve more than two chromosomal breaks. Carriers of balanced translocations such as balanced CCRs are at a higher risk for miscarriages and infertility. Here we report on a man with a CCR who was referred to the Centre of Reproductive Medicine and Andrology (CeRA) because of primary infertility and diagnosed with cryptozoospermia (<100.000 sperm/ejaculate).

Methods: We performed cytogenetic analyses including multicolor Fluorescence-in situ-Hybridization (FISH) as well as SNP-array analysis.

Results: Hormone analyses showed a LH-level of 3.6 U/l (reference range 2-10 U/l), a FSH-level of 4.1 U/l (reference range 1-7 U/l) and a testosterone serum value of 11.0 nmol/l (reference range >12 nmol/l). Cytogenetic analyses found a balanced CCR with a three-break translocation and an additional balanced reciprocal translocation involving two chromosomes. The balanced translocation involved chromosomes Y, 6 and 15 with breakpoints at Yq11.2?1, 6q25 and 15q24. The additional balanced reciprocal translocation involved two breakpoints on chromosome 3 at 3q26.1 and on chromosome 4 at 4q28. SNP-array analysis identified no copy number variation in the area of the breakpoints confirming the balanced nature of the CCR.

Conclusion: Since the presence of a chromosomal rearrangement, especially a CCR such as in this case, can alter the chromosome synapsis during the pachytene stage of meiotic division, it is likely the underlying cause for the severely reduced sperm count.

References:

Grants:

Conflict of Interest: None declared.

EP15.002 recurrent constitutionnal chromosome five inversion revisited

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Background/Objectives: The recurrent pericentric inversion inv(5)(p13q13) is classically considered a polymorphism in Human with no clinical nor reproductive consequences. Indeed this inversion is described and found inherited. Surprisingly pericentric

inversion, same size, on other submetacentric chromosome is described in meiotic rearrangement resulting in segmental duplication / deficiency. Because few genomic data are reported in literature we investigated human families with different ethnic origins in order to update knowledge of this inv(5).

Methods: We report 3 non related families with inv(5)(p13q13). In the first inv(5) was discovered during pregnancy in context of elevated T21 serum markers, and was found paternally inherited. In the second, inv(5) was also diagnosed during pregnancy. In the last family, inv(5) was diagnosed on constitutional blood karyotype in context of blood myelodysplasia diagnosis. Inv(5) was inherited from healthy mother. The breakpoints of inv(5) were mapped in the 3 families. We used Genome and Sanger sequencing, and Optical Genome Mapping.

Results: Both techniques confirmed the breakpoints to be similar with interruption of an OMIM gene at 5q13.

Conclusion: To our knowledge, this study is the first molecular characterization of this inv(5)(p13q13) finding similar breakpoints that will be described. We will discuss the hypothesis between either recurring genomic event or foundation effect. A better knowledge of this entity will be useful to justify the decisions and prenatal genetic counselling in case of its fortuitous prenatal detection. Indeed different practice might occur facing the detection of this inv(5) in the karyotype of a fœtus and its parents.

References:

Grants:

Conflict of Interest: None declared.

EP15.003 Combining cytogenetic techniques to characterize a rare chromosomal rearrangement resulting in pure partial 11q duplication

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Background/Objectives: We report here a case of a 10-year-old girl referred to our department at the age of 35 days because of dysmorphic features with microcephaly and hypotonia in whom cytogenetic analyses showed a pure 11q duplication resulting from a rare and complex paternal chromosomal rearrangement.

Methods: R-banding karyotypes of cultured peripheral blood lymphocytes obtained from the proband and her parents were performed. Fluorescent in situ hybridization (FISH) and array comparative genomic hybridization (aCGH) (Agilent, 180KX4) analyses were performed to characterize the identified chromosomal abnormality.

Results: Karyotype showed a balanced pericentric inversion of the 11p15-q12 region inherited from her father. aCGH was performed and revealed a 19.8 Mb duplication of the 11q22.3-q24.1 region. FISH confirmed aCGH results and revealed that the patient inherited a recombinant chromosome with duplication of the 11q22.3-q24.1 region resulting from a paternal intrachromosomal insertion of this region at the inversion breakpoint 11q12.

Our patient has been followed up for 10 years now and has presented developmental delay including language delay and intellectual disability. Clinical examination showed normal growth and facial dysmorphism.

Conclusion: To date and to the best of our knowledge, only 10 cases of pure partial 11q duplication have been reported in the literature [1]. This chromosomal abnormality resulted from a very rare chromosomal rearrangement associating a pericentric inversion and an intrachromosomal insertion of chromosome 11 characterized by molecular cytogenetic techniques.

References: 1- Kayhan, Gülsüm et al. "Molecular karyotyping of an isolated partial trisomy 11q patient with additional findings." Gene vol. 524,2 (2013): 355-60.

Grants:

Conflict of Interest: None declared.

EP15.004 Genetic heterogeneity of acute leukemias with t(10;11)(p12;q21)/PICALM::MLLT10 in children

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Background/Objectives: Translocation t(10;11)(p12;q14)/*PICALM::MLLT10* is a rare but recurrent rearrangement in some acute leukemias (AL) of various lineage. The reason for such heterogeneity remains unclear. The aim of this work was to analyse the structure of the *PICALM::MLLT10* chimeric gene, as well as the repertoire of *TCR* and *IGH* rearrangements and the spectrum of additional mutations in acute leukemias with t(10;11)(p12;q14) in children.

Methods: The study included 36 patients (3–16 y.o., median 10) diagnosed with T-ALL ($n = 16$, m:f = 3:1), AML ($n = 16$, m:f = 1.29:1) and other ALs (n = 4, m:f = 3:1). All patients underwent karyotyping and FISH. Molecular profiling included RNAseq ($n = 30$), the whole exome analysis ($n = 23$), and the clonal repertoire of *TCR* and *IGH* assessment (n = 24; Komkov et al., 2020).

Results: *PICALM* breakpoints were located in introns 17 to 19, in *MLLT10* – in introns 3 to 9. The structure of *PICALM::MLLT10* was more heterogeneous in AML than in T-ALL (6 vs 3 transcript variants). Analysis of concomitant mutations revealed from 1 to 8 pathogenic exon somatic variants per sample, median 3. RAS pathway was frequently affected ($n = 14$). No association of additional mutations with AL lineage was observed. Clonal rearrangements of *TCR* and/or *IGH* were found in all T-ALLs ($n = 12$ of 12) and in most AMLs ($n = 8$ of 12).

Conclusion: *PICALM::MLLT10*-associated ALs are characterized by high chimeric gene heterogeneity, low mutation load with predominant involvement of RAS pathway, and the presence of *TCR* and *IGH* clonal rearrangements in both T-ALL and AML.

References:

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Conflict of Interest: Elena Zerkalenkova Modest - The study was supported by the Russian Science Foundation grant No. 22-25-00367 (principal investigator), Aleksandra Borkovskaia Modest - The study was supported by the Russian Science Foundation grant No. 22-25-00367 (collaborator), Egor Volchkov Modest - The study was supported by the Russian Science Foundation grant No. 22-25-00367 (collaborator), Anna Kazakova: None declared, Olga Soldatkina: None declared, Evgenii Matveev: None declared, Marat Kazanov: None declared, Yulia Olshanskaya: None declared.

EP15.005 Satellited chromosome 20 - the importance of cytogenetic and FISH analysis

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Background/Objectives: Human acrocentric chromosomes 13, 14, 15, 21, and 22 are characterized with satellited short arms containing three bands. Despite being gene-poor, p-arm of those contain multiple copies of the genes for ribosomal RNA included in the nucleolus organizing region (NOR) in band p12. Sometimes also non-acrocentric chromosomes can appear with satellites – after translocation of p-arm of acrocentrics, including satellites, on subtelomeric or telomeric regions of non-acrocentrics. Gain or loss of satellited short arm doesn't have appearance in phenotype, the example is a balanced carrier of Robertsonian translocation.

Methods: Here we present a case of 31-year-old woman reffered to our lab after genetic counseling for sterility, and initial workup with karyotype 46,XX,add(20)(p13) and aCGH analysis that showed no clinically significant copy number changes. FISH method was performed for identification of additional material on chromosome 20.

Results: Analysis showed normal position of subtelomeric 20p and 20q probes. The whole chromosome 20 probe painted the entire chromosome 20 except the terminal part of the short arm. The locus 20p12.2 probe was also in normal position. Finally, aditional FISH analysis revealed positive Acro-p-arm signal at the terminal end of the short arm of one chromosome 20 and confirmed the additional presence of acrocentric NOR region.

Conclusion: Presented case emphasizes the importance of classical chromosomal cytogenetic and FISH anlaysis for detecting chromosomal abnormalities undetectable with other molecular methods.

References:

Grants:

Conflict of Interest: None declared.

EP15.006 A polygenic risk score predicts mosaic loss of chromosome Y in circulating blood cells

Jonas Mattisson[1;2], *Moeen Riaz*[3;4], *Galina Polekhina*[3;4], *Andrew Bakshi*[3;4], *Jonatan Halvardson*[1;2], *Marcus Danielsson*[1;2], *Adam Ameur*[1;2], *John McNeil*[3;4], *Lars Forsberg*[1;2;5], *Paul Lacaze*[3;4]

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Background/Objectives: Mosaic loss of Y chromosome (LOY) is the most common somatic mutation in circulating white blood cells of older men. LOY in leukocytes is associated with increased risk for all-cause mortality and a range of common disease including hematological and non-hematological cancer, Alzheimer's disease, and cardiovascular events. Recent genome-wide association studies have identified germline variants associated with risk of LOY. The objective of this study was to use these variants to calculate a novel polygenic risk score (PRS) for LOY, and to assess the predictive performance of this score in a large independent population of older men.

Methods: Using previously identified risk variants, a PRS was calculated for LOY in 5131 men aged 70 years and older. Levels of LOY were estimated using microarrays and validated by whole genome sequencing.

Results: After adjusting for covariates, the PRS was a significant predictor of LOY (odds ratio [OR] = 1.74 per standard deviation of the PRS, 95% confidence intervals [CI] 1.62–1.86, $p < 0.001$). Men in the highest quintile of the PRS distribution displayed > fivefold higher risk of LOY than the lowest (OR = 5.05, 95% CI 4.05–6.32, $p < 0.001$). Adding the PRS to a LOY prediction model comprised of age, smoking and alcohol consumption significantly improved prediction (AUC = 0.628 [CI 0.61–0.64] to 0.695 [CI 0.67–0.71], $p < 0.001$).

Conclusion: Our results suggest that a PRS for LOY could become a useful tool for identifying men with increased risk for common disease that might benefit from targeted intervention.

References:

Grants: Funding for this study is detailed in the published article PMID: 34895331.

Conflict of Interest: Jonas Mattisson: None declared, Moeen Riaz: None declared, Galina Polekhina: None declared, Andrew Bakshi: None declared, Jonatan Halvardson: None declared, Marcus Danielsson: None declared, Adam Ameur: None declared, John McNeil: None declared, Lars Forsberg Co-founder and shareholder in Cray Innovation AB, Co-founder and shareholder in Cray Innovation AB, Paul Lacaze: None declared.

EP15.007 Further delineation of the MYT1L duplication syndrome phenotype: a systematic review

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Background/Objectives: Deletions and SNVs of *MYT1L*, a gene implicated in neuronal differentiation, have been described to be associated with a syndromic presentation consisting of developmental delay (DD), intellectual disability (ID) and obesity. If, duplications were rather linked to schizophrenia, to further delineate the phenotypic spectrum, we describe the clinical details of patients with a 2p25.3 duplication including all or a part of *MYT1L*.

Methods: Through national collaboration, DECIPHER database and literature review, we identified a cohort of 54 patients with 2p25.3 duplications identified by chromosomal microarray analysis. We excluded among them 14 patients presenting additional copy number changes or lacking clinical data.

Results: Detailed clinical evaluation of 40 patients with a 2p25.3 duplication revealed variable clinical features. DD and/or ID are the most common clinical phenotype ($N = 14/40$), followed by autism ($N = 11/40$) and schizophrenia ($N = 10/40$). No appreciable neuropsychiatric phenotype was found in 5 patients.

The rearrangement size ranges from 101 kb to 997 kb and includes 1 to 4 genes. All aberrations lead to either partial or complete duplication of *MYT1L*, among which 9 appear to be intragenic. We found 19 duplications that comprise the 5′ region of *MYT1L*, including exons 1–4. Parental testing in 20 subjects showed that 9 duplications were inherited, generally from asymptomatic parents (7 cases).

Conclusion: The 2p25.3 duplication, including *MYT1L*, exhibiting phenotypic diversity and the presence of apparently normal carrier parents suggests an incomplete penetrance and a variable

expression, probably explained by unknown yet additional genetic and nongenetic modifiers.

References:

Grants:

Conflict of Interest: None declared.

EP15.008 Inherited 16p11.2 microdeletion with variable phenotype: Report of a Saudi Arabian family

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Background/Objectives: 16p11.2 microdeletion syndrome is caused by a recurrent chromosomal anomaly and is mainly characterized by neurocognitive developmental delay, intellectual disability and predisposition to obesity. However, phenotypic spectrum of this microdeletion is extremely variable which can be partially explained by the size and location variability of the microdeletion. However, this phenotype variability was also reported in some inherited cases from a parent with a different phenotype. We report here a new inherited case of 16p11.2 microdeletion transmitted from a mother to her son with a completely different phenotype.

Methods: The son is a 7 years old boy presenting Attention-Deficit / Hyperactivity Disorder. Clinical history describes a global developmental delay with speech delay and intellectual disability without seizure or dysmorphic features.

The mother is 45 years old with no identified intellectual or behavioral abnormalities. However, she is obese (BMI = 32), diabetic and presenting dermatological alterations with in particular alopecia and lichen plan pilaris. She reported also reproductive failure with repetitive abortions.

Array CGH was performed using a 180K CGH+SNP microarray for both of them.

Results: Array CGH analysis showed an heterozygote 597,785 Kb microdeletion at 16p11.2 (29,592,783–30,190,568) in both cases.

Conclusion: To the best of our knowledge, this is the first reported case of lichen plan pilaris and recurrent abortions associated with 16p11.2 microdeletion. Variability of clinical outcome in this case, is most likely caused by one or more genes outside the deleted region which are regulators of the phenotype induced by this haploinsufficiency.

References:

Grants:

Conflict of Interest: None declared.

EP15.009 The role of copy-number variation in the pathogenesis of human diseases on Brazilian patients

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Background/Objectives: Genomic variation accounts for a large proportion of human genome and are known to be associated with disease susceptibility. Single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) represent the most popular genomic variations. The objective of this study was to describe CNVs involved in the pathogenesis of human diseases on Brazilian patients.

Methods: We have analyzed 584 patients underwent SNP-array by developmental delay, intellectual disability, dysmorphia, epilepsy and ASD. The SNP-array was performed by the ThermoFisher CytoScan 750k platform at the DNA Laboratory- Central Laboratory for Genetics and Molecular Biology (Salvador-BA/Brazil) between June 2018 and December 2021. The identified CNVs were evaluated using public genetic databases. CNVs were classified according to the criteria of the American College of Medical Genetics and Genomics (ACMG) – (Riggs, et al., 2020).

Results: Eighty-four cases (14.38%) displayed pathogenic or likely pathogenic CNVs and forty-nine cases (8.39%) displayed CNVs which currently have uncertain significance. Pathogenic deletions and duplications were found on all chromosomes, except on chromosomes 6, 19, and 20.

Conclusion: The high prevalence of pathogenic CNVs in this study highlights the importance of microarray analysis on patients with ASD or developmental delay/learning disability and epilepsy. Research on genomic structural variation Is useful to analyze a patient's phenotype and it may also provide essential data regarding his or hers prognosis, which helps physicians to choose the best course of treatment available.

References:

Grants:
No funding.

Conflict of Interest: None declared.

EP15.010 Pitfall in prenatal diagnostic: fetoplacental discordancy of a class I analphoid supernumerary marker chromosome

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Background/Objectives: Neocentromere formation is a rare event reported in more than 100 cases. Here we present a case of a small supernumerary analphoid marker chromosome that could not be detected in CVS but in peripheral blood and fibroblasts from the patient. Prenatally, the fetus presented with an omphalocele, an increased nuchal translucency, a hygroma colli, lateral neck cysts and an anomaly of the cerebellum. Postnatally, the one year old boy presented with global developmentally delay, facial dysmorphism, hirsutism, soft cleft palate, cardiomyopathy, and omphalocele.

Methods: In the prenatal setting, cytogenetic analysis, SNP array and symptom-based panel analysis were performed on chorionic villi. In the postnatal setting, we performed cytogenetic analysis, including FISH, on peripheral lymphocytes and fibroblasts. Further, SNP array was performed on peripheral lymphocytes.

Results: Chromosomal analysis of chorionic villi revealed a normal male karyotype. SNP array and panel analysis on long-term CVS culture showed no pathogenic variants. Postnatally, cytogenetic and molecular cytogenetic analysis of peripheral

lymphocytes showed a de novo analphoid class I marker which is formed by an inverted duplication of the distal part of the long arm of chromosome 3 resulting in a partial tetrasomy of the chromosomal region 3q26.2 – 3qter. This analphoid marker chromosome was present in 94% of the analysed metaphases of peripheral lymphocytes and in 26% of the analysed metaphases of cultured fibroblasts.

Conclusion: This case presents a discrepancy between the analysed chorionic villi sample and the fetus (fetoplacental discordancy). Further, it shows the mosaicism in different tissues of the patient.

References:

Grants:

Conflict of Interest: None declared.

EP15.011 Chromosomal abnormalities in products of conception of first-trimester miscarriages: A Romanian retrospective study

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Background/Objectives: Spontaneous first trimester miscarriages are strongly associated with chromosome abnormalities. The purpose of our study was to investigate the frequency and type of chromosome abnormalities detected by standard karyotyping in first trimester miscarriages in South-Western Romania.

Methods: Chromosome analysis of specimens was performed after long-term culture according to standard cytogenetic methods using G-banding technique. All samples with a normal female karyotype were subjected to QF-PCR testing for maternal cell contamination by comparison of multiple microsatellite markers in maternal blood versus cell culture/fetal samples.

Results: A total of 224 samples were analyzed after rule out of MCC by QF-PCR. Chromosomal abnormalities were detected in 132 cases (58.92%). Single autosomal trisomies accounted for 53.03% of all abnormal karyotypes (70/132)—the most common being trisomies 16, 21 and 22. Monosomy X was detected in 17.42% of cases (23/132), whereas polyploidy was found in 15.9% of cases (21/132). 12 samples (9.09%) presented structural rearrangements and chromosomal mosaicism was found in three cases. In the stratified analysis, women over the age of 35 had a higher rate of chromosomal abnormalities and the association was restricted to trisomies. Women younger than 35 showed a higher incidence of monosomy X and polyploidy.

Conclusion: Our results confirmed previous findings that older women with spontaneous miscarriages have a higher rate of chromosomal abnormalities. Due to the combination of standard karyotyping and QF-PCR, our study can offers reliable information for genetic counseling of patients with first trimester miscarriages.

References:

Grants:

Conflict of Interest: None declared.

EP15.013 A dicentric chromosome 2 due to a 19.7 Mb duplication in a child with normal development, microcephalus and growth retardation

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Background/Objectives: Duplications of the centromere-near regions of chromosome 2 are rarely described, and have been reported to be associated with developmental delay, intellectual disability, feeding difficulties and micro- or macrocephaly for children with partial duplications of 2q11.2(1).

Dicentric chromosomes are rare and their stability during cell division depends on inactivation of one of the two centromeres or by the inter-centromere distance (2).

Methods:.

Results: The case concerns a girl who presented at age 6 months with microcephaly and growth retardation. Currently, at age 10 years, she presents with short stature, microcephaly, hearing loss and normal cognitive development.

Chromosome microarray identified a 19.7 Mb duplication of the centromeric region of chromosome 2 (p11.q12.2). Chromosomal- and FISH-analysis revealed a derivative chromosome 2 with a tandem duplication with two centromeres. A small fraction (2.5 %) showed two apparently normal chromosomes 2. The derivative chromosome 2 was unstable, shattered in some metaphases, with delayed condensation in others and with interphasic micronuclei positive for chr2 signals. The instability was interpreted to be a result of two active centromeres.

Conclusion: We present a 10 years old girl with a dicentric chromosome 2 with a 19.7 Mb gain and discuss her phenotype in relation to the genes involved and the instability of the derivative chromosome 2.

References: (1) Riley, K. et al. Recurrent deletions and duplications of chromosome 2q11.2 and 2q13 are associated with variable outcomes. (2015).

(2) Sullivan, B. et al, Stable dicentric X chromosomes with two functional centromeres. Nat Genet 20, 227–228 (1998).

Grants:

Conflict of Interest: None declared.

EP15.014 Clinical utility of CytoScan Optima for prenatal diagnosis

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Background/Objectives: Prenatal chromosomal microarray (CMA) is known to have 6% of diagnostic yield in fetus with major anomaly and normal karyotype. We investigated clinical utility of CytoScan Optima array as prenatal genetic testing.

Methods: We analysed the results of 1470 cases of prenatal CMA in GC Genome from Jan 2020 to Dec 2021. DNA was extracted from amniotic fluid (AF) or chorionic villus sampling (CVS). Prenatal CMA was performed using CytoScan Optima array (ThermoFisher Scientific, Waltham, MA, USA). The results of prenatal CMA were classified according to ACMG standards for the interpretation and reporting of constitutional copy-number variants (CNVs). Pathogenic/likely pathogenic (P/LP) CNVs were reported as positive and variant of uncertain signicant (VUS) CNVs were reported as inconclusive. Clinical significant absence of heterozygosity (AOH) was also reported. We considered microdeletion/duplication size threshold as 10 Mb.

Results: In total cases, AF was 87% and CVS was 13%. Overall clinical significant results were 13.9%: positive 11.8%, inconclusive 1.7%, and AOH 0.4%. Aneuploidy or large deletion/duplication

cases were 7.8%, which can be detected by conventional karyotyping. Microdeletion/duplication or LOH cases were 6.1%, which cannot be detected by conventional karyotyping. However, in 1.7% of cases with large CNVs, CMA provided more accurate and objective informations such as genomic coordinate, genomic content, and/or additional informations compared to the conventional karyotyping.

Conclusion: CytoScan Optima arrays has effective and sensitive performances in prenatal diagnosis and the diagnostic yield can be 6-8% considering more useful informations compared to the conventional karyotyping.

References:

Grants:

Conflict of Interest: None declared.

EP15.016 Partial trisomy of chromosome 10 in a newborn: a case report

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Background/Objectives: Segmental aneuploidies are the major contributor to neurodevelopmental disorders. We report a case of an infant, currently one and a half years old, with facial dysmorphy (slanted eyes, hypertelorism, saddle nose, flattened face), mild psychomotor retardation, upper extremity hypertonia, hypotonia of the trunk and neck, torticollis and left ventricular hyperthrophy.

Methods: Cytogenetic analyses was performed in the infant, the parents of the infant and the infant's grandmother. Additionally, Array CGH was performed in the infant.

Results: Infant's karyotyping showed a trisomy of the distal part of the long arm of chromosome 10 (46,XY-22,+der(22)t(10;22)(q24;p11)). A chromosomal microarray showed duplication in the region 10q25.1-26.3 of 24.44 Mb. This region includes 144 protein coding genes of which 31 are OMIM morbid. Also, infant's mother and grandmother karyotyping revealed balanced translocation between chromosomes 10 and 22 (46,XX,t(10;22)(q24;p11)) in both cases. Infant's father had normal male karyotype.

Conclusion: We would like to emphasize the importance of a proper genetic counseling and increasing awareness of patients regarding chromosomal rearrangements, in order to avoid occurrence of an unbalanced offspring.

References: No.

Grants: No.

Conflict of Interest: None declared.

EP15.017 A unique de novo partial trisomy 9p23→pter and partial monosomy 22q13.31→qter

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Background/Objectives: The unbalanced rearrangements are not common; however, they have significant and often unique clinical expression. We report a case of the de novo unbalanced translocation between chromosome 9 and 22 in male infant with dysmorphic facial features, tall stature, global developmental delay, hypotonia, periventricular leukomalacia on MRI, patent foramen ovale, hydrocele testis and phimosis, edematose hands and feet and dysplastic nails on his feet.

Methods: The analysis was conducted using Agilent 60K oligonucleotide array-based comparative genomic hybridization. The cytogenetic analysis was performed by conventional high-resolution cytogenetic technology.

Results: Genetic investigation using array-based comparative genomic hybridization revealed a 9p24.3p23 duplication and 22q13.31q13.33 deletion. Chromosome analysis showed an abnormal chromosome 22, suggesting an unbalanced 9;22 translocation with extra 9p-material on terminal 22q. The karyotype was 46,XY,der(22)(q13.3). Both parents had normal karyotype.

Conclusion: To our knowledge, this is the first patient described in the literature with partial trisomy 9p and partial monosomy 22q. Phenotype in patients with segmental aneuploidy and segmental trisomy often vary in their clinical manifestation depending on the size of the chromosomal region involved. In general, determining the genotype-phenotype correlation is often difficult by the presence of concomitant partial trisomy of other chromosome. The presented case shows the importance of classical cytogenetics in determination of the chromosomal rearrangements.

References:

Grants: This study was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project „Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials".

Conflict of Interest: None declared.

EP15.018 A paracentric inversion that disrupts the SHANK2 gene resolved using cytogenomics

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Background/Objectives: A girl of 12 presented with mild ID. Diagnostic analysis demonstrated a *de novo* paracentric inversion; inv (11)(q13.3;q25) as only abnormality. Trio sequencing was negative. Here, we sought to determine the breakpoints in order to identify the causal genetic defect.

Methods: Ultra-high molecular weight DNA was purified from 650µL blood using the SP Blood & Cell Culture DNA Isolation Kit (Bionano genomics). gDNA was labeled using the DLS DNA Labeling Kit. The Saphyr chip was loaded using the Saphyr System User Guide and ran to reach a minimum yield of 320 Gbp. *De novo* assembly and Variant Annotation Pipeline were executed on Bionano Solve V3.7. Reporting and direct visualization of structural variants was done on Bionano Access v1.7. Procedure was performed at the Bionano Services Lab in France. WGS was performed by BGI on a DNBSEQ.

Results: WGS failed to result in the identification of the breakpoints. Subsequently, next-generation cytogenomics with optical genome mapping was performed, which confirmed the presence of an inversion of 63.65 Mb (region: chr11:71,086,636-134,734,015, GRCH38/hg38) and revealed that its breaking point was situated in the *SHANK2* gene (estimated in intron 8 of 25).

Apart from this, a smaller inversion of 335.55 kb situated entirely in *SHANK2* (region: chr11:70,615,351-70,950,900, GRCH38/hg38), which includes exon 11 to 17, was detected.

Conclusion: Here, we elucidated the breakpoints of this large inversion in *SHANK2*. It is possible that a smaller inversion in this gene obscured the detection of the larger inversion on the WGS data.

References:

Grants:

Conflict of Interest: None declared.

EP15.019 Atypical Down syndrome features: Isodicentric chromosome 21

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Background/Objectives: Down syndrome is one of most common chromosomal disorders with an estimated incidence of 1 in 700 live births. Most cases result because of the presence of an extra chromosome 21 due to non-disjunction. Isodicentric chromosome 21 is a rare form of chromosomal rearrangement reported in a few cases in the literature.

Methods: Genomic DNA was fragmented, amplified, and hybridized to the array according to manufacturer's guidelines. The results were analyzed with the chromosomal analysis suite (ChAS, Affymetrix).

Results: We present a premature baby born at 32 weeks of gestation with intrauterine growth restriction. She was found to have atrial septal defect and mitral valve insufficiency. She had subtle dysmorphic features in form of downslanting palpebral fissures with no other features suggestive of Down syndrome. Chromosomal analysis showed isodicentric chromosome 21(46, XX,idic(21)(q22.3)). Array CGH showed concomitant duplication of most of chromosome 21(21p11.2q22.3(7761419_41294939) and 4.5 Mb deletion of long arm of chromosome 21, 21q22.3(41295017_46677460).

Conclusion: The lack of typical Down syndrome phenotype in this case may be attributed to the concurrent large deletion of the 21q22.3. Dissecting this region may help uncover genes with important role in pathogenesis of Down syndrome features. However, the preterm age and low birthweight may obscure some of the typical facial features.

References: Putra M, Surti U, Hu J, Steele D, Clemens M, Saller DN, Yatsenko SA, Rajkovic A. Beyond Down syndrome phenotype: Paternally derived isodicentric chromosome 21 with partial monosomy 21q22.3. Am J Med Genet A. 2017 Dec;173(12):3153-3157.

Grants: None.

Conflict of Interest: None declared.

EP15.020 Challenge in complex chromosomal reorganizations characterization: a family case

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Background/Objectives: Complex chromosomal rearrangements are often difficult to characterize even with a combination of current cytogenetic techniques. We present a family case in which siblings with different phenotypes showed different imbalanced chromosomal alterations inherited from the phenotypically normal mother who carried a complex chromosomal reorganization.

Methods: G-band and FISH cytogenetic analysis; Array-CGH (8x60, ogt, UK).

Results: The index case was an 11-year-old girl with intellectual disability and short stature. Array-CGH study was performed showing two deletions at 9q21.13q21.32 and 9q21.33q22.31 separated by a normal dose region. The 13-year-old brother had mild intellectual disability and the Array-CGH study showed the reciprocal anomaly: two duplications at 9q21.13q21.32 and 9q21.33q22.31 separated by a normal dose region. The mother underwent a cytogenetic FISH study using a 9q21 probe that revealed an insertion of this region in the q arm of chromosome 3. In a later pregnancy, an Array-CGH prenatal study was performed showing a continuous 9q21.13q22.31 duplication not interrupted by any normal region, differing from the alterations observed in the siblings. The prenatal G-band cytogenetic study showed an insertion of material at 3q21 compatible with the insertion of material from chromosome 9 revealed by the mother's FISH study.

Conclusion: The presence of different alterations in the offspring together with the chromosomal insertion observed in the mother, suggest she carries a balanced complex reorganization difficult to identify with the current cytogenetic techniques. New techniques, as optical mapping, may be helpful for its complete characterization.

References:

Grants:

Conflict of Interest: None declared.

EP15.021 The contribution of the Karyotype in the diagnosis of therapy related chronic myelomonocytic leukemia (t-CMML) initially known as Acute Myeloid Leukemia (AML)'relapse: a rare case Tunisian report

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Background/Objectives: Therapy related Chronic myelomonocytic leukemia (t-CMML), an aggressive myeloid neoplasm with overlapping features of myelodysplastic syndrome and myeloproliferative neoplasms, is diagnosed in patients who have been exposed to cytotoxic chemotherapies.

Methods: We describe a rare case report of t-CMML diagnosed as part of AML assessment.

Results: A 51 year old Tunisian man, followed-up for acute myeloid leukemia (AML) since five years and received the AML's protocol. Actually, in the presence of hyperleukocytosis, macrocytic anemia and monocytosis, the AML's relapse is suspected. The bone marrow was hypercellular with megakaryocytic dysplasia, 8% of blasts and monocytes. Chromosome studies revealed monosomy 7.

Conclusion: This is the first a case of t-CMML reported after exposure to chemotherapeutic agents for the treatment of AML. The diagnosis is retained in the presence of cytological and cytogenetic arguments.

References: Therapy Related-Chronic Myelomonocytic Leukemia (CMML): Molecular, Cytogenetic and Clinical Distinctions from ***de novo*** CMML.

Grants:
Conflict of Interest: None declared.

EP15.022 Alfi syndrome in a patient with ring chromosome 9

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Background/Objectives: Ring chromosomes (RC) usually result from two terminal breaks in both chromosome arms, followed by fusion of the broken ends. Phenotypic variability are highly associated with RC, due to the genomic loss, but also to the loss or gain of material and the mosaicism caused by RC instability.

Methods: We report here a case of a two-year old boy presented with Developmental Delay, facial dysmorphism, trigonocephaly, genital abnormalities and heart defect. The patient consulted the department of cytogenetics and reproductive biology of Farhat-Hached-UH for molecular cytogenetic exploration of a mosaic ring chromosome 9.

Results: Array comparative genomic hybridization revealed an isolated terminal deletion of the chromosome 9 short arm of 18Mb in 9p24.3-p22.1. Fluorescence in-situ hybridization confirmed the 9p subtelomeric deletion and the integrity of 9q subtelomeric region. Our patient's deletion encompasses the critical region defined in Alfi syndrome. It includes several genes such as *FREM1, VLDLR, ZDHHC21, and DMRT1*. In fact, *DMRT1* gene is known to be involved in genital development. The haploinsufficiency of the *FREM1* gene is known to be implicated in the premature fusion of the metopic suture causing a trigonocephaly. *VLDLR* and *ZDHHC21* genes have been described respectively responsible of intellectual disability. Heart defects were reported in some Alfi syndrome cases.

Conclusion: In our case, the deletion only included one chromosome arm, which evinces the peculiarity of the mechanism of RC. This leads us to insist on the role of the characterization of RC, in order to establish an adequate genotype-phenotype correlation and for an appropriate genetic counseling.

References:

Grants:

Conflict of Interest: None declared.

EP16 New Technologies and Approaches

EP16.001 Effectiveness of whole genome analysis in detecting patients with compound heterozygosity for deletion and single nucleotide substitution

Mamiko Yamada[1], *Hisato Suzuki*[1], *Taiki Shima*[2], *Hiroyuki Adachi*[3], *Atsuko Noguchi*[3], *Fuyuki Miya*[1], *Tsutomu Takahashi*[3], *Kenjiro Kosaki*[1]

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Background/Objectives: In the diagnosis of autosomal recessive inherited diseases, especially in non-consanguineous families, exome analysis alone has diagnostic limitations. It is desirable to combine whole genome analysis to evaluate not only single nucleotide variants but also structural variants.

Methods: A total of 131 samples that could not be diagnosed by exome analysis alone were additionally analysed by whole genome analysis.

Results: We identified two patients with autosomal recessive disorders caused by a combination of single nucleotide variant and deletion in the corresponding gene. Long read sequencing was also performed to determine the details of the structural variants. Patient 1: A 2-year-old boy had severe intellectual disability and intractable epileptic seizures. Genomic analysis revealed that the patient had a hemizygous variant in NM_017951.4 c.832C>T, p.(Arg278*) of SMPD4 gene of paternal origin and a deletion in SMPD4 gene of maternal origin. Patient 2: A neonatal boy passed away at 15 days of age, and postmortem CT brain imaging showed pontocerebellar hypoplasia. The patient and his brother, who passed away on the day of birth, were found to have hemizygous variant in NM_138773.2 c.385-1G>A in SLC25A46 gene of maternal origin and deletion in SLC25A46 gene of paternal origin.

Conclusion: The combination of exome and whole genome analyses led to definitive diagnoses. In addition, long-read sequencer elucidated the precise structure of the variants. Both patients had severe congenital syndromes with poor prognosis, and preimplantation diagnosis is being planned. Accurate investigation of the genetic causes can contribute to family planning.

References:

Grants: JP21ek0109549 and JP21ek0109485 from AMED.

Conflict of Interest: None declared.

EP16.002 Airway and lung organoids derived from human induced pluripotent stem cells with functionally active CFTR channel

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Background/Objectives: The purpose was to obtain and to characterize airway (AOs) and lung organoids (LOs) from human induced pluripotent stem cells (hiPSCs) from a healthy donor and a donor with cystic fibrosis (CF, CFTR F508del/F508del) for subsequent use in evaluating the efficacy of CFTR channel restoration after genome editing of the F508del mutation in CFTR gene.

Methods: Both types of organoids were obtained from hiPSCs. Immunocytochemical staining, flow cytometry, and confocal microscopy were performed to determine the cellular composition of organoids. Functional activity of the CFTR channel was performed by forskolin-induced swelling (FIS).

Results: AOs and LOs were obtained from healthy and CF hiPSCs. AOs contain the proximal lung epithelial cells, while LOs contain both proximal and distal lung epithelial cells, which include alveolar type I and type II cells. AOs and LOs were characterized for the presence of basal, goblet and Club cells. AOs and LOs from a healthy donor swelled in response to forskolin for 24 hours by an average of 2.5 times ($p < 0.0001$) and 5.6 times ($p < 0.0001$) relative to 0 hours, respectively. AOs and LOs from CF patient swelled for 24 hours by an average of 1.1 times ($p = 0.598$) and 1.08 times ($p = 0.685$) relative to 0 hours, respectively.

Conclusion: The results of the work demonstrate the cellular composition of AOs and LOs, as well as the possibility performing of FIS on both AOs and LOs, which can be used to assess the efficacy of genome editing of the CFTR gene.

References:
Grants:
Conflict of Interest: None declared.

EP16.005 Development of an In-house Long Read Sequencing Clinical Pharmacogenomic Panel

Michelle Moore[1], *Alexandra Traufler*[1], *Shannon Weber*[1], *Mariska Davids*[1], *Shaopeng Gu*[1], *Praveen Cherukuri*[1], *Blake Atwood*[1]

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Background/Objectives: As pharmacogenomics (PGx) more commonly integrates into clinical care, the electronic medical records (EMR) of health care systems become reliant on best practice alerts (BPAs) within the EMR for better support and more accurate prescribing recommendations. Two commonly used laboratory methods to determine star alleles used to run EMR BPAs include quantitative PCR and short read sequencing. While these methods provide reliable genotyping information, neither are able to assess if two SNPs are present on the same or opposite alleles when the SNPs are at a distance that single short reads cannot capture, leaving laboratories to infer their star allele classifications.

Methods: PCR and capture based enrichment methods were compared by using previously published primers to perform long range PCR targeting and sequencing of CYP2D6 (1), then of HLA-A and HLA-B (2) in the PCR method. The capture method had custom probes, designed targeting genes of interest, pulled and sequenced. A bioinformatics pipeline modelled after previously published methods (1) analysed the data.

Results: Quality targeted read sequences from both enrichment methods were visually analysed in single reads using tools like IGV. Each sample was accurately haplotyped using the in-house developed bioinformatic pipeline.

Conclusion: The two developed methods could potentially call star alleles for different pharmacogenomic genes. As shown, the targeted variants were accurately haplotyped on a single read.

References: 1. PMID: 31559921.
2. PMID: 32419382.
Grants: N/A
Word count: 227.

Conflict of Interest: Michelle Moore Full, Sanford Imagenetics, Alexandra Traufler Full Time Sanford Imagenetics, Shannon Weber Full time Sanford Imagenetics, Mariska Davids Full Time Sanford Imagenetics, Shaopeng Gu Full Time Sanford Imagenetics, Praveen Cherukuri Full Time Sanford Imagetics, Blake Atwood Full Time Sanford Imagenetics.

EP16.006 Delivery of transgenes into various cell lines containing lung cells by recombinant adeno-associated viral vectors

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Background/Objectives: Recombinant adeno-associated viral vectors (rAAV) are the most optimal vectors for delivering various genes to target cells in vivo. Three serotypes AAV – 5, 6 and 9, which have tropism to lung cells, were selected for the study. The aim of the work was to evaluate the effectiveness of delivery of the EGFP gene to various cell lines containing lung cells using rAAV.

Methods: The work was carried out on cell lines: CFTE29o- (tracheal epithelium from a cystic fibrosis patient with homozygous F508del mutation in CFTR gene), induced pluripotent stem cells (iPSCs, from a cystic fibrosis patient with the same mutation and a healthy donor), as well as on airway (AO) and lung organoids (LO) derived from iPSCs of a healthy donor. All three rAAV serotypes with EGFP transgene in different MOI (from 500 to 1E +06 vg/cell) were tested on CFTE29o- and iPSC cell lines. AO and LO were transduced by the rAAV9 with EGFP at MOI 1E+09 vg/organoid.

Results: rAAV9 and rAAV6 at MOI 1.0E+06 was the most effective for CFTE29o- (90% and 42% of transduction efficacy, respectively). Transduction efficacy of iPSCs with rAAV6 was 2.5% at MOI 1.0E+06. The efficiency of transduction of AO by the rAAV9 vector was 16%, LO - 2.5%.

Conclusion: Optimal rAAVs were identified in this work for efficient transduction of CFTE29o- cells as well as airway organoids. They can be used in further studies for the delivery of various transgenes, including CRISPR-Cas9 for genome editing in CFTR gene for cystic fibrosis treatment.

References:
Grants:
Conflict of Interest: None declared.

EP16.007 Approach of HRM analysis for ATP7B gene common variants screening

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Background/Objectives: HRM (High Resolution Melting) detects changes in fragment DNA in spite of the type of nucleotide changes. HRM analysis is informative as screens methods to detect unknown variants in PCR products.

Methods: RT-PCR and HRM analysis with EcoStudy software (Illumina, USA) was performed among 30 patients (14 females, 16 men) from Ukraine, with idiopathic hepatobiliary disorders. The age of the patients ranged from 1 to 63 years. All patients had ALT, AST, alkaline phosphatase higher levels, hepatitis infectious and parasitic factors have been excluded. The control group included 30 healthy individuals (11 females, 19 males), aged 25 to 35 years. Validations of the results was carried out by PCR Bi-PASA for c.3207C>T variant, and ARMS PCR for c. 2304dupC.

Results: The ATP7B c.3207C>A (14 exon) and c.2304dupC (8 exon) region were screened. Analysis of the melting curves found that 5 people with idiopathic hepatobiliary disorders have differences in the melting temperature of DNA fragments. In these patient's mutation c.3207C>T was detected by PCR Bi-PASA. There are no differences in exon 8 melting curves. The obtained results coincided with the genotypes obtained by ARMS PCR.

Conclusion: The proposed HRM analysis could be used as a fast test for ATP7B gene mutations screening.

References:
Grants:
Conflict of Interest: None declared.

EP16.008 GRIN Portal: An Interactive Web Application Exploring GRIN Genes and Related Disorders

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Ramsey[9], Tim Benke[8], Stephen F Traynelis[4;5], Dennis Lal[2;6;10;11], Johannes Lemke[1]

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Background/Objectives: GRIN-related disorders represent a rare and neurologically complex genetic disease spectrum caused by pathogenic variants in GRIN genes encoding four different NMDA receptor subunits. We developed the GRIN Portal to unite clinical, genetic, and molecular data, facilitate variant interpretation and empower families and caregivers globally.

Methods: We combine manually curated data from the global GRIN patient registry, functional data from the Center for Functional Evaluation of Rare Variants, and further online resources. Annotation, variant mapping, visualization, and application design were developed using the R programming language and the Shiny framework by RStudio. App deployment and hosting were performed using Google Cloud services.

Results: The GRIN Portal is structured in five interfaces. "Basic Information", "Families" and "GRIN Registry" provide summaries, educational videos, links to family organizations and the GRIN patient registry. "Variant Interpretation" and "Research" rely on pre-annotations of all possible 46,974 single-nucleotide GRIN variants. These include regularly updated genetic and clinical data (n = 596), electrophysiological readouts (n = 605), bioinformatic annotations, sequence alignments, structure viewers and a fully automated variant classifier applying disease-specific ACMG/ClinGen GRIN Variant Curation Expert Panel criteria. The user can evaluate variants through pre-defined filtering or upload variants with real-time, dynamic, and interactive results and visualizations in the user interface.

Conclusion: The GRIN Portal is the first online resource that focuses on a single genetic disorder and represents a novel, interactive, and translational approach to both educate on a rare genetic disorder and expedite research to connect clinicians, researchers, and families. The website is freely accessible at https://grin-portal.broadinstitute.org/.

References:

Grants:

Conflict of Interest: Chiara Klöckner: None declared, Tobias Brünger: None declared, Eduardo Pérez-Palma: None declared, Ilona Krey: None declared, Marie Macnee: None declared, Scott J Myers: None declared, Hongjie Yuan NIH/NICHD (R01HD082373), GRIN2B foundation, Arthur Stefanski: None declared, Patrick May: None declared, Jenifer Sargent: None declared, Kristen Park: None declared, Amy J Ramsey: None declared, Tim Benke GRIN2B foundation, CURE foundation, Ponzio Family Chair in Neurology Research/Children's Hospital Colorado Foundation, Stephen F Traynelis NINDS R35-NS111619, Austin Purpose, Dennis Lal: None declared, Johannes Lemke: None declared.

EP16.009 A method for ABO genotyping by Sanger DNA sequencing

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Background/Objectives: Same ABO blood group matching between donor and recipient decreases graft versus host disease risk in allogeneic solid organ transplantation. A recent publication (Zhou et al. 2020) has documented a case of early graft dysfunction due to an ABO genotype mismatch that was not evident by conventional serotyping. Here we present a research method for genotyping the seven major ABO alleles A101, A201, B101, cis-AB01, O01, O02, O03 by Sanger-based DNA sequencing exons 6 and 7 of the human ABO gene.

Methods: The method entails bi-directional sequencing of four PCR-generated amplicons and analyzing the resulting sequence trace files using Applied Biosystems SeqScape software.

Results: Deciphering the mixed sequencing traces from heterozygous alleles can often be challenging for genotyping complex loci like the ABO gene. The SeqScape analysis software generates a genotype report of the 13 alleles that determine the seven major blood types. The report is then imported into an Excel-based macro that transforms the genotype information to a searchable (query) code. Here the query is aligned and matched by a simple find operation in a look-up table to a list of codes representing the 28 different homo-and heterozygous genotype scenarios.

Conclusion: This workflow enables the determination of common and rare ABO genotypes with possible weak phenotypes that may evade correct typing by serology.

For Research Use Only. Not for use in diagnostic procedures.

References: Zhou et al. Front. Immunol., https://doi.org/10.3389/fimmu.2020.608716.

Grants:

Conflict of Interest: Edgar Schreiber Thermo Fisher Scientific, stock.

EP16.011 Purification of Sanger sequencing reactions using Dynabeads on an automated KingFisher Apex platform

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Background/Objectives: Capillary electrophoresis (CE) is a commonly used tool for determining the base sequence of DNA or RNA molecules. The steps involved include amplification of the target molecule by PCR followed by sequence determination using Sanger dye terminator sequencing. The sequence is revealed by analysis of the sequencing output by CE. The quality of Sanger sequencing data critically relies on removal of excess terminator and buffer salts from the sequencing reaction. While several manual laboratory methods are available, most notably ethanol precipitation, automation of the sequencing reaction clean-up has unique challenges. Here we describe the automated, magnetic bead-based clean-up of samples in 96-well plates using the KingFisher Apex platform.

Methods: Dynabeads are added to 96-well plates containing the completed sequencing reaction which are subsequently placed onto the Kingfisher Apex instrument. This instrument automatically collects, purifies, and releases sequencing product for placing onto the CE instrument.

Results: We have modified and adapted the manual Dynabeads purification protocol for use on the KingFisher Apex platform. As part of this we have modified the wash step to effectively remove dye terminators and salts resulting in high quality sequencing reads. We show that the use of detergents aids in efficient magnetic bead collection from the wells of the bind, wash and elution plates.

Conclusion: A manual protocol using magnetic Dynabeads particles has been modified for automated processing of 96-well PCR plates on a KingFisher Apex automation platform. Purified sequencing reactions using this modified protocol show high quality reads when analysed by CE.

References:

Grants:

Conflict of Interest: Michael Wenz Full time employed with Thermo Fisher Scientific, Mohsen Karbaschi Full time employed with Thermo Fisher Scientific, Achim Karger Full time employed with Thermo Fisher Scientific, Jannes Hauptstein Full time employed with Thermo Fisher Scientific, Frauke Henjes Full time employed with Thermo Fisher Scientific, Merja Mehto Full time employed with Thermo Fisher Scientific.

EP16.012 Using the AlphaFold system to the interpretation of variants of uncertain significance

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Background/Objectives: AlphaFold is an artificial intelligence (AI) system able to correctly depict protein structure for the whole human proteome (Tunyasuvunakool et al. 2021). In this research, AlphaFold was used to evaluate variants of uncertain significance (VUS) found in the *IGFALS* gene of a subject with short stature. This gene encodes the ALS protein, which contributes to stabilization of IGF1 and 2 molecules in the serum. *IGFALS* loss of function (LoF) mutations are rare cause of short stature with a recessive mode of inheritance (Heath et al., 2008).

Methods: A short-stature girl and her parents have been investigated. *IGFALS* mutations have been found by the clinical exome approach and confirmed by Sanger sequencing. The potential presence of other gene alterations was investigated by whole exome and CGH array. AlphaFold was used to analyse ALS protein structure.

Results: Two *IGFALS* variants were found in the proband: c.1349T>C (p.Leu450Pro) and c.1363_1365del (p.Leu455del). Parents analysis demonstrated the in trans position of the two variants. ALS has a horseshoe structure and mutated positions are in the concave side of the protein, likely interfering to interaction with other proteins. According to a LoF effect of the two variants, the proband shows reduced levels of IGF1 and IGFBP-3 proteins as well as GH excess in serum.

Conclusion: Our data constitute a proof of concept that using the AI AlphaFold system can help in interpretation of VUS.

References: Tunyasuvunakool et al., Nature 2021; 596: 590-596. Heath et al., J Clin Endocrinol Metab. 2008; 93: 1616-1624.

Grants:

Conflict of Interest: None declared.

EP16.013 Feasibility of whole genome sequencing in mitochondrial DNA analysis along with comparison of variant callers

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Background/Objectives: It is believed that the interference with nuclear mitochondrial DNA sequences (NUMTs) could be overcome with whole genome sequencing (WGS) since unbiased sequences could be obtained with WGS and mitochondrial genome are abundant in quantity compared to nuclear genome in each cell.

Methods: The datasets were provided by the National Project of Bio Big Data conducted by the Korean Ministry of Health and Welfare where WGS was performed with blood DNA for the enrolled 290 subjects (123 neuromuscular disorder patients and 167 family members of the patients). We evaluated the capability of utilising WGS data in mitochondrial sequence analysis by assessing the quality of the sequences obtained. The read depth was analysed, and three different variant callers (GATK, VarDict, and mtDNA-server) were compared.

Results: All positions exhibited a depth greater than 1,000 with the exception of position 3,107 due to the revised CRS (rCRS) harbouring an artificially inserted "N" residue in position 3,107. Among a total of 17,372 variants called, 9,969 variants were identified by all three callers (57.4%). However, when the comparison was restricted to variants with a variant allele frequency greater than 5%, the agreement among the three callers increased to 99.6% with only 42 variants being called by both GATK and mtDNA-server but not VarDict.

Conclusion: We suggest that WGS is a feasible method for analysing the mitochondrial genome. While all callers demonstrated an almost perfect agreement for variants with allele frequency greater than 5%, variants with low allele frequency exhibited a poor agreement among callers.

References:

Grants:

Conflict of Interest: None declared.

EP16.014 A novel NPHP1 gene variant in combination with a gene deletion at the compound heterozygous state in a patient with Nephronophthisis

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Background/Objectives: Nephronophthisis (NPHP) is a rare autosomal recessive cystic kidney disorder leading ineluctably to renal failure in childhood or early adolescence. To date, 26 different genes have been identified as candidate for NPHP. Homozygous *NPHP1* gene deletion is the most frequent genetic defect responsible for NPHP. We report the clinical observation of a patient with a juvenile form of NPHP and harboring two different anomalies on the *NPHP1* gene at the compound heterozygous state: a novel nonsense variant and a deletion of the *NPHP1* gene.

Methods: We performed a whole-exome sequencing (WES) on the proband's lymphocyte DNA. Targeted Sanger sequencing and comparative genomic hybridization were performed to study the parental segregation.

Results: The proband is a 5-year-old boy, presenting with NPHP, terminal renal failure, and ocular involvement: retinitis pigmentosa, achromatopsia, and nystagmus.

WES showed two anomalies on *NPHP1* gene: a deletion of 81kb in 2q13 (chr2:110881068_110962844) including the whole NPHP1 gene, and a nonsense variation in the exon 17 (NM_000272.4(NPHP1):c.1711del(p.Thr571HisfsTer2). This variation was never described in GnomAD, ClinaVar, or literature, and was considered as pathogenic (PVS1, PM2, PP3) according to the American College of Medical Genetics and Genomics criteria. The study of parental segregation showed that the patient's mother was heterozygous for the deletion while the father was heterozygous for the new variant.

Conclusion: This case highlights the importance of WES in the genetic investigation of NPHP. Accurate diagnosis is essential to give the family the appropriate genetic counseling.

References:

Grants:

Conflict of Interest: None declared.

EP16.015 Cellular diversity of human breast tissue evaluated in pre-templated instant partitions for single-cell RNA sequencing (PIPseq)

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Background/Objectives: Breast tissue comprises a diverse mixture of epithelial, lymphatic, vascular, and immune cell populations, and the structure and composition of breast tissue remodels continuously throughout a woman's lifetime. Fluent BioSciences has developed a scRNA-seq platform (PIPseq) that has a lower cost-per-cell than its competitors and a more flexible workflow. We demonstrate PIPseq on a four-patient cohort of reduction mammoplasty patients to evaluate cell composition and diversity across patients.

Methods: Single-cell suspensions were prepared from breast tissue using PIPseq T20 kits (20,000 cell capture). 8 reactions of 30,000 input cells were processed in duplicate. The resulting libraries were sequenced by Illumina NextSeq 2000. Sequencing data was analyzed using the Fluent Cloud platform for bioinformatics and gene expression matrices were processed using the Seurat package in R.

Results: We demonstrate capture of ~50% of cells from each sample, with a median of ~1000 genes and ~2000 transcripts per cell at 8000 reads per input cell. In total, >80,000 cells were obtained from the 8 samples. UMAP clustering reveals distinct cell types which express markers concordant with previously obtained scRNA-seq data from these tissues processed using the 10x Chromium V3 kit. We demonstrate patient-to-patient variability in immune cell infiltrate and epithelial cell proportions, with high concordance with 10x control samples.

Conclusion: We have demonstrated high resolution cell population profiling in PIPseq with >80,000 captured cells in human breast tissue. PIPseq offers a novel instrument-free, easy-to-use and accessible platform that can be implemented in any molecular biology laboratory.

References:

Grants:

Conflict of Interest: Jesse Zhang Fluent BioSciences, Fluent BioSciences, Kiet Phong: None declared, Zev Gartner Collaborator, Robert Meltzer Fluent BioSciences, Fluent BioSciences.

EP16.017 Fine mapping and functional analysis through Massively Parallel Reporter Assay of MS susceptibility regions containing drug target genes

Lucia Corrado[1], ***Fjorilda Caushi***[1], *Endri Visha*[1], *Beatrice Piola*[1], *Erica Melone*[1], *Diego Cotella*[1], *Laura Follia*[1], *Martina Tosi*[1], *Alessandro Pizzino*[1], *Ferdinando Clarelli*[2], *Domizia Vecchio*[1], *Massimo Filippi*[3;4;5;6], *FEDERICA ESPOSITO*[2;3], *MARTINELLI BONESCHI FILIPPO GIOVANNI*[7;8], *Maurizio Leone*[9], *Nadia Barizzone*[1], *sandra d'alfonso*[1]

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Background/Objectives: Multiple sclerosis (MS) is an autoimmune multi-factorial disease affecting central nervous system (CNS). Through Genome Wide Association Studies (GWAS), 200 non-HLA susceptibility loci were identified, however, for most of these genomic regions we still have to identify the real causative variant and gene and its function.

We aimed to perform a fine-mapping and functional analysis on MS-associated regions selected to contain drug target genes.

Methods: We performed a fine-mapping of these regions and we tested the transcriptional effect of the variants using a Massively Parallel Reporter Assay (MPRA), a high-throughput technique able to test thousands of sequences for their transcriptional regulatory activity.

Results: Basing on fine mapping on an Italian case/control cohort and in-silico prediction (eQTL and differentially expression data), we selected 83 eQTL SNPs for MPRA analysis. After conducting an experimental trial to test the feasibility of the assay, we designed, based on the chromosomic location of each variant, a library suitable for MPRA consisting of sixty probes for each variant, distinguished by a unique 10bp barcode. The library included for each tested SNP: 10 probes complementary to the reference allele of the SNP, 10 complementary to the alternative one, and 10 designed by "scrambling" the sequence including the SNP, each on both forward and reverse strands.

After transfection into SHSY5Y cells the analysis will be performed in terms of barcode ratio between RNA output and DNA input for each variant.

Conclusion: this technique could find application in determining the pathogenic role of gene expression regulation variants.

References:

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Conflict of Interest: Lucia Corrado: None declared, Fjorilda Caushi: None declared, Endri Visha: None declared, Beatrice Piola: None declared, Erica Melone: None declared, Diego Cotella: None declared, Laura Follia: None declared, Martina Tosi: None declared, Alessandro Pizzino: None declared, Ferdinando Clarelli: None declared, Domizia Vecchio: None declared, Massimo Filippi receives research support from Biogen Idec, Merck-Serono,

Novartis, Roche, Teva Pharmaceutical Industries, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA), received compensation for consulting services and/or speaking activities from Alexion, Almirall, Bayer, Biogen, Celgene, Eli Lilly, Genzyme, Merck-Serono, Novartis, Roche, Sanofi, Takeda, and Teva Pharmaceutical Industries, MF is Editor-in-Chief of the Journal of Neurology, Associate Editor of Human Brain Mapping, Associate Editor of Radiology, and Associate Editor of Neurological Sciences; received compensation for consulting services and/or speaking activities from Alexion, Almirall, Bayer, Biogen, Celgene, Eli Lilly, Genzyme, Merck-Serono, Novartis, Roche, Sanofi, Takeda, and Teva Pharmaceutical Industries, FEDERICA ESPOSITO: None declared, MARTINELLI BONESCHI FILIPPO GIOVANNI received research support from Merck, Teva Pharmaceutical Industries, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla and Fondazione Cariplo, has received compensation for consulting services and/or speaking activities from Teva Pharmaceutical Industries, Sanofi Genzyme, Merck-Serono, Biogen Idec, Roche, Medday, Excemed, Maurizio Leone: None declared, Nadia Barizzone: None declared, sandra d'alfonso: None declared.

EP16.018 Application of a novel, instrument-free, single-cell RNA sequencing technology (PIPseq) to assess solid tissue diversity with both low and high cell capture methodologies

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Background/Objectives: Fluent BioSciences has developed a breakthrough single-cell analysis technology that relies on Pre-templated Instant Partitions (PIPseq) that can scale easily from hundreds to millions of individual partitions in a single sample without the need for complex instrumentation or consumables. Here, we compare Fluent's low cell count PIPSeq T2 (2,000 cell capture) and T20 (20,000 cell capture) kits to assay and identify different cell types in breast tissue samples originating from breast reduction surgery patients.

Methods: Previously frozen cells isolated from reduction mammoplasty were captured into PIPs then thermally lysed. Barcoded cDNA was generated and converted to sequencing libraries for Illumina NextSeq 2000. Data was analyzed using the Fluent Cloud Platform to generate gene expression matrices. The Seurat package in R was then used to perform downstream bioinformatic analyses.

Results: Downstream analysis using UMAP clustering allowed for annotation of major cell types within these samples based on known marker genes. We observed specific patient-to patient variability in immune and epithelial cell populations. We demonstrate high concordance between T2 and T20 kit formats, with noted advantages in gene transcription and rare cell identification resolutions, respectively.

Conclusion: PIPseq provides a scalable scRNAseq platform that is adaptable to fit multiple specific research application needs. PIPseq's lack of dependence on capital equipment and complex microfluidic consumables increases accessibility and applicability of scRNAseq.

References:

Grants:

Conflict of Interest: Ahmad Osman Fluent BioSciences, Fluent BioSciences, Jesse Zhang Fluent BioSciences, Fluent BioSciences, Kiet Phong Collaborator, Zev Gartner Collaborator, Robert Meltzer Fluent BioSciences, Fluent BioSciences, Kristina Fontanez Fluent BioSciences, Fluent BioSciences.

EP17 Diagnostic Improvements and Quality Control

EP17.001 Efficacy of conventional fragment analysis and exome sequencing as diagnostic approach for movement disorders- data from a single center study

Benita Menden[1], *Claudia Dufke*[1], *Natalie Deininger*[1], *Florian Harmuth*[1], *Joohyun Park*[1], *Anne S. Soehn*[1], *Maren Rautenberg*[1], *Kathrin Grundmann-Hauser*[1], *Marc Sturm*[1], *German Demidov*[1], *Stephan Ossowski*[1], *Rebecca Schüle*[2], *Matthis Synofzik*[2], *Ludger Schöls*[2;3], *Olaf Riess*[1], *Tobias Haack*[1;4]

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Background/Objectives: Movement disorders (MD) comprise a clinically and genetically heterogenous group of disorders that often overlap on the phenotypic and molecular level. This single center study aimed to evaluate the efficacy of a combined diagnostic approach of conventional fragment analyses (FA) and exome sequencing (ES) in a diagnostic setting. In addition, we benchmarked genome-based diagnostics approaches that enable the detection of repeat expansions (REs).

Methods: Metadata of individuals investigated by diagnostic-grade ES and/or FA between 10/2016 and 12/2020 were identified from in-house databases. Repeat length was determined by FA and systematically validated by genome sequencing (GS) in cases representative for the different RE loci.

Results: The HPO-based query identified 2041 MD index cases including individuals with ataxia ($n = 899$), dystonia ($n = 265$), spasticity ($n = 573$), and combined MD ($n = 304$). Overall diagnostic yield of ES was 19,7% (ACMG class 4/5). Eleven percent of pathogenic findings in ES were intronic and non-coding variants or CNVs.

Of all conducted FA for the diseases SCA1/2/3/4/6/7/8/10/12/17, DRPLA, FXTAS and Friedreich Ataxia ($n = 4726$ tests in 1079 patients) 2.5% of FAs revealed pathogenic findings in 10,8% of all patients with MD. Systematic validation via Expansion Hunter in genome sequencing confirmed all expanded alleles and repeat size was estimated precisely especially for shorter repeats.

Conclusion: Our data suggest that a genome-based diagnostic approach with expanded bioinformatic analyses including SVs, CVs, and especially REs has the potential to replace step-by-step FA and ES. Besides, this unbiased diagnostic approach will help clinicians to diagnose patients with atypical presentation.

References:

Grants:

Conflict of Interest: None declared.

EP17.002 Review of the DNA quantification external quality assessment

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Background/Objectives: Accurate DNA quantification is important for several applications in particular whole genome, exome and next generation sequencing which all rely on specific concentrations to produce reportable results. Samples with concentrations that fall outside the required specification will result in the rejection of the sample or a failed test all of which incurring delays in returning results to patients. The DNA quantification EQA is an external benchmarking exercise designed to evaluate the accuracy of concentration measurements across the different participating laboratories.

Methods: Six DNA samples were provided from four different sources: somatic cell lines, formalin fixed paraffin embedded tissue (FFPE), whole peripheral blood and fresh tissue. Each laboratory was required to measure the samples using their routine double-stranded DNA (dsDNA) specific method. A total of 62 laboratories from 16 different countries participated, with 18 different methodologies used.

Results: The methods can be grouped into three quantification types: Fluorometric, spectrophotometric and qPCR. Variability in concentration measurements was seen within and between methods, with laboratories using spectrophotometric techniques generally reporting higher concentration values compared to fluorometric and qPCR techniques. This was particularly evident for DNA samples sourced from FFPE, potentially due to high contaminants and fragmentation associated with this DNA.

Conclusion: This variability in quantification results highlighted within the EQA may affect downstream processes within a clinical setting and demonstrates a need for continued benchmarking EQAs to achieve consistency and accuracy for DNA concentration measurements to ensure conservative use of patient DNA.

References:

Grants:

Conflict of Interest: None declared.

EP17.003 Preventing sequencing through the insert by quality control analysis

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Background/Objectives: Library preparation in next-generation sequencing (NGS) workflows is a procedure required to convert nucleic acid samples of interest into a platform-specific format. The final library is multiple inserts of various length flanked by adapters. When designing a sequencing experiment, it is important to consider both the library insert size and the desired sequencing read length. Ideally, the fragments composing the library should be optimized to an insert size greater than the anticipated sequencing read length. Exceeding the optimal insert length can result in sequencing through the insert, resulting in increased bioinformatic work and possibly lower quality sequencing results.

Methods: The Agilent 5200 Fragment Analyzer system and the Agilent HS NGS Fragment kit (1-6000 bp) were used for electrophoretic analysis of a final NGS library. Sample preparation was carried out according to the Agilent quick guide instructions. Smear analysis of all samples was performed using the Agilent ProSize data analysis software (version 4.0.0.3).

Results: Optimal and extremely short sizes for an individual library can be easily defined using an intended read length and the lengths of the adapters used. For the library analyzed it was determined that a read length of 150 bp was ideal as 73.7% of the library was of optimal size and 4.1% of the library is extremely short and will sequence entirely through the adapter.

Conclusion: For this particular library a 150 bp read length is optimal as longer read lengths will result in high amounts of the reads sequencing entirely through the adapter.

References:

Grants:

Conflict of Interest: Rainer Dr. Nitsche Agilent Technologies, Annika Dorn Agilent Technologies, Kyle Luttgeharm Agilent Technologies.

EP17.004 CNV interpretation: is there room for change?

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Background/Objectives: Molecular karyotyping yields CNV data, which have to be interpreted. This work aims at analyzing latest publications and free user-friendly software.

Methods: Literature mining, software testing.

Results: Generally, CNV interpretation algorithms are limited to analyzing the following: Database of Genomic Variants, disease association, CNV size and inheritance. Literature mining allowed us to propose the way to expand the CNV interpretation algorithms (Table 1). We found only two online tools which could serve the interpretation task and match our criteria (free and user-friendly): CNVxplorer (http://cnvxplorer.com/), which helps gathering information about CNVs, and AnnotSV (https://lbgi.fr/AnnotSV/), which allows CNV ranking..

Table 1. Expanded CNV interpretation algorithms described in different articles.

Research	Recurrency within method	Enhancers	Expression	Pathways	Imprinting	Introns/exons
[1]	No	Yes	No	No	No	Yes
[2]	Yes	No	Yes	Yes	Yes	Yes
[3]	No	No	No	No	No	No

Conclusion: Free user-friendly CNV interpretation software options are limited. General interpretation decreases potential academic and diagnostic values of CNV analysis. To our mind, interpretation algorithms are to be expanded for creating novel tools for CNV interpretation.

References: 1. Riggs et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the ACMG and the Clinical Genome Resource (ClinGen). Genet Med. 2020; 22:245-57.

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Grants: None.

Conflict of Interest: None declared.

EP17.005 Recommendations for clinical interpretation of variants in non-coding regions of the genome

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Celia Duff-Farrier[7], David FitzPatrick[8], John Greally[9], Jodie Ingles[10], Neesha Krishnan[10], Jenny Lord[4], Hilary Martin[11], William Newman[1], Anne O'Donnell-Luria[12], Simon Ramsden[5], Heidi Rehm[12], Ebony Richardson[10], Moriel Singer-Berk[12], Jenny Taylor[13], Maggie Williams[7], Jordan Wood[12], Caroline Wright[14], Steven Harrison[12], Nicola Whiffin[13]

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Background/Objectives: Clinical genetic testing currently focuses almost exclusively on protein-coding regions of the genome, with globally adopted guidelines for variant interpretation primarily designed for variants in these regions. There is no guidance on how existing guidelines should be adapted for variants identified in other genomic contexts, despite variants in non-coding regions increasingly being identified as causes of penetrant monogenic disorders.

Methods: We convened an expert panel to design a set of recommendations to sit alongside the existing ACMG/AMP guidelines, outlining considerations and adaptations for non-coding region variants. The guidelines were extensively tested by external groups on a set of 30 variants across a range of non-coding regions, and subject to community feedback.

Results: We provide guidance for when variants in non-coding regions are considered 'interpretable', highlight examples of mechanisms through which non-coding region variants can cause disease, detail how candidate regulatory regions should be defined, and outline considerations or adaptations for nine ACMG/AMP rules. Our testers highlighted key barriers to use of the guidelines in a diagnostic setting, including access to epigenetic data, unfamiliarity with functional assays, access to output from in silico prediction tools, and general uncertainty in 'correctness' of classified variants. We are developing workshops to up-skill clinical scientists and increase widespread usability of the guidelines.

Conclusion: These recommendations aim to increase the number and range of non-coding region variants that can be interpreted clinically. This will lead to an increase in new diagnosis for rare disease patients and catalyse the discovery of novel disease mechanisms.

References:

Grants:

Conflict of Interest: Jamie Ellingford: None declared, Joo Wook Ahn: None declared, Richard Bagnall: None declared, Diana Baralle: None declared, Stephanie Barton: None declared, Christopher Campbell: None declared, Kate Downes: None declared, Sian Ellard: None declared, Celia Duff-Farrier: None declared, David FitzPatrick: None declared, John Greally: None declared, Jodie Ingles: None declared, Neesha Krishnan: None declared, Jenny Lord: None declared, Hilary Martin: None declared, William Newman: None declared, Anne O'Donnell-Luria Paid member of the Scientific Advisory Board of Congenica., Simon Ramsden: None declared, Heidi Rehm: None declared, Ebony Richardson: None declared, Moriel Singer-Berk: None declared, Jenny Taylor: None declared, Maggie Williams: None declared, Jordan Wood: None declared, Caroline Wright: None declared, Steven Harrison: None declared, Nicola Whiffin: None declared.

EP17.006 Quality control of NGS libraries with daisy chains

Carmen Rothmund[1], Vera Rykalina[2], Rainer Dr. Nitsche[2], ***Annika Dorn[2]***

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Background/Objectives: DNA library preparation for next-generation sequencing (NGS) demands for accurate qualification and quantification. However, PCR amplification of adapter-ligated DNA fragments can lead to undesired artefacts like 'daisy chains' that cannot be correctly quantified by fluorometric methods.

Methods: The Agilent Bioanalyzer, TapeStation and Fragment Analyzer systems with respective high sensitivity assays were used for the quantitative and qualitative analysis of two KAPA HyperPlus libraries generated with different levels of amplification.

Results: Both libraries demonstrated the desired size distribution, with a pronounced secondary product in the over-amplified library. The Agilent Bioanalyzer system with the High Sensitivity DNA kit, the TapeStation systems with the High Sensitivity D5000 Screen Tape Assay and the Fragment Analyzer systems with the HS Small Fragment kit provided excellent resolution for the simple identification of the desired NGS library and the unwanted daisy chains. Thereby, the consistency between the instruments and reproducibility of analysis could be confirmed.

Conclusion: The Agilent automated electrophoresis product portfolio enables effective and robust quality control of NGS libraries with daisy chain molecules for the Bioanalyzer, TapeStation and Fragment Analyzer systems. All systems offer accurate sizing, which is an essential parameter for the calculation of the library molarity.

References: 1. Quality Control of NGS Libraries with Daisy Chains, Agilent Technologies Application Note, publication number 5994-2233EN, 2020.

Grants:

Conflict of Interest: Carmen Rothmund Roche Diagnostics Deutschland GmbH, Vera Rykalina Agilent Technologies, Rainer Dr. Nitsche Agilent Technologies, Annika Dorn Agilent Technologies.

EP17.007 RNA-based functional characterization of DNA variants in molecular diagnosis: expect the unexpected

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Background/Objectives: Use of NGS in clinical practice to screen an ever increasing number of genes has improved the portion of elucidated cases but has also created a paradoxical increase of unsolved situation/variants. Current variant filtering strategies and interpretation guidelines by the ACMG focus on amino-acid level evidence and filter out non-coding or synonymous variants although they may alter splicing or RNA regulation.

Methods: We implemented an analytical pipeline to assess variants-of-uncertain-significance (VUS) predicted to impact RNA with the aim to decipher their pathogenicity. After selection of pre-filtered variants by an interdisciplinary team, molecular investigations based on fast and cheap techniques were performed on RNA extracted from patients' blood.

Results: Besides confirmation of predicted impact on splicing, sequence or structure of some selected variants, a number of unexpected effects were observed. For example, a variant in an intronic canonical splicing signal of *DNAH11* predicted to induce in-frame exon skipping and/or partial intronic retention ultimately

leads to premature polyadenylation of mRNA. Also, a variant in an intronic cis- regulatory splicing element of *NEB* produced an unexpected broad range of alterations of the sequence, the structure and the balance of alternative isoforms.

Conclusion: This RNA-based analytical approach has the ability to clarify a number of DNA-based interpretation of variants by cost-effective functional characterization of their impact at the RNA level in an accessible patients' tissue (blood). All variants, within various genes, analyzed through this pipeline so far could be re-classified, either upward (pathogenic) of downward (benign) following ACMG guidelines.

References:

Grants:

Conflict of Interest: None declared.

EP17.008 MtDNA as a marker for the quality control of non-invasive prenatal testing

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Background/Objectives.

The accuracy and reliability of non-invasive prenatal testing (NIPT) are largely dependent on the quality and integrity of samples. Maternal cell degradation is the main cause of false-negative NIPT results. In this study, we explore the possibility of using mtDNA as a marker of sample quality.

Methods: The study includes 3089 samples from patients with both normal and pathologic pregnancies subjected to WGS-based NIPT analysis. Samples were transported in Streck Cell-Free DNA BCT blood collection tubes to NIPT laboratory for 1-14 days. MtDNA rate is calculated as the ratio of mtDNA reads to the total read number.

Results: Previously, we showed that fetal fraction size negatively correlates with mtDNA rate, suggesting that mtDNA-enriched samples contain higher levels of maternal DNA thus masking fetal DNA [1]. The mtDNA rate increases during sample transportation, which can be attributed to hemolysis. Moreover, the mean mtDNA rate is about 2 times higher ($p < 0.01$) in samples transported during a cold season, indicating that these samples are more prone to degradation due to unstable temperature conditions.

Conclusion: According to our data, mtDNA has great potential in routine tracking of NIPT sample quality. It's a simple but sensitive measure of blood sample integrity that can help decrease NIPT false-negative error rate.

References: 1. Morshneva, A. et al., Pilot Screening of Cell-Free mtDNA in NIPT: Quality Control, Variant Calling, and Haplogroup Determination. Genes 2021, 12, 743.

Grants:

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Conflict of Interest: None declared.

EP17.009 Using ClinGen standardized scoring system for assessment of gene-disease association in the clinical practice

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Background/Objectives: One of the major challenges for diagnostic laboratories is the evaluation of potentially relevant variants within genes that are not yet conclusively associated to a genetic disease but have been identified in patients. With this study, we aimed to explore the gene-disease association evidence for 38 different genes and significance of large biodatabank for the classification process.

Methods: The ClinGen Clinical Validity Framework for evaluation of gene-disease associations was applied. The assessed genes had no publicly available gene-disease assessment in ClinGen database, and 28 genes had no registered OMIM phenotype. Internal CENTOGENE biodatabank with exome/genome data from previously tested individuals, in addition to data available in the literature or public databases were used for gene classification. According to the framework, definitive, strong, moderate, limited, or no known disease relationship can be reached.

Results: A strong level of evidence has been reached for 26,3% of the genes, moderate for 21,1%, limited for 50,0%, and no known disease relationship for 2,6% of the genes. A higher total number of points has been reached for 57,8% of the genes when using our biodatabank compared with using only externally obtainable data, allowing to increase the final level of classification in 21,1% of the genes.

Conclusion: Our results demonstrate the importance of careful assessment of gene clinical validity data, along with the use of genetic data repositories. Implementation of ClinGen standardized scoring system for assessment of gene-disease association is relatively easy to apply and relevant in a clinical diagnostic setting, besides beneficial for patients, families, and clinicians.

References: PMID:28552198.

Grants:

Conflict of Interest: Emir Zonic CENTOGENE GmbH, Rostock, Germany, Mariana Ferreira CENTOGENE GmbH, Rostock, Germany, Natalia Ordonez-Herrera CENTOGENE GmbH, Rostock, Germany, Deepa Saravanakumar CENTOGENE GmbH, Rostock, Germany, Ligia S Almeida CENTOGENE GmbH, Rostock, Germany, Inês C Fernandes CENTOGENE GmbH, Rostock, Germany, Nishtha Gulati CENTOGENE GmbH, Rostock, Germany, Rebecca Higgins CENTOGENE GmbH, Rostock, Germany, Catarina Pereira CENTOGENE GmbH, Rostock, Germany, Omid Paknia CENTOGENE GmbH, Rostock, Germany, Peter Bauer CENTOGENE GmbH, Rostock, Germany, Jorge Pinto Basto CENTOGENE GmbH, Rostock, Germany, Aida Bertoli-Avella CENTOGENE GmbH, Rostock, Germany.

EP17.011 Neonatal exome sequencing: resource implications for Scottish neonatal Intensive Care

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Background/Objectives: Trio exome sequencing is an effective test for identifying genetic disorders in neonates admitted to intensive or special care. Previous work suggests that a genetic diagnosis can be achieved in up to 13% of neonates fulfilling testing criteria. Locally, routine testing is by chromosome microarray, with Trio exome only used in selected cases. We investigated resource implications, of expanding trio exome sequencing, in the population of a single regional Neonatal Intensive Care unit (NICU) in Tayside, Scotland.

Methods: All admissions between 31/07/2020 and 01/08/2021 were reviewed, identifying cases that would qualify for testing under criteria from French et al. (2019). 2 independent data sources were used to ensure inclusion of all cases.

Results: 464 neonates were admitted to NICU. 48 (10.3%) fulfilled criteria for testing. 18 (37.5% of those eligible) had some genetic testing (16 chromosome microarray, 2 DNA analysis). From testing, 6 diagnoses were made, 5 chromosomal abnormalities and 1 monogenic cause.

5 additional patients had had a genetic diagnosis made before birth (3 chromosomal, 2 monogenic). 8 patients did not meet criteria but had testing sent post discharge. 2 diagnoses were made (2 chromosomal).

Conclusion: Chromosome microarray is an essential economical first test that identifies an abnormality in at least 10.4% of those fulfilling criteria for exome testing on admission to NICU. Trio exome sequencing would be offered to 10.3 % of cases admitted to our NICU, and would be expected to significantly increase the diagnosis of genetic disorder.

References:

Grants:

Conflict of Interest: None declared.

EP17.013 Molecular-genetic diagnosis of helicobacter pylori as a factor of gastric malignant neoplasms

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Background/Objectives: Nowadays, Helicobacter pylori (HP) infection is widespread throughout the world. Early detection and treatment of HP is highly important to prevent the development of malignant neoplasms (MN) of the stomach.

Increasing antibiotic resistance of HP is another significant issue. According to the literature, resistance to levofloxacin and clarithromycin is 30%, thus it is extremely important to quickly and accurately identify HP and its antibiotic resistance.

Methods: A set of primers and a protocol for diagnosing HP and its resistance to clarithromycin and levofloxacin were developed. 10 samples of gastric biopsy with suspected HP were tested and the results were confirmed using a rapid urease test and a serological method.

Results: Sequencing of HP genome regions containing targets, that allow detection of HP infection and identification of its resistance to clarithromycin and levofloxacin was carried out for 10 samples. In all 10 cases HP infection was detected. The method is highly accurate, since it employs the analysis of the HP nucleotide sequence.

Conclusion: The introduction of molecular-genetic diagnostic testing of HP and its antibiotic resistance into clinical practice will allow the doctor to quickly and accurately identify the infection and choose the right therapy, which ultimately will serve as one of the components of the stomach cancer prevention.

References:

Grants:

Conflict of Interest: None declared.

EP17.014 Bioinformatics pitfalls, challenges and opportunities: accumulated experience of over 8,000 exomes at an accredited clinical genetics laboratory

Paulo Silva[1;2], *Filipe Alves*[1;2], *Fátima Lopes*[1;2], *Alexandra Lopes*[1;2], *Sara Morais*[1;2], *Ana Lopes*[1;2], *Ana Filipa Brandão*[1;2], *Joana Sá*[1;2], *Rita Bastos Ferreira*[1;2], *Hélia Ferreira*[1;2], *Maria João Sá*[1;2], *João Parente Freixo*[1;2], *Jorge Sequeiros*[1;2;3], *Jorge Oliveira*[1;2]

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Background/Objectives: The application of "next-generation" sequencing (NGS) technologies, such as whole-exome sequencing (WES), allowed improving the routine genetic diagnosis of rare diseases. CGPP, a ISO15189 accredited laboratory, is a key provider of molecular genetic in Portugal. As genetic testing uptake expands, awareness of WES limitations is fundamental to improve diagnostics yield and guide choices of precise genetic testing.

Methods: Between May 2016 and January 2022, over 8,000 cases required WES, due to referrals for genetic testing of a wide range of genetic diseases. This was accomplished on Illumina certified service providers, while variant identification was done in-house, using a Genome-in-a-Bottle validated pipeline and following current best-practices.

Results: We report the experience accumulated in the day-to-day diagnosis of WES-based multigene panels, and the bioinformatics challenges and pitfalls faced during i) secondary and ii) tertiary analysis. For i), capture limitations, homologous regions (including cryptic pseudogenes) and refractory variants may lead to false positive/negative results. Considering ii), differences in canonical transcript representations, multiple isoforms and their tissue expression, importance of keeping track of population-specific allelic variability, and ever-expanding phenotypes may lead to misinterpretation.

Conclusion: WES-based testing has changed the landscape of medical genetics diagnostics. However, there are important limitations and challenges that must be taken into account, which were unveiled by the risk-assessment process during WES-based test accreditation. Overall, implementation of ISO15189 led to significant improvement in our NGS pipeline, infrastructures and staff training, ultimately allowing enhanced patient diagnosis and genetic counselling.

References:

Grants:

Conflict of Interest: None declared.

EP17.015 Undiagnosed disease program in South Africa: results from first 100 exomes

Shahida Moosa[1;2], *Kimberly Christine Coetzer*[1], *Eugene Lee*[3], *Go Hun Seo*[3]

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Background/Objectives: The Undiagnosed Disease Program in South Africa (UDP) sought to evaluate prospectively evaluate the diagnostic yield of exome sequencing (ES) in a phenotypically-diverse, multi-ethnic cohort of South African patients with suspected rare genetic disorders.

Methods: ES (93 singletons, 3 duos and 4 trios) was undertaken in 100 sequential patients recruited to the UDP at Stellenbosch University during the first year of the program. The data were analyzed through two separate bioinformatics pipelines (EVIDENCE from 3billion and our in-house pipeline).

Results: A definitive diagnosis could be reached in 51% (51/100) patients, with 46% (46/100) patients having either pathogenic or likely pathogenic single nucleotide variants/indels (SNVs/indels) and 5 patients with likely-pathogenic copy number variants (CNVs) (5/100). The CNVs were subsequently confirmed on microarray or MLPA analysis. Detailed phenotyping and HPO terms enabled analysis and variant identification. 26 novel variants in 24 genes are reported here.

Conclusion: We provide data from the first 100 patients enrolled in a UDP in sub-Saharan Africa and show that even amongst mainly singletons from an understudied, diverse African population, ES is a valuable diagnostic tool, especially if it includes CNV analysis.

References:

Grants:

Conflict of Interest: Shahida Moosa: None declared, Kimberly Christine Coetzer: None declared, Eugene Lee 3billion Inc, Go Hun Seo 3billion Inc.

EP17.016 Katarzyna Kamińska, Ewelina Szczerba, Joanna Wojtysiak, Janusz Kowalewski, Marzena Lewandowska

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Background/Objectives: Testing *BRCA1/2* by NGS became essential for treatment decisions. During selection of NGS kit attention should be paid to: certification, availability of analysis solution, limit of detection, gene coverage, possibility of copy-number variants (CNVs) evaluation. Although CNV are frequent changes to *BRCA1/2* (form 3.7% in Polish population[1] to 36% in Dutch[2]), detecting them by NGS remains a challenge.

Methods: We studied 39 samples with cancers associated with BRCA1/2 mutations: 17 blood and 16 FFPE samples from Polish population, as well as 3 germline and 3 somatic samples from EMQN scheme. DNA integrity was determined using qPCR based on fragmentation ratios: 150bp/37bp and 301bp/37bp. BRCAaccuTest™PLUS(NGeneBio) with NGeneAnalySys software were used for library preparation and bioinformatic analysis.

Results: In Polish population we detected five pathogenic variants (15.1%):2 in tissue samples (12.5%) and 3 in blood (17.6%). In EMQN samples we detected 4 pathogenic variants including one CNV: single-copy loss of BRCA promoter (including exon1) and exons2-3.

Conclusion: CNV analysis is easier in germline samples, in contrast to somatic samples with multiple changes in copy number of amplicons due to DNA degradation. We have found that minimum sample integrity is F-ratio150bp = 0.4 and F-ratio300bp = 0.1 and maximum archiving time is 2-3 years which improve uniformity of coverage and sensitivity of somatic variants and CNVs detection. This increases the size of the population eligible for PARPi treatment. Nevertheless, the necessity to assess obligatory the CNV should be considered internally for the country taking into account the different frequency of such CNV changes in the BRCA1/2 genes in different populations.

References:

Grants:

Conflict of Interest: None declared.

EP17.017 Quantification of differences in fetal fraction profiles for male and female fetuses

***Anna Zolotopup**[1], Valeria Leonova[1], Platon Bykadorov[1], Maxim Belenikin[1]*

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Background/Objectives: The fetal fraction (FF) is one of the most important parameters in non-invasive prenatal testing (NIPT). With a higher FF of total cell-free DNA in maternal blood, there is greater statistical separation of aneuploid and euploid pregnancies, providing greater confidence in NIPT results. Approaches of FF estimation for male and female fetuses utilize different genomic regions. This retrospective study is aiming to investigate optimization of FF estimates in NIPT routine.

Methods: We analyzed 20911 clinical NIPT results for difference associated with fetal sex. An advanced method for FF measurement based on differentially methylated genomic regions (FF-QuantSC) was employed for female fetuses.

Results: Total FF of female fetuses (7.3±2.86%) was overall lower compared to male (11.1±4.53%) and had a density spike just above the limit of detection (3.5%-3,6%). Fractions and z-scores of chromosomes were lower for females regardless test result. Incidence of inconclusive ("gray area") results for all three trisomies were multiple times higher among females (324 in total), than males (42 in total). We also estimated chances of gaining a successful test result from the same plasma aliquot.

Conclusion: For our set we found that FF profile and therefore rate of inconclusive NIPT outcomes are different regarding fetal sex. This association affects rate of repetitive testing in routine NIPT for female fetuses. Our findings are useful for an adjustment in the workflow that can be made to compensate the observed FF skew.

References:

Grants: Routine testing of some samples was carried out with the financial support of the Moscow Government.

Conflict of Interest: None declared.

EP17.018 Exome sequencing vs chromosomal microarray for CNV analysis: ES is a reasonable first-tier diagnostic test for CNV detection

***Gloria Leszinski**[1;2], Korbinian M. Riedhammer[1;2;3], Dominik S. Westphal[1;4], Theresa Brunet[1;2], Vera Inka Josephin Graeve[1], Gertrud Eckstein[2], Elisabeth Graf[1], Stephanie Andres[1], Riccardo Berutti[1;2], Thomas Meitinger[1], Matias Wagner[1;2]*

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Background/Objectives: To date, chromosomal microarray (CMA) is recognized as the "gold standard" method for detection of copy number variations (CNVs) in diagnostic routine. We aimed to assess if exome sequencing (ES) is suitable as a first-tier-method for CNV detection.

Methods: First, we evaluated diagnostic yield through CNV analysis in an ES cohort comprising 2,151 individuals. Second, we analyzed the recall rate for CMA-detected CNVs in ES for 19 individuals and cross-checked results with whole genome sequencing (WGS) data (triplet analysis). Third, we evaluated the recall rate of 32 clinical relevant alterations (27 CNVs, 5 aneuploidies) detected via CMA in real.

Results: Overall diagnostic yield in our ES cohort was 43.1%, with yield through CNV analysis of 3.1%: 69 clinical relevant CNVs were found. In our triplet analysis, the recall rate for 41 (via WGS data verified) CMA-detected CNVs was 58.5% (24 of 41) regardless of coverage of the corresponding region in ES, and was 100% (24 of 24) with consideration of sufficient coverage in ES. All clinical relevant CNVs (n = 27) from in-house CMA-cases were recalled in ES.

Conclusion: CNV analysis from ES data is considerably improving diagnostic yield and enables detection of small (< 50kb) CNVs that would probably be missed by CMA. Considering the low proportion of cases with CNVs as underlying genetic defect and high sensitivity of ES for the detection of CNVs, we suggest ES as a comprehensive first-tier diagnostic test for SNV and CNV analysis in individuals with suspected Mendelian diseases without a tentative diagnosis.

References:

Grants:

Conflict of Interest: None declared.

EP17.019 Efficiency of three computer-aided facial phenotyping algorithms (DeepGestalt, GestaltMatcher, D-Score) - comparative diagnostic accuracy study

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Background/Objectives: Genetic syndromes often show characteristic facial features. Computer-assisted tools to phenotype patient faces achieve remarkable sensitivities. However, current machine-learning based models lack the class 'inconspicuous face'. Thus, their specificity remains unclear. Only a few studies compare different approaches.

D-Score is a new tool aiming to distinguish dysmorphic faces from inconspicuous control faces.

Methods: Using 323 images of patients with 17 genetic syndromes and 323 matched inconspicuous control images, we determined the clinical utility of the D-Score, GestaltMatcher, and DeepGestalt algorithms, evaluating sensitivity, specificity, accuracy, number of diagnoses supported, and potential biases such as age, gender, and ethnicity.

Results: Whereas gender and ethnic background had only minor effects on the accuracy, it was higher in younger age groups and for certain syndromes. While D-Score only classifies binarily, DeepGestalt comprises 292 different syndromes and GestaltMatcher 1187 (overlap 276). GestaltMatcher and DeepGestalt showed high top-10-sensitivities (90%, 95%). Syndromes with mild facial dysmorphism were prevalent among false positives (e.g., Angelman: DeepGestalt FPR 84%, GestaltMatcher FPR 92%). D-Score achieved a high discriminatory power (AUROC 0.85), GestaltMatcher and DeepGestalt, not trained to discern dysmorphic faces from controls, achieved lower AUROCs (0.53 and 0.70).

Conclusion: The systems can be instrumental in a stratified manner. Tools such as D-Score could help clinicians with limited syndromological experience or resources decide whether a patient needs further genetic evaluation. Systems like DeepGestalt could support diagnosing "common" genetic syndromes, and programs like GestaltMatcher could help identify rare diagnoses that are unknown to the clinician or not yet defined.

References:

Grants:

Conflict of Interest: None declared.

EP18 Bioinformatics, Machine Learning and Statistical Methods

EP18.001 Exploring lncRNA-mRNA signatures in relapse pediatric AML

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Background/Objectives: Pediatric acute myeloid leukemia (AML) is a type of tumor in which 30-40% of patients have a relapse after the first round of therapy, due to the genetical heterogeneity of initial AML. An altered expression of lncRNAs, which can regulate clusters of "tumor beneficial" genes, plays a role in relapse and therapy resistance.

Methods: RNA-seq results from primary and relapse tumor samples were downloaded from the TARGET-AML project. Differential gene expression analysis was performed using the DESeq2 package in R. Samples were grouped according to origin (primary tumor or recurrent tumor). Genes were filtered by abs(logFC)>1 and adjusted p-value <0.05. Gene ontology analysis was performed by ShinyGO v 0.741. The lncRNA-mRNA network was constructed using undirected network graphs approach based on patterns of gene co-expression with a correlation coefficient >0.7 and a p-value <0.05.

Results: The differential gene expression analysis identified 1073 mRNAs and 612 lncRNAs. GO analysis showed that the most in upregulated transcripts were related to IL-2 and IL-18 receptor complexes, blood cell differentiation genes and inhibitory MHC Ib class receptor activity. Downregulated mRNAs involved in epigenetic regulation and negative regulation of transcription. Genes involved in the lncRNA-mRNA co-expression network were related to tumor response to therapy, drug resistance, inflammation regulation, and immune system escape.

Conclusion: Using bioinformatics analysis, we identified co-expression patterns of mRNA and lncRNA in relapsed pediatric AML, described pathways in which mRNAs are involved, and identified lncRNAs regulated mRNA clusters.

References:

Grants: This work was supported by the "Alpha-Chance" Student Talent Grant.

Conflict of Interest: None declared.

EP18.002 Specific genetic polymorphisms contributing differential binding of gliadin peptides to HLA-DQ and TCR to elicit immunogenicity in celiac disease

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Background/Objectives: Immunogenicity of gliadin peptides in celiac disease (CD) determined by their molecular interactions with HLA-DQ and T-cell receptors (TCR). > 90% of CD subjects carry HLA-DQ2.5 risk allele while the rest carries HLA-DQ2.2/8. We investigated the tripartite (gliadin:HLA-DQ:TCR) interactions to unravel the basis of immunogenicity, variability contributed by the genetic polymorphisms.

Methods: I Insilico docking of eight de-amidated gliadin with HLA-DQ allotypes *and* TCR gene pairs were performed with

ClusPro2.0. Effects of known markers and reported susceptibility SNPs were predicted.

Results: rs12722069A>G, Gln76Arg of HLA-DQ8α; Thr103Lys, rs1130392G>C, Thr109Arg (Arg77) of DQ2β and rs4193G>A,-Gly78Arg (Arg78) of DQA1(α) were identified to form stable H-bonds with gliadin peptides. None of the SNPs were in LD with CD susceptibility markers (r2<0.8) while haplotypic presentation (d'>0.99) was observed in sub ethnic groups. 33-mer elicited the highest binding ($\Delta G = -13.9$,kd = 1.5E-10) with HLA-DQ2.5 in the presence of TRAV26/TRBV7, even higher (−14.3,8.9E-11) when TRAV4 paired with TRBV20. While DQ8 restricted antigen showed stronger binding (−18.5) with DQ2 restricted peptide. Ala112Thr of TRBV7 (rs879003177,G>A) interacts with Glu of gliadin complexed with HLA-DQ2.5.

Conclusion: Polymorphic sites could be utilized for better risk prediction model. Therapeutic strategies by identifying inhibitors/blockers targeting specific gliadin:HLA-DQ:TCR sites could be investigated.

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Ting YT et al.(2020)A molecular basis for the T cell response in HLA-DQ2 2 mediated celiac disease. PNAS.117(6):3063-3073.

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Conflict of Interest: None declared.

EP18.003 Machine learning on genome-wide association study identifies key genes regulating blood pressure loci

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Background/Objectives: Thousands of blood pressure (BP) loci have been identified by genome-wide association studies (GWAS). However, translating these discoveries into advances in precision medicine has proven challenging as determination of within-locus gene causality represents a major bottleneck. Here we triage genes identified by BP-GWAS using tailored machine learning (ML) to optimise functional pattern recognition in genomic data.

Methods: BP-GWAS single nucleotide polymorphisms were divided into training and test sets. We used regression models with nested cross-validation. In the training data, clinically curated genes were scored 1.0, genes with strong BP evidence scored 0.75, and genes with no BP evidence scored 0.1 – with relative frequencies of 21%, 61% and 18% respectively. We benchmarked eleven models (tree-based, ensemble and generalised linear models) using phenotypic and gene expression features. We assessed model performance and investigated prioritised genes in downstream analysis.

Results: The top performing model was extreme gradient boosting (0.84 predicted r^2) which highly prioritised 784 genes that were significantly more haploinsufficient and essential. 730/784 genes were nominally associated with hypertension in the state-of-the-art data aggregation resource, OpenTargets and 135/784 have prior links to BP-related pathways (e.g. renin secretion, cGMP-PKG signaling and aldosterone synthesis).

Conclusion: This research presents ML designed to identify and prioritise evidence-based signals within BP-GWAS associations. It filters genes using similarities with established BP-genes in select features, allowing for better downstream analysis prioritisation and targeted identification of genes and pathways underpinning BP.

References:

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Conflict of Interest: None declared.

EP18.004 Quantitative basis of machine learning models for genomic prediction

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Background/Objectives: Recent and rapid advances in high-throughput sequencing technologies and the development of statistical genetic approaches have provided many novel insights into the basis of common complex diseases. However, despite this immense body of data and its implication for human health, reliable prediction of an individual's risk for heritable diseases remains limited. Non-additive genetic variation, such as epistasis and dominance, are usually neglected in genomic prediction studies of complex human traits, despite their potential to contribute toward prediction accuracy.

Methods: Here, we quantify the theoretical limits of the prediction improvements that can be obtained from machine learning approaches of neural network and Gaussian process regression models, as compared to standard whole-genome regression methods.

Results: & Conclusion: Using the UK and Estonian Biobank data, we provide empirical examples across a range of traits, whilst demonstrating how biological interpretability and a connection to quantitative genetics theory can be maintained, avoiding "black-box" prediction approaches.

Conclusion:.

References:

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Conflict of Interest: None declared.

EP18.005 FABIAN-variant: predicting the effects of DNA variants on transcription factor binding

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Background/Objectives: There are many bioinformatics tools for predicting the effects of coding variants, but the assessment of non-coding variants lags behind. This is especially problematic for promoter variants, which can alter gene expression. Transcription factor binding is often modelled in silico by position-weight matrices (PWMs). These are relatively simple models, and studies have shown that the more advanced transcription factor flexible models (TFFMs) outperform PWMs. TFFMs are based on hidden Markov models and can account for complex positional dependencies as well as variable-length nucleotide patterns.

Methods: Our new web-based application FABIAN-variant uses both PWMs and TFFMs to predict whether or not DNA variants alter transcription factor binding. The application contains 1,224 novel TFFMs and 3,790 classical PWMs for 1,387 different transcription factors. For each transcription factor, the software combines the results of different models for a final prediction. The software is written in C++ for speed. Variants can be entered through a web interface, or alternatively a VCF file can be uploaded to assess all variants identified in an exome or genome.

Results: FABIAN-variant can be accessed at https://www.genecascade.org/fabian/. This website is free and open to all users and there is no login requirement.

Conclusion: Since their inclusion in the JASPAR database for transcription factors, TFFMs are gaining visibility. FABIAN-variant is the first web application that can not only analyse variant effects with position-weight matrices but also with transcription factor flexible models (TFFMs).

References:

Grants:

Conflict of Interest: None declared.

EP18.006 Aggregated genomic data as cohort-specific allelic frequencies can boost variants and genes prioritization in non-solved cases of inherited retinal dystrophies

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Background/Objectives: The introduction of next-generation sequencing techniques in the diagnosis of genetic rare diseases has increased the known repertoire of causal variants and genes involved. As genomic information is produced, cohorts can be studied as a whole, with the capacity of extracting accumulated signal. Here, we aim to build an allelic-frequency database for a heterogeneous cohort of genetic diseases to explore the aggregated genomic information and boost diagnosis in inherited retinal dystrophies (IRD).

Methods: We retrospectively selected 5683 index-cases with clinical exome sequencing tests available, 1766 with IRD and the rest, showing diverse genetic diseases. We calculated sub-cohort's specific allele-frequencies and compare them with suitable pseudocontrols.

Results: Focusing on IRD non-solved cases, we prioritized variants with a significant increment of frequencies, among them 100 variants of uncertain significance, reclassifying 11 to likely pathogenic/pathogenic using ACMG guides. Besides, we developed a method to highlight genes with more frequent pathogenic variants in IRD-non-solved cases than in pseudocontrols weighted by the increment of benign variants in the same comparison. Thus, we identified 27 candidate genes for further studies and partially characterized the phenotype of a syndromic-IRD case with two pathogenic variants in gene ADAMTSL4. Our resource can also help to calculate the carrier frequency of deleterious variants in IRD genes, being the most prevalent ABCA4 (~7%) and USH2A (~3%).

Conclusion: A cohort-specific genomic database and the methods to explore phenotype-specific allele frequencies compared to controls can provide new tools for variants and genes prioritization, hence augmenting disease diagnosis rate.

References: None.

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PI18/00321.
PI19/00579.

Conflict of Interest: None declared.

EP18.007 A fast method to generate hundreds of thousands of synthetic genomes and phenotypes

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Background/Objectives: Synthetic genetic and phenotypic data allows sharing without privacy concerns and simulating multi-ancestry populations currently under-represented in human genetic studies. However, existing methods to simulate synthetic data from existing genotypes are too computationally demanding to simulate biobank-scale datasets. Moreover, a reference synthetic dataset for developing and comparing new common SNP-based methods (e.g. developing polygenic scores) is lacking. We present an efficient tool for generating large-scale, diverse and realistic individual-level datasets.

Methods: For genotype generation, we use a stochastic model of the coalescent, recombination and mutation processes to create new samples from a set of existing reference genomes, where parameters are tuned using an efficient likelihood-free inference technique. Phenotypes are assigned as summation of genetic, covariate, and environmental effects for each individual, where the genetic contribution is additive for causal SNPs.

Both the genotype and phenotype methods can be customised, for example, by specifying population structure and admixture for genotypes, and heritability and polygenicity for phenotypes.

Results: Our pipeline also contains output evaluation modules, including metrics for MAF, LD, population structure (PCA), relatedness, nearest neighbour adversarial accuracy, and GWAS.

Our approach can generate 200,000 genomes (1.1M SNPs) and multiple phenotypes in less than 5 hours. Our synthetic datasets preserve key properties captured in the evaluation metrics, and we are able to identify parameter regimes for which the synthetic datasets are comparable to real data.

Conclusion: We present an efficient tool for generating large individual-level datasets comparable to real data, packaged in Docker/Singularity containers for easy reproducibility.

References:

Grants: Horizon 2020 (101016775).

Conflict of Interest: None declared.

EP18.009 A multi-omics model to predict survival in head and neck squamous cell carcinoma patients: an integrative strategy

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Background/Objectives: Head and neck squamous cell carcinoma (HNSCC) is an aggressive cancer that, despite scientific advancement, presents low survival rates and a lack of clinical molecular biomarkers. This work aimed to create a multi-omics integrative model of survival prediction in order to identify a genetic signature with prognostic value.

Methods: Copy number alteration, methylation, gene expression and clinical data from 410 HNSCC patients was retrieved from The Cancer Genome Atlas (TCGA). Data dimensionality was reduced by performing a Principal Components Analysis (PCA). Two separate clusters were identified when using the first ten principal components, presenting different Kaplan-Meier survival curves. A multi-omics signature that distinguishes between those survival groups was established by variable importance plot and LASSO regression. The selected signature was then used to calculate the model's performance in Random Forest and SVM classifiers with balanced training and test sets.

Results: The determined predictive nine gene multi-omics signature shows excellent ability to distinguish between survival groups, with a mean accuracy of 95.5%. This model includes copy number alterations, methylation profiles or expression of nine genes linked to known signalling pathways related to carcinogenesis mapped to 1q, 3p, 8q, 17q, 19p and 19q. Based on these alterations, we were able to categorize patients with differences in survival of about two years and four months, which translates to differences in clinical prognosis.

Conclusion: This predictive signature based on three omics may positively affect clinical outcome, by predicting survival and, consequently, a better or worse prognosis in HNSCC patients, opening the way to new precision medicine approaches.

References:

Grants:

Conflict of Interest: None declared.

EP18.010 Unbiased estimation of the heritability associated with a binary trait, computed from a case-control study

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Background/Objectives: The heritability, h^2, quantifies the proportion of phenotypic variance explained by genetic variations between individuals. One of the most popular methods estimates the contribution of common variants from genome-wide association studies by restricted maximum likelihood (REML). First developed to study quantitative traits, REML was extended to binary traits through the liability scale. However, in the case-control setting, the REML method provides a biased estimate of h^2 when the proportion *Ke* of cases in the sample differs from the proportion K of cases in the population. It has been suggested that this difference induces an artefactual correlation between genetic and residual components ($G \times E$) of the model resulting in the observed bias.

Methods: We studied this bias and we analytically demonstrate that for fixed K, the $G \times E$ correlation is a parabolic function of *Ke* being null at two points: $Ke_1 = K$ and $Ke_2 = K + K(1-K)\sqrt{2\pi}\, t\, \exp(\frac{t^2}{2})$ where t is the threshold of the liability that separates cases from controls, calculated from K. We thus proposed to associate the REML estimation to a sub-sampling of cases or controls providing a proportion Ke_1 or Ke_2 of cases.

Results: In a simulation study, we show that repeated sub-sampling of controls to respect an empirical proportion of Ke_2 cases provides an unbiased average REML estimate of h^2 whatever the genetic structure of the studied population, with more accuracy than sub-sampling cases.

Conclusion: We obtained an unbiased method for heritability estimation. We are applying it to a large early-onset Alzheimer disease case/control dataset.

References:

Grants:

Conflict of Interest: None declared.

EP18.011 High performance AI based tool for mining and classification of viral genomes in human exposome metadata

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Background/Objectives: The exposome is a measure of all the exposures of an individual in a lifetime and their relation to health. Human papillomavirus (HPV) is the most common viral infection of the reproductive tract and most of the people are infected with it throughout lifespan. Our aim is to develop quick and effective tool to detect and classify HPV samples from the human exposome.

Methods: Our dataset includes 1000 metadata samples that underwent the pipeline from FASTQ raw files including quality trimming and human sequences filtering with diamond blast. The tool is developed on Hopsworks in a Hadoop/Spark environment and uses a variety of methods to speed up the workflow and improve quality sequence detection.

Results: Our tool employs the de novo-assembly to detect highly divergent or yet unknown viruses. Though high-performance computing solutions metagenomic data can be analysed with an increased speedup. The AI tool trained on a large number of sequences can achieve high accuracy in identifying viral sequences outperforming standard virus detection methods. Optimizing the network parameters and application of other tools (e.g. Seq2Vec) further improves performance.

Conclusion: Metagenome data acquired through high throughput sequencing require suitable and user-friendly tools to be analysed by non-informaticians.

References: Merino et al. Human exposome assessment platform. Environ Epidemiol. 2021.

Miao et al. Virtifier: Deep learning-based identifier for viral sequences from metagenomes. Bioinformatics. 2021.

Grants: The results reported herein correspond to specific aims of grant 874662 to the HEAP consortium from the European Union including funding to support open access publishing.

Conflict of Interest: None declared.

EP18.012 MIXER: a Machine-learning method to detect genomic Imbalances exploiting X chromosome exome reads

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Background/Objectives: Whole Exome Sequencing (WES) is rapidly becoming a first-tier test, thanks to declining costs and automatic clinical pipelines. However, while identification of small variants follows standardized workflows, there is no agreement on methods for detection of Copy Number Variants (CNVs). A plethora of WES-based CNV callers have been developed, each showing good performance towards only a limited range of CNV classes/sizes. As clinical CNVs extend from large rearrangements to single genes, more versatile approaches are needed to be of enhanced diagnostic use.

Methods: MIXER is a machine learning method exploiting the naturally occurring presence of one or two copies of the non-pseudoautosomal X-chromosome WES regions in male and female samples, respectively, to simulate deletion/duplication states.

Results: Compared to popular tools (GATK4 gCNV, ExomeDepth, DECoN, CNVkit, EXCAVATOR2), MIXER showed higher stability, identifying in NA12878 WES sample both synthetic deletions and duplications (0.87 and 0.82 F1-score, respectively) encompassing from 2 to >50 target regions. Evaluated on a collection of WES data ($n = 251$) sequenced by the Epi25 collaborative, MIXER correctly discovered all clinical exonic CNVs previously identified by SNP-arrays.

Conclusion: CNVs are an important source of clinical variation. Providing the WES diagnostic setting with robust and flexible methods is essential, but current tools have biases that prevent their ready use in clinical practice. MIXER, introducing an original machine-learning solution, establishes itself as a way to reduce this gap towards higher accuracy and wider applicability.

References:

Grants: This study partly uses WES and SNP-array data generated by Epi25 Collaborative, https://epi-25.org.

Conflict of Interest: None declared.

EP18.013 Evaluation of TCRγ generation probability using Bayesian statistics

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Background/Objectives: T cell receptors (TCRs) have an essential role in adaptive immunity against tumor cells and viral infections. They are formed during V(D)J recombination, which includes a selection of the genomically encoded segments (V, D, and J for α/γ chains; or V and J for β/δ), their trimming and insertions of random nucleotides on the junctions. Previously, Murugan's group described this process with Bayesian methods and suggested the models to estimate the probability of recombination event distribution for TCRβ and TCRα [1]. In this study, we present the first computational model for TCRγ with proven robustness and reliability.

Methods: As a DNA source, we used PMBC samples obtained from healthy donors and enriched with TCRγ sequences using multiplex PCR. Raw NGS data was processed with MiXCR toolkit to extract TCR repertoires. To obtain rearrangement models we used IGoR.

Results: We assumed on frequencies of observed and expected V-J combinations for DNA- and RNA-based repertoires, that V and J usage are cross-dependent. Basing on Kullback–Leibler divergence between two rearrangement models we evaluated the minimal number of clonotypes necessary to calculate model parameters. The generated TCRγ dataset had similar probability distribution to the real one obtained from healthy individuals.

Conclusion: Our model is able to estimate TCRγ generation probability with a high accuracy.

References: [1] Murugan, A., Mora, T., Walczak, A. M., & Callan, C.G. (2012). Statistical inference of the generation probability of T-cell receptors from sequence repertoires. Proceedings of the National Academy of Sciences, 109(40), 16161-16166.

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Conflict of Interest: None declared.

EP18.014 GeneTree: an innovative solution to build simultaneously a pedigree, downloadable in BOADICEA and CanRisk files, and the clinical history of a family

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Background/Objectives: To date, no free solution allows the generation of a family pedigree that can be quickly modified and exported with the corresponding text.

Methods: I build a web application host by GitHub servers.

Results: This application is an integrated tool to help doctors and genetic counsellors in their genetic counselling. The application is available without priori installation in French and English: https://jimouse.github.io/GeneTree/.

It has a specific mode for oncogenetic consultation and a mode using HPO phenotypes. The family can be loaded (JSON or BOADICEA files) or created from a standard or custom structure, then completed via a table or a graphical interface, both interconnected.

The pedigree can be exported in several file formats and modified by a vector editor (PDF, SVG) or can be printed directly.

Finally, this application allows the automatic generation of text based on the content of the table.

Moreover, a second interface, specifically designed for patients, allow them to fill their familial information prior to the consultation.

Conclusion: This tool is extremely time-saver and has been particularly optimised for oncogenetic consultations to avoid triple entry (text-tree-boadicea risk score). This is the first application allowing text-generation based on a pedigree.

References: CanRisk Tool—A Web Interface for the Prediction of Breast and Ovarian Cancer Risk and the Likelihood of Carrying

Genetic Pathogenic Variants. Carver, T. et al. Cancer Epidemiol Biomarkers Prev (2020). https://doi.org/10.1093/bioinformatics/btx705.

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Grants:

Conflict of Interest: None declared.

EP18.015 New opportunities for gene-based association analysis using GWAS summary statistics

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Background/Objectives: Gene-based association analysis is an effective gene mapping tool. Many gene-based methods have been proposed recently. Since their power depends on the underlying genetic architecture, which is rarely known, it is likely that a combination of such methods could serve as a universal approach. Several frameworks have been developed combining different gene-based methods. We extended their opportunities in the new sumSTAAR framework.

Methods: We included in the framework two additional methods based on the principal component analysis (PCA) and the functional linear model analysis (FLM). We also included the polygene pruning procedure to guard against the influence of the strong GWAS signals outside the gene. This procedure excludes some variants within the gene being in LD with outside GWAS-identified variants from gene-based analysis.

Results: We used summary statistics for neuroticism and coronary artery disease obtained from the UK biobank data for gene-based association analysis. We applied three methods (SKAT-O, PCA and ACAT-V) and their combination using the aggregated Cauchy omnibus test. For both traits, the new PCA method showed the best results for many genes. This allowed us to identify several additional genes. We applied the polygene pruning procedure to the genes initially identified by gene-based analysis. About half of the genes lost significance after this procedure, because the association was explained by strong association signals outside the gene.

Conclusion: We extended the opportunities of the gene-based association analysis in our sumSTAAR framework, which is a flexible and comprehensive tool for performing state-of-the-art gene-based analyses using GWAS summary statistics.

References:

Grants:

Conflict of Interest: None declared.

EP18.016 Comparison of different copy number variations (CNVs) detection tools using whole-genome sequencing (WGS) data

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Background/Objectives: The identification and characterization of genetic variants is of increasing relevance in genetic medicine. A significant percentage of genome variability is caused by copy number variations (CNVs), which encompass unbalanced rearrangements that increase or decrease DNA content. Whole genome sequencing (WGS) has become a widely used method for studying genetic variability, being a viable and sensitive method to detect CNVs of various sizes. Here we present the comparation of seven tools for CNVs detection from WGS data to determine the most suitable for use in genetic diagnosis of Mendelian diseases.

Methods: More than 70 tools have been reviewed, and seven tools (BreakDancer, CNVpytor, Delly, Lumpy, Manta, Pindel and SvAba) have been selected to evaluate their performance in terms of sensitivity and accuracy using 20 samples included in HGSVC2.

Results: Good performances have been obtained in deletion detection with Lumpy being the best performing tool. However, the performance in gain detection was not as good, with the best performing tool being Manta. When differentiating by variant size, higher yields are obtained. In addition, sensitivity and accuracy can be modified to achieve better performance by filtering according to the minimum number of reads supporting each variant and combining the tools.

Conclusion: This work has allowed us to identify the best performing tools in different scenarios, as well as strategies for modifying sensitivity and accuracy parameters if necessary.

References:

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Conflict of Interest: None declared.

EP18.017 Comparison of genetic admixture in different approaches in the preparation of ancient DNA libraries

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Background/Objectives: To date, there are several different methods for preparing genomic libraries for aDNA, and they can affect the statistical results in different ways in further analysis. In this work, we compare the results of ADMIXTURE analysis for sample of the Bronze Age from the burials of the Klada burial from Caucas based on different approaches to the preparation of genomic libraries.

Methods: We used 4 methods for preparing ancient DNA libraries: a standard protocol for library preparation with the Swift biosciences kit and two enrichment methods for a target set of 1,237,207 SNPs using the Agilent kit and myBaits Expert Human Affinities Kit (Arbor) and additional variant was created including DNA repair step with uracil-DNA glycosylase (UDG) and endonuclease VIII (endo VIII).

Results: We have shown that in samples without UDG treatment, an additional component in the ADMIXTURE analysis associated with post-mortem changes can appear and can provide a basis for the overall analysis. We propose a bioinformatics approach to reduce this basis. We also demonstrate a significant negative correlation (-0.5844) between the additional ADMIXTURE component and the presence of UDG processing for 3284 European samples from the Allen Ancient DNA Resource (AADR) database.

Conclusion:.

References:

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Conflict of Interest: None declared.

EP18.018 Design and management of a custom mitochondrial DNA variants database and its application to genetic diagnosis of mitochondrial diseases

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Background/Objectives: Mitochondrial diseases (MD) are clinically heterogeneous disorders caused by a wide spectrum of pathogenic variants in genes encoded by either the nuclear or the mitochondrial genome (mtDNA). Thus, many MD could be undiagnosed when sequencing the exome without mtDNA analysis.

Methods: 450 patients' DNA, captured by xGen Exome Research Panel v2 with a spike-in xGen Human mtDNA Hyb Panel (IDT), were sequenced by Illumina NextSeq-550 (PE:2x75pb). After FASTQ aligning (BWA-mem) and genotyping (GATK-HaplotypeCaller) steps, all mitochondrial variants in VCF files were annotated with a custom bash script, using files from mitochondrial resources (MitoMap, HelixDB, MitImpact…). Then, VCFs were merged into a common VCF that was also annotated. All annotated files were uploaded to a MySQL database (12OVar-Mitoc) that also includes the patient's phenotypic data. Data can be managed with a custom web-app (JNOMICS), letting the user query variants using different filters.

Results: 1628 variants were identified (876 singletons, 6 homoplasmic novels). Five variants of clinical interest (according to Clinvar/Mitomap) were detected:

Variant	Heteroplasmy(%)
MT-RNR1:m.1555A>G*	100;15
MT-TK:m.8344A>G	35
MT-ATP-6:m.9185T>C;(p.L220P)	100
MT-ND5:m.13042G>A;(p.A236T)	100
MT-ND6:m.14484T>C;(p.M64V)	84

*2 patients.

Conclusion: A custom database of mitochondrial variants has been developed. This database can grow with new data, characterizing the mtDNA of our population. Five variants of clinical interest were detected, that without mtDNA analysis would have been lost, improving the genetic diagnosis of these patients, giving them a more accurate genetic counseling.

References:
Grants:
Conflict of Interest: None declared.

EP18.019 Automated secondary analysis of multiple Sample Sheets in a single NGS Sequencing run

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Background/Objectives: Demultiplexing and bcl-conversion of Illumina sequencers' output is the crucial first step to analyze the sequenced data. This -- often time consuming – 'translation' into fastQ files needs a carefully created Sample Sheet, especially if you want to fully automate the secondary analysis. Presuming you also perform different analysis -- perhaps with different index lengths -- in one sequencing run, this automation will get more challenging due to splitting the different analyses into several Sample Sheets.

Methods: It is hardly possible to process multiple Sample Sheets at the same time, if they are facing the data of a single sequencer run, or a limited amount of compute is available. In that case, the automation has to prioritize the Sample Sheets regarding importance in a time saving manner. Also, invalid Sample Sheets or conflicts between all Sample Sheets have to be detected ahead of demultiplexing in order to prevent running into errors during processing, or in further analyses.

Results: The script improves the processing time as well as the monitoring of a single, or multiple sequencing runs and their further processes significantly, independent of the number of their respective Sample Sheets. Additionally, the hands-on time is reduced whereas the data generation is reproducible with ease.

Conclusion: At first, Sample Sheet handling is challenging, but the time that will be saved in the future is worth implementing such a task into the routine. Furthermore, additional quality checks are implemented and can be expanded -- almost -- limitless.

References:
Grants:
Conflict of Interest: None declared.

EP18.020 Musta: end-to-end pipeline to detect, classify and interpret mutations in cancer

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Background/Objectives: Accurate detection and comprehensive analysis of somatic variants are a major task in cancer sample data analysis, which is routinely carried out combining different software packages with specific software dependencies and with the need of laborious and time-consuming data format conversions. To overcome these limitations, we developed Musta, an end-to-end pipeline to detect, classify and interpret mutations in cancer.

Methods: Musta is a Python command-line tool that easily handles matched tumor-normal or tumor-only samples, from variant calling to the deconvolution of mutational signatures, through variant annotation, driver genes detection, pathway analysis, tumor heterogeneity. Musta's core is *Snakemake-based* following the GATK Best Practices for variant calling and annotation, relying on SigProfiler and deconstructSigs for mutational signatures deconvolution and exploiting mafTools to analyze results and plots.

Results: Musta, whose reliability was extensively tested on different cohorts from The Cancer Genome Atlas (TGCA), is currently used for cancer sample data analysis at the CRS4-NGS Core (http://next.crs4.it), one of the largest sequencing facilities in Italy. Musta was conceived for an easy installation through the Docker platform. A simple Makefile bootstraps Musta, taking care of the installation, configuration and running steps and allowing the execution of the entire pipeline or any individual step depending on the starting data.

Conclusion: Musta, both in tests and routine analysis, has proven to be a robust and flexible pipeline for accurate detection and comprehensive analysis of somatic variants in cancer and is freely available by contacting the authors.

References:

Grants:

Conflict of Interest: None declared.

EP18.023 Prospective MALDI-TOF analysis of blood plasma peptidome to predict the onset and progression of hereditary transthyretin amyloidosis

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Background/Objectives: Diagnosis in the early stages of hereditary transthyretin amyloidosis (ATTRv) is essential for a timely treatment to halt disease progression. Early recognition remains a challenge, resulting in a delayed diagnosis often due to misdiagnosis [1-2]. Based on MALDI-TOF proteomic profiling, the goal is to build, assess and validate a predictive model to classify unknown serum samples as belonging to ATTRv asymptomatic carriers, patients or healthy individuals.

Methods: A 12-month study is carried out in the Majorcan endemic focus, including patients, asymptomatic carriers, and healthy volunteer controls. Proteomic analysis of blood samples using MALDI-TOF.is carried out. The data is processed and analyzed using linear discriminant analysis (LDA) and predictive models (machine learning techniques).

Results: Blood samples have been collected from 90 individuals and obtained the MALDI-TOF spectra. The three groups have been processed, compared, and classified using ADL.

Conclusion: The LDA of the MALDI-TOF spectra shows no conclusive differences between the three groups despite revealing a greater separation of the patient group.

References: 1. Cappellari M, Cavallaro T, Ferrarini M, et al. Variable presentations of TTR-related familial amyloid polyneuropathy in seventeen patients. J Peripher Nerv Syst 2011; 16:119-129.

2. Koike H, Hashimoto R, Tomita M, et al. Diagnosis of sporadic transthyretin Val30Met familial amyloid polyneuropathy: a practical analysis. Amyloid 2011; 18:53-62.

Grants: This project has been co-financed through Pfizer (IRR ID#64764667) and Sobi.

Conflict of Interest: Eugenia Cisneros-Barroso The project has been founded by Pfizer and Sobi, Juan Gonzalez Moreno The project has been founded by Pfizer and Sobi, Participated as speaker in several meetings founded by Pfizer, Alnylam and Sobi., Member of several advisory boards on behalf of Pfizer, Alnylam and Sobi., Inés Losada López The project has been founded by Pfizer and Sobi, Participated as speaker in several meetings founded by Pfizer, Alnylam and Sobi., Member of several advisory boards on behalf of Pfizer, Alnylam and Sobi., Adrián Rodríguez: None declared, Tomás Ripoll-Vera The project has been founded by Pfizer and Sobi, Participated as speaker in several meetings founded by Pfizer, Alnylam and Sobi., Member of several advisory boards on behalf of Pfizer, Alnylam and Sobi., Ivan De Paul: None declared, Francisca Orvay: None declared, Jaume Segura: None declared, Rosa Gomila: None declared.

EP18.024 Epigenomics of Malignant Pleural Mesothelioma: a structural equation modeling

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Background/Objectives: Recently, asbestos exposure, aging, single CpGs DNA methylation (DNAm) and white blood cells (WBCs) composition have been individually associated with Malignant Pleural Mesothelioma (MPM). No study has shown the simultaneous effect combining all these predictors with the aim to test the epigenomic pathway using a statistical approach.

Methods: Structural equation modeling (SEM) is a largely confirmatory, rather than exploratory, technique; It is used to determine whether a model is valid than to find a suitable model. Asbestos exposure levels were extracted considering a quantitative measure; DNAm profiles have been used as single CpGs and to compute WBCs estimation and biological age measures.

Results: Causal Graph was used to encode assumptions about the model including asbestos exposure, aging, single CpGs DNAm and WBCs composition measures. The SEM showed that all ten relationship (4 four associations and six covariances) included in the graph model were statistically significant (P value <0,05).

Conclusion: Our results suggest the potential use of a suite of peripheral blood DNA methylation measures to better characterize the MPM biological path. This will allow to increase the knowledge about the epigenetics contribution in MPM and more in detail to develop non-invasive tests for asbestos-exposed subjects with the aim to monitor early detection indicators during the risk assessment.

References:

Grants: This work was supported by the AIRC Foundation for Cancer Research in Italy [grant number IG21390] and partly by Ministero dell'Istruzione, dell'Università e della Ricerca - MIUR project "Dipartimenti di Eccellenza 2018–2022".

Conflict of Interest: None declared.

EP18.025 Identification and characterization of putative fusion genes in esophageal squamous cell carcinoma RNA-seq profiles

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Background/Objectives: Esophageal cancer (EC) is the sixth most common cause of cancer deaths worldwide and sixth in Kazakhstan. Esophageal squamous cell carcinoma (ESCC) is the prevalent histological subtype of EC. Chromosomal rearrangements and fusion transcripts play important roles in tumorigenesis and fusion genes are involved in the pathogenesis of ESCC. The aim of the study was to detect fusion genes from RNA-Seq data of Kazakhstani patients and understand their role in disease.

Methods: Tissue samples were obtained from 25 ESCC-affected individuals immediately after Ivor-Lewis esophagectomy from Multidisciplinary Medical Center in Nur-Sultan. Whole transcriptome sequencing was performed following the TruSeq RNA Protocol on Illumina HiSeq 2000 platform. FuSeq and FusionCatcher was used to identify putative fusion genes. Real-time quantitative RT-PCR were performed for validation of a reliable set of primers.

Results: In this study, 8 pairs of fusion genes were identified (GABRP-SCGB3A2, NPDC1-CACNA1B, ADAMTS2-AC136628.4, SCRN3-FGGY, HAND2-SCRG1, LINC02457-MAP1LC3B2, CTSC-RAB38, RNASE10-CD38) in 18 samples. We have designed more than 10 pairs of primers for validation of the presence of fusion genes in tumor samples. The presence of fusion genes HAND2-SCRG1 were identified in two samples with combination of 5r1-5f1 primers.

Conclusion: Recent studies reveal that fusion transcripts may actively associate with the ESCC pathogenesis. Findings of this study will expand the genetic spectrum of putative fusions in ESCC and may provide additional insights into promotion and progression of pathogenesis.

References: None.

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Conflict of Interest: None declared.

EP18.026 Making Discoveries with Kids First Variant WorkBench

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Background/Objectives: The Gabriella Miller Kids First Pediatric Research Program (Kids First) aims at facilitating researchers to uncover new insights into the biology of structural birth defects (SBD) and childhood cancers (CC). A recently released tool Variant WorkBench (VWB) enables users to query, analyze and visualize germline genomic variants, in Apache Zeppelin notebooks with scripting languages. We intended to screen reported renal disease related genes in a Kids First cohort to demonstrate the utility of VWB.

Methods: We selected a cohort of Congenital Anomalies of the Kidney and Urinary Tract (CAKUT; n = 1054). In addition to variant calls and phenotypic information, VWB hosts rich external variant annotations in the public domain, as well as gene-phenotype links. Users can visualize analysis results, import custom datasets, and export analysis results to local drives. Harnessing a series of PySpark/SQL scripts in VWB, we first restricted gene symbols to a gene list[1] and required most variant consequence predictions to be damaging, then checked the variants' genotype status in other family members and confirmed their rarity in public databases.

Results: We identified a *TRAP1* variant that is homozygous in a proband and heterozygous in both parents. This variant has been linked to CAKUT[2,3]. The whole process took less than one hour.

Conclusion: VWB is efficient and powerful in terms of phenotype-relevant genomic variant identification across a large cohort of whole genome sequencing data.

References: 1. https://medicine.uiowa.edu/humangenetics/kidneyseq.
2. https://pubmed.ncbi.nlm.nih.gov/24152966/.
3. https://pubmed.ncbi.nlm.nih.gov/30143558/.

Grants: The Gabriella Miller Kids First Data Resource Center is funded by the NIH Common Fund and collaborates with member institutions.

Conflict of Interest: None declared.

EP18.027 1 patient/1 sequencing per life

FIlip Zembol[1], *David Stejskal*[1], *Monika Koudová*[1], *Martina Bittoova*[1], *Lenka Bich Nguyen Thi Ngoc*[1], *Michael Němec*[1]

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Background/Objectives: One sequencing per life for one patient has been big project with one main objective to create universal NGS panel. Whole exome panel (WES) combined with clinical significant regions (spike-in) and stable bioinformatics background with clinical accuracy NGSIS may solve this issue.

Methods: We used WES panel form Twist VCGS and after that we added probes from Illumina to create universal NGS panel. These probes covered clinical significant regions. Data was analysed by DRAGEN and by in-house accredited bioinformatics pipeline. Universal output Vcf files were uploaded to NGSIS (Checkbase) developed in our clinic GENNET. Whole workflow was validated on 200 hundred samples with known SVs and CNVs and supplemented by array analysis.

Results: By combining NGS with array technologies we could attain complete clinical solution for one patient per life. WES sequencing that targets the protein-coding regions of the genome plus specific spike-in could provide SVs and CNVs in diagnostically relevant regions. Array will complete these results from coding regions with information about SVs and CNVs information in intron and intergenetic regions. Once prioritized, identified variants require intensive scrutiny at a biological level and require judicious assessment alongside the clinical phenotype. For this scrutiny it is necessary to load variants into robust NGSIS. This solution could be the answer for one sequencing for one patient per life.

Conclusion: One sequencing per life for patients has been a big challenge for many clinics. Creation of an omnipresent panel containing all clinical significant regions in combination with robust NGS was the main objective.

References:

Grants:

Conflict of Interest: FIlip Zembol full, David Stejskal full, Monika Koudová full, Martina Bittoova full, Lenka Bich Nguyen Thi Ngoc full, Michael Němec full.

EP18.028 Investigating interaction between instrumental variables in Mendelian randomization study using Lasso for hierarchical interactions model

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Background/Objectives: Many complex diseases are resulted from genetic factors and interactions. Mendelian randomization (MR) enables us to overcome problems of confounding and reverse causality. Limited MR studies consider the interaction between instrumental variables. This study was aimed to apply a Lasso for hierarchical interactions model to investigate the interaction between instrumental variables in MR.

Methods: Significant genetic markers (with *p*-value threshold $= 1 \times 10^{-5}$) obtained from the genome-wide association study (GWAS) were entered into a Lasso for hierarchical interactions model to select significant interaction terms (with *p*-value threshold $= 5 \times 10^{-2}$). Two-stage least square instrumental variable regression was used to evaluate the causal effects between exposures and outcomes in MR. This method was illustrated using a GWAS data of smoking cessation. The R software was used for investigating the interactions between instrumental variables in MR study.

Results: A total of thirteen genetic markers were found to be associated with Fagerstrom test for nicotine dependence (FTND) scores and nine gene-gene interactions associated with FTND score were identified from the Lasso for hierarchical interactions model. After adjusting for gender, age, and duration of smoking, MR analysis showed that smokers with higher FTND scores were less likely to quit smoking at six month (OR = 0.88, 95%C.I. = [0.76, 1.03]) and at twelve month (OR = 0.93, 95%C.I. = [0.80, 1.08]), respectively.

Conclusion: This study provided a novel approach for identifying interactions between instrumental variables in Mendelian randomization study.

References:

Grants: MOST 109-2314-B-010-045.

Conflict of Interest: None declared.

EP18.029 Genetic variants database formation founded on the healthy Russian Federation residents

Gaukhar Zobkova[1], *Elena Baranova*[1], *Olesya Sagaidak*[1], *Elena Zelenova*[1], *Maxim Belenikin*[1], *Evgeniy Albert*[2]

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Background/Objectives: There are no genetic variants databases founded on whole genome sequencing (WGS) in Russian Federation. The goal was to develop our own database.

Methods: DNA was extracted from 8,000 peripheral blood samples of healthy Russian Federation residents. WGS was performed using DNBSEQ-G400 and DNBSEQ-T7 platforms. Data processing was carried out using GATK pipeline. Processing vcf files for counting frequencies was performed with Plink program.

Results: The database was characterized on the questionnaires information containing questions about the health and place of residence of participants and their relatives. As a result of the study, for the period from 2019 to 2022, the frequencies of more than 100 million genetic variants were determined. When looking for our frequency differences with European non-Finnish data (gnomAD v3.1.2) special attention was paid to pathogenic variants, likely pathogenic and uncertain significance variants for genes associated with the most common hereditary diseases. Differences were noted in the frequencies of some pathogenic and likely pathogenic variants. For example *PAH* rs62642945 (0.00001470 and 0.0006037), *BRCA1* rs80357906 (0.00005879 and 0.0017247), *BLM* rs200389141 (0.0003676 and 0.0021559) frequencies in the European population and Evogen database, respectively.

Conclusion: We are planning to regularly update and refine the database, as number of participants will increase. Establishing the frequencies of genetic variants of healthy residents of the Russian Federation will improve the efficiency of diagnosing a wide range of hereditary diseases.

References:

Grants:

Conflict of Interest: Gaukhar Zobkova LLC Evogen, Elena Baranova LLC Evogen, Olesya Sagaidak LLC Evogen, Elena Zelenova LLC Evogen, Maxim Belenikin LLC Evogen, Evgeniy Albert Moscow Institute of Physics and Technology.

EP18.030 Using polygenic risk scores to determine age-related macular degeneration risk in a Polish group of patients

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[1]Genomed S.A., Warszawa, Poland; [2]Medical University of Silesia, Chair and Clinical Dept. of Ophthalmology, Katowice, Poland.

Background/Objectives: Age-related macular degeneration (AMD) is an ophtalmological, degenerative disorder leading to severe visual impairment and often a complete loss of vision. Up to date, based on genome-wide association studies, several genetic variants have been linked to the AMD susceptibility with two most common ones: Y402H and A69S. As the pathogenesis remains complex, calculating the cumulative individual risk in terms of polygenic risk scores (PRS) may be beneficial in AMD diagnostics and treatment guidance.

Methods: Results of the targeted sequencing of flanked coding sequences of 30 AMD-related genes of a cohort of 471 AMD patients and 167 healthy controls served as the target data, while the summary statistics of GWAS performed by Yan et al. constituted the base data. The initial data QC was perfromed using PLINK1.9. The PRS were calculated using PRSice-2 in three prediction models including (I) three genetic multidimensional scaling components (MDS), (II) sex, BMI and smoking pack-years, (III) both covariate sets combined.

Results: The highest predictive value was achieved for a model of 12 variants located in 6 genes with threshold p-value of 0.0094005. The polygenic risk model including three genetic MDS components explained 18.4% of variation in AMD with $p < 0.01$ and AUC = 0.729. The median PRS for cases was higher by 1.36 than for controls.

Conclusion: The established polygenic risk model achieved an intermediate predictive ability, comparable to PRS results on other diseases. The established PRS model may become one of risk assessment tools in diagnostics of AMD in future.

References:

Grants:

Conflict of Interest: None declared.

EP18.031 FexSplice: LightGBM Modeling for predicting the splicing pattern of Single-Nucleotide Variants (SNV) at exon first nucleotide

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Background/Objectives: SNVs at the first nucleotide of an exon cannot be accurately predicted using any current splicing analysis tool. Several factors, including the AG-dependent 3′ splice site (ss) and interaction with U2AF35, affect the splicing pattern of SNVs at the first nucleotide G of an exon (1,2). Furthermore, recent databases lack information on functional studies for splicing.

Methods: SNVs at the first exon nucleotide of 379 papers were scrutinized individually to ensure that functional studies were included. Our data set included 65 splicing affecting SNVs from the Human Gene Mutation Database (HGMD) that were functionally analyzed in their corresponding paper and 94 neutral SNVs from the dbSNP database considering minor allelic frequency ($0.1 \leq MAF < 0.50$). We considered 124 features (3), linear and non-linear models, including Gradient-Boosting, Random-Forest, and Support-Vector-Classifier (SVC). GridsSearch optimized hyperparameters and cross-validation was performed rigorously to avoid overfitting.

Results: Gradient Boosting had the best model evaluation, the area under the receiver operating characteristic curve (AUROC) and the area under the precision-recall curve (AUPR) were on average 0.88 and 0.86, respectively.

Conclusion: The performance of our model is higher than other available tools such as "SpliceAI"(4) and "CADD"(5). Then, we generated a web service (FexSplice) that accepts a genomic coordinate according to either GRCh37/hg19 or GRCh38/hg38. This program automatically generates three possible SNVs at the coordinate and predicts a probability of aberrant splicing.

References: 1- Fu, 2011.
2- Yoshida, 2020.
3- Takeda, 2021.
4- Jaganathan, 2019.
5- Rentzsch, 2021.

Grants: JST SPRING.

Conflict of Interest: None declared.

EP18.032 A cloud-scalable integrated data processing and visualization suite for data analysis and interpretation of large scale proteogenomics studies

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Background/Objectives: Comprehensive assessment of the flow of genetic information through multi-omic data integration can reveal the molecular consequences of genetic variation underlying human health. Integration of proteomics and genomics data require a variety of tools, many of which require command-line interfaces and operating system-specific requirements that can act as a barrier for researchers to adapt new data analysis tools.

Methods: The Proteograph™ Product Suite leverages physiochemically distinct nanoparticles to enable unbiased, deep, and rapid plasma proteome analyses at scale, followed by an intuitive, scalable proteogenomic data analysis platform called Proteograph Analysis Suite (PAS). Here, we demonstrate the utility of PAS by analyzing data from non-small cell lung cancer samples to show how this software enables proteogenomic data analyses.

Results: PAS facilitates automated LC-MS/MS data upload, search-engine processing (Data Independent Analysis and Data Dependent Analysis from all major proteomics vendor instrumentations), statistical filtering and protein quantification, and analysis visualization tools (e.g., PCA, hierarchical clustering, differential abundance analysis, GO enrichment, etc.) for deriving functional and biological insights. Further, PAS is compatible with variant call format files from NGS workflows to enable personalized database searches to identify peptide variants. Using a cloud-based architecture, computational tasks are distributed for efficient and rapid analysis, significantly improving time to results. The integrated proteogenomic viewer allows variant IDs to be interpreted in the context of genomic coordinates, protein sequence, and functional domains.

Conclusion: Together, these results show the utility of PAS for seamless and fast proteogenomic data analysis.

References: NA

Grants: NA

Conflict of Interest: None declared.

EP19 Personalized Medicine and Pharmacogenomics

EP19.001 Genetic profile of pediatric acute myeloid leukemia using the custom panel mut4Child

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Background/Objectives: Acute myeloid leukemia (AML) constitutes 20% of pediatric leukemias and is considered to have a poor prognosis. Genetically, it is a heterogeneous disease in which, thanks to recent advances in molecular techniques, new criteria for patient stratification and new therapeutic strategies have been developed. This study aims to evaluate the clinical utility of a customized NGS panel, in order to improve the diagnosis, prognosis, and treatment of pediatric AML.

Methods: A total of 22 AML patients younger than 18 years were enrolled in this study. Clinical information was collected, and genetic characterization was performed from DNA extracted from bone marrow at diagnosis or relapse. The DNA was analyzed by NGS for the presence of somatic variants using the mut4Child panel and an in-house bioinformatics pipeline. Finally, classification and interpretation of the identified variants were performed following the AMP guideline.

Results: A total of 65 somatic variants were identified. All the variants considered oncogenic or likely oncogenic were classified as clinically relevant regarding the diagnosis ($n = 25$), prognosis ($n = 20$), or therapy ($n = 18$). Despite mainly being a retrospective study, two of the high-risk group patients received targeted treatment based on the identified genetic alterations and achieved complete remission in both cases.

Conclusion: The genetic study by NGS using our customized mut4Child panel allows the identification of different types of mutations. In addition, it provides relevant information regarding the classification into risk groups, prognosis, and treatment of patients, especially in those considered high-risk patients without therapeutic alternatives.

References:

Grants:

Conflict of Interest: None declared.

EP19.002 DPYD*6 as a risk factor for drug toxicity in patients treated with 5-fluorouracil – preliminary results

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Background/Objectives: 5-Fluorouracil (5-FU) is an antineoplastic agent widely used in the treatment of many solid cancers. Despite their clinical benefit, fluoropyrimidines are associated with adverse drug reactions (ADRs), including gastrointestinal, hematological toxicities and hand-foot syndrome. ADRs may limit treatment effectiveness, because they impose modification of treatment schedules and/or their discontinuation. The aim of our study is to detect mutations in the DPYD gene that can be associated with a higher rate of 5-FU toxicity.

Methods: The study included 15 patients with colorectal cancer treated with chemotherapy regimens including 5-FU. Circulating tumor DNA (ctDNA) was extracted from blood plasma and DNA sequencing was performed.

Results: DPYD*6 was detected in 7 patients, all of whom suffered hematological toxicity during chemotherapy treatment.

Dihydropyramidine dehydrogenase (DPD) participates in the 5-FU metabolism by converting up to 80% into inactive metabolites and thus it is responsible for its elimination. The major cause of DPD deficiency is the presence of mutations within the encoding gene DPYD, affecting splicing process, gene transcription and enzyme activity.

Conclusion: Our study found a significant correlation between the non-synonymous SNV c.2194G>A (DPYD*6) and the ADRs in patients treated with fluoropyrimidines but further investigations are necessary to be performed in order to confirm this interaction.

References: Del Re, M. et al. DPYD*6 plays an important role in fluoropyrimidine toxicity in addition to DPYD*2A and c.2846A>T: a comprehensive analysis in 1254 patients. Pharmacogenomics J 19, 556–563 (2019).

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Conflict of Interest: Nelly Miteva-Marcheva Project BG05M2OP001–1.002-0005 /29.03.2018 (2018–2023) - Center for Competence "Personalized Innovative Medicine (PERIMED)"., Hristo Ivanov Project BG05M2OP001–1.002-0005 /29.03.2018 (2018–2023) - Center for Competence "Personalized Innovative Medicine (PERIMED)"., Gabriela Raycheva: None declared, Janet Grudeva-Popova: None declared, Dimitar Dimitrov Project BG05M2OP001–1.002-0005 /29.03.2018 (2018–2023) - Center for Competence "Personalized Innovative Medicine (PERIMED)"., Ivan Zheliazkov Project BG05M2OP001–1.002-0005 /29.03.2018 (2018–2023) - Center for Competence "Personalized Innovative Medicine (PERIMED)"., Aleksandar Linev Project BG05M2OP001–1.002-0005 /29.03.2018 (2018–2023) - Center for Competence "Personalized Innovative Medicine (PERIMED)"., Momchil Topalov: None declared, Peter Shopov: None declared, Vili Stoyanova Project BG05M2OP001–1.002-0005 /29.03.2018 (2018–2023) - Center for Competence "Personalized Innovative Medicine (PERIMED)".

EP19.003 Exome sequencing of patients with extreme toxicities to uncover pharmacogenomics of 5-fluorouracil-based chemotherapeutics in colorectal cancer

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Background/Objectives: Chemotherapeutic treatment for colorectal cancer (CRC) allows for increased patient overall survival. However, current therapeutic regimens are often associated with the development of adverse drug reactions (ADRs), which represent a morbidity, mortality and economic issue. We propose to identify novel germline markers that allow us to predict ADR development after CRC chemotherapy.

Methods: Exomes from 163 CRC patients with severe ADRs (CTCAE grades 3-4) and 52 controls were exome sequenced. We performed data analysis focusing on rare variants (MAF<=1%) that were present in >=2 cases but were absent from the controls.

Results: We have identified 10 novel genes associated haematological and digestive toxicity, diarrhoea, and nausea.

Conclusion: This is the first description of a possible association between these genes and ADRs caused by CRC chemotherapy. We hope that further validation and functional studies may unveil the mechanisms by which these genes confer susceptibility to ADRs and therefore offer preventive strategies to avoid toxicity to these patients.

References: Latremouille-Viau, D.; Chang, J.; Guerin, A.; Shi, S.;Wang, E.; Yu, J.; Ngai, C. The economic burden of common adverse events associated with metastatic colorectal cancer treatment in the United States. J. Med. Econ. 2016, 20, 54–62.

https://gatk.broadinstitute.org/hc/en-us/articles/360035535932-Germline-short-variant-discovery-SNPs-Indels-.

Seunggeun L, et al., Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. AJHG. 2012, 91, 224–237.

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Conflict of Interest: None declared.

EP19.004 Identification of a novel SERPING1 indel variant causing Hereditary Angioedema type I in a patient from Madeira

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Background/Objectives: Hereditary angioedema (HAE) is a rare disease caused by C1 inhibitor (C1-INH) deficiency or dysfunction or dysregulation of the kinin cascade (HAE-nC1-INH). International HAE management guidelines strongly recommend genetic testing to improve clinical diagnosis. Unfortunately, this practice is still uncommon in the diagnosis routine. Here we describe the genetic analysis of a patient from Madeira (Portugal) with clinical family history of type-I HAE.

Methods: C4 and C1-INH protein levels and activity determinations, and whole-exome sequencing data were obtained from a 23-year male with facial and cutaneous attacks, including airways affection. Causal variant prioritization was conducted with the HAE Database Annotation (HADA) tool.

Results: Biochemical analysis supported a HAE type-I diagnosis (C4 = 3.0 mg/dl; C1-INH < 6 mg/dl; C1-INH activity = < 1%). HADA prioritized a 1-pb insertion in SERPING1 gene (c.336_337insC; p.Ser113LeufsTer20) absent from genomic databases and reference datasets and never reported in literature. Integrated information supported a likely pathogenic variant under ACMG guidelines (PVS1 very strong, PM2 moderate).

Conclusion: We describe a novel variant for HAE type-I. A fast and precise identification of the underlying genetic cause was reached by HADA.

References: Germenis et al. J. Allergy Clin. Immunol. Pract. 2019, in press.

Mendoza-Alvarez et al. J. Med. Internet. Res. 2020; 22:e19040.

Grants: Instituto de Salud Carlos III (CD19/00231) and Ministerio de Ciencia e Innovación (RTC-2017-6471-1; AEI/FEDER), co-financed by the European Regional Development Funds, "A way of making Europe" from the EU; ITER agreement (OA17/008); SEAIC Foundation (18_A01); FIISC (FPIFIIS19/48); ACIISI (TESIS20 20010002) co-funded by European Social Fund; and Cabildo Insular de Tenerife (CGIEU0000219140).

Conflict of Interest: None declared.

EP19.005 Understanding of pharmacogenomics testing and pain management in relation to total cost of care

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Background/Objectives: Medication selection in pain management presents challenges to providers which may result in trial/fail prescribing. This may impact total cost of care (TCOC). One study found the estimated cost of managing pain was ~$261- $300 billion.[1] Response to pain medications is multifactorial with one factor being an individual's genetic variations.[2] Pharmacogenomics (PGx) can be utilized to help understand the potential implications. Analysis of *CYP2D6* and *CYP2C9* genes may aid providers in identifying patients with risk phenotypes (phenotypes with actionable Clinical Pharmacogenetics Implementation Consortium (CPIC) considerations). Objectives:1) Determine knowledge of PGx in association with pain medications 2) Observe the frequency of risk phenotypes in *CYP2D6* and *CYP2C9* retrospectively.

Methods: A survey of 11 questions was created/distributed to assess PGx knowledge. Retrospectively analyzed 13,822 and 13,913 patients genotyped for *CYP2C9* and *CYP2D6*, respectively, (2020 and 2021).

Results: A total of 31 survey responses were received. Up to ~90% of respondents agree with the potential healthcare system savings with PGx utilization. The retrospective analysis showed up to ~14% of patients having risk phenotypes associated with specific NSAID/Opioid CPIC guidelines.

Conclusion: Assessment of the results indicate PGx education is needed. More studies are needed to understand the potential positive impact on TCOC.

References: 1. Gaskin, Darrell J., and Patrick Richard. "The economic costs of pain in the United States." *The journal of pain* 13.8 (2012): 715-724. https://www.ncbi.nlm.nih.gov/books/NBK92521/.

2. Loh, Feng-Hua, et al. "Pharmacogenomic Testing and Patient Perception Inform Pain Pharmacotherapy." *Journal of personalized medicine* 11.11 (2021): 1112. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8621784/.

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Conflict of Interest: Ghada Elnashar Full time, Full time employee of OneOme LLC, Cathryn Jennissen Full time, Full time employee of OneOme LLC, Ellie Jhun Full time, Full time employee of OneOme LLC, Victor Tam Full time, Full time employee of OneOme LLC, Eimear O'Mahony Full time, Full time employee of OneOme LLC, Julie England Full time, Full time employee of OneOme LLC.

EP19.006 Induced pluripotent stem cell reprogramming of lymphoblastoid cell lines from Estonian individuals

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Background/Objectives: Protocols for generating iPSCs from blood cells, including immortalised lymphoblastoid cell lines (LCLs), are well established. Given the potential utility of LCLs resources to make patient specific iPSCs, we made a pilot project to reprogram three LCL lines derived from Estonian individuals into iPSCs.

Methods: LCL lines were reprogrammed into iPSCs using episomal plasmids, encode reprogramming factors OCT4, Sox2, Klf4, L-Myc, LIN28 and shRNA against p53. We compared pluripotency marker expression against a positive control human embryonic stem cell line, H9 (hESC H9), with fluorescence-activated cell sorting (FACS). For plasmid integration qPCR was performed with primers against either endogenous or plasmid-encoded reprogramming factors.

Results: We isolated 30 independent clones based on their hESC-like morphology. FACS results showed that LCL-iPSC lines expressed pluripotency markers (94.2 % SOX2, 90.2% NANOG) at a rate greater than the human embryonic stem cell line H9 (83.2%

SOX2, 60.2% NANOG). LCL-derived iPSCs were free from episomal plasmids as early as the 4th passage, with no evidence of integration into the host genome.

Conclusion: Reprogrammed LCLs could offer an unlimited source of patient-specific iPSCs that can be used to create specific cell types for use in different studies. We have successfully made LCL derived iPSCs that were free of plasmids used for reprogramming and expressed pluripotency markers similar to H9 cell line. This would give the chance to leverage samples in Estonian Biobank to make patient-specific iPSC lines.

References: Loh et al. 2009, Blood 113:5476–5479.

Grants: EU Horizon 2020 grant 810645 and ERDF grant MOBEC008.

Conflict of Interest: None declared.

EP19.007 Analysis of the association between IL-1β and IL-6 gene polymorphisms and acute ischemic stroke patients' recovery after thrombolytic therapy

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Background/Objectives: Recombinant tissue plasminogen activator (rtPA) is the thrombolytic drug for acute ischemic stroke (AIS) that improves functional recovery in patients treated shortly after AIS. There are evidences of the roles of interleukin (IL)-6 and IL-1β in the pathogenesis of cerebral ischemic events. Moreover, there is a link between high plasma IL-6 levels and early neurological deterioration after stroke. Polymorphisms -174 G/C (rs1800795) within the IL-6 and -511 T/C (rs16944) within IL-1β genes could affect IL-6 and IL-1β genes expression and subsequently AIS patients' recovery, therefore we aimed to analyze the influence of the IL-6 (rs1800795) and IL-1β (rs16944) genes polymorphism genotypes on AIS patients' recovery.

Methods: The study involved 166 patients with AIS treated with rtPA. Modified Rankin Scale (mRS) three months after AIS was used for determining patients' recovery. The favorable recovery has been defined with scores 0-1 and unfavorable by scores 2-6. Genotypisation was performed using the Real-Time PCR method.

Results: Patients who had unfavorable recovery after rtPA therapy were significantly more likely to have the GG genotype of the IL-6 -174 G/C polymorphism ($p = 0.034$; odds ratio (OR)=2.31; 95% confidence level (CI) 1.10–4.83). We have observed no association between the IL-1β -511 T/C polymorphism genotypes and patients' recovery after rtPA.

Conclusion: GG genotype of the IL-6 -174 G/C polymorphism may be associated with unfavorable outcome after AIS treated with rtPA.

References:

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Conflict of Interest: Marija Dusanovic Pjevic Ministry of Education, Science and Technological Development of the Republic of Serbia [grant numbers 175087, Milka Grk: None declared, Ljubica Vojvodic: None declared, Tatjana Damnjanovic Ministry of Education, Science and Technological Development of the Republic of Serbia [grant number 175091, Milica Rasic Ministry of Education, Science and Technological Development of the Republic of Serbia [grant number 175091, Biljana Jekic Ministry of Education, Science and Technological Development of the Republic of Serbia [grant number 175091.

EP19.008 Identification of deregulated miRNAs in active and non-active multiple sclerosis patients

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Background/Objectives: Multiple sclerosis (MS) is a chronic, progressive neurological disorder. Given its marked clinical heterogeneity, MS is a typical condition where the identification of molecular markers predictive to disease outcome would be highly beneficial. Our aim is to identify miRNAs that may be relevant for MS disease activity.

Methods: Two cohort of treatment-naïve MS patients were included: i) 67 MS patients recruited at the IRCCS San Raffaele Scientific Institute (Italy); ii) 86 MS patients recruited at the Centre Hospitalier Universitaire de Toulouse (France). At 2-year follow-up, patients were classified as NEDA (No Evidence of Disease Activity, defined as the absence of clinical relapses, MRI activity and confirmed disability progression assessed) or EDA (Evidence of Disease Activity). PBMCs were collected in absence of concomitant treatment and total RNA were extracted. MiRNA sequencing profiles were obtained (TruSeq® Small RNA Libraries Prep Kit). MiRNA-seq data were transformed into counts per transcript (Cutadapt, miRDeep2) and comparison between EDA and NEDA patients was performed (DESEq2).

Results: 76 miRNAs were found to be differentially expressed in the Italian cohort ($P < 0.05$, $adjP < 0.2$). Among them, 5 were confirmed in the French cohort: hsa-miR-10400-5p, hsa-miR-5787, hsa-miR-1246, hsa-miR-664b-5p, hsa-miR-331-3p, showing a higher expression in NEDA patients. Shared miRNA targets include genes involved in synaptic function and myelination (NEURL1, ARL8A, RNF40) as well as immune function (ADCY9, BCL9L, FURIN).

Conclusion: Five miRNAs were identified to be potentially relevant for MS disease activity. Co-expression analyses are ongoing to characterize their molecular mechanisms.

References:

Grants: ERAPERMED2018-233..

Conflict of Interest: Melissa Sorosina: None declared, Silvia Santoro: None declared, Béatrice Pignolet: None declared, Kaalindi Misra: None declared, Antonino Giordano: None declared, Elisabetta Mascia: None declared, Ferdinando Clarelli: None declared, Miryam Cannizzaro: None declared, Laura Ferrè: None declared, Ettore Mosca: None declared, Roland Liblau: None declared, Massimo Filippi from Bayer, Biogen Idec, Merck-Serono, Novartis, Roche, Sanofi Genzyme, Takeda, and Teva Pharmaceutical Industries, from Bayer, Biogen Idec, Merck-Serono, Novartis, Roche, Sanofi Genzyme, Takeda, and Teva Pharmaceutical Industries, FEDERICA ESPOSITO from Merck Serono and Novartis, Merck Serono. MF from Bayer, Biogen Idec, Merck-Serono, Novartis, Roche, Sanofi Genzyme, Takeda, and Teva Pharmaceutical Industries.

EP19.009 Genetic associated thrombosis after gastric bypass

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Background/Objectives: One of the methods of effective treating of morbid obesity is bariatric surgery. We present a clinical observation of a rare complication in the form of venous thrombosis after gastric bypass surgery.

Methods: A 42-year-old patient, 7 months after the treatment (gastric bypass) for grade 2 obesity (BMI 42 kg/m2), complaining of the abdominal volume increase. Thrombosis of the portal vein, which caused the development of ascites, was detected. Mutations were found in the FGB, PAI-1, MTR and MTRR genes.

Results: Venous thrombosis after gastric bypass surgery occurs in 13.8% of cases, within six months after treatment it corresponds to 4%. The typical manifestations of complications are abdominal pain (82.7%), vomiting (38.2%), fever (12.7%), which was absent in the patient described in the article [1]. Known risk factors include: oral contraceptives, injuries, obesity, smoking, coagulopathy, which also did not occur in our patient [2]. However, mutations associated with the development of thrombosis due to impaired fibrinolysis and hyperhomocysteinemia have been identified.

Conclusion: Our data highlight the importance of a comprehensive risk assessment of complications in bariatric patients, including determination of genetic predisposition.

References: 1. Carrano, F. M., Weiner, S. et al. (2021). Portomesenteric Vein Thrombosis after Bariatric Surgery: An Online Survey. Journal of Clinical Medicine, 10(17), 4024. https://doi.org/10.3390/jcm10174024.

2. Campello, E., Spiezia, L. et al. (2019). Thrombophilia, risk factors and prevention. Expert review of hematology, 12(3), 147–158. https://doi.org/10.1080/17474086.2019.1583555.

Grants:

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EP19.010 Distributed cognition and process management for genomic medicine

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Background/Objectives: Integration of genomic medicine into clinical practice is key to achieving precision medicine. However, the guiding principles, constraints, obstacles and solutions to achieving a consistent workflow within genomic medicine practice have not been described. Our objective was to develop a process map of the operations and key players in delivering care, and to provide guidelines for the practice of genomic medicine.

Methods: A process map was generated through identifying tasks and decision-points from referral to end-of-care, using data captured across multiple platforms within a single centre. Case examples were selected to demonstrate workflow.

Results: We identify the enactors of the care process and describe their roles.

1. We define the core biological and utilitarian principles governing genomic medicine practice. We observed that Bayesian probability theory is used in clinical decision making and detail considerations for referral, triage, patient intake, phenotyping, testing, variant analysis and interpretation, counselling, surveillance, and therapy.
2. Based on our observations, we generate guidelines for consistent patient-specific variant interpretation. These are demonstrated with case examples.
3. A prototyped electronic data management system within REDCap was designed to further support and automate key aspects of genomic care.

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Conclusion: The practice of genomic medicine reduces to a series of decisions that are governed by biological, utilitarian and Bayesian thinking. We show that this can be modelled in a flexible but standardized workflow that necessitates technology and distributed cognition. We discuss the future of genomic medicine and comment on areas for continued efforts.

References: N/A

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EP19.011 The polymorphisms of UGT1A6, ACSM2A and PTGS1 and their associations in heart failure patients with implanted LVAD

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Background/Objectives: Aspirin is an effective antiplatelet drug which is prescribed for patients with congestive heart failure (HF) after implantation of the Left Ventricular Assist Device (LVAD). It prevents platelet aggregation. Genetic variations of UGT1A6, ACSM2A and PTGS1 enzymes influence to the aspirin metabolism. Our research aim was to investigate influence of gene polymorphisms of rs2070959 in UGT1A6, rs1133607 in ACSM2A and rs3842787 in PTGS1 in HF patients with implanted LVAD devices.

Methods: Venous blood samples were recruited from n = 98 patients (52.7±11.0 years old) with implanted LVADs in the JSC "National Research Cardiac Surgery Center". Study also included healthy control group (n = 95). Patients were prescribed with aspirin (100mg/day) according to the clinical protocol. Genomic DNA samples were genotyped for polymorphisms rs1133607, rs3842787 and rs2070959 by the real-time PCR with TaqMan probes.

Results: The distributions of allelic and genotype frequencies of SNPs rs1133607, rs3842787 and rs2070959 were not significantly different between HF patients and healthy control groups ($p > 0.05$). Carriers of AA genotype for polymorphism rs2070959 in UGT1A6 A>G gene and CC genotype for rs1133607 in ACSM2A C>T gene were higher in HF and healthy control groups (40.8% vs. 45.3% and 60.2% vs. 75.8%, $p > 0.05$). TT genotype for rs3842787 in PTGS1 C>T gene was not identified in both groups.

Conclusion: According to the results, research needs to increase number of participant and SNPs which will allow us to

identify genetic influence in the metabolism of aspirin and dosage for HF patients with implanted LVADs.

References:

Grants:

Conflict of Interest: None declared.

EP19.013 Genetic factors influence serum calretinin levels in asbestos-related diseases

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Background/Objectives: Malignant mesothelioma (MM) is an aggressive cancer with poor prognosis, often associated with asbestos exposure. MM diagnosis is confirmed with immunohistochemical analysis of several markers, including calretinin. Increased circulating calretinin was also observed in MM, but there is substantial interindividual variability. Our aim was to determine if *CALB2* polymorphisms or polymorphisms in genes encoding miRNAs and transcription factors regulating calretinin expression are associated with serum calretinin or MM susceptibility.

Methods: Our study included 616 occupationally asbestos-exposed subjects without MM and 288 MM patients. All subjects were genotyped for seven polymorphisms in *CALB2*, *E2F2*, *MIR335*, *NRF1* and *SEPTIN7* genes using competitive allele-specific PCR. Serum calretinin was determined with enzyme immunoassay in 545 subjects. Nonparametric tests, logistic regression and ROC curve analysis were used for statistical analysis.

Results: Carriers of two polymorphic *E2F2* rs2075995 alleles were less likely to develop MM (OR = 0.64, 95% CI = 0.43-0.96, P = 0.032), but the association was not significant after adjustment for age (P = 0.093). In all subjects, carriers of at least one polymorphic *CALB2* rs889704 allele had lower calretinin levels (P = 0.036). In subjects without MM, carriers of two polymorphic *MIR335* rs3807348 alleles had higher calretinin (P = 0.027), while carriers of at least one polymorphic *NRF1* rs13241028 allele had lower calretinin levels (P = 0.034). Optimal calretinin cut off values predicting MM differed according to *CALB2*, *NRF1*, *E2F2*, and *MIR335* genotypes.

Conclusion: Our results suggest genetic variability could affect serum calretinin levels. This could contribute to a better understanding of calretinin regulation and potentially to earlier MM diagnosis.

References:

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Conflict of Interest: None declared.

EP19.014 Blood transcriptomic network analysis reveals key drivers of response to TNF inhibitors in patients with rheumatoid arthritis

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Background/Objectives: Although TNF inhibitors have widely been used for the effective treatment for rheumatoid arthritis (RA), one-third of patients do not respond well, subsequently showing poor prognosis. Our study aimed to uncover the transcriptomic dynamics under anti-TNF treatments and identify biomarkers to stratify potentially well-responding patients.

Methods: RNA-seq data from peripheral blood cells and clinical disease activity indices of 62 patients with RA were obtained right before and 6 months after treatment of TNF inhibitors. Response-associated genes were defined by a paired sample differential expression analysis followed by a heterogeneity test between EULAR-defined good-responders with >70% ACR improvement and null-responders in both the EULAR and ACR20 response criteria. Coexpression-network-based key driver analyses were conducted to identify key response-associated genes responsible in network-level expression changes upon treatment.

Results: We identified 161 response-associated genes that were significantly enriched in coexpression networks involved in the response to type I interferon, NK cell-mediated immunity, B cell proliferation, and nucleosome assembly. There were 24 network-specific key drivers that potentially drove the down-regulation of the type I interferon signaling network and the up-regulation of the NK cell-mediated immunity and B cell proliferation networks in responders upon treatment. The treatment-induced expression changes of key drivers in the type I interferon signaling and B cell proliferation networks were significantly correlated with the change of erythrocyte sedimentation rate in the cohort.

Conclusion: This study provides the landscape of response-associated transcriptomic changes in peripheral blood cells from RA patients that can be used in response prediction of anti-TNF biologics.

References:

Grants:

Conflict of Interest: None declared.

EP19.015 Routine DPYD genotyping prior fluoropyrimidine therapy in Slovene cancer patients

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Background/Objectives: Fluoropyrimidine toxicity is strongly associated with reduced activity of the enzyme dihydropyrimidine dehydrogenase (DPD). The most prevalent cause of DPD deficiency is the presence of four deleterious variants in its encoding gene DPYD. We aimed to assess the frequency of four DPYD clinically important variants among Slovene cancer patients attending fluoropyrimidine therapy.

Methods: In 2020 we started routine DPYD genotyping, and till the end of 2021, 780 cancer patients were genotyped. Four clinically relevant DPYD-variants c.1905+1G>A (rs3918290, DPYD*2A), c.1679T>G (rs55886062, DPYD*13), c.2846A>T (rs67376798), and c.1129-5923C>G (rs75017182, HapB3) were determined in patients' DNA isolated from peripheral blood. Genotyping was performed using in-house allele-specific RT-PCR (TaqMan Genotyping Assays). Based on patient's DPYD genotype the fluoropyrimidine dosing was recommended according to CPIC guidelines for each patient.

Results: Clinically important DPYD-variants were detected in 31 (4.0 %) among 780 genotyped cancer patients. The most frequent DPYD-variant was c.1129-5923C>G determined in 3.2 % (n = 25) of patients, followed by c.1905+1G>A determined in 0.8 % (n = 7) of patients. One patient had both mentioned DPYD-variants. The variants c.1679T>G and c.2846A>T were not detected in our patients.

Conclusion: Our results are in accordance with published data, where 3-7 % of the European population have partial or complete DPD deficiency due to four deleterious DPYD-variants. We can conclude that DPYD genotyping was successfully implemented in our routine clinical practice. The possible severe toxicity of fluoropyrimidine therapy was reported in 4.0 % of genotyped patients and the recommendation for fluoropyrimidine dosing was adjusted according to detected DPYD-variant.

References:

Grants:

Conflict of Interest: None declared.

EP19.016 Comparison of breast cancer transcriptomes in Arab, African American, and European American women

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Background/Objectives: Breast cancer is the most common type of cancer among women worldwide. It is the major cause of morbidity and mortality among females in Saudi Arabia. Clinical observations indicate that 60% of all female breast cancers in Saudi Arabia developed before the age of 50 years, compared to 23% in the USA. Moreover, Breast cancer diagnosed in young women is more aggressive in nature with a poorer prognosis compared to older counterparts. In this study, we aimed to identify racial differences in genomic characteristics of breast cancer in young and elderly women.

Methods: We used genome-wide gene expression profiling datasets from young and elderly Arab (KSA), African American (Black) and European American (White) women with breast cancer and compared the transcriptomic profiles to age- and race-matched normal controls to characterize the underlying biology of breast tumors in different racial groups and to identify population-specific gene signatures and activated pathways.

Results: Our results revealed distinct genomic features in young as well as in elderly women across the three population cohorts. Intriguingly, African American women displayed more similar molecular characteristics to Arab women than the non-Hispanic White American women did. We also identified population-specific upstream regulators, canonical pathway and gene networks.

Conclusion: The genomics analyses demonstrate that cancer appearing in women with different ethnic/racial backgrounds contains distinct biological characteristics and deregulated signaling pathways and may explain the racial discrepancies in disease aggressiveness and outcome.

References: None.

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Conflict of Interest: None declared.

EP19.017 Schizophrenia polygenic risk score as a pharmacogenomic predictor: Insights from a retrospective analysis of clozapine prescription patterns

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Background/Objectives: Clozapine is currently the treatment of choice in treatment-resistant schizophrenia (TRS); however, titrating its dose and optimizing its administration can be quite complex. For this reason, it is of clinical interest to identify predictive factors that can influence clozapine response, including genetic liability to schizophrenia. In this study, we investigate whether a polygenic risk score (PRS) for schizophrenia is associated with a specific therapeutic choice in clozapine treatment: the highest dose ever prescribed.

Methods: Two independent multi-ancestry cohorts of individuals with TRS recruited in the UK were used: CLOZUK2 (N = 3133) and CLOZUK3 (N = 909). Schizophrenia PRS was calculated using the latest GWAS summary statistics from the Psychiatric Genomics Consortium (2021), and the relationship between PRS and highest dose of clozapine (a potential proxy phenotype for poorer treatment response) was assessed via regression modelling.

Results: Schizophrenia PRS was correlated with highest clozapine dose in both CLOZUK2 ($\beta = 12.217$, $P = 0.001$), and CLOZUK3 ($\beta = 12.730$, $P = 0.034$). Furthermore, the schizophrenia PRS was specifically associated with the probability of taking a clozapine dose >600 mg/day (OR = 1.279, $P = 0.006$), as individuals with high genetic liability for schizophrenia were twice as likely to have been prescribed doses on this range than those on the lower end of the schizophrenia PRS spectrum.

Conclusion: Schizophrenia PRS was associated with highest clozapine dose in two independent multi-ancestry cohorts, suggesting that factors associated with genetic susceptibility to schizophrenia might be associated with clozapine treatment decisions. These findings may inform future pharmacogenomic strategies for personalized clozapine prescribing in TRS.

References:

Grants:

Conflict of Interest: Djenifer Kappel: None declared, Sophie Legge: None declared, Michael Owen Dr Owen is investigator on a grant from Takeda Pharmaceuticals paid to Cardiff University, Michael O'Donovan Dr O' Donovan reported grants from the National Institutes of Health, UK Medical Research Council, Commission of the European Union, and Takeda Pharmaceuticals., James Walters Dr Walters has received grants from Medical Research Council, National Institutes of Health, and European Union 7th Framework Programme for Research, and Takeda Pharmaceuticals., Antonio Pardiñas Dr Pardiñas was supported by an Academy of Medical Sciences Springboard Award (SBF005\1083).

EP19.018 Polygenic risk for age at diabetes onset and diabetic complication in Taiwan Han population

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Background/Objectives: More adults develop type 2 diabetes (T2D) at younger age. Previous studies suggest that early age at onset is an ascertained trait that reflects an inherited predisposition for raised glucose. Also, studies demonstrated that early-onset

T2D is a more progressive disease which with more complications and other diseases and offers important prognostic information in assessing an individual's comorbidities risk. This study aimed to examine if onset age and diabetic complications can be attributed to polygenic architecture of T2D in Taiwan Han population.

Methods: A total of 9,878 T2D cases were identified from Taiwan Biobank and the onset age were identified via linked to the databases of National Taiwan Insurance Research Database (NHIRD). For each subject, the diabetic polygenic risk score (PRS) was calculated using information from the East Asian type 2 diabetes (T2D) meta-analyses which performed with 77,418 T2D cases participating in the Asian Genetic Epidemiology Network (AGEN) and the Diabetes Meta-analysis of Trans-ethnic Association Studies (DIAMANTE).

Results: The diabetic PRS was positively associated with onset age (OR = 1.11 (95%CI = 1.05-1.17), $P < 0.001$). We conducted sensitivity analyses with different P value thresholds for PRS, and the results remain similar. Furthermore, the diabetic PRS was positively associated with diabetic retinopathy (OR = 1.14 (1.06, 1.22, $P < 0.001$) and diabetic foot (OR = 1.18 (1.05, 1.34), $P = 0.007$) with P value equal to 0.0005 threshold for PRS.

Conclusion: Our findings indicated that diabetic susceptibility variants effect the onset age and modify the diabetic complications in diabetic patients.

References:

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Conflict of Interest: None declared.

EP19.019 Circulating plasma microRNAs as potential biomarkers for type 2 diabetes and its complications

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Background/Objectives: MicroRNAs (miRNAs) is a class of small non-coding RNA molecules involved in regulating gene expression. There is some evidence that the circulating microRNAs can play important role in the pathogenesis of type 2 diabetes (T2D). The aim of this study was to assess the microRNA expression in plasma samples in a cohort of patients with complicated or non-complicated T2D, and healthy controls.

Methods: We studied the plasma samples from 44 T2D patients (22 patients had a myocardial infarction in anamnesis, 22 patients had no myocardial infarction in anamnesis), and 22 healthy individuals using the next-generation sequencing method. T2D was diagnosed based on the World Health Organization criteria. All patients and controls included in this study had the Russian ethnicity and were recruited in the hospitals of Saint-Petersburg.

Results: MicroRNAs expression profile analysis revealed 198 differentially expressed plasma circulating microRNAs compared to that of the control group (adj. $P < 0.05$). The top eighth microRNAs with significant expression change were verified by RT-PCR. When comparing the MI and non-MI T2D patients, 2 differentially expressed microRNAs (hsa-miR-224-5p, hsa-miR-7704) were found (adj. $P < 0.05$).

Conclusion: Circulating microRNAs in body fluids are promising biomarker candidates for T2D and its complications.

References:

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Conflict of Interest: None declared.

EP19.020 Estimation of genetic and phenotypic parameters influencing response to metformin treatment

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Background/Objectives: Metformin is an oral hypoglycemic agent widely used for the treatment of patients with type 2 diabetes. The purpose was to analyze the association between genetic variants in *ATM, SLC22A1, SLC47A1, SLC2A2* genes, anthropometric, anamnestic, biochemical parameters, and glycemic response to metformin in diabetic patients of Russia.

Methods: Study subjects consisted of 464 diabetic patients and 129 healthy volunteers. The metformin treatment response was estimated by assessing the decrease in HbA1c level after 6 months of monotherapy and achieving of HbA1c target. Polymerase chain reaction with restriction fragment length polymorphism was used for genotyping. To predict the response to metformin therapy, a set of machine learning models was constructed based on the following parameters: fasting glucose, HbA1c, and creatinine levels in plasma, age and sex of the patient, BMI, WHR, familial background, and genotypes at 5 variant sites: rs11212617 (*ATM*), rs628031, rs12208357 (*SLC22A1*), rs2289669 (*SLC47A1*), and rs8192675 (*SLC2A2*).

Results: We found an association between rs12208357 polymorphism in *SLC22A1* and poor metformin response. The risk factors for treatment failure were male gender, rs12208357 polymorphism, familial background, and abdominal obesity. There were no statistically significant differences in the allele frequencies of the analyzed polymorphisms between the group of diabetic patients and the control group.

Conclusion: Genotypic and phenotypic information can predict the response to metformin therapy.

References:

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Conflict of Interest: None declared.

EP19.022 Investigation of cytochrome P450 genotypes in treatment-resistant major depression patients by next generation sequencing method: Preliminary findings

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Background/Objectives: Despite continuous advances in drug discovery, polymorphic differences between individuals cause inadequate drug treatments. Interindividual differences in drug efficacy are associated with polymorphisms in drug-metabolizing

enzymes, the most important of which is CytochromeP450 (CYP450) family (1). The persistence of depressive symptoms despite the use of at least two different antidepressants in sufficient doses for a sufficient period of time is defined as Treatment-Resistant Depression(TRD) (2).

Methods: It's aimed to discuss treatment response of 12 TRD patients by pharmacogenetic analyses. All exons and exon-intron boundaries of 13 CYP genes which are associated with drug metabolism were sequenced using NGS method (Illumina Miseq) and analyzed in SOPHIA DDM™ platform. Pathogenicity was determined according to ACMG classification.

Results: In one patient, a pathogenic variant of CYP2D6, c.1333G>A (p.Gly445Arg) and a rare variant of CYP2B6; in another patient, a pathogenic frameshift variation of CYP2C9, c.353_362del (p.Lys118Argfs*9) was detected. Four different benign/likely benign rare variants were detected in three patients, whereas no variations were detected in seven cases.

Conclusion: While pathogenic variants in CYP genes may effect drug response in TRD, it is suggested that besides other drug-metabolizing enzymes, microbiota may have an effect, especially in cases which these mutations are not present (10/12). In order to contribute to the literature on TRD, pharmacogenetic and microbial analyzes of 50 patients will be examined and compared in the continuation of the study.

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Grants:

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EP19.023 ABCG2 and SLCO1B1 polymorphisms in the Croatian population

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Background/Objectives: Data on the frequency of pharmacogenetic polymorphisms of drug transporters in the Croatian population are limited. We analysed allele and genotype frequencies of the most common variants of polymorphic drug transporters genes *ABCG2* and *SLCO1B1* in the Croatian population.

Methods: This study included 761 subjects of Caucasian origin, all Croatian ancestry from different parts of Croatia. Genotyping was performed using TaqMan DME Genotyping Assays for *ABCG2 c.421C>A* (rs2231142) and *SLCO1B1 c521T<C* (rs4149056) by real-time PCR. We analysed genotyping data from routine pharmacogenetic testing in patients on cardiovascular drugs therapy.

Results: For *ABCG2 c.421C>A*, the variant allele frequency was $A = 0.09724$. 620 subjects (81.5%) were carriers of 421CC, 134 (17.6%) were carriers of 421CA and 7 (0.9%) were carriers of 421AA genotype. For *SLCO1B1 c.521T>C*, the variant allele frequency was $C = 0.24456$. 468 subjects (61.5%) were carriers of 521TT genotype (normal function phenotype). 264 subjects (34.7%) were carriers of 521TC (intermediated function phenotype), while 29 subjects (3.8%) were carriers of 521CC genotype (low function phenotype). Analysing the combination of *ABCG2 c.421C>A* and *SLCO1B1 c.521T>C* revealed that 379 subjects were carriers of at least one decreased transport function allele (49.8%), while 79 (10.4%) were carriers of two or more decreased function alleles.

Conclusion: The allele and genotypes frequencies of the *ABCG2 c.421C>A* and *SLCO1B1 c.521T>C* in Croatian population are in accordance with the other European populations, and may be used for preemptive actionable pharmacogenetic information.

References:

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EP19.024 Global biobank analyses provide lessons for computing polygenic risk scores across diverse cohorts

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Background/Objectives: With the increasing availability of biobank-scale datasets that incorporate both genomic data and electronic health records, many associations between genetic variants and phenotypes of interest have been discovered. Polygenic risk scores (PRS), which are being widely explored in precision medicine, use the results of association studies to predict the genetic component of disease risk by accumulating risk alleles weighted by their effect sizes. However, limited studies have thoroughly investigated best practices for PRS in global populations across different diseases.

Methods: In this study, we utilize data from the Global-Biobank Meta-analysis Initiative (GBMI, $N = 2.1$ million), which consists of individuals from diverse ancestries and across continents, to explore methodological considerations and PRS prediction performance in 9 different biobanks for 14 disease endpoints. Specifically, we constructed PRS using classic P+T and Bayesian (PRS-CS) methods.

Results: We found that the genetic architecture, such as SNP-based heritability and polygenicity, varied greatly among endpoints. For both PRS construction methods, using a European ancestry LD reference panel resulted in comparable or higher prediction accuracy compared to several other non-European based panels; this is largely attributable to European descent populations still comprising the majority of GBMI participants. PRS-CS overall outperformed P+T, especially for endpoints with higher SNP-based heritability. Notably, prediction accuracy is heterogeneous across endpoints, biobanks, and ancestries, especially for asthma which has known variation in disease prevalence across global populations.

Conclusion: Overall, we provide lessons for PRS construction, evaluation, and interpretation using the GBMI and highlight the importance of best practices for PRS in the biobank-scale genomics era.

References:

Grants:

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EP20 Population Genetics and Evolutionary Genetics

EP20.001 Koṅkaṇ population along the southern route of earliest human migration

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Background/Objectives: Koṅkaṇ region on the west coast of India, forms parts of the southern route of earliest migration out of Africa. This littoral region bordered by the Western Ghats represents a meeting point for two of the world's most populous language families i.e. Indo-European and Dravidian. Yet the ethnolinguistic dynamics of this ancient corridor of human migration remains understudied.

Methods: To understand the maternal ancestral landscape of the Koṅkaṇ, 333 individuals from this region were grouped based on known history and social structure. The mitochondrial hypervariable segment variation was analysed and compared with ancient and extant West Eurasian and African population samples.

Results: Overall results showed that the Koṅkaṇ population is genetically diverse and there is a correlation between the social structure and maternal lineage. Besides the dominance of M haplogroup, Non-Agrarian group had higher West Eurasian component (subclades of H, HV, J, T and W) and admixture compared to other groups including agrarian and fishing communities which are less diverse, carrying mostly aboriginal haplogroups (subclades of M, R and U).

Conclusion: The presence of both pure and admixed clusters with African and West Eurasian components allows us to infer that Koṅkaṇ was not just a corridor for the earliest waves of migration, but also a place of Pleistocene settlement along the southern coastal route. Higher diversity and occurrence of West Eurasian component in Non-Agrarian group suggests historical back migration and admixture in this group and its absence in other groups hints at autochthonous peopling.

References:

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EP20.002 Detection of recent positive selection signatures in the cohort of the Lithuanian Chernobyl catastrophe clean-up workers

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Background/Objectives: Ionizing radiation (IR) is one of the factors that is known to affect genomes and, therefore, challenge organisms to adapt and acquire new traits. Lithuanian Chernobyl catastrophe clean-up workers (LCCWs) are an exclusive object of research, because not only they survived extreme conditions, but also adapted to the life-lasting effects of IR. This study focuses on adaptation and the search for the recent positive selection signatures in the genomes of LCCWs.

Methods: Whole-genome sequencing of 15 LCCWs and 15 control individuals (all males) was performed. Control group included men of Lithuanian descent, who were not involved in the clean-up work of Chernobyl catastrophe. To identify genomic regions which may be under recent positive selection analysis was performed using RAiSD tool. It uses μ values as a predictor for the selective sweep signatures. Top 1% of μ values, of which were higher than a median value in a chromosome, was set for further analysis. These values and selective sweep signatures were compared between the two groups.

Results: Eleven genomic regions under recent positive selection have been identified throughout autosomes. These regions are unique to the LCCWs compared to the control group and have the highest selective sweep values.

Conclusion: The signatures of recent positive selection differ between LCCWs and the control group individuals. The analysis of these genomic regions might explain LCCWs adaptive abilities and why some of the LCCWs survived and age relatively healthy despite large IR doses experienced.

References:

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EP20.005 The impact of archaic genomes on the Lithuanian gene pool

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Background/Objectives: Sequencing the archaic and modern human genomes has demonstrated that archaic hominins left their signatures in present-day human genomes. To understand functional consequences of introgression we need to identify the genetic variants inherited form archaic hominin ancestors. The main objective of this work was to identify introgressed archaic DNA segment in the genome of present-day Lithuanians.

Methods: We analysed genome-wide SNP genotyping data of 425 Lithuanian individuals, and 100 outgroup Yoruba individuals. PLINK v1.07 was used for data quality control. Introgressed archaic fragments were inferred using statistical models ArchIE and Sprime. Annotation performed with Ensembl Variant Effect Predictor (VEP).

Results: ArchIE detected seven 50 kb archaic fragments in 6 and 13 chromosomes, with probability of being archaic ≥90 %. After comparing putative archaic segments to archaic genomes, among the introgressed segments detected we identified genes related with the adaptive immune response in chromosome 6 (HLA-DOB, TAP2 and PSMB8) and gene (ABCC4) in chromosome 13, which regulates intracellular cyclic nuecletodie levels.

Conclusion: Comparative analysis of detected introgressed archaic fragments in the Lithuanian population with Vindija Neanderthal genome identified matching SNPs, which are residing in genes, related to adaptive immunity, mitochondrial translation and DNA reparation.

References:

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Conflict of Interest: None declared.

EP20.007 Understanding the structure of regional populations with founder effects

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Background/Objectives: Quebec is an example of a young and non-uniform founding population. By combining genetic and genealogical data from different regions in Quebec, it's possible to study in detail the regional subdivisions and better understand the associated founder effects.

Methods: The genetic and genealogical data are available for 708 individuals from 8 Quebec regions. We have characterized the identity-by-descent (IBD) sharing, the genealogical kinship and the ancestors' number of occurrences per generation using bioinformatic tools such as genlib R package and refinedIBD.

Results: The IBD length distribution analysis reveals longer segments and greater sharing in individuals from Saguenay and Acadians from Gaspésie. The genealogical information, such as kinship and ancestors' number of occurrences, demonstrate that despite the similar IBD distribution, the demographic and historical phenomena behind are specific to each subpopulation. Also, with the genealogical kinship of ancestors, we could follow the evolution of the population structure at each generation from the beginning of the colony until today.

Conclusion: These results demonstrate a fine and specific regional genetic structure in Quebec subpopulations. Sagueneans and Acadians from Gaspésie possess different historical particularities that lead to a similar genetic sharing: a powerful founder subeffect for Saguenay and more ancestors' kinship for Acadians. This difference is however observable within the genealogical data.

Finally, this approach integrating population genetics and genealogical structure will be used for studying complex diseases, such as epilepsy and schizophrenia, in future projects.

References:

Grants:

Conflict of Interest: None declared.

EP20.008 Analysis of the association between IL10RA gene haplotypes and the occurrence of Sjögren (SLE-SS) syndrome in patients with Systemic Lupus Erythematosus

Milka Grk[1], *Rada Miskovic*[2], *Marija Dusanovic Pjevic*[1], *Biljana Jekic*[1], *Nela Maksimovic*[1], *Milica Rasic*[1], *Danijela Miljanovic*[3], *Ivana Lazarevic*[3], *Andja Cirkovic*[4], *Ivica Jeremic*[5], *Milica Basaric*[5], *Ana Banko*[6]

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Background/Objectives: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by the altered activity of the immune system which leads to variable clinical symptoms. Function and expression of interleukin 10 receptor (IL10R), as a part of the IL10 antiinflammatory pathway, could play important role in the pathogenesis of SLE. Potentially Interleukin 10 receptor subunit A (IL10RA) gene haplotypes could alter signalling through this pathway in SLE patients and affect the course of the disease. We aimed to examine the potential difference in the frequency of IL10RA gene haplotypes between patients who had lupus associated with Sjögren (SLE-SS) syndrome and patients who had SLE only.

Methods: The study included 100 SLE patients diagnosed and treated at the Clinic of Allergology and Immunology, Clinical Center of Serbia in Belgrade. Genotyping of 6 polymorphisms within the IL10RA gene (rs10892202, rs4252270, rs3135932, rs2228055, rs2229113, rs9610 were determined using TaqMan assays. Haplotype analysis was performed using Haploview software.

Results: Among our SLE patients, 38 (38%) had SLE-SS. Patients with Systemic Lupus Erythematosus were harbouring IL10RA gene GCAAGA, GCAAAG, GCGAAG, CTAAGG, GCAGGG, GCAAGG haplotypes (0.475; 0.181; 0.169; 0.075; 0.055; 0.039, respectively). There was a statistically significant difference between the frequency of GCGAAG haplotype in patients with SLE only and SLE-SS patients (0.226 vs 0.072, respectively; $p = 0.006$).

Conclusion: According to our study, IL10RA gene haplotypes could influence the course of disease in patients with SLE in sense of the development of SLE-SS.

References:

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EP20.009 Population genomics and evolutionary gene order preservation elucidate major differences between cytoplasmic actin isoforms on genomic level

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Background/Objectives: ACTB and ACTG1 encode two ubiquitously expressed, non-muscular cytoplasmic actins (γ and β), differing by only 4/375 amino acids. Despite this similarity, murine Actg1 cannot compensate LoF mutations in Actb. When, however, the endogenous Actb alleles are engineered to produce γ- instead of β-actin, the resulting mice are normal, suggesting that nucleotide motifs in Actb rather than the β-actin itself are essential for actin isoform function [PMID 30012594].

Methods: Comparison of the ACTB and ACTG1 genomic variation landscape from public databases; analysis of gene synteny.

Results: A) Population databases demonstrate consistent absence of recurrent synonymous variants in the ACTB locus, whereas the paralogous positions in ACTG1 contain variants with high population frequency, despite identical nucleotide sequence context in both gene isoforms. These striking differences show neither correlation with the conservation score in 100 vertebrate species nor with codon usage. B) Unlike population databases depicting germline variability, number and type of somatic variants were comparable between both loci, indicating that both

actin loci have equal mutation acquisition potential, but selective pressure is different for systemic variants. C) In every vertebrate with a reliable genomic reference sequence, the ACTB gene is syntenic with FSCN1, whereas ACTG1 is flanked by different genes in non-mammalian vertebrates.

Conclusion: Although not altering the amino acid sequence, many ACTB nucleotides appear to be crucial for reproductive fitness, probably due to involvement in gene expression regulation of ACTB itself and/or its tightly coupled FSCN1 neighbor. In contrast, there appears to be much less selective pressure on non-coding or silent ACTG1 variations.

References:

Grants:

Conflict of Interest: None declared.

EP20.010 Genetic markers of predisposition to professional sports activities in Belarusian men and women

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Background/Objectives: Athletic performance is a complex trait that is influenced by many factors including genetic. The aim of our study was to identify specific allele variations in a number of genes associated with predisposition to professional sports activities in men and women.

Methods: The study included 459 elite athletes and 546 people not involved in professional sport. Genotyping of VEGFA rs2010963, UCP2 rs660339, AMPD1 rs17602729, ACE rs4646994, EPO rs1617640, BDKRB2 rs5810761, NOS3 rs61722009 and rs1799983, ACTN3 rs1815739, MB rs7293, PPARG2 rs1801282 and SERPINE1 rs1799762 variants was performed by real-time PCR. The statistical differences among groups were compared using the Fisher exact test.

Results: In cyclic sports athletes, we revealed statistically significant differences in frequencies of BDKRB2 D/D genotype (33,3% vs. 16,1%, $p = 0{,}02$), eNOS T/T genotype (23,3% vs. 7,4%, $p = 0{,}01$) and PPARG2 Ala/Ala genotype (13,3% vs. 0,9%, $p = 0{,}002$) between women in sport and control groups. Men-athletes in this subgroup under-represented ACE I/I genotype (9,8% vs. 26,6%, $p = 0{,}02$). In speed-strength sports, statistically significant differences in BDKRB2 D allele (51,7% vs. 40,8%, $p = 0{,}04$) and NOS3 4a-allele (31,6%vs. 17,2%, $p = 0{,}001$) frequencies were observed between women in sport and control groups. In coordination sports athletes, we revealed statistically significant differences in frequencies of PPARG2 Ala/Ala genotype (12,5% vs. 0,9%, $p = 0{,}02$) and NOS3 4a/4a genotype (25,0% vs. 3,6%, $p = 0{,}005$) between woman in sport and control groups.

Conclusion: BDKRB2, NOS3 and PPARG2 gene variants might be associated with professional sports activities in women.

References:

Grants:

Conflict of Interest: None declared.

EP20.014 Incidence of the congenital microcephaly in the Czech Republic

Natalie Friedova[1;2;3], *Antonin Sipek Jr*[2;3], *Frantisek Liska*[3], *Vladimir Gregor*[2], *Antonin Sipek Sr*[2;4;5], *Jan Klaschka*[6], *Marek Malý*[6;7]

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Background/Objectives: Congenital malformations of the central nervous system represent a diverse group of congenital malformations with a variable phenotype and varying clinical impact. Microcephaly is a rare condition characterized by the head size reduction, mostly defined as the head circumference under the 3rd percentile for specific age and sex of the particular individual.

Methods: The data were obtained from the National Registry of Congenital Anomalies. The registration process is population-wide and compulsory. All cases of (Q02 code in ICD-10) microcephaly in children (born between 2000-2017) were included. Additional variables (maternal age, gestation week, anthropometric values, sex and associated anomalies) were also collected.

Results: During the selected period there were 218 children (215 live-births and 3 stillbirths) born with microcephaly (1,14 per 10.000 of births in relative numbers). Out of those 131 were females and 85 males, the difference in female/male ratio (compared to F/M ratio in children born without a congenital anomaly) is statistically significant ($p < 0.001$). In 111 cases the microcephaly was associated with another type of congenital anomaly, 107 cases were isolated.

Conclusion: The Zika virus outbreak in Brazil pointed to the need for more accurate registration of the congenital malformations especially in case of microcephaly. Although overall incidence in the Czech Republic is low—compared to the data from other population based registries, it is necessary to register all the cases, mostly the severe ones with neurological symptoms.

References: none.

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EP20.015 Repurposing antidiabetic drugs for rheumatoid arthritis: results from a two-sample Mendelian randomization study

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Background/Objectives: Thiazolidinedione could decrease disease activity and inflammation among non-diabetic rheumatoid arthritis (RA) patients in crossover trials. We aimed to explore the repurposing potentials of thiazolidinedione for RA using Mendelian randomization (MR) to investigate the effect of genetically predicted glucose-lowering through the thiazolidinedione pathway on RA.

Methods: MR uses genetic variants randomized at conception as instruments to estimate unconfounded associations between an exposure and outcome and may thus be a viable method to gauge the potential of repurposing thiazolidinedione for RA. To proxy the pharmacological modulation of thiazolidinedione, this study identified the gene encoding the protein target of thiazolidinedione from ChEMBL and Drugbank databases and chose independent variants from the gene region as instruments. Summary statistics were from genome-wide association studies on blood glucose (UK Biobank) and RA, respectively. The effect of genetically predicted glucose-lowering through thiazolidinedione on RA was estimated by the inverse-variance weighted method. Sensitivity analyses included MR-Egger, weighted median and weighted mode methods.

Results: Twenty-three variants were chosen from the PPARG gene that encodes the protein target of thiazolidinedione. Lower genetically predicted glucose through thiazolidinedione was inversely associated with RA risk (odds ratio: 0.54 per 0.1 mmol/L decrease in glucose; 95% confidence interval: 0.35-0.83). Sensitivity analyses showed robust results. The test of the MR-Egger intercept did not suggest horizontal pleiotropy (intercept = 0.006 and $P = 0.884$).

Conclusion: Using MR analysis, we provide genetic evidence supporting thiazolidinedione as a potential drug to reduce RA risk.

References:

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Conflict of Interest: None declared.

EP20.016 Origins and diffusion of mitochondrial haplogroup J in Scandinavia

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Background/Objectives: We examine human mitogenome sequences since the Neolithic periods to infer the genetic history of haplogroup J in Scandinavia.

Methods: We analyzed haplogroup J sequences ($n = 2245$) gathered from GenBank and Viking Age sequences ($n = 54$) retrieved from the European Nucleotide Archive. Genetic differentiation was assessed using Principal Component Analysis. Sequences were also projected onto a maximum likelihood rooted phylogenetic tree and dating was calculated using the Least Squares Derivative program.

Results: Our results support a Neolithic farmer source for haplogroup J into Europe, dating to 42.6 kya (95% CI: 30.0-64.7 kya) in Western Asia. We found 13.3% ($p < 0.001$) genetic differences between several regions. Incorporating the geographic location of each sequence and subclade age estimates enabled human migratory inferences to be made about haplogroup J diversification. Each of the three major haplogroup J subclades were found in all regions, with J1b being the most predominant in the Near East and Arabian Peninsula; J1c most predominant in the western hemisphere of Europe, and J2b most predominant in the Mediterranean region. The earliest haplogroup J sequence, part of J1c subclade, dates to the 4th century CE in northern Norway. Variations including early-branching J sequences were also found in Denmark and Sweden among remains from the medieval period that share branches with sequences from Eastern and Southern Europe.

Conclusion: Although haplogroup J is not studied very much in Scandinavia, its origins can be traced back to the agricultural revolution.

References:

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Conflict of Interest: None declared.

EP20.017 An overview of the maternal genetic affinity of Sinhalese and Tamils in relation to Eastern and Western populations

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Background/Objectives: Sri Lanka's central geographical location in the Indian Ocean may have played a vital role in the out of Africa migration (1) by anatomically modern humans to populate the rest of the world. We aimed to understand the maternal genetic affinities of two Sri Lankan ethnic groups Sinhalese (living in Sri Lanka) and Tamils of Sri Lankan origin from the United Kingdom (SLT/UK) to several populations in the Western and Eastern regions using complete mitochondrial sequences.

Methods: All population sequences except for Sinhalese were retrieved from the 1000 Genome Database. Sinhalese sequences ($N = 60$) were generated in our laboratory. Haplogroups were determined from Haplogrep. Thereafter, Haplogroup frequencies were calculated, and the Principal Component (PC) Analysis was performed with R studio.

Results: We observed four distinct clusters related to each geographical region. Sinhalese and SLT/UK were separated along the PC2. Among the two populations, Sinhalese showed a greater affinity to other South Asian populations in the 1000 Genome Database than SLT/UK. Sinhalese were closer to the Bengali than Gujarat and Punjabi populations.

Conclusion: Common maternal ancestry of Sinhalese in Sri Lanka and Tamils of Sri Lankan origin in UK appears to be more distant than that of some other South Asian populations. Maternally Sinhalese are closely related to Indian populations than to other populations in the Eastern and the Western world used in the present study.

References: 1. Marrero P, et al. Carriers of human mitochondrial DNA macrohaplogroup M colonized India from southeastern Asia. BMC Evol Biol. 2016; 16:246.

Grants: National Research Council (NRC17042).

Conflict of Interest: None declared.

EP20.018 Ancient DNA study of predisposition to neurodegenerative disorders

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Bulgaria.

Background/Objectives: Knowledge about the historical prevalence of genetic variants associated with human diseases might advise how past adaptations have consequences for contemporary human health and medicine. No studies have yet been attempted to evaluate the genetic susceptibility to neurodegenerative disorders in ancient human communities.

Methods: We examined 2729 publicly available genomes [1] obtained from ancient human DNA samples from different geographical regions and dated 100–15,000 BP. Selected were variants whose clinical significance as monogenic defects or risk factors for neurodegenerative diseases has been determined using the Varsome platform [2]. Their spatiotemporal prevalence was subsequently established.

Results: Four variants in genes associated with neurodegenerative disorders in modern populations were selected and their historical and geographic prevalence was assessed. These variants are two rare variants in the *LRRK2* gene associated with Mendelian Parkinson's disease, a pathogenic variant in the *CRH* gene, associated with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), and a rare variant in the *TREM2* gene, a possible risk modifier associated with Alzheimer's disease. Established were differing spatiotemporal frequency dynamics of these clinically significant variants.

Conclusion: Data on molecular predisposition to neurodegenerative disorders in ancient genomes is instructive to modern medical diagnostic and therapeutic practices.

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Grants: KP-06-N33/5 from 13.12.2019 - National Science Fund of Bulgaria.

Conflict of Interest: None declared.

EP20.019 Prevalence of myotonic dystrophy type 1 in Iceland is three times the world average with 77% of cases identified through indirect family-mediated cascade testing

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Background/Objectives: Myotonic Dystrophy type 1 (DM1) is a multisystemic disease. Inheritance is autosomal dominant with anticipation due to increased number of CTG tandem repeats in the *DMPK* gene. The prevalence of DM1 differs between countries, with an average general world estimate of 12,5 per 100.000.[1] We previously documented in 2005 a high prevalence of DM1 (28,2 per 100.000).[2] The current study re-examined the prevalence and diagnosis.

Methods: Data from Landspitali University Hospital, Akureyri Hospital, Medical Director of Health and independent clinics were accessed. Five ICD-9 and seven ICD-10 relevant numbers were searched for. Additionally, results of *DMPK* genetic testing were obtained from Landspítali. Inclusion criteria were Icelandic residency, alive on January 1, 2021 and any of the following: Clinical diagnosis of DM1, positive *DMPK* genetic test or positive EMG test.

Results: In Iceland, 178 individuals had been diagnosed with DM1, of which 136 were alive, giving a point prevalence of 37 per 100.000. In 111 cases the reason leading to diagnosis was documented. Most cases (77%) were diagnosed with indirect family-mediated cascade testing and 23% due to symptoms.

Conclusion: Thorough acertainment of diagnosed cases found the prevalence of DM1 in Iceland to be three times the world average. Our data demonstrates the importance of cascade testing in this disease.

References: 1. Harper (2001) *Myotonic Dystrophy*. 2. Leifsdóttir et al. (2005) Prevalence of myotonic dystrophy in Iceland. *Icelandic Med J*.

Grants: The Icelandic Student Innovation Fund and Myotonic Dystrophy Association of Iceland.

Conflict of Interest: Haukur Svansson Academic. The Icelandic Student Innovation Fund and Myotonic Dystrophy Association of Iceland. Erla Guðbjörg Hallgrímsdóttir Academic. The Icelandic Student Innovation Fund and Myotonic Dystrophy Association of Iceland., Hildur Ólafsdóttir: None declared, Ólafur Árni Sveinsson: None declared, Vigdis Stefansdottir: None declared, Eirikur Briem: None declared, Sigurlaug Sveinbjörnsdóttir: None declared, Jon J. Jonsson Academic. The Icelandic Student Innovation Fund and Myotonic Dystrophy Association of Iceland.

EP20.021 Population-specific transcriptome patterns of the decidua cells in Preeclampsia

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Background/Objectives: Comprehension the processes regulation of gene expression at the population level is fundamental importance for biomedical research and for evolutionary biology. Preeclampsia (PE) is one of the leading obstetric complications with racial and ethnic differences in the frequency of occurrence in human populations. Important pathogenic links of PE are disturbances of decidualization and maturation of decidual stromal cells (DSCs).

Methods: Using laser capture microdissection and high-throughput sequencing (RNA-seq) for the first time we study the transcriptomic profile of native single DSCs from the placenta of Caucasian ($n = 8$) and Mongoloid ($n = 8$) women with PE.

Results: During analysis we obtained 19749 transcripts (CPM>1). Comparison the expression profiles of DSCs in the examined groups revealed significant differences (FDR < 0.05) for 61 transcripts. Annotation of GO-categories these transcripts indicates the connection with the processes of the viral immune response, interferon signaling pathway and the effectors of cellular response. Analysis of the 1000 most highly-expressed genes by the geneXplain platform revealed 101 master regulators (MR) for Buryats and 89 MRs for Russians. The unique MR (ranks sum < 100) for the Buryats were TP53, TBL1XR1 which are known as markers of cancer and are involved in the regulation of cell invasion. For Russians, a unique MR was PKN2 witch mediates endothelial NOS activation and vascular tone regulation.

Conclusion: Thus, through the action of the identified population-specific MRs works of many pathways can be disrupted and lead to the disease development, therefore, these MRs can be considered as potential markers of PE.

References:

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Conflict of Interest: None declared.

EP20.022 Frequency of GM1-gangliosidosis in the regions of Ukraine

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Background/Objectives: GM1-gangliosidosis is a lysosomal storage disease due to a deficiency of β-galactosidase activity. According to various studies, the average incidence of GM1-gangliosidosis in the world is 1:100,000–1:200,000.

Methods: Data from the official website of the State Statistics Service of Ukraine were used to calculate the incidence of GM1-gangliosidosis for the period 2004-2020. Genetic studies were performed in 25 patients from different regions of Ukraine.

Results: It is shown that 60% of identified patients with GM1-gangliosidosis (15 out of 25) lived in the western regions of Ukraine. A high-frequency areas are Zakarpattia region - 1:35,080 and Ivano-Frankivsk region - 1:48 871, where the incidence is much higher than worldwide. The lowest frequency is in Kyiv region—1:601,578. The total incidence of GM1-gangliosidosis in Ukraine is 1:228 472. Analysis of the incidence of mutations found in the GLB1 gene (15 types identified) in patients with GM1-gangliosidosis in Ukraine showed that 74% of alleles with p.His281Tyr (14 of 19 alleles) were found in the western regions—in Zakarpattia, Ivano-Frankivsk and Lviv regions, and all alleles with p.Gly255His (5 out of 5)—in Zakarpattia region.

Conclusion: Such features of the spread of mutations in the GLB1 gene in the western regions of the country provide grounds for further research on the origin of these mutations in these areas. The obtained data are the basis for the individual choice of the strategy of laboratory examination of patients with GM1-gangliosidosis in Ukraine.

References:

Grants:

Conflict of Interest: None declared.

EP20.023 The role of MAPT H1 and H2 haplotypes as factors for Alzheimer's disease in the Bulgarian population

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Background/Objectives: Autosomal dominant mutations in the Tau gene, *MAPT*, are present in a variety of clinical conditions such as Alzheimer's disease, frontotemporal dementia and others. Linkage disequilibrium throughout the gene results in two major haplotypes, H1 and H2, with H2 characterized by a deletion of a 238 bp sequence upstream of Tau exon 10 and being found primarily in Europe and southwest Asia. Our aim was to establish the haplotype frequencies among the Bulgarian population and possible associations between a specific haplotype and healthy controls vs at-risk patients.

Methods: A total of 304 Bulgarian individuals (131 male and 173 female) were divided into three groups—at risk (46), healthy (60) and general population (198), which were genotyped for the H1 and H2 haplotypes via allele-specific PCR to detect the presence of a 238 bp deletion between exons 9 and 10, characteristic of the H2 haplotype. Fisher's exact test and Chi-squared test were performed using SPSS v26.0.0.0.

Results: The H2/H2 haplotype was observed only in samples from the general population and healthy controls, with a frequency of 0.02 and 0.05, respectively. No H2/H2 carriers were found in the at-risk group. Using GnomAD allele frequencies data for Bulgarian individuals showed a statistically significant corellation for the H1/H1 haplotype and the risk group.

Conclusion: Further functional evaluations of the H1 and H2 haplotypes are required to determine their contribution to an individual's risk of dementia.

References:

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Conflict of Interest: None declared.

EP20.024 Epidemiological estimates of Autosomal Dominant Polycystic Kidney Disease (ADPKD) diagnosed cases in Malta

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Background/Objectives: Estimating the prevalence and incidence rates of ADPKD is challenging due to the highly variable expression of the disease. Here we investigate the epidemiology among ADPKD diagnosed cases in an isolated small island of Malta.

Methods: A total of 59 unrelated cases (38 males, 21 females) over 18 years with clinical features of ADPKD were studied. A detailed three-generation pedigree was generated where possible.

Results: The estimated point prevalence of ADPKD for the Maltese adult population was 2.1 (95% CI 1.7–2.5) per 10,000 inhabitants and the estimated annual incidence rate of ADPKD was 1.6 per 100,000 person-years (95% CI 0.78–3.1). The annual incidence rate of ESRD in ADPKD patients was 0.78 (95% CI 0.21–1.98) per 100,000 person-years with a male-to-female ratio of 2.8:1. Moreover the crude percentage of clinically diagnosed ADPKD adult patients on renal replacement therapy (RRT) was 9.6%. Data on the Maltese population was retrieved from the National Statistics Office (NSO).

Conclusion: Labelled as a rare disease, ADPKD is an underestimated cause of ESRD. The high percentage of patients on RRT stimulates the need of further studies to delay ESRD. Moreover, this study highlights the need of genetic testing in order to confirm the genetic cause of renal cysts.

References: N/A

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& Development Trust (RIDT) and by the Tertiary Education Scholarship Scheme.

Conflict of Interest: None declared.

EP20.025 Association between longevity-related SNPs and reaching 90.0 years of age among the elderly Croatian population

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Background/Objectives: Human longevity is influenced both by genetic and non-genetic factors, where genetic variability accounts for 25% of human life expectancy variation (1). We aimed to elucidate SNPs that are significantly related to longevity as defined by cut-off age of 90.0 in a sample of elderly persons of European origin.

Methods: 42 SNPs—selected due to strong or repeatedly found association with human longevity in other studies—were genotyped in 314 individuals aged 85.0+ from Croatia. Univariate and multivariate logistic regressions were performed with genotypic data coded as: 2 = longevity allele homozygotes; 1 = heterozygotes; 0 = non-longevity allele homozygotes.

Results: 16 SNPs that reached inclusion criteria ($p < 0.2$ in univariate logistic regression) were selected for a series of multivariate logistic regression analyses. The best model, explaining 20.5% of variance for survival to the age of 90.0, has 9 SNPs. Significant association with longevity has been shown for genotypes containing longevity alleles of TERC rs16847897 and GHRHR rs2267723 ($p < 0.01$), as well as of APOE rs7412 and TNF-α rs1800629 loci ($p < 0.05$), while the same effect was found in TP53 rs1042522 heterozygotes ($p = 0.046$). Loci FOXO3 rs12206094, KLOTHO rs9536314, ERCC2 rs50871, TXNRD1 rs17202060 also contribute to the quality of the model.

Conclusion: Our study points to TERC rs16847897, GHRHR rs2267723, APOE rs7412, TNF-α rs1800629 and TP53 rs1042522 as predictive genetic factors for reaching longevity (defined by cut-off age 90.0) in the Croatian elderly population.

References: 1. Passarino et al. Human longevity: Genetics or Lifestyle? It takes two to tango. Immun Ageing, 2016;13:12.

Grants: CSF IP-01-2018-2497 (HECUBA).

Conflict of Interest: None declared.

EP20.026 Associations of telomere length related genetic loci with metabolic syndrome using both cross-sectional and longitudinal designs

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Background/Objectives: Telomeres are the tips of chromosomes and play a critical role in maintaining chromosomal stability by protecting chromosome ends from damage and degradation. Several studies reported significant associations between shorter TL and deregulated metabolic syndrome (MetS) components. Several genetic loci associated with telomere length have been identified. The study was aimed to evaluate if the TL related genetic loci were associated with metabolic syndrome and the components.

Methods: A total of 3441 adults were recruited from the Matsu community-based integrated health screening project and 1291 participants had been followed-up for ten years. We selected 43 SNPs on 17 TL related genes for genotyping. Multiple logistic regression, Cox proportional hazard model and generalized estimating equation adjusting for other covariates were performed for data analysis.

Results: We found the rs10831229 in *MRE11* gene was significantly associated with MetS and three SNPs (rs10792447 in *CDC42BPG* gene, in rs1684149 in *BICD1* gene, rs55691416 in *CSNK2A2* gene) were significantly associated with the increase of the number of abnormal components. Polygenic risk score was also significantly associated with increase of the number of abnormal components ($\beta = 0.044$, 95%CI: 0.008, 0.081). For longitudinal study, we found the rs72732496 in *DCAF4* gene was associated with the incidence risk of MetS (HR = 1.38, 95% CI = 1.03, 1.86) and the rs3827026 of *ZBTB46* gene was associated with increase of the number of abnormal components ($\beta = 0.021$, 95% CI: 0.006, 0.036).

Conclusion: Our results identified several TL related genetic variants were significantly associated with metabolic syndrome and the increase of the number of components.

References:

Grants: MOST 106-2314-B-010-020-MY3; MOST 109-2314-B-010-045.

Conflict of Interest: None declared.

EP20.027 Association of drug-resistant tuberculosis with the TLR2 gene polymorphism

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Background/Objectives: Kazakhstan is on the list of thirty countries with high rates of multi-drug resistant tuberculosis in the world (1). Various publications reveal association of *TLR2* gene polymorphism with development of drug-resistant tuberculosis (TB). Aim of this study is to estimate association of *TLR2* gene polymorphism with development of drug-resistant tuberculosis in Kazakhstani patients and determine *M. tuberculosis* families involved in drug-resistance.

Methods: 80 TB patients diagnosed with primary pulmonary tuberculosis from Kazakhstan participated in this study. Patients were genotyped for *TLR2* gene polymorphism (rs5743708) using TaqMan probe on 7900HT Fast System. Resistance of *M. tuberculosis* isolates to first-line antibiotics was identified by absolute concentration method. *M. tuberculosis* samples were divided into two groups: drug-resistant (35 samples) and susceptible (45 samples). Genotyping of isolates was conducted by 15 MIRU-VNTR method.

Results: Tendency of association of GG genotype of *TLR2* with development of drug-resistant TB was notified – 100% ($p = 0.09$). AG genotype of the gene showed association with infection by

susceptible TB form. However, obtained results did not reveal statistical significance ($p > 0.05$). Five families of *M. tuberculosis* were identified among all TB samples. Beijing family isolates prevailed among drug-resistant isolates (77.1%).

Conclusion: *TLR2* gene polymorphism (rs5743708) was not associated with development of drug-resistant TB in our study. Beijing genotype was the most spread among drug-resistant isolates. In future, sample size should be expanded to establish the obtained results.

References: Global tuberculosis report 2019. Geneva: WHO. Licence: CCBY-NC-SA3. 0IGO. 2019; 58-268.

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Conflict of Interest: Ainur Akhmetova This study was supported by Grant 11022021CRP1511 and Grant AP09259750, Dauren Yerezhepov This study was supported by Grant 11022021CRP1511 and Grant AP09259750, Ainur Akilzhanova This study was supported by Grant 11022021CRP1511 and Grant AP09259750, Ulan Kozhamkulov This study was supported by Grant 11022021CRP1511 and Grant AP09259750.

EP20.028 New genetic variants (Factor V R2, MTHFR A1298C and PAI-1) and inherited thrombophilia in Georgian population

Ketevan Kartvelishvili[1;2], *Nino Pirtskhelani*[1;2;3], *Nino Kochiashvili*[1], *Tea Mukhuradze*[4;5], *Nugzar Pargalava*[5], *Mamuka Bokuchava*[4;5]

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Background/Objectives: Inherited thrombophilia is a genetic disorder, resulting in a hypercoagulable state, which is a possible cause of thromboembolism and pregnancy complications. Thrombosis is a complex, multifactorial disease caused by a combination of numerous, often unknown, environmental and genetic factors. According to our previous studies distribution of three genetic variants (Factor V Leiden, Prothrombin/Factor II G20210A, MTHFR C677T) in Georgian population was high and resembled upper data of Caucasians. The aim of the study was to evaluate the frequency of new pathogenic variants and their impact on inherited thrombophilia.

Methods: 72 unrelated Georgians with thromboembolism and/or pregnancy complications were genotyped by PCR for simultaneous detection of six genetic risk factors associated with thrombophilia Factor V (G1691A; H1299R), Prothrombin (G20210A), MTHFR (C677T; A1298C) and PAI-1/SERPINE1 (4G/5G).

Results: Results of our study, represented in table, showed importance of simultaneous detection of six genetic risk factors of inherited thrombophilia, compare to previous studied 3 factors in group of Georgian patients with thromboembolism and/or pregnancy complications..

Table. Distribution of mutations

Total number of patients	**72**	
Pathogenic genetic variants	**3 factors**	**6 factors**
Patients with Inherited Thrombophilia	16(22%)	60(83%)
Double/Triple Mutations	4(5.6%)	46(63%)
MTHFR C677T and A1298C Compound Heterozygotes	-	18(25%)
MTHFR A1298C Homozygotes	-	8(11%)
PAI-1 4G/5G	-	56(78%)
FV R2 H1299R	-	12(17%)

Conclusion: Significant prevalence of three above mentioned new genetic factors in Georgian patients, showed importance of identification of new candidate gene variants associated with thrombophilia and its implementation in diagnostic gene panel for successful management of thrombotic disorders.

References:

Grants:

Conflict of Interest: None declared.

EP20.030 Extending the genetic landscape and recent demographic history of Britain and Ireland

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Background/Objectives: Recent population genetics studies have identified subtle but discrete genetic groups across Britain and Ireland. However, these studies did not explore the impact of recent changes in effective population size or movement on this structure within Britain and Ireland. To address this, we have assembled the largest haplotype sharing dataset for Ireland and Britain with associated regional ancestry and applied innovative methods to improve our understanding of fine-scale population structure and recent demographic change in Britain and Ireland.

Methods: We assembled genotype data from 6,724 individuals. Using patterns of Identity-By-Descent (IBD) segment-sharing, we applied the Leiden community detection algorithm to identify genetic clusters in this dataset. Additionally, we inferred regional effective population sizes across time using IBDNe, and estimated recent migration rates using MAPS to reveal insights into recent demographic history.

Results: We identified fine-scale genetic population structure which stratifies by geography and detected new subgroups in Ireland and South-East England. Using IBD, we provide novel insights into changes in regional effective population sizes over time, with all population clusters showing patterns of recent exponential growth. We have also identified evidence of gene flow barriers through estimated migration surfaces.

Conclusion: Our findings extend our knowledge of fine-scale population structure across Britain and Ireland and help disentangle the relative effects of population structure and demographic history when examining rare variant-mediated genetic architecture of complex diseases.

References:

Grants: Science Foundation Ireland (Grant# 18/CRT/6214).

Conflict of Interest: None declared.

EP20.031 Genetically determined levels of the human plasma proteome in the UK Biobank and risk of cardio-metabolic diseases: a Mendelian Randomisation study

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Background/Objectives: Proteins are the effector molecules of biology and are the target of most drugs. In this study, we investigated the causal association of plasma proteins with cardio-metabolic diseases (CMDs) through Mendelian randomisation (MR).

Methods: GWAS were performed for each of 1472 plasma proteins (measured using Olink assays), in up to ~43,000 participants from the UK Biobank (UKB). Conditionally independent variants associated with each protein were used as instrumental variables and their associations with CMDs, *i.e.* type 2 diabetes (T2D), coronary artery disease (CAD), Stroke, chronic kidney disease (CKD), non-alcoholic steatosis hepatises (NAFLD), and Obesity, were obtained from publicly available GWAS summary statistics. We used Inverse-variance-weighted (IVW) MR and evaluated model assumptions using MR-Egger and MR-PRESSO. Proteins were considered to have an effect on CMDs if $P < 3.4 \times 10^{-5}$ (=0.05/1472 proteins) after accounting for multiple testing.

Results: We identified a total of 93 genetically proxied proteins associated with risk of at least one CMD: 16 for T2D, 20 for CAD, 6 for Stroke, 3 for CKD, 1 for NAFLD and 55 for obesity (BMI and WHR). Eight of these proteins influenced at least 2 diseases/traits, while 80 proteins had a specific effect on a CMD outcome. Our analyses highlight CMD relevant proteins not known previously and underscore the role of established therapeutic targets on CMD risk (e.g. IL6RA on CAD: OR[95% CI]=0.964[0.950-0.978] $P = 8.58 \times 10^{-7}$).

Conclusion: By harnessing the largest available GWAS results to date of circulating proteins and CMDs, we have identified a catalogue of proteins with a central role in CMD.

References:

Grants:

Conflict of Interest: Lingyan Chen Novo Nordisk Ltd., Thomas Richardson Novo Nordisk Ltd., Matthew Traylor Novo Nordisk Ltd., Sile Hu Novo Nordisk Ltd., Cyrielle Maroteau Novo Nordisk Ltd., Lina Cai Novo Nordisk Ltd., Joanna Howson Novo Nordisk Ltd.

EP20.033 Mitochondrial DNA polymorphism in the populations of indigenous peoples of Dagestan

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Background/Objectives: The Dagestan Republic (North Caucasus) is the region of Russia with the most ethnic diversity, and interethnic marriages are still uncommon. The unique population structure of Dagestan peoples is yet to be properly described while being important for the goals of medical genetics and human evolutionary genetics.

Methods: MtDNA D-loop HVS1 was sequenced for approximately 1200 individuals representing 23 ethnic groups belonging to the Avar, Ando-Tsez, Lezgin, Lak language groups of the Nakh-Dagestan language family, as well as to the Turkic language group (Kumyks). The informed consent was obtained from all participants. With additional restriction sites, mtDNA haplogroups were identified. Analysis of molecular variance (AMOVA) was performed in Arlequin 3.5.

Results: Most of mtDNA lineages in Dagestan populations are of West-Eurasian origin. East-Eurasian lineages were rare and belonged to the haplogroups B, C, D, F, G2a, M, N9a. Haplogroups H, T, U4, U5a were registered in almost all ethnic groups. Haplogroups J, I, W, U2, U1, U7, X, HV4 were found in several populations in particular linguistic groups. The absence of the haplogroup U5b was the notable characteristic of all studied populations. Considering haplogroup frequencies, AMOVA results attribute 93.1% to the variability within populations, 4.3% between populations within the linguistic groups, and 2.6% between the groups. Regarding HVS1 haplotypes, most of them were population-specific.

Conclusion: Dagestan populations are characterized by a high differentiation of mtDNA polymorphism that partially corresponds to the linguistic classification.

References:

Grants: The study was supported by RFBR grant no. 19-04-1322-A.

Conflict of Interest: None declared.

EP20.035 Agent-based modeling of autosomal recessive deafness 1A (DFNB1A) prevalence in isolated human population under various intensity of selection pressure

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Background/Objectives: An increase in the prevalence of autosomal recessive deafness 1A (DFNB1A) in populations of European descent was shown to be promoted by assortative marriages among deaf people. Assortative marriages became possible with the widespread introduction of sign language, resulting in increased genetic fitness of deaf individuals and, thereby relaxing selection against deafness. However, the effect of this phenomenon was not previously studied in populations with different genetic structure.

Methods: Using an agent-based computer model, we simulated an isolated human population under different modelling scenarios representing various intensity of selection pressure by deafness over 400 years.

Results: Modelling of the "purifying" selection pressure on deafness ("No deaf mating" scenario) resulted in a decrease in the proportion of deaf individuals and the pathogenic allele frequency. Modelling of the "relaxed" selection ("Assortative mating" scenario) resulted in an increase the proportion of deaf individuals which quickly reaches plateau with a sub-sequent decline and a decrease in the pathogenic allele frequency. The modelling of neutral selection pressure ("Random mating" scenario) showed no changes in the proportion of deaf individuals or the pathogenic allele frequency.

Conclusion: Initially low genetic fitness of deaf people in an isolated human population can be significantly increased in the presence of assortative mating by deafness, resulting in a higher prevalence of DFNB1A. On the other hand, random mating is not expected to increase the initial prevalence of hereditary deafness.

References:

Grants: Federal State Projects (FSRG-2020-0016, FWNR-2022-0021); The Research Project of the YSC CMP; RFBR grants (18-05-60035_Arctika, 20-015-00328_A).

Conflict of Interest: None declared.

EP21 Functional Genomics and Epigenomics

EP21.001 Spatial regulation of the ischemic process in the rat brain at the transcriptome level

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Background/Objectives: Ischemic stroke is one of the most severe multifactorial diseases. The genomic component occupies a significant place in the pathogenesis, diagnosis and treatment of ischemic stroke. It was shown that focal ischemia can lead to metabolic changes not only in the damaged (ipsilateral) hemisphere (IH) but also in the opposite (contralateral) hemisphere (CH) of the brain[1]. However, the genome reactions of CH cells remain not fully understood. Here, we analyzed series of rat brain samples from IH and CH after the transient middle cerebral artery occlusion (tMCAO) model and corresponding sham-operated samples using genome-wide RNA sequencing (RNA-Seq).

Methods: Wistar rats, tMCAO, magnetic resonance imaging, RNA-Seq, real-time RT-PCR, bioinformatics.

Results: We revealed 164 differentially expressed genes (DEGs) with cut-off>1.5 and $p_{adj} < 0.05$ that were associated with subcortical structures of CH at 24h after tMCAO. Among them, 34 genes were DEGs for CH but non-DEGs for IH. Moreover, 16 genes (Hrh3, Chst15, Drd2, Rasd2, Drd1, Hpca, Lrrc10b, Slc24a4, Scn4b, Neu2, Gng7, Adora2a, Asic4, Syndig1l, Rgs9, and Gpr6) had oppositely changed mRNA level in two brain hemispheres after tMCAO. These genes were predominantly associated with the functioning of neurosignaling system.

Conclusion: Complex spatial regulation of the ischemic process in the rat brain was revealed at the transcriptome level. We believe that specific genome responses in CH and IH can be the key for the study of regeneration potential of brain cells after stroke.

References: Ruan et al., Neural Regen Res. 2017;12(6):931–937.

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Conflict of Interest: Ivan B. Filippenkov Full employment, Institute of Molecular Genetics of National Research Center "Kurchatov Institute", Kurchatov Sq. 2, 123182 Moscow, Russia, The research was funded by RFBR and Moscow city Government according to the project № 21-34-70048., Julia A. Remizova Part-time employment, Institute of Molecular Genetics of National Research Center "Kurchatov Institute", Kurchatov Sq. 2, 123182 Moscow, Russia, Alina E. Denisova Part-time employment, Pirogov Russian National Research Medical University, Ostrovitianov str. 1, 117997 Moscow, Russia, Vasily V. Stavchansky Full employment, Institute of Molecular Genetics of National Research Center "Kurchatov Institute", Kurchatov Sq. 2, 123182 Moscow, Russia, Ksenia D. Golovina Part-time employment, Institute of Molecular Genetics of National Research Center "Kurchatov Institute", Kurchatov Sq. 2, 123182 Moscow, Russia, Leonid V. Gubsky Part-tine employment, Pirogov Russian National Research Medical University, Ostrovitianov str. 1, 117997 Moscow, Russia;.

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EP21.002 RAMP1 gene promoter and female migraine susceptibility: new clues in epigenetic processes

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Background/Objectives: Migraine is a complex neurovascular disorder, characterized by attacks of moderate to severe headache pain, affecting mainly females.

Receptor activity modifying protein (RAMP1) is part of the Calcitonin Gene Related Peptide (CGRP) receptor, a pharmacological target for migraine.

DNA methylation occurs mostly in the gene promoter and can control gene expression, playing a role in the clinical presentation of various diseases.

We aimed to investigate the RAMP1 promoter methylation status in female samples to find epigenetic biomarkers that can predict migraine risk in an accessible body fluid.

Methods: We investigated the methylation state of the RAMP1 promoter in 104 female blood DNA samples: 54 migraineurs and 50 controls. We treated DNA with sodium bisulfite and performed PCR, Sanger Sequencing, and Epigenetic Sequencing Methylation (ESME) software analysis.

Results: We identified 51 CpG dinucleotides, 5 showing methylation variability. Migraineurs showed a higher ratio of individuals with the five CpG methylated (26%) than controls (16%), but statistical significance was not reached ($\alpha = 0.05$, p value $= 0.216$). We also found that CpG -284bp, related to the transcription start site (TSS), showed significantly higher methylation levels in cases ($p = 0.017$, OR $= 1.06$; 95% C.I: 1.01–1.12).

Conclusion: We identified a novel CpG unit in the RAMP1 promoter, significantly methylated in migraineurs. This CpG may play a role in migraine affecting RAMP1 transcription or receptor malfunctioning and/or altered CGRP binding.

References:

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Conflict of Interest: None declared.

EP21.003 Do assisted reproductive technology (ART) procedures influence DNA methylation on the X chromosome?

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Background/Objectives: The X chromosome constitutes 5% of the human genome and may help explain sex difference in prevalence for various phenotypes. Here, we compared DNA methylation (DNAm) on the whole X chromosomes of children conceived by ART and those conceived naturally to identify epigenetic changes that may be related to ART.

Methods: DNAm data were available for 963 ART trios and 982 non-ART trios from the Study of Assisted Reproductive Technology (START) project (a sub-study of the Norwegian Mother, Father, and Child (MoBa)). We tested four statistical models and adjusted for various combinations of covariates in the EWAS: mother's age, smoking status, BMI, and parity, parental DNAm, and child's birth weight and gestational age. We also searched for differentially methylated regions (DMRs).

Results: Overall, CpGs were hypomethylated in girls and hypermethylated in boys. The significantly differentially methylated CpGs and regions differed between girls and boys in number and chromosome location. Adjusting for covariates did not affect the results of the girls-only analyses, but it did affect the boys-only analyses. Genes that co-localized with the significant CpGs/DMRs were associated with important biological processes (e.g., neurodevelopment) and diseases (e.g., autism). The most significant CpG in the boys-only analysis was located in UBE2DNL, a gene expressed in testis but without known function.

Conclusion: ART procedures influenced X-linked DNAm differently in boys and girls. Adjusting for parental DNAm did not affect the observed associations in girls, ruling out parental subfertility as a reason for the associations.

References:

Grants: Research Council of Norway, grant 262700.

Conflict of Interest: None declared.

EP21.004 Model Matchmaking via the Rare Diseases Models & Mechanisms Network (RDMM-Europe)

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Background/Objectives: In medical genetics, there is a critical need for model organism research to assess new disease-gene associations, to understand pathophysiology, and to test potential therapies.

Methods: Within the Solve-RD project we established the European Rare Disease Models & Mechanisms Network (RDMM-Europe) to facilitate collaborations of model organism researchers and clinicians with a focus on patients with rare diseases (RD). The principle of this brokerage service is to fill the gap between RD gene discovery and functional validation. For that purpose, we connect Solve-RD clinicians that have discovered new disease-causing genes with model organism investigators (MOIs) that can study the mechanistic role of the given genes in health and disease by using an appropriate model organism or cell culture system. Solve-RD supports these validation projects with 50 Seeding Grants of 20,000 EUR each.

For the selection of candidate genes and model matchmaking, a two-committee process and a registry were set up using the structures of the successful Canadian RDMM Network as role model.

Results: To date, we have awarded 29 Seeding Grants to MOIs and have linked Solve-RD scientists to model researchers in eight European countries, UK, Canada, USA, Qatar, Japan, and Australia.

Conclusion: Linking scientists across borders via the RDMM network and supporting these joint projects will advance RD research and will be of benefit to patients and families living with RD.

References:

Grants: The Solve-RD project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 779257.

Conflict of Interest: None declared.

EP21.005 Functional characterization of GJB2 cis-regulatory elements in non-syndromic hearing loss

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Background/Objectives: Three-dimensional chromatin organization plays a key role on gene expression. Gene regulation depends on cis-regulatory elements (CREs) which can interact with gene promoter by chromatin loop. Alteration of chromatin architecture and/or CREs can lead to enhanceropathies. Hearing loss is the most common sensory pathology, affecting about 1–2 in 1000 newborns. In industrially countries, congenital deafness has a genetic origin in 80% cases. This deafness can be syndromic or not (associated or not with other pathology or malformation) and represents respectively 10% and 90% of cases. Among this deafness, we found the first type of autosomal recessive non-syndromic hearing loss and deafness (DFNB1). The GJB2 (Gap Junction Beta 2) gene is implicated on DFNB1 and is responsible for more than 30 % according to study population. Several unelucidated nonsyndromic hearing loss and deafness 1 (DFNB1) cases are monoallelic, led to strongly suggest the presence of distant cis-regulation.

Thanks to chromatin conformation capture carbon copy (5C) study, we previously identified the first GJB2 CREs, with enhancer and silencer effect on GJB2 expression. Analysis of CTCF binding allowed to purpose a DFNB1 3D looping model.

Methods: To confirm an endogenous enhancer activity, we used CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) with good results.

Then, we use circular chromosome conformation capture (4C) technique to study all interactions possible between the GJB2 promoter and all the genome, and between GJB2 CREs and the genome.

Results:.

Conclusion: This allows to determine new chromatin interactions inside DFNB1 locus, and control cooperative interactions.

References:

Grants:

Conflict of Interest: None declared.

EP21.007 MicroRNA and piRNA profiles during pregnancy in serum and plasma

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Background/Objectives: MicroRNAs and piRNAs play a regulatory role by suppressing gene expression and are potential early biomarkers of pregnancy complications. In our study, we investigated miRNA and piRNA profiles during healthy pregnancy in plasma and serum.

Methods: Total of 42 serum and plasma blood samples from seven women across pregnancy were collected. Small RNA isolation and library preparation for sequencing was performed using miRNeasy Serum/Plasma Kit (Qiagen) and QIAseq miRNA Library Kit (Qiagen). Libraries were sequenced on a HiSeq 2500 (Illumina). Bioinformatic data analysis was performed using the GeneGlobe Data Analysis Center and DESeq2 R Package.

Results: We found 2 miRNAs in plasma (hsa-miR-7853-5p and hsa-miR-200c-3p) and 10 miRNAs in serumv (hsa-miR-203a-5p, hsa-miR-495-3p, hsa-miR-4435, hsa-miR-340-5p, hsa-miR-4417, hsa-miR-1266-5p, hsa-miR-4494, hsa-miR-134-3p, hsa-miR-5008-5p, and hsa-miR-6756-5p), that exhibit level changes during pregnancy. It was shown that the content of the 7 piRNAs in plasma increases from the first to the third trimester (piR 000765, piR 020326, piR 019825, piR 020497, piR 015026, piR 001312, piR 017716). We observed differences for 36 miRNAs and for 9 piRNAs between plasma and serum which should be taken into consideration when comparing the results between studies performed using different biosample types.

Conclusion: This study can become the basis for the search for miRNA and piRNA biomarkers of various pregnancy complications.

References:

Grants: This study was financially supported by a Russian Science Foundation grant 19-75-20033.

Conflict of Interest: None declared.

EP21.008 Expression of the NUP153 and YWHAB genes from canonical and alternative LINE-1 promoters in the placenta of the first trimester of pregnancy

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Background/Objectives: The placenta has aunique hypomethylated epigenetic landscape. During the first trimester of pregnancy, the activity of retrovirus- and retrotransposon-derived regulatory elements potentially increases, which are suppressed by DNA methylation in the adult body. The aim of this study was to analyze the expression of the NUP153 and YWHAB genes, which are highly active in the placenta, from canonical and alternative LINE-1 promoters.

Methods: Analysis of the genes expression was performed using real-time PCR in adult lymphocytes ($n = 10$), chorionic villi and extraembryonic mesoderm from miscarriages (normal karyotype – $n = 10$, trisomy 16 – $n = 8$, and monosomy X – $n = 6$), and induced abortions ($n = 10$). LINE-1 methylation index was assessed in chorionic villi from miscarriages by targeted bisulfite massive parallel sequencing.

Results: The level of expression of both genes from canonical promoters was higher in the blood lymphocytes than in the placental tissues (NUP153 – 8.4-fold, YWHAB – 2.5-fold, $p < 0.05$). However, the level of expression from the alternative LINE-1 promoters was higher in chorionic villi (NUP153 – 64-fold, YWHAB – 20.3-fold, $p < 0.05$) and extraembryonic mesoderm (NUP153 – 44.9-fold, $p < 0.05$). LINE-1 methylation index negatively correlated with the level of gene expression from both canonical (NUP153 – $R = -0.59$, $p < 0.003$; YWHAB – $R = -0.52$, $p < 0.01$) and alternative LINE-1 promoters (NUP153 – $R = -0.46$, $p = 0.03$; YWHAB – $R = -0.66$, $p = 0.001$).

Conclusion: An increase in the LINE-1 methylation index in placenta of miscarriages is associated with decrease in gene expression not only from alternative promoters, but also from canonical ones, which can affect normal embryogenesis.

References:

Grants: This study was supported by Russian Science Foundation, grant 19-74-10026.

Conflict of Interest: None declared.

EP21.009 Identification of epigenetic determinants of obesity by comparing ancient and modern human epigenomes

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Background/Objectives: The aim of the study is to identify epigenetic loci associated with obesity, the mechanisms of which are not fully understood. Genetic loci associated with the risk of this disease only account for a few percent of the variance in obesity, thence attention has turned into investigating the role of epigenetic changes in the etiology of this disease. We supposed that the diet and lifestyle of modern humans influence their epigenome and make them more susceptible to obesity. The specificity of our study is the use of ancient human methylation profile as a healthy norm, assuming that the lifestyle of archaic humans was significantly different from the modern one.

Methods: We compared epigenomes of ancient and modern humans and identified genomic regions that have different DNA methylation patterns. We supposed that these differences may contribute to the development of obesity in modern humans. To verify this hypothesis, we simulated the methylation status of the genome loci by using the CRISPR/dCas9 system fused with TET1CD/DNMT3A in the NIH 3T3-L1 cell line and performed a differentiation into adipocytes with parallel analysis of the gene expression.

Results: Effects of the epigenetic editing on adipogenesis will be discussed.

Conclusion: The results of the study will provide new information about the epigenetic component of the molecular nature of obesity. The discovered epigenetic loci can potentially be used as promising targets for the search for new drugs.

References:

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Conflict of Interest: None declared.

EP21.010 LINC00493/SMIM26 gene and its dual functioning

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Background/Objectives: The study of new lncRNAs is important to improve our knowledge of intracellular processes and may help to find possible therapeutic targets. In the present study, we investigate the widely expressed lncRNA LINC00493. We determine the structure of the LINC00493 transcript, its localization, protein coding potential, and its impact on cell physiology..

Methods: Rapid amplification of cDNA ends used to define the exact structure of the transcript. Soft lysis method was used for subcellular localization understanding. Knockdown and overexpression experiments were used to investigate influence LINC00493 on cell physiology . Cell proliferation was measured using MTT assay. Cell migration capability was measured by wound healing assay.

Results: We investigated exon-intron structure of LINC00493 and showed the existence of two isoforms that differ by three nucleotides. (GenBank:MW979249, MW979250). LINC00493 contains a small open reading frame that could be translated to SMIM26 protein. Sequence analysis showed that the LINC00493/SMIM26 gene is evolutionarily conserved among mammals at both the RNA and protein levels. It has been established that LINC00493 is expressed at a high level in most tissues and is predominantly localized in the cytoplasm. Knockdown of LINC00493/SMIM26 demonstrated an effect on cell viability depending on the cell type. On the other hand, wound-healing assay revealed that LINC00493 knockdown did not affect cell migration. Overexpression experiments have shown that even in the absence of translation of the SMIM26 protein, the LINC00493 transcript itself increases cell viability in cell lines.

Conclusion: Our results show that the LINC00493 transcript has an intrinsic function independent of the SMIM26 protein.

References:

Grants:

Conflict of Interest: None declared.

EP21.011 Interleukin-10 gene polymorphism rs1800896 and IL-10 serum level in Cystic Fibrosis

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Background/Objectives: Airway inflammation in cystic fibrosis (CF) is associated with increased production of pro-inflammatory cytokines (IL-1, IL-8, IL-6) and reduced levels of anti-inflammatory cytokines such as Interleukin-10 (IL-10) [1]. In this study we investigated a polymorphism located in the 5′-flanking region of the IL-10 gene, at a position −1082 A>G, which is known to be involved in regulating the production of IL-10, and evaluated the serum levels of IL-10 in patients with CF.

Methods: The study was approved by the ethics committee of the TSMU. 30 CF patients were genotyped for the IL-10 −1082 A>G polymorphism using a TaqMan assay (Thermo Scientific, USA). The serum level of IL-10 was determined using an ELISA kit (Qiagen, USA).

Results: The results demonstrated that the IL-10 genotype frequencies in CF patients were: 37% AA genotype and 63% AG+GG genotypes. Allele frequency of major allele (A) and minor allele (G) in IL10 −1082 A>G variant was found to be 0.63 and 0.37, respectively. In addition, the −1082 AG and GG genotypes were associated with higher serum levels of IL-10 compare to AA genotypes.

Conclusion: We can conclude that the IL-10 −1082 G allele and the AG+GG genotypes can be considered as a modifier of the CF disease severity.

References: 1. Courtney J.M., Ennis M., Elborn J.S., Cytokines and inflammatory mediators in cystic fibrosis, Journal of Cystic Fibrosis, Volume 3, Issue 4, 2004, Pages 223-231..

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Conflict of Interest: None declared.

EP21.012 Imprinting disorders in Egyptian patients

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Background/Objectives: Imprinted genes characterized by monoallelic expression either maternal or paternal, this is important for normal growth and development, disruption of their monoallelic expression leads to imprinting disorders (IDs). We aimed for proper diagnoses of IDs in Egyptian patients, depending on clinical evaluation and genetic testing, and through emphasis on certain clinical manifestation that may indicate IDs o give proper diagnosis and genetic counseling.

Methods: Methods: Clinical evaluation and selection of patients with suspected IDs. We applied methylation-multiple ligation-dependent robe amplification (MS MLPA) and Array CGH.

Results: Results: Forty-four patients were selected from the outpatient's clinics, National Research Center Egypt. They were subjected to MS MLPA, we found five patients with methylation defect, two patients with hypomethylation of KCNQ-CR and clinically presented with Beckwith Wideman, one patient with hypermethylation of SNPRN with Prader-Willi, one patient with hypermethylation of MEST-1 on chromosome 7 and had Silver-Russel syndrome (SRS) and one patient with hypermethylation H19 with manifestations of SRS. We used SNP array to exclude uniparental disomy. In patient with maternal hypermethylation of chromosome 7, we found uniparental disomy (UPD) in chromosome7. It is important to do MS MLPA test for patients with manifestations suspected imprinting disorders like hemi-hypertrophy, growth retardation, neonatal feeding problems, obesity, precocious puberty or history of assisted reproduction.

Conclusion: it is important to diagnose IDs, Knowing the exact cause of the IDs is important in genetic counseling. Whole genome methylation helps to identify more imprinting disorders.

References:

Grants:

Conflict of Interest: None declared.

EP21.014 Association of breastfeeding and genome-wide DNA methylation in child blood

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Background/Objectives: Breastfeeding has short- and long-term beneficial effects on child health. Epigenetic modifications are a potential molecular mechanism explaining the association of breastfeeding with infant development. We aimed to study the association between breastfeeding and genome-wide DNA methylation in children's blood.

Methods: The analysis was conducted in 788 children of the Human Early Life Exposome (HELIX) project. Breastfeeding practices were reported by the mothers during the first year of life: any breastfeeding (never/ever), any breastfeeding duration (in months), any breastfeeding duration in categories (never, ≤4 months, >4–12 months; and ≥12 months). Blood DNA methylation was assessed with the Illumina 450K array at the mean age of 8 years. Associations were tested using robust linear regressions, adjusting for confounders.

Results: Six-hundred and sixty (83.8%) children were ever breastfed. Median duration of any breastfeeding was 4.9 months (interquartile range = 0.99; 10.84). Categories of any breastfeeding duration were: 125 (15.9%) never; 250 (31.7%) ≤ 4 months; 278 (35.3%) >4–12 months; and 135 (17.1%) ≥ 12 months. Lambda inflation factors ranged from 0.95 to 0.99. None of the associations passed multiple-testing correction. The lowest p-value was observed for cg17700453 in *XRRA1* (effect = −0.017, p value = 1.82e-07) when comparing children breastfed for ≤4 months vs never breastfeed.

Conclusion: HELIX results will be meta-analyzed with data from an additional 11 cohorts of the Pregnancy And Childhood Epigenetics (PACE) Consortium.

References:

Grants: FP7/2007-206 - 308333; H2020-EU.3.1.2. - 874583; JPI HDHL and Instituto de Salud Carlos III - AC18/00006.

Conflict of Interest: None declared.

EP21.015 Diversity and differential expression of miRNAs in the human skeletal muscle with distinct fiber type composition

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Background/Objectives: The ratio of fast-twitch and slow-twitch fibers in human skeletal muscle tissue can vary significantly depending on individual characteristics and type of physical activity. In this study we investigated the contribution of miRNAs in skeletal muscle fiber type composition.

Methods: Muscle biopsies of vastus lateralis were taken from ten male athletes under the age of 40 years divided into two groups with a significant predominance of fast-twitch or slow-twitch muscle fibers identified by immunohistochemical analysis. Transcriptomes were previously obtained from all samples by rRNA-depleted RNA sequencing. Small RNA sequencing was used to obtain miRNomes for this study.

Results: On average, 365 miRNA species were detected in a single sample. Five miRNAs - miR-499a-5p, miR-206, miR-208b-3p, miR-501-3p, and hsa-miR-185-5p - were differentially expressed between the study groups ($p < 0.05$, log2FC>1). Four of these miRNAs were originating from miRtrons and two of them (miR-208b-3p and miR-499a-5p) had strong correlations in expression with their host genes (MYH7 and MYH7B, respectively). The correlation analysis of miRNA expression with the expression of their mRNA targets revealed 26 miRNA-mRNA interactions with strong correlation. Notably, three of these interactions belonged to miR-206 suggesting its regulatory relationships with ESR1, NR1H3 and ANXA1 expression.

Conclusion: Joint analysis of miRNomes and transcriptomes in human skeletal muscle samples with distinct fiber type composition revealed the diversity of miRNAs, their differential expression and potential interactions between miRNAs and their host or target genes.

References:

Grants: The study was supported by the grant from the Russian Science Foundation No. 21-15-00362.

Conflict of Interest: None declared.

EP21.016 Comparative study of small RNA library preparation kits for biomarker analysis in plasma, cell-free saliva and thereof isolated extracellular vesicles

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Background/Objectives: The aim of the study was the comparison of commercially available library preparation approaches for small RNA sequencing from liquid biopsies including plasma, cell-free saliva and thereof isolated extracellular vesicles (EVs).

Methods: Using a synthetic miRNA reference sample as well as a common pool of total RNA derived from i) plasma, ii) cell-free saliva, iii) plasma exosomes and iv) saliva exosomes we compared various small RNA library preparation kits. Starting off with 5 ng total RNA, library preps were done according to manufacturers' recommendations and finally applied to 50 nt single read sequencing.

Results: We identified the small RNA library kit from Qiagen as the best kit since it not only resolved the miRNA reference sample in the best way but also showed the best results in almost any variant of liquid biopsy sample investigated. However, the kit from Somagenics came very close and appeared to work best for saliva exosomes. Though every tested kit did not detect the exact same panels of miRNAs, there was still quite some miRNA overlap between the three best performing kits for which a detailed data analysis was performed.

Conclusion: The small RNA library kit from Qiagen turned out to be the in our hands best working approach for small RNA profiling from plasma, saliva and thereof isolated EVs. Some differences in the spectrum of identified miRNAs were found, dependent on the chosen library kit, but more significant

dependent on the liquid biopsy sample source. Our findings lay a sound basis for future small RNA biomarker studies.

References:

Grants:

Conflict of Interest: None declared.

EP21.018 Functional genetic approach towards personalized healthcare in preventive medicine – a case study

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Background/Objectives: Functional genetics studies how genes and intergenic regions contribute to various polygenic-multifactorial disorders (PMD).

Methods: Thirty-tree subjects from Bulgarian origin were tested for functional genetic variants related to cardiovascular disorders (DNALYSIS, Biotechnology®, Denmark): LPL, CETP, APOC3, APOE, MTHFR1, MTHFR2, MTR, MTRR, CBS, IL6, TNFA, ENOS, MNSOD/SOD2, CYP1A2, FADS1, ACE, AGT. All subjects filled in a questionnaire. Dietary recommendations and lifestyle changes were given after the results analysis by trained clinical geneticist and dietitian. Monitoring for the cardiovascular disease continues.

Results: Variants predisposing to elevated plasma levels of cholesterol and homocysteine in more than half of the subjects were found. Moreover, high predisposition to the chronic inflammation is observed. Four subjects were found to be carriers of the E4 APOE gene. Twenty-nine were carriers of the risk G allele in the FADS1 gene in rs174537. Twenty-tree subjects were carriers of the risk alleles in salt-sensitivity related genes ACE and AGT and seventeen were slow caffeine metabolizers. After receiving the results, the study participants were given personalized recommendations for specific diet and lifestyle changes. Monitoring for the cardiovascular disease is performed yearly in the adult subjects only.

Conclusion: The integration of risk allele variants with epidemiological risk factors could improve the stratification of individuals, potentially resulting in more effective PMD prevention and clinical interventions.

References: N/A

Grants: N/A

Conflict of Interest: None declared.

EP21.019 Western diet causes molecular and physiological changes in the circadian clock system of oral tissues

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Background/Objectives: The circadian clock regulates most body functions following 24-hours physiological cycles. Numerous studies have shown that circadian clock disruption is directly linked to disease trigger and outcomes. Diet modifications and resultant epigenetic changes are among the causes of circadian clock disruption[1]. This study aims to elucidate the potential links between diet, circadian clock disruption and oral cancer.

Methods: Mice for this pilot study were separated randomly in two groups ($n = 12$). One group was fed with Normal Diet (ND) and the other group was fed with Western Diet (WD). Both groups were kept in a circadian cabinet to continuously monitor circadian physiology traits. At week 20, mice were sacrificed at 4 time-points with 6h intervals, and oral tissues were collected for DNA/RNA and protein expression. Saliva was collected for metabolomics.

Results: The expression of clock genes in two different time points significantly decreased in the WD group compared to the ND group. Circadian cabinets measurements indicate significant activity reduction in WD group compared to ND group.

Conclusion: This data suggests that WD results in significant changes in circadian clock genes expression in oral epithelium. Additional studies are needed to characterize the precise molecular changes and potential epigenetic modifications involved. Nevertheless, our data suggest that diet is a key modifier of the circadian clock system and may predispose to oral cancer development.

References: 1. Adeola et al., Front Physiol; 2019.

Grants: Supported by Saskatchewan Centre for Patient-Oriented Research–Saskatchewan Health Research Foundation, Centennial Enhancement Chair in One Health and University of Saskatchewan Dean's Scholarships.

Conflict of Interest: None declared.

EP21.020 Interaction map of cis-regulatory elements controlling ABCA4 in human retina

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Background/Objectives: Stargardt disease is a genetic degeneration of the macula that causes loss of vision, especially in the central visual area, and represents the most common hereditary macular degeneration in young patients. This project aims to identify and functionally characterize cis-regulatory elements (CREs) controlling *ABCA4* expression, the gene affected in the most common form of the disease, both in adult human retina and retinal pigment epithelium (RPE).

Methods: Regulatory elements that are active in human retina and RPE were located by means of ChIPseq and ATACseq experiments. Subsequently, we used the HiChIP technique, which allows to determine 3D chromatin structure, to identify the target genes of these CREs in each tissue. Finally, we tested these enhancers in transgenesis assays in zebrafish, and compared the expression driven by them with the zebrafish endogenous *ABCA4* expression, determined by in situ hybridization.

Results: We found that the regulatory landscape of *ABCA4* gene encompasses 400 kb in which we could identify several active enhancers, some of them specific for retina or RPE. Preliminary data in F0 injected zebrafish embryos show that they drive expression in domains compatible with the *ABCA4* endogenous expression, specifically in the eye.

Conclusion: We conclude that these enhancers may be critical for the correct expression of the gene, and therefore for the development of the disease. Further characterization of these enhancers needs to be done in the future in order to determine their particular role and involvement in the development of the Stargardt disease.

References: Cherry et al., 2020.

Grants: Marie-Curie no 813490 StarT.

Conflict of Interest: Soraya Kalayanamontri phD student, phd student, Ana Neto Post Doc, Pedro Manuel Martinez-Garcia Post Doc, Víctor López Soriano phD student, Jose Luis Gomez-Skarmeta principal investigator, Elfride De Baere principal investigator, Juan Ramón Martínez Morales Martínez-Morales principal investigator, Juan Jesus Tena-Aguilar PI, principal investigator.

EP22 New Treatments for Genetic Disorders

EP22.001 Tauroursodeoxycholic acid and sodium 4-phenylbutyrate treatments diminish low-molecular-weight-proteinuria in a Clcn5 knock-in mouse model for Dent disease-1

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Background/Objectives: Dent disease-1 (DD1) is a rare X-linked tubular disorder characterized by low-molecular-weight-proteinuria (LMWP), hypercalciuria, nephrolithiasis/nephrocalcinosis. It is caused by mutations in the CLCN5 gene, which encodes the voltage-gated ClC-5 chloride/proton antiporter. Currently, the treatment of DD1 is only supportive and focused in delaying the progression of the disease. Our group has generated a Clcn5 knock-in (KI) mouse carrying the pathogenic mutation p.V523del, that presents the main clinical manifestations of DD1.We aimed to assess the ability of two small chemical chaperones, sodium 4-phenylbutyrate (4-PBA) and tauroursodeoxycholic acid (TUDCA), to ameliorate those symptoms in our DD1 mouse model.

Methods: Twelve-weeks old male mice, Clcn5-KI and controls, were selected for treatments. Mice selected for 4-PBA treatment received 250 mg/kg/day in drinking water for 31 days, whereas mice selected for TUDCA treatment received 250 mg/kg/day IP in saline solution for 20 days. Urinary ß2-microglobulin and serum and urinary creatinine were measured by ELISA. Calcium and phosphate concentrations in urine were estimated using colorimetric kits.

Results: We observed a significant reduction of ß2-microglobulin urinary excretion in Clcn5-KI mice treated with 4-PBA and TUDCA ($p < 0.05$). The treatment with TUDCA was more effective in mice showing higher levels of ß2-microglobulin excretion. In addition, urine production and urinary calcium and phosphate levels showed significant differences depending on treatment.

Conclusion: The reduction of LMWP in Clcn5 KI mice treated with 4-PBA and TUDCA suggest that both treatments represent promising therapeutic options for some DD1 patients.

References:

Grants: ASDENT (D18-003), ISCIII-ERDF (PI17/00153, PI20/00652) "Another way to build Europe".

Conflict of Interest: None declared.

EP22.002 Antisense oligonucleotides targeting exon 11 are able to partially rescue the Neurofibromatosis Type 2 phenotype in vitro

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Background/Objectives: Neurofibromatosis type 2 (NF2) is a neoplasia predisposing syndrome caused by loss of function variants in *NF2*. The clinical presentation of the disease is variable and related to the type of the inherited variant (1). Here, we evaluated the use phosphorodiamidate morpholino oligomers (PMOs), to reduce the severity of *NF2* splicing and truncating variants to milder hypomorphic forms in vitro.

Methods: Variant-specific PMOs were designed targeting four splicing variants. In addition, a pair of PMOs were designed to induce the skipping of exons 4, 8 or 11 of *NF2* harboring truncating variants. PMOs were tested in primary fibroblasts of *NF2* patients. Functional studies assessed Merlin levels, actin cytoskeleton organization and proliferation capacity.

Results: PMOs designed for variants located at +/- 13 from the intron-exon boundary region interfered in the correct splicing signalling of *NF2* wild-type and mutated alleles. Regarding truncating variants, only the skipping of exon 11 produced an hypomorphic Merlin able to partially rescue the phenotype observed in primary fibroblasts harboring a pathogenic germline variant on *NF2*.

Conclusion: Although it has not been possible to recover Merlin levels for pathogenic variants located at exons 4 or 8, or to correct splicing signals in the variants located near canonical splice sites tested, the encouraging result on exon 11 is an in vitro proof of concept of the use of antisense therapy to develop personalized therapies for NF2.

References: (1) Evans DG, Orphanet J Rare Dis. 2009 Jun 19;4:16.

Grants: CTF-2019-05-005, Neurofibromatosis project foundation, AcNeFi, PI20/00215, 2017 SGR 496.

Conflict of Interest: None declared.

EP23 Genetic Counselling/Services/Education

EP23.001 By improving knowledge in genetics, we spread out the understanding of the genetics impact on the prevention and treatment of oncology patients - an example of good cooperation practice between institutions and civil society organizations

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Background/Objectives: Recent years have witnessed a growing need to educate oncology patients about genetics.

Methods: From 2014, we have educated 500 oncology patients and their family members through the civil association "Everything for her". Through 13 lectures and 150 individual consultations we explained, in a simple and understandable way, the connection between genes and cancer development as well as the impact of targeted treatment on these processes with the aim to look at genetics from another, patient-friendly, side.

Results: All of the above resulted with the Genetic testing guidelines for hereditary cancers published in 2017, as well as a number of educational materials on the "Everything for her" digital media. The knowledge gained during these activities was shared with 1418 medical students and 30 students of post-graduate oncology study. The activities also led to the development of the Outpatient Clinic for Hereditary Cancer Patients at the University Hospital Center Zagreb, which performs 16 tests for hereditary cancers monthly. All acters cooperate in sharing knowledge, information, and the possibility of genetic testing for hereditary cancers in both oncology patients and their family members.

Conclusion: Based on these results we are convinced that spreading knowledge about genetics through lectures and individual consultations would significantly improve the understanding of genetics in our country and make this aspect of the organism functioning closer to oncology patients.

References:

Grants: Co-financed by the EU through the Europe Regional Development Fund, Operational Programme Competitiveness, and Cohesion, grant No. KK.01.1.1.01.0008, Reproductive and Regenerative Medicine – Exploring New Platforms and Potentials.

Conflict of Interest: None declared.

EP23.004 Co-design, implementation, and evaluation of a plain language family report for genomic testing

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Background/Objectives: Interpreting and communicating genomic results can be challenging for families and clinicians. Plain language genomic reports are emerging in the literature, however generally lack co-design or rigorous evaluation outside hypothetical scenarios. Rather than modifying existing laboratory reports, co-designing new reports provides opportunity for a family-centred approach.

Methods: Following review of literature and international best practice, 'family report' templates for a range of genomic test results were produced via iterative co-design involving genetic health professionals, patient groups, educators and plain language experts. The templates were implemented in a national study providing ultra-rapid genomic testing to critically ill paediatric patients. Family and genetic health professional experiences with these reports were explored using surveys deployed March 2021–February 2022. Domains included layout, information provided, and report use.

Results: Of 153 families who received a family report, and 108 clinicians who used them, 43 families and 51 clinicians responded (RR = 28% and 47%, respectively). The majority were satisfied with the layout (92% and 100%, respectively). Families mostly (80%) found the report helpful in understanding their result. Most families (62%) had shared the report, predominantly with family members (70%) or health professionals (67%). Clinicians (34%) reported adapting the report for use in other clinical and research settings.

Conclusion: In a real-world setting we show that, through co-design, genomic test reports can be tailored to meet family needs. Provision of clear plain-language reports successfully supported families and health professionals to interpret and communicate genomic test information more broadly within healthcare teams.

References:

Grants: Australian Government GHFM76747.

Conflict of Interest: None declared.

EP23.005 Too many questions, too little information: Supporting families who have a child with severe genetic epilepsy

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Background/Objectives: Developmental and Epileptic Encephalopathies (DEE) are a clinically severe and complex group of rare conditions. Parents of a child with DEE report higher levels of anxiety and depression than population norms. Unmet information needs may be contributing to these outcomes. To address this, we partnered with families to co-design 'GenE Compass'. GenE Compass invites parents to submit questions about their child's diagnosis, such as about expected comorbidities, natural history and current research. Our multidisciplinary team prepares personalised, evidence-based reports to respond to parents' questions.

Methods: We are conducting a pre-post pilot of GenE Compass with parents who have a child (<18 years) with a suspected or confirmed DEE diagnosis. We will assess acceptability, feasibility, and impact of GenE Compass on parents' wellbeing. Parents will complete a survey at enrolment (Q1), receive 6-months of access to GenE Compass, and then complete a second survey at 6-months (Q2). Parents will also provide feedback on each report at the time of receiving it.

Results: Recruitment commenced January-2022. We anticipate ~50 families will have completed Q1 by June-2022. In this presentation, we present on the development of GenE Compass, early feasibility data and any report-specific feedback.

Conclusion: 'GenE Compass' is an innovative model of care within childhood rare disease, specifically DEE, which will provide the highest-quality, understandable and relevant information to families. We will use reports as the basis of a 'living information resource' that will be freely available to families and clinicians internationally via the PENNSW website.

References: NA

Grants: Philanthropic funding.

Conflict of Interest: None declared.

EP23.008 THL Biobank´s genomic tools are drivers for new research discoveries

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Background/Objectives: Biobanks provide a valuable resource for detailed descriptions of disease risk factors and genetic data to support the research era of personalized medicine. To promote new research solutions for health improvement and disease prevention, THL Biobank established two new services for researchers to get easy access to genomic data collections: polygenic risk score (PRS) service and the public Beacon variant discovery service.

Methods: PRS service is provided for all biobank sample collections including >100 000 individuals with imputed genomic data. Researchers may choose a GWAS of interest and PRS is calculated with PRS-CS method. The genomic dataset featured in THL Biobank's Beacon was created as part of many international research collaborations and imputed by the FinnGen project against a Finnish population specific whole genome sequence.

Results: We offer ready-to-use PRSs for several diseases/traits such as T2D, T1D, BMI, ADHD, BPD etc. that are calculated in our population specific sample collections. Beacon service is available as part of the European wide ELIXIR Beacon Network. THL Biobank ´s Beacon includes a population of 105 000 Finnish sample donors with frequencies of 20 million genomic variants, making it one of the largest Beacon resources in the world.

Conclusion: Identification of not only disease specific variants but also genome-wide disease fingerprints are the key features of precision medicine. The genomic data can be combined with rich biomarker and lifestyle data to drive new research discoveries.

References:

Grants:

Conflict of Interest: None declared.

EP23.009 ERN-ITHACA: European Reference Network on congenital malformations and rare neurodevelopmental disabilities

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Background/Objectives: ITHACA stands for Intellectual disability, TeleHealth, Autism and Congenital Anomalies. The name also echoes the diagnostic odyssey experienced by so many patients with developmental anomalies. ITHACA is a coordinated network

of more than 70 clinical genetics department across EU academic hospitals.

Methods: This ERN brings together experts in rare multiple congenital anomalies and rare neurodevelopmental disorders, the latter field mainly covering intellectual disability and autism spectrum disorder. ITHACA's field of expertise covers the clinical and biological/genetic diagnosis of these developmental anomalies, the coordination of their multidisciplinary treatment, and also their prenatal diagnosis and fetal pathology. A very large number of children and adults are affected by rare developmental anomalies: Congenital malformations affect one in 40 babies. Many malformations occur together as part of complex 'syndromes' that often show also NDD. Over 5 000 rare syndromes have been described.

Results: ERN-ITHACA networks patient representatives and medical experts with the aim to develop best practices and coordinate guidelines production, to provide a collaborative support for clinical research and to generally improve early diagnosis, care and cure of patients with rare developmental anomalies.

Conclusion: ITHACA has established the patient registry ILIAD dedicated to disorders falling under its scope of expertise. It also encourages the development of telemedicine and tele-expertise to allow collegial discussion of complex situations between referring doctors and RD experts who are scattered in the EU. ITHACA will produce advanced training and e-learning tools dedicated to health professionals, lay persons and Patients Advocacy Groups.

References:

Grants: 3rd Health Programme, grant nr 869189 ERN-ITHACA..

Conflict of Interest: None declared.

EP23.010 Educational Programme on Genetics, Lifestyle Behaviours and Cancer Prevention: A Multidisciplinary Consensus Study

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Background/Objectives: Most common hereditary cancers in Europe have also been associated with lifestyle behaviours. There is a scarcity of interventions to promote healthy lifestyle behaviours in people with high risk of cancer. We aimed to reach multidisciplinary consensus on knowledge and skills needed to enable European nurses to understand the importance of cancer and genetics and to promote healthy lifestyle behaviours for people living with inherited mutations.

Methods: It was conducted a Delphi study with an international panel of experts in cancer and genetics. In round 1, experts assessed the relevance of topics found on the systematic review and suggested additional terms. The following rounds aimed to bring consensus on the important topics by including or excluding those topics that reached 75% of consensus. Ethics approved by University committee.

Results: A total of 72 experts (nurses, physicians, psychologist, geneticist, genetic counsellors…) from all around the world participated. All but two initial topics were considered relevant. Twenty additional topics were proposed. Experts agreed on topics for an educational programme including knowledge and abilities competencies in genetics, lifestyle behaviours and communication and barriers.

Conclusion: All professionals agreed in the importance of increasing awareness of cancer and genetics and prevention. This multidisciplinary consensus will be the base to build an educational programme to increase cancer nurses' skills to support the complex care of people living with a higher risk of cancer, and to be able to give advice and support and motivate changes in lifestyle behaviours.

References: (Bruno et al., 2020) (Visser et al., 2017).

Grants: N/A

Conflict of Interest: Celia Díez de los Ríos de la Serna Collaborator in research projects with EONS (on breast cancer), Paz Fernandez-Ortega Collaborator in research projects with EONS, Teresa Lluch-Canut: None declared.

EP23.011 The Wessex mainstreaming cancer genetic testing programme (MCGT)

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Background/Objectives: Approximately 10% of common cancers arise on a background of an inherited high-risk gene variant. Family history-based guidelines miss a significant number of BRCA gene carriers amongst breast and ovarian cancer patients. Until recently cancer patients required a referral to Clinical Genetics for counselling and to assess eligibility for testing.

The identification of high-risk gene carriers informs cancer treatment and provides options for risk reduction and surveillance for patients and their families. The MCGT programme has streamlined genetic testing for ovarian and breast cancer patients, increasing access to genetic testing and providing quicker results.

Methods: The MCGT programme included a gap analysis of current service provision, the development of new test pathways and patient resources including an online decision aid (Breast Cancer choices), training and education programmes for cancer clinicians and an evaluation of the new service.

Results: Over 300 patients have been tested in the MCGT programme with a detection rate of BRCA1/2 or PALB2 variants of 11%. Test result timeframes have halved, and clinicians are using results to direct cancer treatment. To date 66 cancer clinicians have attended the online mainstream training programme. Updated data will be presented.

Conclusion: The MCGT programme has resulted in rapid and equitable genetic testing for patients by an educated and motivated workforce. Ongoing developments are (1) the clinical evaluation of the breast cancer choices decision aid (2) increasing online training resources and (3) widening access to patients with other cancers, such as prostate cancer.

References:

Grants: Wessex Cancer Alliance, NHS England.

Conflict of Interest: None declared.

EP23.012 ¨Beyond the improvement family genetic testing in rare diseases- Fabry disease¨

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Laboratorio de Biología Molecular., Alicante, Spain; [5]Universidad de Alicante, Biotecnología, Alicante, Spain.

Background/Objectives: Diagnostics delay in Fabry disease can be due to the non-specificity of early symptoms. Subsequent, the condition can go unnoticed. The most severe consequence the patient does has not access to early drug and clinical treatment can lead to end-stage renal disease, cardiac arrhythmias, and stroke.

A practical implementation of pedigree analysis in Fabry disease may display a greater number of new testing candidates, new carriers and affected individuals, often at a younger age before the appearance of the first symptoms.

Methods: The subject matter is reviewing the Fabry probands family pedigree of the rare disease department of the Hospital General Universitario de Alicante (Spain).

The analysis has been carried out on how many relatives per Fabry proband were tested and declared positive for GLA mutation, and finally, how many family members are yet to be tested per proband.

These values expressed on average can be compared with data from bibliographic reviews. They will be analyzed demographically to identify the population type and the barriers to the Fabry genetic testing into the consultation.

Results: Of that 8 probands with Fabry disease, 27 affected family members were identified through family genetic testing using genetic assessment interventions (average of 3,37 affected family members per proband).

This mean is 1,43 less than the average of 4,8 according to the review of papers in the consulted bibliography.

Conclusion: We propose to delve into a strategy study of the barriers of family genetic testing in our Fabry community through a validated survey.

References:

Grants:

Conflict of Interest: None declared.

EP23.013 Polyvalence of genetic counselling services in current clinical practise

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Background/Objectives: Genetic counseling, including pre- and post-analytical one, is an ever-expanding segment of daily clinical practice. With the increase in knowledge gained regarding genetic diseases and respective one of testing possibilities, the demand for such a specialized service naturally rises. However, the number of specialists to provide it is not of reciprocal growth.

Methods: We have performed a retrospective analysis of genetic specialized services, provided for two periods—2012–2016 and 2017–2021, in the Laboratory of Medical genetics, UMHAT St. Marina in Varna, Bulgaria. We stratified these services into several sections with the main ones being reproductive, pediatric, neuro- and oncogenetics. Noteworthy, during the first period there were 2 genetic specialists versus 4 specialists for half of the second period and 2 - later on.

Results: There was an overall increase of 29% in consultations provided for the second period compared to the first one. Steadily, laboratory testing performed onsite increased with 17% (fully or partially conducted by medical geneticists performing counseling). Distribution of indications showed an 89.6% increase in consultations regarding oncogenetics, 42.7%—for reproductive, 14.7%—for pediatric genetics, and 25.5%—for paternity testing.

Conclusion: Genetic counseling is extremely heterogenous in means of indications. While this suggests a need for progressive subspecialization, countries with still-developing economics cannot yet afford such staff distribution. Thus, future practice of genetic specialists will be defined by the prioritization by national healthcare and higher education systems. Currently, it is a matter of delicate balance between number of fellows and demand for counseling and analyses.

References:

Grants: None received.

Conflict of Interest: None declared.

EP23.014 Teaching medical genetics during the pandemic

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Background/Objectives: In the pandemic years I approached my students using asynchronous remote teaching. Testing their acquired knowledge in Medical Genetics proved difficult. To more accurately assess students' performance in the future, I asked them to state their behavior when answering test questions.

Methods: At the end of their training in Medical Genetics, at the end of the first term, first year students of the Carol Davila Medical and Pharmacy University in Bucharest were asked to state how they chose to solve tests. 99 students answered the questionnaire in the academic year 2020/2021 and only 39 in 2021/2022.

Results: Students' outlook about their approach to answering test questions

I answered the test questions	Number of students in the academic year 2020/2021 (single choice)	Number of students in the academic year 2021/2022 (multiple choice)
By myself	17 (17.2%)	18 (46.2%)
After reviewing the study material	58 (58.6%)	32 (82.1%)
After searching the answer online	4 (4%)	10 (25.6%)
After discussing with somebody (one person)	8 (8.1%)	5 (12.8%)
After discussing with my colleagues	12 (12.1%)	10 (25.6%)
At random	0	6 (15.4%)

Although fewer students accepted to answer the questionnaire in this academic year, it is still possible to better understand students' behavior.

Conclusion: In case of online learning the assessment of performance and mastery was biased by the existence of multiple options of behavior that students chose to use.

References:

Grants:

Conflict of Interest: None declared.

EP24 Ethical, Legal and Psychosocial Aspects in Genetics

EP24.001 Reproductive genetic carrier screening for deafness: views of those with a lived experience

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Background/Objectives: Many commercial carrier screening panels include genes associated with non-syndromic hearing loss, however there is little research exploring the ethical acceptability of their inclusion. Although some couples wish to avoid having a deaf child, with effective interventions and supports available there is currently no consensus on whether deafness is a health condition appropriate to include in screening.

This study explored views of people with an experience of deafness on carrier screening for deafness.

Methods: We interviewed 27 participants: 14 who identified as deaf and 13 parents with a child who is deaf. Thematic analysis was undertaken on interview transcripts.

Results: This study reveals the complexity of attitudes within these groups. There is tension between wanting to support prospective parents' reproductive autonomy, and concerns about potential harms, especially negative messages about living with deafness and an existential threat to Deaf culture. While some felt carrier screening could help prospective parents prepare for a deaf child, prenatal screening and termination of pregnancy had little support. Participants who supported carrier screening emphasised the need for accurate and current information on the lived experience of deafness. Many participants felt deafness was not as severe as other conditions included in carrier screening, and most did not consider deafness as a disability.

Conclusion: People with experience of deafness, personally or as parents, have diverse attitudes toward carrier screening for deafness that are informed by their own identity and experience, and many have concerns about how deafness would be discussed and included in carrier screening.

References:

Grans:.

Conflict of Interest: None declared.

EP24.002 Patrolling portals: the regulation of online genetic data sharing

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Background/Objectives: Online portals are being increasingly used to disseminate genetics data. While portal-facilitated sharing might have significant scientific value, it is also likely to raise difficult legal and ethical questions. Many of these questions were highlighted during the Covid–19 pandemic, as the urgency of efficient data sharing became apparent. Responding to these emerging concerns, regulators around the world have implemented regimes for 'software used as a medical device.' It is unclear how these regimes affect online portals for genetic data sharing. This study addresses how online data sharing may be shaped by new forms of regulatory oversight.

Methods: We performed an international comparative analysis of advisory documents applicable to software as medical devices prepared by medical regulators in Canada, the United States, France, and the United Kingdom. We selected 20 documents for review.

Results: We found that regulatory agencies are likely to regulate online tools as medical devices only when they are intended to perform a medical purpose. Online portals for genetic data sharing will not usually meet this threshold. Nevertheless, regulatory guidance provides significant insight into the kinds of issues to which regulators are likely to be attentive in addressing online data sharing. We identify eight normative and logistical issues: efficiency, equity, transparency, confidentiality, communication, empowerment, training, and safety.

Conclusion: This review clarifies how the regulation of medical software might apply to portals for genetic data sharing. We offer recommendations to portal developers and researchers.

References:

Grants: Government of Canada and the Ministère de l'économie et de l'innovation du Québec: Génome Québec Grant 13528.

Conflict of Interest: None declared.

EP24.003 An emotionalised debate: newspaper reporting on reimbursing drugs for rare diseases in Germany, Switzerland, and the UK

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Background/Objectives: Orphan drugs are intended to treat patients with rare conditions, most of them being genetic. An increasing number of high-priced orphan drugs are being approved every year. To ensure fair and equitable access to these drugs, it is urgent to find a fair and sustainable reimbursement system. Some scholars have argued that, in publicly-funded healthcare systems, the public should be involved in this process. This study's aim is to compare public debates in newspapers around orphan drug reimbursement from Germany, German-speaking Switzerland, and the United Kingdom.

Methods: Articles from six newspapers about orphan drug reimbursement and published between 2000-2020 were analysed quantitatively and qualitatively.

Results: A total of 239 newspaper articles were identified. Coverage increased importantly since 2014. An increasing number of articles took the perspective of individual patients by presenting patient stories and calls for crowdfunding. Other articles took a broader public health perspective; they tended to frequently cite companies (health insurance, pharmaceutical) and to cover issues about pricing and country-specific reimbursement policies.

Conclusion: The growing importance of personalised stories emotionalises the public debate on orphan drug reimbursement. It bears justice issues if rare disease patients have to rely on crowdfunding campaigns to get treatment. The prominent standing of the pharmaceutical industry and insurance companies indicate active lobbyism of win-oriented entities. This opposes the basic principle of solidarity that underlies current healthcare policy in publicly-funded healthcare systems.

References:

Grants: This work was supported by the Swiss Academy of Medical Sciences' Käthe Zingg Schwichtenberg grant (grand no KZS 33/19).

Conflict of Interest: None declared.

EP24.004 Healthy children's assessments of their experiences of blood collection with topical anesthesia

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Background/Objectives: Injections for blood collection elicit fear and pain in children. Although informed assent is an important component of medical research involving children, they are unlikely to agree to the procedure at first.

Methods: This study investigated factors that affect satisfaction with health check-ups that included blood collection in healthy seven-to-eight-year-old children who underwent blood collection with topical anesthesia. Two studies, one involving the use of illustrated surveys and the other structured interviews, were conducted to gather insights and understand the emotions of 492 and 20 children, respectively.

Results: We found that the following six points can be applied to encourage children to assess their experience of blood collection positively: (1) prior information using a pamphlet; (2) telling the children that the volume of blood drawn will be small; (3) carefully explaining the risk and benefit of topical anesthesia; (4) conducting the blood collection process swiftly; (5) praising and thanking the children's effort and cooperation; and (6) explaining the results of the research to the children if their blood is going to be used for research.

Conclusion: The findings indicate that with appropriate measures to reduce pain and fear, children's initial negative feelings toward blood collection can be replaced by positive feelings after the procedure.

References: https://www.env.go.jp/chemi/ceh/en/index.html.

Grants: This study was funded by the Ministry of Environment, and was supported by AMED under Grant Number JP21bm 0904002, Japan.

Conflict of Interest: None declared.

EP24.005 Working with "severity" in reproductive genetic carrier screening

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Background/Objectives: Severity is an important factor in genomic health when determining genes to include in reproductive genetic carrier screening (RGCS). The concept lacks clarity, and there is no consensus on how to classify severity of conditions in screening.

Methods: We analyse the concept of severity, drawing on bioethics and genomics literature. We identify how ethically robust RGCS programs can respond to severity and its fundamental complexity.

Results: Our analysis found that severity is contestable and often depends on context and perspective. Gene selection for RGCS must be undertaken using a transparent and robust process that incorporates a variety of perspectives, including those who live with the condition and their families. Focusing on conditions with a significant impact enables consideration of how severity can be used as a criterion in RGCS to facilitate robustly informed reproductive decisions. Screening for milder or highly variable conditions adds complexity to decision-making. Reproductive decisions based on carrier screening results should be made with access to psychosocial support and high quality information about the condition.

Conclusion: Severity is an important criterion for RGCS. Programs must be designed and implemented to account for the different ways severity can be construed by different stakeholders.

References: Boardman, F.K. (2017) 'Experience as knowledge: disability, distillation and (reprogenetic) decision-making', *Soc Sci Med*, 191, pp. 186–193.

Newson, A.J. and Dive, L. (2021) 'Taking seriousness seriously in genomic health', *Eur Journal Hum Genet*, pp. 1–2. https://doi.org/10.1038/s41431-021-01002-9.

Grants: Mackenzie's Mission is funded by the Australian Medical Research Future Fund, grant GHFM73390.

Conflict of Interest: Lisa Dive Research Fellow, University of Sydney (0.8 FTE), Alison Archibald Group Leader – Reproductive Genetic Counselling (part-time), Fragile X Association of Australia Scientific Advisory Committee, Lucinda Freeman Head of Discipline, Genetic Counselling at University of Technology, Sydney (part time), Ainsley Newson Professor, University of Sydney (full time), 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 honorarium 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.

EP24.006 Challenges for the ELSI in rare disease research: a qualitative study of Japanese stakeholders

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Background/Objectives: The IRDiRC, an international consortium of national funding agencies, drug development companies, and rare disease patient communities, has organized an ELSI working group to identify issues concerning rare diseases that countries should address (Hartman et al. 2020). Prior studies have indicated that rare diseases generate more complex ethical and social issues than common diseases and thus require more sensitive consideration of risk and privacy.

Methods: We conducted a semi-structured interview survey with stakeholders of rare disease research (patient organizations, researchers, and companies; $N = 9$) to explore the perceptions and concepts regarding ELSI in Japan, starting in November 2021. The transcripts were coded in MAXQDA and subjected to qualitative analysis.

Results: The qualitative analysis revealed several significant axes of analysis regarding the rare disease challenges for ELSI in Japan. One aspect is that the difficulties created by this rare disease reinforce the ethical challenges of patient engagement. The lack of treatment opportunities and social vulnerabilities are also associated with extreme altruism and a tendency to underestimate the risks of clinical trials. These results suggest that additional rare disease–specific considerations are necessary to address therapeutic misconception, which states that patients tend to expect clinical trials to have a therapeutic effect.

Conclusion: Through this research, we identified several aspects of ELSI that should be taken into consideration and that suggest the need to build an ethical ecosystem that supports patients and families.

References: Hartman et al. ELSI in rare diseases. *Eur J Hum Genet* 28:174-81, 2020.

Grants: This research was supported by AMED under Grant Number 21ek0109494h0002.

Conflict of Interest: None declared.

EP24.007 Intergenerational relations matter: exploring health-related roles in families with transthyretin-related familial amyloid polyneuropathy (TTR-FAP) and Huntington disease (HD)

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Background/Objectives: The importance of an intergenerational perspective to understand family adaptation to hereditary diseases is recognized. This study examines how older generations influence health management in younger ones, in Portuguese families with TTR-FAP or HD. Both are progressive, incurable, late-onset neurological diseases, though some therapeutical measures are already known to slow down TTR-FAP progression.

Methods: This exploratory, qualitative study used the critical incidents technique, in semi-structured interviews with 28 participants (18 TTR-FAP, 10 HD families). Data were analysed thematically.

Results: Findings showed recent disease awareness in HD families, whereas in TTR-FAP families the disease was well known to previous generations. In both cases, older family members played influential roles towards younger generations: through their testimony, providing information and as role models(1,2). In TTR-FAP families, older relatives were active influencers towards pre-symptomatic testing decisions(1). Providing support (emotional/instrumental) stands out in HD families(2).

Conclusion: Older family members play relevant roles to help younger relatives dealing with disease-imposed psychosocial challenges. Our results are relevant for provision of psychosocial support to these families, particularly by highlighting key intergenerational exchanges that may improve individuals and families' psychosocial outcomes.

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Grants: CINTESIS (UIDB/4255/2020; UIDP/4255/2020); FCT (SFRH/BD/131925/2017); FCT (CEECIND/02615/2017).

Conflict of Interest: None declared.

EP24.008 Inbreeding and genetic disorder in children

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Background/Objectives: Consanguineous marriages results in decreased biological fitness of a population, in homozygosity, which can increase the chances of genetic diseases. Objective: analysis of morbidity incidence in children born in consanguineous marriages.

Methods: We present data on the 12 children from consanguineous marriages. The analysis of life history, complaints, clinical signs, laboratory, instrumental studies and whole exome sequencing were carried out.

Results: Two children with hepatosplenomegaly and transferasemia had a mutation characteristic of Wilson-Konovalov disease, two children from the same family with severe skeletal deformity had Brooke syndrome; a patient with malabsorption and defects of mitochondrial beta-oxidation posthumously had Alpers syndrome. A patient with nephrotic syndrome was diagnosed with Schimke disease, with hepatosplenomegaly - glucose-galactose malabsorption, hyperlipoproteinemia, type I, with virilization and hypertension – congenital dysfunction of the adrenal cortex, non-classical form. One child with hepatosplenomegaly was diagnosed with acute myeloblastic leukemia, one child with malabsorption died from multiple organ failure. Total 83% patient had autosomal recessive disease and 40% of the mutant alleles occurred de novo.

Conclusion: In this study, out of 12 children born in inbred marriages, 11 revealed genetic diseases that require expensive diagnostics and rehabilitation measures, leading to disability and in some cases to early death of patients.

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Grants: None.

Conflict of Interest: Gadzhikerim Gadzhikerimov Student, Olga Gumeniuk: None declared.

EP24.009 Study on shared decision making in oncogenetics

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Background/Objectives: Shared Decision Making (SDM) is a model used in the clinical context, whereby the health professionals and the patients actively exchange information and explore treatment and/or prevention options together with the aim of making a decision jointly. To achieve this objective, the health professionals must create a climate of trust that allows the patient to express him/herself freely. The SDM approach allows the patient to learn more about their disease, encourages them to collaborate and helps them to formulate their preferences, including whether or not to undergo genetic testing. We decided to evaluate the SDM in oncogenetics by interviewing patients following their appointment with a genetic counsellor. We aim to understand which strategies are available to optimise and improve decision making in oncogenetic.

Methods: We realised an anonymised questionnaire with 17 questions on decision making, which we proposed to our patients over a 2-month period.

Results: 50 patients agreed to participate in this study which took place in Switzerland. It appears that, in general, decision making is an act that must be reflected upon, but which is not easy from the patient's point of view. We described the patient's

satisfaction with the help received by the health professionals, the motivations that influence the decision on the process of genetic tests.

Conclusion: Although the decision-making approach is ethically appropriate, patients are rarely involved in "decision-making" process as they would like. Its implementation depends primarily on the attitude and skills of the health professional, and on family and cultural factors.

References:

Grants:

Conflict of Interest: None declared.

EP24.010 Equity, justice and genomics

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Background/Objectives: The development and practical implementation of genomic technologies has the potential to exacerbate pre-existing health inequities and social injustices. We consider the opportunities that we ESHG members have, as professionals and practitioners in medical genetics, to ensure that genomics works to redress inequities in health and to counter injustice.

Methods: These thoughts have been developed over two years by e-mail discussion among the authors and interested colleagues.

Results: The potential for the implementation of genomics to exacerbate pre-existing problems will take multiple forms:.

Difficulties in accessing healthcare may apply more strongly to genomics;.

Lack of representativeness in population genetics studies;.

Potential discrimination against individuals through misuse of stored personal data;.

'Hype': exaggerated claims for genomic applications;.

Abuse of the genetic dissection of intelligence, personality and psychiatric disease risk in diagnostic testing and direct-to-consumer applications;.

The process of 'genomicisation', in which genomics is presented as the solution to many health and social problems, focuses inappropriately on individuals. This distracts attention from collective solutions to communal problems;.

Introduction of population screening programmes that may exacerbate inequities and lead to discrimination against individuals and groups.

Understanding transgenerational and early life effects of malnutrition and poverty could allow public health action to break vicious cycles of deprivation.

Conclusion: The opportunities for the genetics community to promote greater justice in healthcare will vary among professional groups and between countries. It is important for us to be self-critical and encourage reflection about how - and for whose benefit - our technologies are applied.

References:

Grants:

Conflict of Interest: Angus Clarke Adviser to Pfizer on introduction of gene therapies (modest), Carla van El: None declared.

EP24.011 Vulnerabilities and sensitivities concerning human germline gene editing in patients and carriers of heritable diseases

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Background/Objectives: Human germline gene editing (HGGE) is a topic of public debates worldwide. Within these debates, however, the voice of potential future users of HGGE, e.g. patients and carriers of heritable diseases, is barely audible. Although their perspectives as direct stakeholders are very important, it might be difficult to find the right tone to engage them in deliberation. This study therefore aims to ascertain and understand the vulnerabilities and sensitivities concerning HGGE in patients and carriers of heritable diseases, and to explore their perspectives regarding potential applications.

Methods: We included five patients and carriers of heritable diseases via patient organisations, in semi-structured interviews about their thoughts and feelings concerning HGGE. Interviews are ongoing. Whenever relevant, we included mail conversations ($N = 8$) to explore sensitivities in first reactions.

Results: Mail conversations showed that HGGE is a sensitive topic, especially for those who have reproductive problems associated with their heritable condition. Patients revealed that they experienced tension between their feelings of guilt over passing on the condition, and their value of taking responsibility for voicing the patient perspective. If applied to eliminate severe genetic conditions, participants indicated they would prefer HGGE over pre-implantation genetic testing and prenatal diagnosis, because they see HGGE as a less disruptive, more acceptable, and more effective opportunity.

Conclusion: Patients and carriers of heritable diseases endorse the approach of an open invitation that is spread by patient organisations, to engage in the deliberation on HGGE that emphasizes that the choice to participate is fully voluntary.

References:

Grants:

Conflict of Interest: Jeanne Arnold: None declared, Diewertje Houtman Part-time employment at Erasmus MC, Sam Riedijk Fulltime employment at Erasmus MC, PI in prenatal genetics and social genomics.

EP24.012 Genetic data sharing for research in Europe: consent not only a legal basis under GDPR

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Background/Objectives: Today, genetic data sharing is presented as necessary to improve health research. Nevertheless, the specificity of this data - due to its high sensitivity - raises questions when considering how to protect data subjects while encouraging sharing.

Methods: There are several legal bases for processing sensitive data - including genetic data – in the GDPR such as explicit consent, public interest in the area of public health and scientific research, which are used differently by Member States. In order to preserve the autonomy of individuals, some national laws use consent for the (re)use of genetic data. However, in practice, the specific requirements for consent in the GDPR are perceived as impairing research activities so that the scientific research exemption is often preferred.

Results: We show that even if consent is not chosen as a legal basis, it should continue to be envisaged as an ethical requirement for expressing clear choices on data reuses, when possible. A more flexible practice of consent would allow both to improve data subjects' involvement in the reuse of their data and to facilitate accountable practice from the scientific community. In this perspective, we show that the GDPR offers rooms for innovating in consent practices, in line with recognised ethical standards.

Conclusion: In a context where trust of individuals in the re-use of their data remains a continuous challenge, reconsidering consent rather than waiving it could reinforce the data subject autonomy and active contribution to research.

References: GDPR.

Grants: CINECA, GA No 825775 - Easi-Genomics, GA No 824110 – GeneInfoKid INCa n°2018-127.

Conflict of Interest: None declared.

EP24.013 Hereditary transthyretin amyloidosis: a person-centred occupational therapy project

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Background/Objectives: The aim of this project is to develop a specific Occupational Therapy project for Hereditary transthyretin amyloidosis Val50Met (ATTRv) patients.

To develop an Occupational Therapy project in patients diagnosed with hereditary Val50Met transthyretin amyloidosis.

Methods: An experimental study is carried out to implement a specific Occupational Therapy project in the patients included in the study. For this purpose, a semi-structured interview was applied in which items such as clinical, socio-demographic, psychological and occupational aspects were evaluated. Of the 42 patients interviewed, 16 were included in the study.

Results: Our study revealed that, the disease had a significant impact on most of the activities of daily living. When asked if and how the illness had affected their lives, 18 out of 42 participants (42.8%) responded that the illness had affected their basic activities. In 22 out of 42 participants surveyed (52.4%), the disease had affected their instrumental activities. While 42 of the 42 participants (100%) referred that the disease had affected in advanced activities.

Conclusion: These results highlight the psychological and occupational impairment of patients. The impact of this disease is mainly on quality of life and mental health, so a person-centred occupational therapy project could be beneficial for patients.

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Grants: This project has been funded through the Pfizer Independent Research Grants Program ID#64965375.

Conflict of Interest: Aina Isabel Gayá Barroso This project has been funded through the Pfizer Independent Research Grants Program ID#64965375, Juan Gonzalez Moreno This project has been funded through the Pfizer Independent Research Grants Program ID#64965375, Pfizer, Sobi, Alnylam, Advisory of Pfizer, Sobi, Alnylam, Adrián Rodríguez This project has been funded through the Pfizer Independent Research Grants Program ID#64965375, Eugenia Cisneros-Barroso This project has been funded through the Pfizer Independent Research Grants Program ID#64965375, Inés Losada López This project has been funded through the Pfizer Independent Research Grants Program ID#64965375, Pfizer, Sobi, Alnylam, Advisory of Pfizer, Sobi, Alnylam.

EP24.014 Reshaping the language of clinical genetics in modern practice

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Background/Objectives: The language of clinical genetics is, at times, misleading, inaccurate and pejorative. Many of its clinically descriptive terms have also been co-opted into the play-ground lexicon, often for use as insult. Examples abound, and we seek to highlight a few exemplars; with suggestions for alternative approaches, that are more accurate and less likely to be hurtful.

Methods: The authors conducted a review of the literature, seeking to identify commonly used terms in clinical practice and correspondence, that do not fit with current day values and are either misleading, inaccurate or pejorative.

Results: Clinical descriptors, such as 'dwarf' and 'cherubism', may have once been considered illustrative, but are now problematic. Accurate and appropriate use of terms such as 'mutation', 'variant' and 'carrier' warrant discussion. For instance, mutation, simply meaning a non-wildtype sequence variant in the DNA, is often interpreted to imply pathogenicity, especially in mainstream use in clinical genetics.

Conclusion: This review examines the clinical definition and cultural understanding of a range of terms, still evident in practice and publications, where there is inexactitude or where descriptors fall short of modern day sensitivities; with considered recommendations for alternative phraseology.

References: nil.

Grants: nil.

Conflict of Interest: Russell Gear Royal Melbourne Hospital, Recipient of the Royal Australasian College of Physician's Dr Helen Rarity McCreanor Travelling Fellowship for 2022 ($10,000 to attend an international conference or similar), Neil Rajan: None declared, Ingrid Winship: None declared.

EP24.015 Oncogenetic for children: ethical and legal analysis of consent forms in the French context

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Background/Objectives: Paediatric genetic cancers represent a huge challenge where improving diagnosis and treatment is

needed. Development of new genomic technologies raises hope for children and their families but also challenges that appropriate consent is delivered. The medical relationship in this regard implies oncologist, geneticist, genetic counsellor and psychologists. Parents, who are the legal guardians and the child, whose rights must be respected under international law (Convention on the Rights of the Child, 1989) and national law (Public Health Code), must contribute to the decision to perform a genetic test. Even these rights are reaffirming child's autonomy, practices on what should be required and how, are varying according to medical centres.

Methods: We conducted an ethical and legal study, aiming at mapping the consent practices in French paediatric oncogenetic units. We confronted the legal obligations required by the French law for the "consent" of minors and that of the guardians, with the consents forms we collected from the GENINFOKID project partners and consents available on internet. We coded the documents and built an analysis grid.

Results: Our analysis focused on the scope and design, the semantic and the topics of the consents' forms (including reuse of previously collected biological material in care for research). We discuss these issues from a legal and ethical perspective, highlighting the heterogeneity in the local practices regarding the respect of the French legal framework.

Conclusion: We concluded on the prejudice these practices have on the child's autonomy and on the need to implement means for better harmonisation.

References:

Grants: (Inca *N° 2018-127*).

Conflict of Interest: None declared.

EP24.016 The assessment of ethical problems arising at the reception of doctors of various specialties

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Background/Objectives: The ethical aspects of genetic care in Russia are poorly investigated. Our study aims to attempt to estimate ethical problems arising during the reception of a geneticist and other specialists.

Methods: We have developed a questionnaire containing 28 questions including questions about age, gender, marital status, religion, and other parameters characterizing the consultant himself and hypothetical situations in which the doctor is invited to make a choice and explain it. Most of the questions required responses on a 5-point Likert scale, ranging from "strongly disagree" to "strongly agree".

Results: Preventing the birth of children with genetic diseases is very or extremely important for all respondents. 28 respondents reported that the most important goal of medical genetics is to reduce harmful genes in the population and also agreed to introduce mandatory screening for heterozygous carriage of mutations that lead to hereditary diseases such as cystic fibrosis (which is in line with ACMG recommendations), but 27 respondents also agreed to conduct a study on the carriage of mutations, including those causing diseases with a late-onset and lack of effective treatment and prevention measures, for children under 13 years old. 15 respondents believe that the patient should in any case learn about the results of their genetic testing (denial of the right to "not know").

Conclusion: The majority of physicians in the Russian Federation adhere to a directive approach which can have a great impact on the patient's future reproductive behavior.

References:

Grants: The research was supported by RSF (project No. 19-18-00422).

Conflict of Interest: Elena Baranova This work has been supported by the grants the Russian Science Foundation, RSF 19-18-00422. I am a collaborator., Vera Izhevskaya This work has been supported by the grants the Russian Science Foundation, RSF 19-18-00422. Vera Izhevskaya is a collaborator.

EP24.017 Searching for closure in prenatal diagnosis: Parental experiences of recontacting for extended genetic testing after a terminated pregnancy for fetal malformations

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Background/Objectives: Genome-wide analysis approaches have recently been introduced in clinical genetics because of a potential increased diagnostic yield. In case of couples who had a termination of pregnancy (TOP), these techniques might meet the parent's need to know. The aim of this qualitative study is to explore experiences of being recontacted for additional diagnostic testing. In addition, why are these couples still opting for genetic testing several years after the termination?.

Methods: This study included parents of a retrospective cohort of 85 foetuses that underwent a TOP for congenital malformations between January 2015 and December 2018 in UZ Brussel. After selection, 31 participants were eligible for recontacting, given that a clinical diagnosis is suspected though a molecular diagnosis remains unclear. After receiving a standardized letter, 14 couples agreed to come to the Genetics department to participate. All interviews were transcribed verbatim, anonymized and coded by thematic analysis of Braun & Clarke (2006).

Results: Despite the years passed since the TOP, these participants were still motivated to perform new genetic testing. Both intrinsic (searching for answers for themselves and their children) and external motivators (contributing to science and helping parents) played an important role as a driver. All participants were pleased that the medical team took initiative with this sensitive approach, since they would not have taken the initiative themselves.

Conclusion: These results showed an interest in being recontacted which has important implications for clinical practice.

References: Braun, V. & Clarke, V. (2006). Using thematic analysis in psychology. *Qualitative Research in Psychology*, 3 (2), 77–101.

Grants: Innoviris.

Conflict of Interest: None declared.

EP24.018 Ethical values in relation with Open Science and FAIR data principles applied to human genetics research

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Background/Objectives: The growing movement of Open Science is entering research practices widely and it is reflected in institutional requirements, for example by the European Commission. This challenges the way research outputs are identified, recognised, classified and valued. The ethical dimension of sharing data and more generally of Open Science has been previously analysed. A major facet of Open Science is represented by the

FAIR principles as regards research data, i.e. "Findable, Accessible, Interoperable, Reusable" data. Much work is being done on the requirements to make data FAIR. The legal restrictions to openness of research data when they are personal or sensitive data such as health and genetic data have been put forward. However, the ethical and societal values that underpin the FAIR principles have less been explored.

Methods: We have conducted such an analysis. We propose below a non-exhaustive list of values *per* principle, based on multiple grounds such as ethics, law, societal, economics, and environment.

Results: Findable: Inclusivity, Transparency, Sustainability, Efficiency, Accuracy, Research Integrity.

Accessible: Accountability, Trust and Reciprocity, Pursuit of the Common Good, Human Well-being.

Interoperable: Inclusivity, Reproducibility, Novelty, Quality.

Reusable: Reciprocity and Recogtition, Benefit sharing, Non-maleficence, Scientific Freedom and Legitimacy of research, Human Dignity and Autonomy.

An articulation of the different levels addressed by such values will be presented.

Conclusion: In the field of healthcare and human genetics, data FAIRification may present multiple advantages not only for research and health care development but also for society as a whole.

References: Wilkinson et al. (2016) S*ci Data*, 2016;3:160018.

https://fairplus.github.io/the-fair-cookbook/content/recipes/introduction/FAIRplus-values.html.

Grants: https://fairplus-project.eu/.

Conflict of Interest: Anne Cambon-Thomsen IMI project FAIRplus, European Commission, Alejandra Delfin EU IMI project FAIRplus (employed on the grant during completion of the work reported, Emmanuelle Rial-Sebbag Collaborator on the EU IMI project FAIRplus.

EP24.019 Genes as a defense to homicide in Italy and the United States: comparative legal and ethical issues

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Background/Objectives: It has long been recognized that relationships exist among certain genotypes and human behavior. For example, several studies have concluded that there exists a link between genetic variation as to monoamine oxidase A (MAO-A), involved in neurotransmitter metabolism, and aggressive responding. Alterations in MAO-A genes result in Brunner syndrome which manifests as global cognitive deficits, impulsivity, and mood lability and has been associated with antisocial personality disorder. Recently, the criminal courts worldwide have seen a rise in the use of neuroscientific evidence, particularly with regard to defense of homicide. The MAO-A gene features prominently. This research is an exploration of the legal and ethical issues associated with the use of behavioral genetic evidence at criminal trial.

Methods: Two prominent legal databases, Westlaw and LexisNexis, were searched using search terms MAO-A, MAO, MAOA and monoamine oxidase from 1995 to August 16, 2021.

Results: Thirteen cases were retrieved and categorized by case outcome. Results how that Italian and U.S. courts diverge with respect to the use of genetic evidence in homicide defenses, with the former much more amenable to considering genetic make-up, at least in sentencing.

Conclusion: While some of the differences may relate to the different legal traditions in place, the divergence largely relates to evidentiary procedures. Specifically, Italian courts have exhibited a willingness to incorporate behavioral genetics in sentence reduction on appeal. Conversely, U.S. opponents of the gene-as-a-defense-to-homicide approach cite flawed scientific methodology and the ethics of linking complex behavior to singular genetic defects.

References:

Grants:

Conflict of Interest: None declared.

EP25 GWAS

EP25.001 Investigating the effect of sexual behaviour on oropharyngeal cancer risk: a methodological assessment of Mendelian randomization

Mark Gormley[1;2], *Tom Dudding*[2], *Linda Kachuri*[3], *Kimberley Burrows*[1], *Amanda Chong*[1], *Richard Martin*[1], *Steven Thomas*[2], *Jessica Tyrrell*[4], *Andrew Ness*[5], *Paul Brennan*[6], *Marcus Munafo*[7], *Miranda Pring*[2], *Stefania Boccia*[8], *Andrew Olshan*[9], *Brenda Diergaarde*[10], *Rayjean Hung*[11], *Geoffrey Liu*[12], *Eloiza Tajara*[13], *Patricia Severino*[14], *Tatiana Toporcov*[15], *Martin Lacko*[16], *Tim Waterboer*[17], *Nicole Brenner*[17], *George Davey Smith*[1], *Emma Vincent*[1], *Rebecca Richmond*[1]

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Background/Objectives: Human papilloma virus infection is known to influence oropharyngeal cancer (OPC) risk, likely via sexual transmission. However, sexual behaviour has been correlated with other risk factors including smoking and alcohol, meaning independent effects are difficult to establish. We aimed to evaluate the causal effect of sexual behaviour on OPC risk using Mendelian randomization (MR).

Methods: Genetic variants robustly associated with age at first sex (AFS) and number of sexual partners (NSP) were used to perform both univariable and multivariable MR analyses with summary data on (2,641 OPC cases and 6,585 controls), obtained from the largest available genome-wide association studies (GWAS). Given the potential for genetic pleiotropy, we performed

MR on positive and negative control outcomes, Causal Analysis Using Summary Effect estimates (CAUSE) and multivariable MR to account for the effects of smoking, alcohol, risk tolerance and educational attainment.

Results: In univariable MR, we found evidence supportive of an effect of both later AFS (IVW OR = 0.4, 95%CI (0.3, 0.7), per standard deviation (SD), $p = <0.001$) and increasing NSP (IVW OR = 2.2, 95%CI (1.3, 3.8) per SD, $p = <0.001$) on OPC risk. However, negative control analysis suggested potential violation of the core MR assumptions and subsequent CAUSE analysis implicated genetic pleiotropy. Finally, there was attenuation of the univariable MR results in the multivariable models.

Conclusion: Despite using genetic variants strongly related sexual behaviour traits, we found evidence for correlated pleiotropy. This emphasises a need for multivariable approaches and the triangulation of evidence when performing MR of complex behavioural traits.

References:

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EP25.003 LDAK-GBAT - a powerful and efficient tool for gene-based analysis of GWAS data

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Background/Objectives: Genome-wide association studies (GWAS) start by testing each SNP individually for association with the outcome. However, it is now recognized that gene-based analyses - which jointly test sets of SNPs within a gene for association with the outcome - can complement single-SNP analysis and provide additional insights for the genetic architecture of complex traits.

Here we propose LDAK-GBAT, a new tool for gene-based association analysis that requires only summary statistics and a reference panel.

Methods: We compared the performance of LDAK-GBAT and alternative methods such as MAGMA, GCTA-fastBAT, VEGAS2 and sumFREGAT (which implemented SKAT-O, PCA and ACAT methods) using 14 traits from UK Biobank, 9 traits from Psychiatric Genomics Consortium and 18 traits from Million Veteran Program.

Results: LDAK-GBAT is computationally efficient, on average taking less than 1 min to analyse genotyped data (>606k SNPs) and ~4 min to analyse imputed data (>7.1 million SNPs) using a reference panel of 404 individuals. It also produces p-values that are well-calibrated under the null and is robust to the choice of reference panel.

We find that LDAK-GBAT is consistently more powerful than alternative methods. In particular, LDAK-GBAT finds on average 94% more significant genes (range 0% to 200%) than MAGMA, the second-best method. Finally, we show that compared to single-SNP analysis, LDAK-GBAT is equivalent to a 40% increase in effective sample size.

Conclusion: Our proposed tool, implemented in our freely available software LDAK (https://www.ldak.org/), has the potential

to identify additional novel disease-susceptibility genes for complex diseases from GWAS datasets.

References:

Grants:

Conflict of Interest: None declared.

EP25.004 Fully automatic landmarking of 2D photographs identifies novel genetic loci influencing facial features

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Background/Objectives: We have previously reported genetic loci impacting facial features in Latin Americans based on categorical phenotyping and measurements derived from manual landmarking of the profile photographs [1,2]. Here we conducted a GWAS of measures derived from automatic landmarking of the frontal 2D photographs.

Methods: 106 landmarks were placed automatically on 2D frontal photographs using the cloud service platform Face++. After Procrustes superposition, we calculated inter-landmark distances for 34 landmarks and GWAS was performed for 301 distances.

Results: We detected significant association at 42 genome regions, and 9 regions have been previously reported. For 26 of the 33 novel genome regions, we detected replication in East Asians or Europeans. The replicated regions include 1q32.3, 3q21.1, 8p11.21, 10p11.1 and 22q12.1 all of which comprise strong candidate genes with evidence for a role in craniofacial development.

Conclusion: These results establish that automated landmarking of 2D photographs is a simple and informative approach for the genetic analysis of facial variation, suitable for the rapid analysis of large population samples.

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EP25.005 National genome initiatives in Europe and United Kingdom in the era of whole-genome sequencing

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Background/Objectives: Overview of result from and local genome sequencing project projects from United Kingdom and Europe and also future plans and perspectives to newly announced ones.

Methods: Google, PubMed, EGA (The European Genome-phenome Archive), HGV (Human Genome Variation) and EMBL-EBI data archives were searched in December 2021 for gathering of information about national genomic initiatives by using the search parameters (<country name> [Title]) and (human genome project) OR (national genome initiative).

Results: Up to date, results of 9 national genome projects are published (United Kingdom, Iceland, Sweden Finland, Denmark, Lithuania, Netherlands, Italy, Russia). 12 countries in Europe have their projects ongoing in various stages and with various aims raging from development of scientific infrastructure to de novo genome assembly of local population. Further development for local genome projects in Europe should also bring ´1+ Million Genomes (+1MG)' European initiative. The goal of 22 signatory EU countries is to obtain sequenced genomes from more than 1 million individuals by 2022 in to cover analysis of genomic and health data both inside and across national boundaries in Europe.

Conclusion: Current version if human genome assembly GRCh38/hg38 and its predecessors covered genetic variability of local European population only briefly and thus there is strong need for further genomic data from local or native populations. This data provided by various countries world-wide should lead to rapid improvement in the area of precision and/or personalized medicine and thus bring another important tool to the clinical diagnostics of the diseases.

References:

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EP25.006 Genetic components of chronic back pain may underpin distinct subtypes of the disease

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Background/Objectives: Chronic back pain (CBP) is a common debilitating condition with a considerable socioeconomic impact and complex genetic architecture. There is now raising evidence for shared genetic background of various pain conditions. Simultaneously, an existence of unshared genetic factors attributing to specific chronic pain types can be assumed. Here we aimed to study the genetic factors of CBP shared and unshared by various chronic pain phenotypes.

Methods: We used UK Biobank GWAS data on chronic pain at various locations (back, neck, hip, knee, stomach, head) and split into a discovery ($N = 265{,}000$) and a replication sample ($N = 191{,}000$). We applied the SHAHER framework and obtained GWAS results for shared genetic impact trait (SGIT) and unshared genetic impact trait (UGIT) of CBP. Utilizing COJO, we identified

the loci associated with SGIT or UGIT of CBP and performed the replication. For functional annotation of the results, we used LDSC regression, DEPICT, VEP, FUMA, SMR-HEIDI and PRS analysis.

Results: We revealed nine loci associated with SGIT, four of them considered novel with two replicated. We also found one locus associated with UGIT of CBP reported previously. The functional analyses shown relatedness of SGIT to the nervous system structure, functioning and impairment, while UGIT of CBP seemed to be attributed to the musculoskeletal and immune systems.

Conclusion: SGIT is likely to underpin the neurogenic and psychogenic subtypes of CBP and chronic pain traits in general, while UGIT primarily reflects the nocigenic CBP.

References:

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EP25.007 Possibly protective genomic variants against ionizing radiation in DNA homologous recombination repair genes

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Background/Objectives: Catastrophe at the Chernobyl power plant claimed many lives and left long-term effects for humanity and nature. However, some Lithuanian Chernobyl catastrophe clean-up workers (LCCW) who experienced high doses of ionizing radiation (IR) have been able to survive and have been still relatively healthy. This led to a hypothesis that these people might have specific reaction to IR. The aim of this study was to find possibly protective genomic variants in genes responsible for biological processes such as DNA homologous recombination repair, oxidative stress, inflammation, apoptosis, and tumour suppression.

Methods: This study was performed by using genome-wide SNP genotyping Illumina beadchip technology. The study group included 127 male LCCW. The control group consisted of 211 unrelated, self-reported healthy males of Lithuanian descent. Quality control of the genotyping data was examined using GenomeStudio 2.0, PLINK v1.9 and KING software. Association analysis for the selected 21 gene-set was performed using PLINK v1.9 software.

Results: The study identified statistically significant ($p \leq 0.05$) genomic variants in DNA homologous recombination repair genes BRCA2 (rs4987117, $p = 0.001$, OR = 4.351), RAD51B (rs3784121, $p = 0.003$, OR = 1.675; rs17755657, $p = 0.009$, OR = 1.558; rs746663, $p = 0.041$, OR = 1.386; rs7359088, $p = 0.047$, OR = 1.394), ATM (rs11212570, $p = 0.008$, OR = 0.4878).

Conclusion: The results suggest that DNA homologous recombination repair genes of LCCW might have possibly protective genomic variants to IR. Further studies are needed.

References:

Grants:

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Conflict of Interest: None declared.

EP25.008 Genome-wide association studies of serum VEGFR1 protein at different times after sepsis diagnosis

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Background/Objectives: The acute respiratory distress syndrome (ARDS) is a pulmonary dysfunction with a high mortality burden that mainly develops because of sepsis. The *FLT1* gene, encoding a main VEGF receptor (VEGFR1) involved in angiogenesis and immunity, has been identified in sepsis-associated ARDS susceptibility. Here, we conducted genome-wide association studies (GWAS) on VEGFR1 serum measures to identify additional loci of interest for ARDS risk.

Methods: We collected serum samples from 284 Intensive Care Unit patients within the first 24 (T1) and 72 (T2) hours after sepsis diagnosis. Protein levels were measured by VEGFR1/Flt-1 DuoSet ELISA kit. Variants were genotyped using the Axiom Genome-Wide CEU1 array and 7.7 million variants were obtained after imputation. Association testing was conducted at variant (EPACTS, linear model) and gene-level (MAGMA, 17700 genes).

Results: The most significant signals were an intergenic variant for *PARL* and *ABCC5* genes at T1 (OR[95%CI]=0.85[0.81-0.91], $p = 3.3e\text{-}7$), and an intronic variant for *TCF20* at T2 (OR[95%CI] =0.80[0.74-0.87], $p = 1.5e\text{-}7$). The gene-level analysis prioritized the *ABCC5* ($p = 3.5e\text{-}5$, T1) and *TCF20* ($p = 1.8e\text{-}5$, T2) genes. Interestingly, variants linked to these genes have been associated with blood cell count.

Conclusion: We prioritized two loci associated with VEGFR1 serum levels that could be of interest for ARDS susceptibility.

References:

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EP25.009 Common genetic variability associated with years of education and cognitive performance predicts language outcomes at two

Harriet Cullen[1;2], *Konstantina Dimitrakopoulou*[3], *Hamel Patel*[4], *Charles Curtis*[4], *Andrew Chew*[1], *Shona Falconer*[1], *Chiara Nosarti*[1], *Stephen Smith*[5], *Daniel Rueckert*[6], *Joseph Hajnal*[1], *A David Edwards*[1]

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Background/Objectives: Educational attainment is an important, heritable behavioural measure associated with numerous health and socioeconomic outcomes. Here, we explore whether common genetic variability, associated with Years in Education or Cognitive Performance, is predictive of neurodevelopmental outcome at age two.

Methods: Data were obtained as part of the Developing Human Connectome Project. Our European subsample comprised 167 unrelated term-born infants. Infant saliva DNA was genotyped for SNPs genome-wide. Standard quality control was performed and the dataset was imputed to the Haplotype Reference Consortium panel.

The genotype data were used to compute genome-wide polygenic scores (GPS) for years of education (EduYears) and cognitive performance (CP) for each of the cohort using the results of recently published Genome Wide Association Studies.

Neurodevelopmental assessment was undertaken at approximately 2 years of age using the Bayley Scales of Infant and Toddler Development—Third Edition. We used a linear regression model to explore association between standardised scores for receptive and expressive language and cognition and EduYears or CP GPS. Gender, gestational age at birth, Index of Multiple Deprivation rank (a proxy for socioeconomic status) and ancestry principal components were included as covariates.

Results: We found a statistically significant association between EduYears GPS and expressive language scores ($\beta = 0.233$, se $= 0.075$, $p = 2.17 \times 10^{-3}$) and CP GPS and receptive language ($\beta = 0.299$, se $= 0.073$, $p = 6.69 \times 10^{-5}$). Greater GPSs were associated with higher scores in Bayley's language scales.

Conclusion: Whilst we cannot exclude confounding from parental environmental factors, these findings suggest that variability in language outcomes at two, may in part be explained by common genetic variability.

References:

Grants:

Conflict of Interest: None declared.

EP25.010 Association of HLA genes with acute respiratory distress syndrome susceptibility in patients with sepsis

Tamara Hernandez-Beeftink[1;2], *Megan Paynton*[3], *Eva Suarez-Pajes*[4], *Beatriz Guillen-Guio*[5], *JOSÉ MIGUEL LORENZO-SALAZAR*[6], *Almudena Corrales*[5;7], *Miryam Prieto González*[8], *Aurelio Rodríguez-Pérez*[9;10], *Demetrio Carriedo*[11], *Jesús Blanco*[7;12], *Alfonso Ambrós*[13], *Elena González Higueras*[14], *Elena Espinosa*[15], *Arturo Muriel-Bombin*[12], *David Domínguez*[15], *Abelardo García de Lorenzo*[16], *Jose Manuel Añon*[16], *Marina Soro*[17], *Louise Wain*[3;18], *Jesús Villar*[2;7], *Carlos Flores*[5;6;7]

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Background/Objectives: Acute respiratory distress syndrome (ARDS), frequently caused by sepsis, is a pulmonary dysfunction with a disproportionate death toll in the Intensive Care Units. Despite its central role in inflammatory and immunological diseases, the human leukocyte antigen (HLA) locus has not been tested for ARDS association yet. Here we assessed the association of common variants from this locus with sepsis-induced ARDS.

Methods: The genotypes from 831 sepsis patients (318 with ARDS) were phased, and classical alleles from eight HLA genes, amino acids, and SNPs were derived based on Impute2 and SNP2HLA references. Logistic modelling adjusting for gender, age, and the two main principal components of genetic variation were used for association testing with EPACTs. Significance thresholds were set at $P < 2.52e\text{-}4$ for HLA alleles, $P < 4.86e\text{-}5$ for amino acids, and $P < 1.5e\text{-}5$ for SNPs.

Results: A total of 198 classical alleles, 1,028 amino acids, and 10,931 SNPs from eight HLA genes were confidently analyzed. None of the classic alleles (lowest P value $= 7.66e\text{-}3$), amino acids (lowest P-value $= 1.26e\text{-}3$), or SNPs (lowest P value $= 5.51e\text{-}4$) were associated with sepsis-induced ARDS in this cohort.

Conclusion: No HLA variants of large effect were detected. Larger sample sizes may be needed to determine the contribution of variants with modest effect sizes.

References:

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Conflict of Interest: None declared.

EP25.011 Exploring the multi-ethnic genome landscape of hypertensive disorders during pregnancy in a North Carolina-based cohort

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Background/Objectives: Hypertensive disorders of pregnancy (HDP) are defined as gestational hypertension with preeclampsia/eclampsia. There are racial/ethnic disparities in the prevalence and severity of HDP, and non-Hispanic Black women have higher odds of experiencing more severe disease in the United States. This study assesses genetic contributions to HDP.

Methods: We used whole-genome sequencing data to assess genetic variation related to self-reported HDP in the Personalized Environment and Genes Study (PEGS) cohort. We performed a genome-wide association study (GWAS) using Firth-corrected logistic regression modeling for previously pregnant case and control groups, assuming an additive genetic model. Participants who reported no complications during pregnancy were assigned to the control group. Adjusting for population stratification, covariates included the first 10 principal components in addition to self-identified race/ethnicity and phenotype-specific covariates.

Results: We identified a significant novel candidate region associated with HDP in the maternal genome (202 cases and 1,569 controls) near *RARB (retinoic acid receptor beta)* on chromosome 3 (3:24669983-25182073), and near *LRP1B (LDL Receptor Related Protein 1B)* on chromosome 2 (139728665-140228665). The top variant is at 3:24930847 (minor allele frequency: 4.8%; odds ratio: 3.16; 95% confidence interval: (2.16, 4.64); $p = 3.87 \times 10^{-9}$). A proximal variant (3:25011263; $p = 7.04 \times 10^{-6}$) related to proteinuria in the fetal genome was recently reported in a Peruvian sample.

Conclusion: GWAS revealed novel loci associated with HDP in the maternal genome across an ethnically diverse population.

References:

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EP25.013 GWAS and X chromosome eQTLs identify TMEM187 as a functional candidate in celiac disease

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Background/Objectives: Celiac disease (CeD) is a complex, immune-mediated enteropathy that develops in genetically predisposed individuals. CeD prevalence is higher in women, suggestive of a role of the X chromosome in its pathogenesis. Mendelian Randomization (MR) analyses combining GWAS and QTL information have proven useful to disentangle functional mechanisms in CeD.

Methods: We constructed a cis-eQTL database of the X chromosome with QTLtools using HRC-imputed genotypes and expression data from unstimulated ($n = 226$) and LPS-stimulated ($n = 167$) monocytes of women [1]. Results were replicated in unstimulated, male monocytes. We applied summary-data-based MR to integrate CeD Immunochip [2] results with eQTLs, to identify X chromosome genes functionally involved in CeD.

Results: The expression of *TMEM187* is regulated by CeD-associated, eQTL SNPs rs7350355, rs5945386 and rs80208125, and CeD-risk alleles result in lower *TMEM187* expression (SMR $\beta = [(-0.47) - (-0.63)]$; pSMR $< 5\times10^{-3}$). *TMEM187* was downregulated in LPS-stimulated monocytes. Scrutiny of GEO dataset GSE113469 [3] showed that *TMEM187* is upregulated in PBMCs of CeD individuals on gluten-free diet compared to controls ($p < 10^{-14}$; logFC = -0.589).

Conclusion: SMR analysis of monocyte eQTLs has identified *TMEM187* expression as a putative effector of the CeD-associated SNPs on the X chromosome. Further studies of the X chromosome are needed to understand the implication of the sex chromosomes in the gender bias in CeD prevalence.

References: 1. B.P Fairfax et al. 343;:1246949 (2014).
2. G. Trynka et al. 43:1193–1201 (2011).
3. M.Sangineto et al. 13:e0197915 (2018).

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Conflict of Interest: None declared.

EP25.014 Two-sample mendelian randomization detects bidirectional causality between HLA-DQ2 and gut microbiota in celiac disease

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Background/Objectives: Celiac disease (CeD) is an autoimmune enteropathy triggered by gluten intake in genetically predisposed individuals. The major genetic determinant is the HLA-DQ2 heterodimer present in 90-95% of celiac individuals. In addition, the host's gut microbiota, influenced by environmental and genetic factors, might play a pivotal role in CeD. Observational studies have shown that individuals carrying HLA-DQ2 haplotypes have a distinctive gut microbiota composition. Whether these alterations are cause or consequence of the disease remains unknown.

Methods: To determine the putative causality between CeD and the gut microbiome, we performed a bidirectional Two-Sample Mendelian Randomization analysis. For CeD, we used data from the Immunochip CeD study[1] considering only high-risk celiac population carrying at least one copy of the HLA-DQ2 alleles (2587 cases, 334 controls). For the microbiome, we employed public data from MiBioGen[2] consortium ($n = 18340$).

Results: When assessing the effect of gut microbiota over CeD, we identified five main taxon from the Firmicutes phylum, including *Ruminococcaceae*, as previously reported. When

assessing the effect that CeD has over gut microbiota, we identified other bacteria from the Firmicutes, Proteobacteria, and Bacteroidetes phyla. Our main hit was the *Pasteurellaceae* family, which belongs to the Proteobacteria phylum.

Conclusion: The existing relationship between CeD and gut microbiota is highly complex and bidirectional.

References: [1] G. Trynka et al., 43, 1193–1201 (2011). [2] A. Kurilshikov et al., 53, 156–165 (2021).

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EP25.015 Whole genome sequencing in African-American families identifies candidate genes for myopia risk

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Background/Objectives: Myopia is one of the most common eye diseases in the world. Previous studies using genotype data in a set of African-American families with apparent autosomal dominant myopia had shown linkage of myopia risk to 7p but the causal gene(s) could not be identified (1). Here, we analysed whole genome sequence data from these African-American families to find genes that may be associated with myopia risk.

Methods: We performed whole genome sequencing on 124 individuals in fifteen African-American families using PCR-free libraries and the NovaSeq 6000 (illumina). Data were aligned to hg19 using BWA and genotypes called using the GATK4 pipeline best practices. Myopia was measured as mean spherical equivalent (MSE) and converted to a binary phenotype. Parametric two-point linkage analysis was performed between myopia and each genotype using TwoPointLods, assuming an autosomal dominant mode of inheritance, disease allele frequency of 0.01 and penetrance of 0.9 for disease allele carriers and 0.1 for noncarriers.

Results: Genetic linkage found genome-wide significant signals in the noncoding RNA gene *LOC401324* (HLOD = 4.1) on chromosome 7 and in KCNA5 (HLOD = 3.3) on chromosome 12. Further suggestive signals were found at these loci on chromosomes 7 and 12, as well as chromosome 3. Good candidate genes at these loci were identified, including the eye organogenesis gene *BMPR*.

Conclusion: This study identified two genome-wide significant signals on chromosomes 7 and 12. There are excellent candidate genes at these loci, and further functional analysis and fine-mapping to determine the causal variant is ongoing.

References: https://doi.org/10.1167/iovs.62.9.16.

Grants: None.

Conflict of Interest: None declared.

EP25.016 Large-scale genomic meta-analysis on math abilities in cohorts of 20,000 participants

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Background/Objectives: Dyscalculia is a heritable condition ($h^2 = 0.65$) characterized by impaired number processing skills which affects ~3–6% of children (Kucian et al., 2015). The disorder can be considered as the lower extreme of the mathematical abilities in the general population.

Few genomic studies have been performed for dyscalculia and math abilities.

Methods: We performed GWAS meta-analysis on arithmetic skills based on national standardised tests across 12 cohorts from 7 countries (N_{Total} ~ 20,000). We followed standard protocols and tools for GWAS through linear mixed modelling (BOLT-LMM and GEMMA) and downstream analyses (inverse variance-weighted meta-analyses: METAL, functional mapping: FUMA, genetic correlation analyses with neurodevelopmental disorders: LDSC).

Results: Preliminary results in individual cohorts have highlighted a genome-wide significant locus in HSPD1P9 (chr13q13.3). Other lead variants were found in genes known to be also associated with other neurodevelopmental disorders. Results of the meta-analysis conducted across all cohorts will be presented at the conference.

Conclusion: This study is the largest GWAS meta-analysis for arithmetic abilities recruited in different countries. We aim to shed new light on the genetics of dyscalculia and the link with other neurodevelopmental disorders.

References: Kucian, K. & von Aster, M. Developmental dyscalculia. Eur. J. Pediatr. 174, 1–13 (2015).

Grants:

Conflict of Interest: None declared.

EP25.017 Integration of exome sequences from non-European ancestries enhances the detection of gene-biomarker associations in the UK Biobank

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Background/Objectives: The UK Biobank (UKB) bioresource has genetic data linked to diverse phenotypic measurements, including blood biomarkers. The availability of exome data allows for the exploration of rare coding variants that regulate blood biomarker levels.

Methods: Using exome sequence data from 412,394 unrelated participants across four ethnically diverse ancestries in the UKB [Europeans (N = 394,695), Africans (N = 7,412), South Asians (N = 8,078) and East Asians (N = 2,209)], we performed gene-level collapsing analysis to test the aggregate effect of rare functional variants in genes in relation to 30 clinical blood biomarkers. Two distinct analyses were conducted: a 'European-only' analysis and a 'pan-ancestry' analysis that combined all ancestral groups.

Results: We identified 323 significant gene-biomarker relationships ($p < 1 \times 10^{-8}$) in the European-only analysis across 10 different collapsing models, with an additional 22 (7%) detected in the pan-ancestry analysis. Creatinine, HbA1c, and IGF-1 were the biomarkers with the highest number of gene associations ($N = 2$) specific to the pan-ancestry analysis. One such noteworthy association was between homozygous or putative compound heterozygous carriers ($N = 5$) of rare nonsynonymous variants in *SYT7* and creatinine levels: this association was not significant in the European-only analysis (beta = 1.17 [0.06,2.28], p = 0.04), but it reached statistical significance in the pan-ancestry analysis given 3/5 carriers were of African or South Asian ancestry (beta = 2.17 [1.46,2.87], $p = 1.6 \times 10^{-9}$). Consistent with biomarker findings, the *SYT7* variant carriers showed a strong but suggestive association with increased risk of glomerular disease (OR = 92.1 [12.1,713.2], $p = 2.6 \times 10^{-5}$).

Conclusion: By using rare-variant analysis of blood biomarkers as an exemplar, we demonstrate that diversifying ancestral representation beyond Europeans can enhance discoveries in genetic studies.

References:

Grants:

Conflict of Interest: Abhishek Nag Is an employee of AstraZeneca, Quanli Wang Is an employee of AstraZeneca, Dirk Paul Is an employee of AstraZeneca, Katherine Smith Is an employee of AstraZeneca, Slavé Petrovski Is an employee of AstraZeneca.

EP26 COVID-19

EP26.002 Association of polymorphic variants in human genome with the COVID-2019 severity and post-COVID syndrome development

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Background/Objectives: Despite intensive research of the novel coronavirus SARS-CoV-2 and COVID-2019 caused by it, factors affecting the severity of the disease remains poorly understood. Clinical manifestations of COVID-2019 may vary from asymptomatic form to pneumonia, acute respiratory distress syndrome (ARDS) and multiorgan failure. Features of individual genetic landscape of patients can play an important role in development of the pathological process of COVID-19. In this regard the purpose of this study was to investigate the influence of polymorphic variants in genes (ADD1, CAT, IL17F, IL23R, NOS3, IFNL3, IL6, F2, F13A1, ITGB3, HIF1A, MMP12, VEGFA), associated with cardiovascular, respiratory and autoimmune pathologies, on the severity of COVID-19 and post-COVID syndrome in patients from Russia.

Methods: The study included 200 patients recovered from COVID-19. Two groups of patients were formed in accordance with clinical manifestations: with mild and moderate forms of the disease. The polymorphic variants were analysed with real-time PCR using commercial kits (Syntol).

Results: 13 SNPs (rs4961; rs1001179; rs612242; rs11209026; rs2070744; rs8099917; rs1800795; rs1799963; rs5985; rs5918; rs11549465; rs652438; rs699947) were genotyped and comparative analysis of allele frequency distribution was carried out in two groups of patients recovered from COVID-2019.

Conclusion: Identification of polymorphic variants in genome associated with severity of pathological processes in patients infected with SARS-CoV-2 can contribute to the identification of individuals with an increased risk of severe infection process and can also serve as a basis for developing personalized therapeutic approaches to the treatment of post-COVID syndrome.

References:

Grants:

Conflict of Interest: None declared.

EP26.003 NR3C1 rs41423247: genetic predictor of complications in pneumonia caused by SARS-CoV-2

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Background/Objectives: Runaway inflammation is a key feature of COVID-19. NR3C1 gene encodes for glucocorticoid receptor which plays an important role in inflammation reaction. The variant rs41423247 cause increased glucocorticoid receptors sensitivity. This study aimed to investigate the impact of variants of NR3C1 gene *on* the course of COVID-19 pneumonia in patients with necessarily artificial lung ventilation.

Methods: The study group included 20 patients (9 women and 11 men) with diagnosis "viral COVID-19 pneumonia" on artificial lung ventilation at the intensive care unit. All patients underwent daily standard examinations according clinical protocols. Determination of NR3C1 gene variants was carried out by using PCR-RFLP.

Results: There were found the significant negative correlations between NR3C1 gene variants and level of SpO2 ($rS = -0.601$, $p = 0.008$), *Glasgow* Coma *Scale score* (rS = -0.523, $p = 0.026$). Also, it was defined a protective effect of genotype CC at risk of development acute respiratory distress syndrome in this patients ($\chi 2 = 4.38$, $p = 0.037$, OR = 0.05 (CI:0.01–0.66)).

Conclusion: The investigated variant rs41423247 of the NR3C1 gene may be the genetic predictor of complicated course of COVID-19 pneumonia.

References:
Grants:
Conflict of Interest: None declared.

EP26.004 Genotype–phenotype correlations in five patients harboring SERPINA1 mutations and previously showing a severe COVID-19 infection

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Background/Objectives: During COVID-19 pandemic, it is essential to detect patients potentially at risk of life-threatening complications, due to possible specific genetic mutations. The aim of our work is to show a practical application of genetic testing, allowing a diagnosis of alpha 1 antitrypsin deficiency in cases with a severe clinical course during COVID-19 infection.

Methods: During hospitalization for COVID-19, we identified 5 patients (3 female, 2 males from two different families, age range 18–47 years) with a severe course of COVID-19 infection, requiring high pressure ventilation with high volume oxygen supply. Two months after discharge, those patients were reevaluated with respiratory function tests, biochemical tests, genetic counselling and genetic testing. A peripheral blood sampling for *SERPINA1* genetic testing has been performed, using Sanger sequencing.

Results: Two months after discharge, in all 5 patients respiratory function tests were consistent with a dysventilatory obstructive syndrome, in contrast with usual findings related to COVID-19 infection. Blood test still showed increase plasmatic transaminase concentration in 3 out of 5 patients, one having increased serum bilirubin as well. We performed *SERPINA1* genetic testing showing homozygosity for *SERPINA1* pathogenic mutations (c.193del and c.875C>T, respectively) in all 5 patients.

Conclusion: These cases showed the importance of genetic testing for patients with unexplained severe COVID-19 infection. Genetic testing allowed the diagnosis of cases affected by alpha 1 antitrypsin deficiency, associated with dysventilatory obstructive syndrome, that may worsen the short and long term prognosis of COVID-19.

References: PMID 33485406.
PMID 34010739.
PMID 33964815.

Grants: No.

Conflict of Interest: Emanuele Micaglio Full time, Sara D'Imperio Full time, Michelle Monasky Full time, Cecilia Marcolin Part - time, Emanuela Teresina Locati Full time, Carlo Pappone Full time.

EP26.005 Presence of rare potential pathogenic variants in subjects under 60 years old with very severe or fatal COVID-19

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Background/Objectives: Genetic variants affecting host defense against pathogens may help explain COVID-19 fatal outcomes. Our aim was to identify rare genetic variants related to COVID-19 severity in a selected group of patients under 60 years who required intubation or resulting in death.

Methods: Forty-four very severe COVID-19 patients were selected from the Spanish STOP_Coronavirus cohort, which comprises more than 3,500 COVID-19 patients. Genotype was performed by whole exome sequencing and variants were selected by using a gene panel of 867 candidate genes (immune response, primary immunodeficiencies or coagulation, among other). Variants were filtered, priorized and their potential pathogenicity was assessed following ACGM criteria.

Results: We detected 44 different variants of interest, in 29 different patients (66%). Some of these variants were previously described as pathogenic (26%). Mostly, the candidate variants were located in genes related to immune response (38%), congenital disorders of glycosylation (14%) or damaged DNA binding genes (9%). A network analysis, showed three main components, consisting of 25 highly interconnected genes related to immune response and two additional networks enriched in carbohydrate metabolism and in DNA metabolism and repair processes.

Conclusion: The variants identified affect different, but interrelated, functional pathways such as immune response and glycosylation. Further studies are needed for confirming the ultimate role of the new candidate genes described in the present study on COVID-19 severity.

References:

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Conflict of Interest: None declared.

EP26.006 Genetic associations from the Long COVID-19 Host Genetics Initiative contribute to understanding of the genetics of prolonged symptoms in COVID-19

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Background/Objectives: One of the most remarkable features of SARS-CoV-2 infection is that a large proportion of individuals are asymptomatic while others experience progressive, even life-threatening acute respiratory distress syndrome, and some suffer from prolonged symptoms ("long COVID"). The contribution of host genetics to susceptibility and severity of infectious disease is well-documented, and include rare monogenic inborn errors of immunity as well as common genetic variation. Studies on genetic risk factors for long COVID have not yet been published.

Methods: We compared long COVID (1534) to COVID-19 patients (96,692) and population controls (800,353) using both questionnaire and EHR- based studies. First meta-analysis of 11 GWAS studies from 8 countries did not show genome-wide significant associations.

Results: Testing 24 variants earlier associated to COVID-19 susceptibility or severity by COVID-19 Host Genetics Initiative showed genetic variation in rs505922, an intronic variant in *ABO* blood group gene, to be associated with long COVID compared to population controls (OR = 1.16, 95% CI: 1.07–1.27, $p = 0.033$). (Within-COVID analysis gave similar OR, but was not significant after conservative Bonferroni correction (OR = 1.17, 95% CI: 1.06–1.30, $p = 092$)).

Conclusion: The first data freeze of the Long COVID Host Genetics Initiative suggests that the O blood group is associated with a 14% reduced risk for long COVID. The following data freezes with growing sample sizes will possibly elucidate long COVID pathophysiology and pave the way for possible treatments for long lasting COVID symptoms.

References:

Grants:

Conflict of Interest: None declared.