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PLENARY SESSIONS

PL1 OPENING PLENARY

PL1.3 Highly accurate protein structure prediction and its implications for understanding human biology

John Jumper

Predicting a protein's structure from its primary sequence has been a grand challenge in biology for the past 50 years, holding the promise to bridge the gap between the pace of genomics discovery and resulting structural characterization. In this talk, we will describe work at DeepMind to develop AlphaFold, a new deep learning-based system for structure prediction that achieves high accuracy across a wide range of targets. We demonstrated our system in the 14th biennial Critical Assessment of Protein Structure Prediction (CASP14) across a wide range of difficult targets, where the assessors judged our predictions to be at an accuracy "competitive with experiment" for approximately 2/3rds of proteins. The talk will cover both the underlying ideas of AlphaFold and the implications for biological research.

Conflict of Interest: None declared.

PL2 WHAT'S NEW? HIGHLIGHT SESSION

PL2.1 Single cell, whole embryo phenotyping of pleiotropic disorders of mammalian development

Jana Henck[1;2], *Xingfan Huang*[3], *Chengxiang Qiu*[3], *Varun Sreenivasan*[1], *Sascha Ulferts*[4], *Rose Behncke*[4], *Nils Hansmeier*[4], *Rene Haegerling*[4], *Alexandra Despang*[2], *Daniel Ibrahim*[2], *Alessa Ringel*[2], *Michael Robson*[5], *Lars Wittler*[2], *Vera Kalscheuer*[2], *Uwe Kornak*[6], *Natja Haag*[7], *Ingo Kurth*[7], *Wing-Lee Chan*[4], *David Beier*[8], *Lauren Saunders*[3], *Cole Trapnell*[3], *Diane Dickel*[9], *Axel Visel*[9], *Len Pennacchio*[9], *Junyue Cao*[10], *Jay Shendure*[3], *Malte Spielmann*[1;2]

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Background/Objectives: Mouse models represent a critical tool to study human diseases, as well as for advancing our general understanding of developmental biology. However, current phenotyping technologies lack the necessary throughput and resolution for the investigation of mutations at the organismal scale. In this study, we set out to establish single cell RNA sequencing as a tool for large scale standardized and comprehensive phenotypic analysis of whole mouse mutant embryos.

Methods: In a multiplexed experiment, we applied single cell RNA sequencing to profile 104 whole mouse embryos from 22

different mutants and 4 wild type strains at embryonic stage E13.5. The mouse mutants range from established multisystem disorders to single enhancer deletions. We developed and applied several analytical frameworks for detecting differences in composition and gene expression changes across all cell types and trajectories.

Results: We present a mouse mutant cell atlas (MMCA) consisting of 1.9 million single cell RNA-seq profiles and identify 300 significantly changed cell type proportions from 52 sub trajectories across the 22 mutants. Some mutants exhibited changes in dozens of trajectories (*e.g.*, the pleiotropic consequences of altering the *Sox9* regulatory landscape) whereas the impact of others was considerably more restricted. We further identified differences between widely used wild type strains, compared gain vs. loss of function mutants, and identified changes that are shared by heretofore unrelated models.

Conclusion: Our findings show how single cell profiling of whole embryos can enable the systematic molecular and cellular phenotypic characterization of mouse mutants with unprecedented breadth and resolution.

References:

Grants:

Conflict of Interest: None declared.

PL2.2 Balanced chromosomal rearrangements offer insights into coding and noncoding genomic features associated with developmental disorders (virtual)

Chelsea Lowther[1;2;3], *Mana Mehrjouy*[4], *Ryan Collins*[1;2;5], *Mads Bak*[4], *Olga Dudchenko*[6;7], *Harrison Brand*[1;2;3], *Zirui Dong*[8], *Malene B. Rasmussen*[4;9], *Peter Jacky*[10], *Erez Lieberman Aiden*[2;6;7], *Iben Bache*[4;9], *Michael Talkowski*[1;2;3], *Niels Tommerup*[9]

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Background/Objectives: Balanced chromosomal rearrangements (BCRs) reorganize large sections of the genome and contribute substantial risk for developmental disorders (DDs). However, the rarity of BCRs in the population has precluded unbiased analyses of the features and mechanisms associated with risk for DDs.

Methods: We aggregated 1,420 BCR breakpoints from 406 DD cases and 304 control BCR carriers.

Results: We found that BCRs were not more likely to disrupt genes in DD cases than controls but were seven-fold more likely to disrupt genes associated with dominant DDs (21.3% of cases vs. 3.4% of controls; $P = 1.60 \times 10^{-12}$). Moreover, BCRs that did not disrupt a known DD gene were significantly enriched for breakpoints that altered topologically associated domains (TADs) containing dominant DD genes in cases compared to controls (odds ratio [OR] = 1.43, $P = 0.036$). We discovered six TADs enriched for noncoding BCRs that contained known DD genes and were collectively disrupted by 7.4% of the DD cohort. Phased Hi-C analyses of five cases with noncoding BCR breakpoints localized to the TAD encompassing *MEF2C* confirmed extensive disruption to local 3D chromatin structures and reduced frequency of contact between the *MEF2C* promoter and enhancers. We further identified six genomic features enriched in TADs preferentially disrupted by noncoding BCRs in DD cases and used these features to build a model to predict TADs at risk for LRPEs across the genome.

Conclusion: These results emphasize the potential impact of noncoding structural variants to cause LRPEs in unsolved DD cases, as well as the complexity of predicting TAD intolerance.

References:

Grants:

Conflict of Interest: Chelsea Lowther: None declared, Mana Mehrjouy I am an employee of Illumina Inc., Ryan Collins: None declared, Mads Bak: None declared, Olga Dudchenko: None declared, Harrison Brand: None declared, Zirui Dong: None declared, Malene B. Rasmussen: None declared, Peter Jacky: None declared, Erez Lieberman Aiden I receive in-kind support from IBM and Illumina Inc., Iben Bache: None declared, Michael Talkowski I receive research support and/or reagents from Levo Therapeutics, Microsoft Inc, and Illumina Inc., Niels Tommerup: None declared.

PL2.3 Long-read sequencing reveals heritable large structural variants induced by CRISPR-Cas9

Ida Höijer[1], *Anastasia Emmanouilidou*[1;2], *Lars Feuk*[1], *Ulf Gyllensten*[1], *Marcel den Hoed*[1;2], *Adam Ameur*[1]

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Background/Objectives: Genome editing by CRISPR-Cas9 has become an invaluable research tool and has the potential to cure many genetic disorders. However, for clinical applications it is crucial to minimize the risk of adverse effects due to unintended CRISPR-induced mutations. To increase our understanding of unintended CRISPR-Cas9 genome editing in vivo, we investigated the genomes of >1100 genome edited zebrafish larvae, juveniles and adults from two generations.

Methods: We used four guide RNAs (gRNAs), previously used in functional studies, to edit the genome of fertilized zebrafish eggs at the 1-cell stage. Off-target sites were detected in vitro by Nano-OTS. We then used long-read sequencing to determine the CRISPR-Cas9 outcomes at on- and off-target sites.

Results: In founder larvae, on-target editing of the four gRNAs was 93-97% efficient, and three sites across two gRNAs were identified with in vivo off-target editing. Six percent of the CRISPR-Cas9 editing outcomes corresponded to structural variants (SVs), including deletions <4.8 kb and insertions <1.4 kb. The F1 generation contained high levels of genome editing, with all alleles of 46 examined F1 juvenile fish affected by on-target mutations, including four cases of SVs. In addition, 26% of the juvenile F1 fish carried off-target mutations.

Conclusion: We demonstrate that large SVs and off-target mutations can be introduced in vivo and segregate to the next generation. Our results have important consequences for clinical applications, and we outline a validation method based on long-read sequencing to reduce the risk of unanticipated effects with potentially large implications.

References:

Grants:

Conflict of Interest: None declared.

PL2.4 Loss-of-function of AMFR causes autosomal recessive hereditary spastic paraplegia by altering lipid metabolism

Ruizhi Deng[1;2], *Eva Medico Salsench*[1], *Anita Nikoncuk*[1], *Niko Kühn*[1], *Reshmi Ramakrishnan*[3], *Kristina Lanko*[1], *Leslie Sanderson*[1], *Herma C*

van der Linde[1], Soheil Yousefi[1], Ivan Capo[4], Evita Medici van den Herik[5], Marjon van Slegtenhorst[1], Rick van Minkelen[1], Rob Willemsen[1], Walter de Valk[1,2], Geert Geeven[1,2], Kay Metcalfe[6], Lenika De Simone[7], Nancy Kuntz[7], Arjan Bouman[1], Lies Hoefsloot[1,2], Maha Zaki[8], Joseph Gleeson[9], Stefan T Arold[3,10], Tjakko Van Ham[1], Reza Maroofian[11], Stefan Barakat[1,2]

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Background/Objectives: Hereditary spastic paraplegia (HSP) is a group of rare, inherited neurodegenerative or neurodevelopmental disorders which mainly present with lower limb spasticity and muscle weakness, due to dysfunction of the motor neurons.

Methods: Whole genome sequencing identified an early bi-allelic truncating *AMFR* variant, subsequent international collaboration recognized more patients with different bi-allelic truncating *AMFR* variants. Primary patient fibroblasts, neural stem cells (NSCs) and in vivo modeling using zebrafish disease mechanisms were studied including initial pre-clinical experiments for possible therapy development.

Results: We identified bi-allelic truncating variants in *AMFR*, which encoding a RING-H2 finger E3 ubiquitin ligase anchored at the membrane of the endoplasmic reticulum in 16 individuals from 7 unrelated families. Patients segregated with a phenotype of both pure as well as complex HSP consisting of global developmental delay, intellectual disability, motor dysfunction, and progressive spasticity. We show that absence of *AMFR* causes altered lipid metabolism leading to the accumulation of lipid droplets in NSCs, a finding which is also recapitulated in patient derived fibroblasts, both using light microscopy and electron microscopy studies. *amfra-/-* zebrafish larvae show similar lipid accumulation and altered locomotor activity likely phenocopying spasticity observed in patients. Interestingly, administration of an FDA approved drug partially improves the observed locomotor phenotype in *amfra-/-* larvae, which promises potential therapeutic interventions for this newly defined disorder.

Conclusion: Our study identifies bi-allelic truncating mutations in *AMFR* as a cause of a novel autosomal recessive HSP. Functional studies further implicate *AMFR* as the disease-causing gene by altering lipid metabolism which potentially could be therapeutically modulated.

References:

Grants:

Conflict of Interest: None declared.

PL2.5 An integrated approach using multi-omics data to dissect cis-regulatory role of ultraconserved non-coding elements (UCNEs) in human retina

Víctor López Soriano[1], *Alfredo Dueñas Rey[1], Stijn Van de Sompele[1], Frauke Coppieters[1], Andrew Webster[2], Miriam Bauwens[1], Elfride De Baere[1]*

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Background/Objectives: Whole genome sequencing (WGS) has revealed an increasing number of pathogenic non-coding variants in inherited retinal diseases (IRD), with a majority of deep-intronic variants. We focus on the role of ultraconserved non-coding elements (UCNEs) defined as genomic regions >200 bp characterized by at least 95% human-chicken conservation, and their association with gene regulation and disease. Using an integrated multi-omics approach, we set out to assess a potential cis-regulatory role for active UCNEs in human retina.

Methods: To predict UCNEs with active enhancer-like marks, we integrated publicly available transcriptomic (bulk and scRNA-seq) and epigenomic (ATAC-seq, DNase and histone modifications for active enhancers) datasets derived from human retinal material. WGS data from ~3,331 probands of the ophthalmological disease cohort of the 100,000 Genome Project Genomics England was mined to retrieve sequence variants (SNVs) and structural variants (SVs) within UCNEs.

Results: Interrogation of multi-omics data derived from human retina revealed a total of 1,349 retina-active UCNEs. Interestingly, 40 genes under putative UCNE control are linked to an eye-disease phenotype. A total of 79 rare SNVs and 10 SVs related to genes implicated in IRD or other eye phenotypes were identified.

Conclusion: We identified 1,349 retina-active UCNEs potentially acting as CREs and representing an understudied target of non-coding variants that may explain missing heritability in IRD. Deciphering retinal UCNEs and their cis-regulatory landscapes will contribute to functional genome annotations in the retina and to the non-coding morbid genome of IRD. Ultimately, they may represent novel targets for treatment.

References:

Grants: StarT-H2020-MSCA-ITN-2018-N°81349, FWO1802220N.

Conflict of Interest: None declared.

PL2.6 Noncoding regulatory mutations as driving event for the oncogenic core regulatory circuitries of neuroblastoma

Vincenzo Aievola[1], *Vito Alessandro Lasorsa[2], Annalaura Montella[3], Ferdinando Bonfiglio[3], Matilde Tirelli[4], Achille Iolascon[3], Mario Capasso[3]*

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Background/Objectives: Neuroblastoma, a paediatric cancer composed of mesenchymal and adrenergic-like cells, can arise from impaired activity of Core Regulatory Circuitries (CRCs), Transcription Factors (TF) sets that determine the cell identity. We hypothesize that noncoding somatic single nucleotide variants (SNVs) altering TF binding sites (TFBSs) can affect cellular differentiation promoting cancer initiation. Our aim is to identify noncoding somatic SNVs that drive tumorigenesis by altering NB CRCs.

Methods: Active TFBSs in adrenergic-like SKNBE2C neuroblastoma cells, identified through ChIP-seq and ATAC-seq, were integrated with SNVs obtained by whole genome sequencing of 151 neuroblastomas. Mutation rates of core/flanking regions were modeled using chi-square distribution. SNVs selected on CADD, FuncSeq2 and motifbreakR tools scores, were functionally tested with Luciferase assay. Expression of TFBSs target-genes, identified through HiC analysis in SKNBE2C, was evaluated using RNA-seq data of 89 patients with matched WGS. Target-genes expression values were correlated with clinical and survival data.

Results: We found that transcriptionally active binding sites of TFs (PHOX2B, TBX2 and ISL1) belonging to adrenergic CRC were enriched of SNVs (FDR < 0.05). Among the selected variants, the chr7:126481446:A>C mutation was predicted to disrupt a PHOX2B motif and showed allele-specific transcriptional activity in Luciferase assay. HiC data showed significant PHOX2B motif interactions with GRM8 and PAX4, whose expression was reduced in mutated samples compared to wild types (P < 0.05). Low PAX4 expression correlated with stage 4 and reduced overall survival (P < 0.01).

Conclusion: This work demonstrates that combined noncoding somatic mutations affecting the CRC activity can promote neuroblastoma tumorigenesis.

References:

Grants: This work was founded by AIRC IG 2021 ID 25796.

Conflict of Interest: None declared.

PL3 ELPAG AWARD LECTURE

PL3.1 ELPAG Award Lecture

Martina Cornel

On the occasion of the ELPAG lecture I will briefly summarize my work..

We stand on shoulders of giants, and for me Leo ten Kate, Ségolène Aymé and Bartha Knoppers were amongst those giants. The latter cited as a legal basis for the The Global Alliance for Genomics and Health (GA4GH) Article 27 of the 1948 Universal Declaration of Human Rights. This guarantees the rights of every individual in the world "to share in scientific advancement and its benefits".

That attitude was already in the back of our minds when I started working with Leo ten Kate in the Northern Netherlands EUROCAT registration in 1984 in the Groningen Human Genetics department. As MD/epidemiologist I collected data on congenital anomalies, and offered physicians who notified the children to receive information, so they could use the scientific insights for the families they cared for. Also, we joined forces with patient- and parents organizations to inform mothers-to-be on the protective effects of folic acid against neural tube defects. I was scientific advisor on a national information campaign and visited the EU Parliament with spina bifida patients in wheelchairs and their assistance dogs.

I later moved to Human Genetics Amsterdam as professor of Community Genetics and Public Health Genomics, and supervised PhD students on e.g. Genetics Education for Primary Care Physicians and Translating the Dynamics of Genetics into Health Care Practice. In those years Ségolène Aymé built an even more professional information service "Orphanet" including encyclopaedia, centres of expertise and information on trials and I was happy to start that in The Netherlands. I am involved in the national neonatal screening program and in the cascade screening in families for cardiogenetic conditions. As of June 1st 2022 all newborn infants in the Netherlands are offered screening for 26 conditions. The latest condition added is spinal muscular atrophy. Curative treatments are becoming available for an increasing number of conditions, and early diagnosis is needed for optimal effectiveness.

Listening to patients' needs and trying to use genetic knowledge in their interest, taking into account their values is the common thread through my work.

I would like encourage all ESHG visitors to apply our knowledge in the interest of future generations.

Conflict of Interest: None declared.

PL5 ESHG AWARD LECTURE

PL5.1 ESHG Award Lecture

Aarno Palotie

Using the Finnish population structure and nationwide health records to understand the genetic basis of diseases.

Population isolates such as Finland provide benefits in genetic studies because the allelic spectrum of damaging alleles in any gene is often concentrated on a small number of low frequency variants (0.5% ≤ minor allele frequency < 5%), which survived the founding bottleneck, as opposed to being distributed over a much larger number of ultra-rare variants. This has been well documented for Mendelian diseases, including numerous studies on recessive diseases more common in Finland than any other population, often called as Finnish Disease Heritage. The value of the bottleneck effect in common diseases has only lately been explored. On the other hand, human genetics has matured to a point where the assessment of genotype-phenotype relationships in large populations can inform basic disease mechanisms and even benefit drug discovery efforts by identifying novel targets and informing on their potential safety profile. Finland provides special opportunities for such large-scale studies. In addition to the unique population structure, it has centralized medical records that cover comprehensive health care service usage of every resident over lifetime, like all Nordic countries. Partially stimulated by the national health care system and its registries, there is a strong tradition of epidemiological studies. These epidemiological studies have paved the way for biobank research and subsequently for large-scale genome studies. This tradition is further supported by legislation for biobanks and secondary usage of health register data. Consequently, several large-scale genomic studies have been performed or are in progress. These include the SUPER study (https://www.superfinland.fi) that collected over 10 000 psychosis cases, the Generisk study(https://www.generisk.fi) studying means how to return genetic information for participants and the FinnGen (https://www.finngen.fi), a public-private partnership project, combining genetic data with life long electronic health register data in 500 000 participants. Along with other large international projects these studies have provided insight for the field how common and rare variants contribute to disease susceptibility. The enrichment of selected, coding variants in the Finnish population provide advantages for understanding the genetic background of diseases. In the FinnGen project, among over 400 Finnish enriched diseases associations, more than 150 are coding variants. The disease associated coding variants enables to interpret functional consequences and design follow-up studies more readily than for non-coding variant associations. The FinnGen project was initiated in 2017 and Finnish biobanks have already collected over 500 000 participants for the project. FinnGen produces data freezes every six months. They consist of GWAS data generated with a population specific array and imputed against 8000 Finnish deep whole genome sequences

enabling reliable identification of variants down to population frequencies around 1/1000. A safe computing environment that fulfils all data protection requirements has been built in the Google Cloud environment where partners can access individual level data but cannot download it. Results can be browsed in the FinnGen PheWeb browser. Results are made public for the entire research community after a 12 month embargo. Requests to download summary statistics can be placed here: https://www.finngen.fi/en/access_results. So far data has been downloaded by over 2000 scientists around the world. To shed light to the functional consequences of the Finnish enriched, disease associated variants various omics and cellular assays have been initiated along with deeper phenotyping.

Conflict of Interest: None declared.

CONCURRENT SESSIONSC01 NEW GENES IN NDDS

C01.1 Germline variants in tumor suppressor FBXW7 lead to impaired ubiquitination and a novel neurodevelopmental syndrome

*Sarah Stephenson[1;2], Gregory Costain[3;4;5], Laura Blok[6], Michael Silk[7;8], Thanh-Binh Nguyen[7;8], FBXW7 International consortium (multiple authors)[9], Sze-Chern Lim[10], Sue White[2;10], David Ascher[7;8;11], Annette Schenck[6], Paul Lockhart[1;2], John Christodoulou[2;10], **Tiong Yang Tan**[2;10]*

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Background/Objectives: Neurodevelopmental disorders are highly heterogenous conditions resulting from abnormalities of brain architecture and/or function. *FBXW7* (F-box and WD repeat domain containing 7), a recognised developmental gene and tumour suppressor, has been shown to regulate cell cycle progression, cell growth and survival by targeting substrates including CYCLIN E1/2 and NOTCH for degradation via the ubiquitin proteasome system.

Methods: We used a genotype-first approach, global data sharing platforms and clinical deep phenotyping to identify individuals with *FBXW7* variants. We undertook in silico protein modelling, expression of recombinant *FBXW7* missense variants in cultured HEK293T cells, and pan-neuronal knockdown of the *Drosophila* ortholog, *ago*, to understand their functional impact.

Results: We identified 35 individuals harbouring germline *FBXW7 de novo* and inherited monoallelic chromosomal deletions, nonsense, frameshift, splice site and missense variants associated with a neurodevelopmental syndrome. The *FBXW7* neurodevelopmental syndrome is distinguished by global developmental delay, borderline to severe intellectual disability, hypotonia and gastrointestinal issues. Brain imaging detailed variable structural abnormalities affecting the cerebellum, corpus callosum and white matter. A crystal structure model of FBXW7 predicted missense variants clustered at the substrate-binding surface of the WD40 domain and may reduce FBXW7 substrate binding affinity. Expression of recombinant *FBXW7* missense variants in cultured cells demonstrated impaired CYCLIN E1 and CYCLIN E2 turnover. Pan-neuronal knockdown of the *Drosophila* ortholog, *ago*, impaired learning and neuronal function.

Conclusion: Collectively, we present evidence of another F-Box protein-related phenotypically variable neurodevelopmental disorder associated with monoallelic variants in *FBXW7*, expanding the genetic landscape of intellectual disability.

References: Multiple.

Grants: Multiple.

Conflict of Interest: None declare.

C01.2 Biallelic variants in GTF3C3 result in an autosomal recessive intellectual disability

***Kerith-Rae Dias**[1;2], Anais Begemann[3], Laura Blok[4], Jingyi Long[4], Lachlan De Hayr[5;6], Carey-Anne Evans[1;7], Ying Zhu[7], Mike Field[8], Alan Ma[9;10], Melanie Leffler[8], Lesley Ades[9;10], Sarah Josephi-Taylor[9;10], Rolph Pfundt[4], Maha Zaki[11], Hoda Tomoum[12], Henry Houlden[13], Clarissa Rocca[13], Stephanie Efthymiou[13], Anne Gregor[14;15], André Reis[15], Sateesh Maddirevula[16], Markus Zweier[3], Fowzan Alkuraya[16;17], Michael Buckley[7], Reza Maroofian[13], Joseph Gleeson[18;19], Mireia Coll-Tané[4], David Koolen[4], Robert Harvey[5;6], Christiane Zweier[14;15], Annette Schenck[4], Tony Roscioli[1;7;20], Anita Rauch[3;21;22]*

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Background/Objectives: *GTF3C3* encodes a critical subunit of transcription factor TFIIIC, which has conserved roles in the regulation of RNA Polymerase III mediated transcription, chromatin insulation and chromosome organisation. We delineate a novel

autosomal recessive intellectual disability disorder, caused by variants in the *GTF3C3* gene.

Methods: An international collaboration, exome sequencing, molecular modelling and a knockdown *Drosophila* model were used to characterise the *GTF3C3* variants.

Results: We identified fifteen affected individuals from nine unrelated families harbouring homozygous and compound heterozygous variants in *GTF3C3*. Of these, thirteen individuals have recurrent variants at four positions, of which one variant is hyper-recurrent in seven individuals. The cohort manifests a core neurodevelopmental phenotype including intellectual disability and developmental delay, variable non-familial facial features and congenital anomalies, and seizures in nine individuals. Molecular modelling shows the variants cluster in a hotspot in the tetratricopeptide repeat structural motif, an ancient protein-protein interaction module. Neuronal knockdown of the *Drosophila GTF3C3* ortholog causes deficits in habituation learning and seizure-like behaviour. These findings support a causal relationship of *GTF3C3* variants with intellectual disability and epilepsy.

Conclusion: We present combined genomic and functional evidence for disruption of *GTF3C3* as the aetiology for an autosomal recessive form of syndromic intellectual disability.

References:

Grants: Australian NHMRC, Centre for Research Excellence APP1117394.

Conflict of Interest: None declared.

C01.3 A novel neurodevelopmental syndrome caused by loss-of-function of the Zinc Finger Homeobox 3 (ZFHX3) gene

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Background/Objectives: Neurodevelopmental disorders result from impaired development and functioning of the brain. Here, we identify loss-of-function variation in *ZFHX3* as a novel cause for syndromic intellectual disability (ID). ZFHX3, previously known as ATBF1, is a zinc-finger homeodomain transcription factor involved in multiple biological processes including cell differentiation and tumorigenesis.

Methods: Through international collaboration, we collected clinical data of 34 individuals with premature truncating variants or (partial) deletions of *ZFHX3*. Via data-mining and multiple in vitro models we identified the subcellular localization and binding partners of ZFHX3. We used a reverse genetic approach to characterize the ZFHX3 orthologue in *Drosophila melanogaster*.

Results: Loss-of-function variation of *ZFHX3* consistently associates with (mild) ID, postnatal growth retardation, feeding difficulties, and recognizable facial characteristics as supported by artificial intelligence (Face2Gene). Publicly available and in-house generated expression data show increased expression of ZFHX3 during human brain development and neuronal differentiation. Immunoprecipitation followed by mass spectrometry in neural stem cells and SH-SY5Y shows that ZFHX3 interacts with the chromatin remodelling BRG1/Brm-associated factor complex and the cleavage and polyadenylation complex. In addition, we identified a specific DNA methylation signature in leukocyte-derived DNA. In *Drosophila melanogaster*, ZFH2 is considered the ZFHX3 orthologue. We show that ZFH2 is expressed in the third instar larval brain and that its knockdown results in an adult lethal phenotype suggestive for a key role in development.

Conclusion: Loss-of-function variants in *ZFHX3* are a novel cause for syndromic ID. Our results indicate a role for ZFHX3 in chromatin remodelling and mRNA processing.

References:

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Conflict of Interest: None declared.

C01.4 Pathogenic variants in U2AF2 and PRPF19 encoding essential spliceosomal subunits cause neurodevelopmental disorders with autistic features

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Background/Objectives: The pre-mRNA splicing is a highly coordinated and precise process that involves numerous trans pre-mRNA binding protein factors. We describe pathogenic variants in two such factors, U2AF2 and PRPF19, guiding the early stage of splice-site choice and activating spliceosome, respectively.

Methods: By exome sequencing and international matchmaking, we identified 35 unrelated individuals with 17 different *de novo* novel missense variants in *U2AF2* and four individuals with *de novo* novel *PFPF19* variants.

Results: Four out of the 17 *U2AF2* variants were recurrent in 20 individuals, representing mutation hotspots of U2AF2. Detailed clinical assessment of the affected individuals with *U2AF2* or *PFPF19* variants showed consistent presentations. In vitro assays demonstrated ten *U2AF2* variants affected splicing and RNA binding affinity. Crystal structures of two U2AF2 mutants were determined on X-ray, which indicated that hydrogen bonds with the polypyrimidine tract nucleotides were disrupted by the bulky residue replacement or the shortened length of the side chain. Multiple neural-specific RNAis of *Drosophila* orthologues *U2af50* and *Prp19* led to mushroom body (MB) patterning defects, seizures, and social deficits. These phenotypes mimic the salient features observed in two patient cohorts. Remarkably, these phenotypes seen in flies can be rescued by wild type human *U2AF2* or *PRPF19* but not the variants identified in humans, highlighting the pathogenic nature of the identified variants in the two genes. RNA-Seq of fly RNAi brains revealed three downstream effectors which could rescue reduced MB volume phenotype.

Conclusion: In conclusion, this study extends the tally of causative genes in neurodevelopmental disorders to include two splicing factors.

References:

Grants:

Conflict of Interest: None declared.

C01.5 De novo missense variants in the E3 ubiquitin ligase adaptor KLHL20 cause a developmental disorder with intellectual disability, epilepsy and autism spectrum disorder

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Background/Objectives: KLHL20 is part of a CUL3-RING E3 ubiquitin ligase involved in protein ubiquitination. KLHL20 functions as the substrate adaptor that recognizes substrates and mediates the transfer of ubiquitin to the substrates. Although KLHL20 regulates neurite outgrowth and synaptic development in animal models, its role in human neurodevelopment remains unknown. We delineate a novel neurodevelopmental disorder caused by *de novo* missense variants in *KLHL20*.

Methods: Patients were ascertained by investigators through GeneMatcher. Deep phenotyping of patients with *de novo* missense variants in *KLHL20* was performed.

Results: We studied 14 patients with *de novo* missense variants in *KLHL20*, delineating a novel genetic syndrome with patients having mild to severe intellectual disability, epilepsy, autism spectrum disorder and attention deficit hyperactivity disorder. Associated clinical features were spasticity, dystonia, strabismus, distal joint laxity, pectus excavatum, scoliosis, kyphosis, vascular anomalies, brachycephaly and subtle dysmorphic facial features. We observed a recurrent *de novo* missense variant in 11 patients ([NM_014458.4]: c.1069G>A, p.Gly357Arg). All missense variants clustered in the Kelch-type β-propeller domain of the KLHL20 protein, which shapes the substrate binding surface. Protein structure analysis revealed that two out of four missense variants destabilize the interaction of KLHL20 with DAPK1, a substrate associated with upregulated expression in the brain of patients with temporal lobe epilepsy.

Conclusion: Our findings implicate *KLHL20* in a novel neurodevelopmental disorder characterized by intellectual disability, epilepsy, autism spectrum disorder and attention deficit hyperactivity disorder.

References:

Grants:

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C01.6 Bi-allelic variants in the neuronal cell adhesion molecule NRCAM lead to a novel neurodevelopmental disorder characterized by developmental delay, hypotonia, peripheral neuropathy or spasticity

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Background/Objectives: Cell adhesion molecules (CAMs) are predominantly expressed in the CNS along principal axonal pathways and play key roles in nervous system development, neural cell differentiation and migration, axonal growth and guidance, myelination, and synapse formation. To date, only L1CAM and NFASC have been associated with neurodevelopmental disease.

Methods: Patient cohort was assembled using GeneMatcher; exome/genome sequencing were used for variant identification. Protein structure analyses and mutant zebrafish studies were performed.

Results: We describe 10 patients (eight families) with bi-allelic variants in the NRCAM, presenting with a neurodevelopmental syndrome of varying severity, characterized by developmental delay/intellectual disability, hypotonia, peripheral neuropathy and/or spasticity. Computational analyses of NRCAM variants, mostly clustering in the third Fn-III domain, strongly suggest a deleterious effect on protein structure and function, potentially hindering its protein-protein interactions. Studies on zebrafish nrcamaΔ mutants, revealed that mutant larvae display significantly altered swimming behavior in darkness compared to wild type larvae ($p < 0.03$). Moreover, nrcamaΔ larvae displayed a trend towards increased amounts of α-tubulin and 5-HT fibers in the dorsal telencephalon, suggesting an alteration in white matter tracts and projections.

Conclusion: Our study provides evidence that bi-allelic NRCAM disruption causes a variable form of a neurodevelopmental disorder, and broadens the knowledge on NRCAM function in nervous system development, as part of the growing role of the CAM superfamily in the CNS.

References:

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C02 HEREDITARY CANCER SYNDROMES AND RISK ASSESSMENT

C02.1 Estimating the prevalence of pathogenic germline variants in cancer susceptibility genes in 1,336 cases of renal cell carcinoma

Bryndis Yngvadottir[1], *Avgi Andreou*[1], *Laia Bassaganyas*[1], *Alexey Larionov*[1], *Alex J Cornish*[2], *Daniel Chubb*[2], *Charlie N Saunders*[2], *Philip Smith*[1], *Huairen Zhang*[1], *Yasemin Cole*[1], *Genomics England Research Consortium*[3], *James Larkin*[4], *Lisa Browning*[5], *Samra Turajlic*[6], *Kevin Litchfield*[7], *Richard S Houlston*[2], *Eamonn R Maher*[1]

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Background/Objectives: Renal cell carcinoma (RCC) occurs in a number of cancer predisposition syndromes but the genetic architecture of susceptibility to RCC remains unclear. This study investigates the frequency of pathogenic germline variants in a large series of unselected RCC patients.

Methods: Whole genome sequencing data on 1,336 RCC cases and 6,214 controls recruited to the UK 100,000 Genomes Project, a nationwide multicentre study, was analysed to identify rare pathogenic or likely pathogenic (P/LP) short variants (SNVs and INDELs) and structural variants in 121 cancer susceptibility genes (CSGs).

Results: Among 1,336 RCC participants (mean 61.3 years [±12SD], range 13-88 years; 64% male), 85 participants (6.3%; 95% CI [5.1, 7.8]) had one or more P/LP germline variant in a wider range of CSGs than previously recognised and most patients with pathogenic variants in well-established RCC-CSGs were aged <50 years.

Conclusion: Approximately 6% of patients with RCC unselected for family history have a germline variant requiring additional follow-up analysis and possible genetic counselling (including cascade testing). To improve diagnostic yield we suggest expanding the panel of RCC-CSGs tested to include CHEK2 and all SDHx subunits and raising the eligibility criteria for age-based testing.

References:

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C02.2 Short and long read targeted RNAseq approaches to detect complex abnormalities for cancer susceptibility genes

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Background/Objectives: Current molecular diagnostic approaches for hereditary breast and ovarian cancer (HBOC) are based on DNA short read sequencing of exons and flanking regions. These approaches hardly detect complex events nor characterize large genomic deletion/duplication breakpoints. Only 10% of molecular abnormalities are found within families at risk of breast and ovarian cancer. But some variants may be missed in the explored genes by this usual modality. We aim to explore these unexplained inheritances by new RNAseq approaches.

Methods: We selected highly susceptible families with negative or inconclusive DNAseq HBOC panel results. We developed an innovative targeted capture of long read RNAseq analysis. This new analysis was combined with a short read RNAseq assay to explore the expression and splicing profiles of complex molecular alterations. From lymphoblast cells, long read sequencing was performed on an Oxford Nanopore Technologies® platform. The capture probes of our custom panel were designed by Integrated DNA Technologies®. Analyses were performed by a custom bioinformatics pipeline.

Results: Different molecular alterations were characterized in 5 families. Retrotransposon insertions in intron 10 (2 families) and intron 13 (1 family) of *BRCA1* were detected. PCR tests were designed to perform genetic counseling. A complex transcript corresponding to an intronic retention combined with an intronic genomic deletion was found in *BRCA1* (1 family). Finally, we characterized tandem duplication in *BRCA1* exon 8 (1 family).

Conclusion: Combined short and long read targeted RNAseq analyses elucidate some unexplained hereditary for highly susceptible families through the identification of complex events. These new methods optimize preventive and therapeutic cares.

References:

Grants:

Conflict of Interest: None declared.

C02.3 Unveiling the differential disease spectrum in carriers of Pathogenic and Likely Pathogenic germline CTNNA1 variants

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Background/Objectives: Hereditary Diffuse Gastric Cancer (HDGC) predisposes to diffuse gastric cancer (DGC) and/or lobular breast cancer (LBC) development. HDGC is mainly caused by *CDH1*/E-cadherin variants, and by rare *CTNNA1*/αE-catenin germline variants for which tumour spectrum and variant-type associated causality is unknown.

Methods: We collected clinical data on *CTNNA1* germline variant carriers/relatives from ERN-GENTURIS collaborating Institutes, others worldwide, and the literature. We classified variants molecularly/clinically; according to HDGC-criteria; analysed genotype-phenotype associations; and developed a Drosophila melanogaster αE-catenin-knockdown model to study *CTNNA1* tissue-specific impairment.

Results: Fifty-two families carried rare *CTNNA1* germline variants, 35% Pathogenic (PV) and 37.5% Likely Pathogenic (LPV), all considered truncating variants. Most frequent phenotypes among PV/LPV-carriers were DGC (26%, age-of-onset 43 ± 16), Gastric Cancer of unknown-histotype (GC) (6.3%, age-of-onset 53 ± 16), Breast Cancer of unknown-histotype (BC) (27%, age-of-onset 55 ± 15) and LBC (3.5%, age-of-onset 54 ± 18). Non-classical HDGC cancers had frequencies between 0.7-3.5%. In PV-carriers, DGC and LBC were dominant (respectively 38% and 4.3%), but novel phenotypes coexist in similar frequencies to LBC (e.g. thyroid cancer (3.2%)); while in LPV-carriers, non-classical HDGC phenotypes dominated (BC-62%, melanoma-2.8%). Phenotypes differed significantly between PV and LPV-families ($p < 0.00001$). Most PV-carrier families fulfilled HDGC criteria (81%), while no LPV-carrier family did. αE-catenin knockdown in Drosophila wing/eye created aggressive phenotypes and comorbidities in adult flies, compatible with a broad disease-spectrum.

Conclusion: Early-onset DGC/LBC are recurrent and frequent cancers in *CTNNA1* PV-carriers, however, disease spectrum extends beyond HDGC classical phenotypes. *CTNNA1* LPV-carriers disease spectrum diverges from classical-HDGC. Current data claims for phenotype-driven *CTNNA1*-specific variant classification rules, supported by robust in vivo testing models.

References:
Grants:
Conflict of Interest: None declared.

C02.4 Colorectal cancer risk: the interplay of polygenic background, high-impact monogenic variants, and family history

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Background/Objectives: Summarized in polygenic risk scores (PRS), the effect of common genetic variants for colorectal cancer (CRC) can be used for risk stratification.

Methods: To assess the combined impact of the PRS and other main factors on CRC risk, 163,516 individuals from the UK Biobank were stratified according to: carrier status for germline pathogenic variants (PV) in CRC susceptibility genes (APC, MLH1, MSH2, MSH6, PMS2); low (<20%), intermediate (20-80%), or high (>80%) PRS; and family history (FH) of CRC. Multivariable logistic regression and Cox proportional hazards models were applied to compare odds ratios (OR) and to compute lifetime incidences, respectively.

Results: Depending on the PRS, the CRC lifetime incidence for non-carriers ranged between 6% and 22%, compared to 40% and 74% for carriers. A positive FH of CRC was associated with an increase in the cumulative incidence to 26% in non-carriers and 98% in carriers. In non-carriers with a negative FH, but a high PRS, the CRC risk was doubled, whereas a low PRS even in the context of a FH resulted in a decreased risk. The full model including PRS, carrier status, and FH improved the area under the curve for risk prediction (0.704).

Conclusion: Irrespective of a sporadic or monogenic background, the CRC risk was strongly influenced by the PRS. FH, PV, and common variants all complementary contributed to CRC risk. The implementation of PRS in routine care would likely improve personalized risk stratification, which will guide tailored preventive surveillance strategies in high, intermediate, and low risk groups.

References:
Grants:
Conflict of Interest: None declared.

C02.5 Coping with incidental findings in cancer susceptibility genes after exome sequencing in paediatric patients

***Estela Carrasco López**[1;2], Adrià López-Fernández[1;2], Marta Codina-Sola[3], Sara Torres-Esquius[1], Guillermo Villacampa[4], Victor Navarro[4], Irene Valenzuela[3], Belen Perez Dueñas[5], Anna Maria Cueto-González[3], Orland Diez Gibert[1;3], Maite Torres[1], Dolors Palau[3], Mara Cruellas[1;2], Constantino Sábado[6], Elena Garcia Arumi[3], Eduardo Tizzano[3], Lucas Moreno[6], JUDITH BALMAÑA[1;2]*

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Background/Objectives: Exome sequencing may identify pathogenic variants (PV) unrelated with the main purpose of the analysis. We investigated the frequency of secondary and incidental findings (IF) in cancer susceptibility genes (CSG), listed by the American College of Medical Genetics (ACMG SF V2), clinical actionability, and psychological impact in families with an IF (cases) compared with families with cancer history and studied with CSG panel (controls).

Methods: We analysed 533 exomes to identify IF. Medical records of all carriers were reviewed. Psychological impact was analysed with MICRA scale 2-6 months after results disclosure. Propensity score weighting method was used to adjust imbalance in prognostic factors in the comparison in cases ($n = 32$) and controls ($n = 576$).

Results: IF's frequency in CSG was 2% (11 index cases): 1.8% from ACMG list, three BRCA2, three PMS2, two SDHB, one BRCA1 and one MLH1; and one in RAD51C (0.2%). Predictive testing was performed in 42 relatives (18 were carriers). Out of 29 carriers, 20 enrolled for surveillance, that yielded a cancer diagnosis in four (20%): paraganglioma in three SDHB carriers, breast cancer in one BRCA2 carrier. Concerning psychological impact, MICRA mean scores in cases and controls were 21.3 versus 16.2 (total), 6.3 versus 4.2 (distress), and 8.3 versus 6.6 (uncertainty), all $p < 0.001$.

Conclusion: PVs in CSG were identified in 2% of patients who underwent exome testing for reasons unrelated to cancer predisposition. The psychological impact was higher when variant was identified as an IF. However, IF allowed early detection and prevention in families without a prior cancer diagnosis.

References:

Grants:

Conflict of Interest: Estela Carrasco López: None declared, Adrià López-Fernández: None declared, Marta Codina-Sola: None declared, Sara Torres-Esquius: None declared, Guillermo Villacampa GV has received research honoraria for speakers bureau from MSD and Pierre Fabrer and advisory role from Astrazeneca, Victor Navarro: None declared, Irene Valenzuela: None declared, Belen Perez Dueñas: None declared, Anna Maria Cueto-González: None declared, Orland Diez Gibert: None declared, Maite Torres: None declared, Dolors Palau: None declared, Mara Cruellas Rio Ortega Grant.

AECC Grant, Constantino Sábado: None declared, Elena Garcia Arumi: None declared, Eduardo Tizzano: None declared, Lucas Moreno data monitoring committees for clinical trials sponsored by Novartis, Actuate Therapeutics, Shionogi, Incyte, the University of Southampton and the Royal Marsden NHS Foundation Trust; had a consulting role for Novartis, Norgine, Boehringer, Ymabs and Shionogi and participated in educational activities organized by Bayer and Eusa Pharma.

s member of the Executive Committee of SIOPEN (European neuroblastoma research cooperative group), an organisation which receives royalties for the sales of dinutuximab beta.

, My institution receives funding from sponsors for DMC participation, advisory role or conducting industry-sponsored clinical trials., JUDITH BALMAÑA Consultant or advisory board for AZ and Pfizer.

C02.6 Cancer risks by cancer type, gender and PTEN variant type in a large cohort of PTEN Hamartoma Tumour Syndrome patients

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Background/Objectives: PTEN Hamartoma Tumour Syndrome (PHTS) is rare with diverse phenotypes, including increased risks for breast (BC, 67-78% at age 60), endometrial (EC, 19-28%) and thyroid cancer (TC, 6-38%). Current risks are likely overestimated due to ascertainment bias. We aimed to provide more accurate and personalized cancer risks.

Methods: A European, adult PHTS cohort study with data from medical files, registries and/or questionnaires. Cumulative lifetime (CLTRs) and hazard ratios were assessed with Kaplan-Meier and Cox regression analyses, and standardized incidence ratios (SIR) were calculated. Ascertainment bias was addressed by excluding cancer indexes and incident case analyses.

Results: 446 patients were included, 48% indexes, 374 with prospective follow-up years (median 6, IQR:3-10)), 159/278 females and 39/168 males had cancer. CLTRs at age 60 ranged from 68% to 85% for females (95%CI-width: 23% and 25%) and 16% to 37% for males (95%CI-widths: 25% and 62%). Female BC CLTRs ranged from 54% to 73% (95%CI-width: 24% and 30%), with two-to-three-fold increased risks by *PTEN* variant type and domain. EC CLTRs ranged from 5% to 18% (95%CI-widths: 16% and 25%). TC CLTRs ranged from 9% to 21% (95%CI-widths 10% and 35%). Colorectal, renal cancer and melanoma risks were <10% each.

Conclusion: Females have genotype-related BC risks. Highest risks include BC, EC, and TC for females, and TC for males. Risks for colorectal, renal cancer and melanoma should not be an important focus of surveillance. The lower, more accurate and personalized cancer risks from this study provide guidance for optimized risk management.

References:

Grants: PTEN Research foundation 17-001

Conflict of Interest: None declared.

C03 CELLULAR DIFFERENTIATION AND REGULATION

C03.1 Mapping the functional impact of immune-associated regulatory elements through single-cell CRISPR-based screens

Celia Alda Catalinas[1], *Ximena Ibarra-Soria*[2], *Jorge Esparza Gordillo*[2], *Christina Flouri*[1], *Adrian Cortes*[2], *Alison Acevedo*[3], *Gerard Drewes*[1], *Radu Rapiteanu*[1]

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Background/Objectives: Drug targets with human genetic evidence are twice as likely to lead to approved drugs. Yet, translating disease-associated genetic variants into functional knowledge remains a fundamental challenge of early drug discovery. GWAS and QTL studies are powerful tools to address these challenges, however, a majority of all disease associated variants cannot be mapped to causal genes by current methods.

Methods: We developed a CRISPR-based, single-cell functional screening approach in primary human CD4+ T cells that enables interrogation of transcriptomic changes induced by modulation of regulatory elements at scale. We first implemented and optimized highly efficient CRISPRi in primary CD4+ T cells, and we showed that these perturbations can be read-out by single-cell transcriptomics using a CROP-seq-based approach. Subsequently, we performed a proof-of-concept pooled screen targeting previously-validated intergenic regulatory elements and transcription start sites (TSSs), profiling approximately 280,000 CD4+ T cell single-cell transcriptomes.

Results: After systematically interrogating different analytical approaches to map regulatory elements to genes (SCEPTRE, Limma-Voom, MAST and Wilcoxon rank sum test), we were able to robustly detect the expected target gene downregulation for >90% of the control TSSs and the expected cis effects for >75% of the intergenic elements perturbed, validating our method.

Conclusion: We present a functional genomics approach that enables the generation of enhancer-gene maps at scale in a disease-relevant in vitro mode, opening up exciting new avenues for disease target identification with genetic evidence.

References: Gasperini, M. et al. (2019). A genome-wide framework for mapping gene regulation via cellular genetic screens. Cell 176, 377–390.e19.

Grants: None.

Conflict of Interest: None declared.

C03.2 Extreme long-range gene regulatory perturbation drives a human craniofacial disorder

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Background/Objectives: Increasingly, mutations in the non-coding genome are implicated in Mendelian developmental disorders and complex disease. Mutations far upstream of the *SOX9* gene have been identified in patients with isolated Pierre Robin sequence (PRS), a congenital disorder characterised by underdevelopment of the lower jaw (micrognathia). We set out to explore disease mechanisms for PRS, hypothesising that patient mutations may ablate distal-acting regulatory elements such as enhancers.

Methods: We leverage directed differentiation of human embryonic stem cells (hESCs) to cranial neural crest cells (CNCCs), progenitor cells that give rise to the majority of the vertebrate face. Using genome editing in hESCs and mouse we model PRS mutations to explore both impact on *SOX9* expression and facial development.

Results: We identify and characterise two enhancer clusters that regulate *SOX9* expression over extreme genomic distances (up to 1.45Mb), act during a restricted window of craniofacial development and are conserved in activity down to Coelacanth fish. Mouse models of PRS provide evidence for a novel disease mechanism and demonstrate how small changes in gene dosage can lead to morphological variation. Using imaging-based approaches, we explore dramatic changes in 3D conformation of the *SOX9* locus during CNCC differentiation and identify a structural element required for locus topology and robust *SOX9* expression.

Conclusion: Together, we functionally characterize some of the most extreme long-range regulatory elements implicated in human disease, propose mechanisms driving tissue-specificity in an enhanceropathy, and uncover a role for locus topology in robust gene activation at a human disease locus.

References: Long et al, Cell Stem Cell, 2020.

Grants: 106051/Z/14/Z.

Conflict of Interest: None declared.

C03.3 Leveraging interindividual variability of regulatory activity refines genetic regulation of gene expression in schizophrenia

Maris Alver[1,2], *Nikolaos Lykoskoufis*[1], *Anna Ramisch*[1], *Halit Ongen*[1], *Emmanouil Dermitzakis*[1]

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Background/Objectives: Schizophrenia is a heritable and polygenic psychiatric disorder with limited understanding about the mechanistic changes in gene expression regulation.

Methods: To elucidate on this, we integrated interindividual variability of regulatory activity (ChIP-seq for H3K27ac) with gene expression and genotype data captured from the prefrontal cortex of 272 cases and controls.

Results: By systematically measuring interindividual correlation between proximal chromatin peaks, we show that regulatory element activity is structured into 10,936 and 10,376 cis-regulatory domains (CRDs) in cases and controls, respectively, of which 42% are shared. Differentially expressed genes were significantly enriched at differentially active CRDs (Fisher $P = 8.72 \times 10\text{-}6$, $OR = 1.60$). In SCZ cases, at 5% FDR, we discovered in cis 867 and 796 expression QTLs (eQTLs) and CRD activity QTLs (aCRD-QTLs), respectively, of which 130 eQTLs and 40 aCRD-QTLs showed effect only in SCZ. To study the interplay among genetic variants, gene expression and CRDs, we applied Bayesian Networks to infer the most likely causal relationship for QTLs affecting both CRDs and genes. While 2/3 of the studied triplets displayed the same direction of effect from QTL onto gene/CRD in both groups, 1/3 of triplets showed change in directional effect from QTL variant onto gene expression in cases vs controls. These directional change-associated genes cluster in molecular functions related to synaptic function and dendritic spine morphology.

Conclusion: We show that accounting for coordinated regulatory activity provides a novel mechanistic approach to reduce

the search space for unveiling genetically perturbed regulation of gene expression in schizophrenia.

References:

Grants: NCCR 51NF40-158776; ETAg PUTJD901.

Conflict of Interest: Maris Alver: None declared, Nikolaos Lykoskoufis: None declared, Anna Ramisch: None declared, Halit Ongen: None declared, Emmanouil Dermitzakis E.T.D is currently an employee of GSK. The work presented in this manuscript was performed before he joined GSK.

C03.4 Sex differences in gene expression in human induced pluripotent stem cells

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Background/Objectives: Human induced pluripotent stem cells (hiPSCs) are used for biological modeling in relevant human cell types. Previously reported X chromosome inactivation (XCI) erosion in prolonged hiPSC cultures has raised a concern about their utility to recapitulate trait-relevant sex differences. To assess the utility of hiPSCs in the study of sex effects, we examined the gene expression differences (sex-DE) in male and female lines and estimated the impact of XCI erosion to the inference of sex-DE.

Methods: We examined sex-DE genes using RNA-seq data of hiPSCs generated from fibroblasts of 122 male and 173 female healthy donors from Human Induced Pluripotent Stem Cell Initiative (HipSci). We used allele-specific expression of X chromosome genes and *XIST* expression levels to identify eroded-XCI. We compared the sex-DE genes in hiPSCs with human tissues using data from GTEx.

Results: Compared to males and normal females, the XCI-eroded female lines (~30%) had increased expression of X chromosome genes and additional effects to autosomal gene expression. Excluding the eroded lines improved the specificity of sex-DE analysis, yielding over 4000 sex-DE genes in hiPSCs. Comparison to postmortem human tissues revealed marked overlap in pattern of sex-DE with the highest resemblance with fibroblasts (~15% shared sex-DE).

Conclusion: Our results demonstrate that sex-DE is abundant in hiPSCs including both shared and distinct effects compared to somatic tissues. The closest resemblance in sex-DE was with the cell-type of origin, suggesting potential somatic memory in hiPSCs. Additionally, inclusion of XCI-eroded lines may hamper detection of sex-DE.

References:

Grants:

Conflict of Interest: None declared.

C03.5 Systematic reconstruction of cellular trajectories across mouse embryogenesis (virtual)

Chengxiang Qiu[1], *Junyue Cao*[2], *Beth Martin*[1], *Tony Li*[1], *Ian C. Welsh*[3], *Sanjay Srivatsan*[1], *Xingfan Huang*[1], *Diego Calderon*[1], *William Stafford Noble*[1], *Christine M. Disteche*[1], *Stephen A. Murray*[3], *Malte Spielmann*[4;5], *Cecilia B. Moens*[6], *Cole Trapnell*[1], *Jay Shendure*[1]

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Background/Objectives: Mammalian embryogenesis is characterized by rapid cellular proliferation and diversification. Within a few weeks, a single cell zygote gives rise to millions of cells expressing a panoply of molecular programs, including much of the diversity that will subsequently be present in adult tissues. Although intensively studied, a comprehensive delineation of the major cellular trajectories that comprise mammalian development in vivo remains elusive.

Methods: Here we set out to integrate several single cell RNA-seq datasets (scRNA-seq) that collectively span mouse gastrulation and organogenesis. To bridge technologies, these datasets were supplemented with new, intensive profiling of ~150,000 nuclei from a series of E8.5 embryos in 1-somite increments with an improved combinatorial indexing protocol. Overall, we define cell states at each of 19 successive stages spanning E3.5 to E13.5, heuristically connect them to their pseudo-ancestors and pseudo-descendants, and for a subset of stages, deconvolve their approximate spatial distributions.

Results: Despite being constructed through automated procedures, the resulting trajectories of mammalian embryogenesis (TOME) are largely consistent with our contemporary understanding of mammalian development. We leverage TOME to nominate transcription factors (TF) and TF motifs as key regulators of each branch point at which a new cell type emerges. Finally, we apply the same procedures to single cell datasets of zebrafish and frog embryogenesis, and nominate "cell type homologs" based on shared regulators and transcriptional states.

Conclusion: We believe that this framing provides a useful entry point for analyses that benefit from a global view of developmental processes.

References:

Grants:

Conflict of Interest: Chengxiang Qiu The Bonita and David Brewer Fellowship, This work was supported by the Bonita and David Brewer Fellowship to C.Q., Junyue Cao The Rockefeller University, Beth Martin University of Washington, Tony Li: None declared, Ian C. Welsh The Jackson Laboratory, Sanjay Srivatsan: None declared, Xingfan Huang: None declared, Diego Calderon: None declared, William Stafford Noble University of Washington, Christine M. Disteche University of Washington, Stephen A. Murray The Jackson Laboratory, Malte Spielmann Max Planck Institute for Molecular Genetics, Cecilia B. Moens Fred Hutchinson Cancer Research Center, Cole Trapnell University of Washington, Jay Shendure University of Washington, This work was supported by Paul G. Allen Frontiers Foundation, the National Institutes of Health (UM1HG011531). J.S. is an investigator of the Howard Hughes Medical Institute.

C03.6 Identification of genetic variants that impact gene co-expression relationships using large-scale single-cell data

Shuang Li[1], *Katharina Schimid*[2;3], *Dylan de Vries*[1], *Maryna Korshevniuk*[1], *Morris Swertz*[1], *Harm-Jan Westra*[1], *Monique van der Wijst*[1], *Matthias Heinig*[2;3], *Lude Franke*[1]

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Background/Objectives: Expression quantitative trait loci (eQTL) studies have shown how genetic variants (SNPs) affect downstream gene expression. To understand the underlying upstream regulatory processes, single-cell data offers the unique opportunity to reconstruct personalized co-expression networks. By exploiting the large number of cells per individual, we can identify

SNPs altering co-expression patterns (i.e.co-expression QTLs, co-eQTLs) using a limited number of individuals.

Methods: To tackle the large multiple testing burden (SNP-Gene1-Gene2 combinations) associated with a genome-wide analysis, we conducted a co-eQTL meta-analysis across 3 scRNA-seq PBMC datasets (177 participants, 1M cells) with a novel filtering strategy, followed by a permutation-based approach.

Results: Using this strategy, we identified a robust set of cell-type-specific co-eQTLs for 73 independent SNPs, affecting 946 gene pairs. These co-eQTLs provide novel insights into how disease-associated variants alter regulatory networks. For instance, the type I diabetes (T1D) SNP rs1131017 affects the co-expression between RPS26 and other ribosomal genes. Interestingly, the comparison across cell types showed distinct co-eQTLs related to T cell activation specifically within T-lymphocytes, revealing a previously overlooked process that could explain its association with T1D. This example highlights the importance of studying gene regulation at the context-specific level to understand the biological implications of genetic variation.

Conclusion: With the expected growth of sc-eQTL datasets, our strategy will soon identify many more co-eQTLs that help elucidate unknown disease mechanisms.

References: van der Wijst, Nature Genetics 2018.
Oelen, bioRxiv, 2021.

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NWO Vici, Oncode Investigator grant (LF).

Conflict of Interest: Shuang Li University Medical Center Groningen, Katharina Schimid Helmholtz Center Munich, Dylan de Vries University Medical Center Groningen, Maryna Korshevniuk University Medical Center Groningen, Horizon2020 №860895, Morris Swertz University Medical Center Groningen, NWO VIDI grant number 917.164.455, Harm-Jan Westra University Medical Center Groningen, Monique van der Wijst University Medical Center Groningen, NWO Veni 192.029, Matthias Heinig Helmholtz Center Munich, BMBF computational life science 031L0203A, CZI heart seed network 2019-202666, Lude Franke University Medical Center Groningen, NWO Vici, Oncode Investigator grant.

C04 UNRAVELLING CAUSES AND MECHANISMS OF MULTIPLE CONGENITAL ANOMALIES

C04.1 Solving patients with rare diseases within Telethon Undiagnosed Disease Program through reanalysis of exome-phenome data

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Background/Objectives: The Telethon Undiagnosed Diseases Program (TUDP) is a national program with the objective of identifying the causes of rare pediatric-onset single-gene disorders. Through whole exome sequencing (WES) in trio, TUDP reaches a genetic diagnosis in 50 percent of a total of 660 families. As the periodic WES variant-level data re-evaluation, and case-level data re-analysis increases the diagnostic yield, due to the constantly expanding of technical developments and scientific understanding, we then re-analyzed the cases that remained undiagnosed within the TUDP.

Methods: Sequencing raw data (FastQ) derived from still-undiagnosed patients and their parents were processed using the VarGenius analysis pipeline[1] based upon the updated Genome Analysis Tool-Kit (GATK4.0.5). Furthermore the phenotypic and genomic data sets were processed by the pipeline of the Horizon 2020 Solve-RD project[2], and were shared internationally on matchmaker exchange platforms.

Results: We found pathogenic variants in 30% of patients. For 23% of cases, disease-causing variants were identified in genes that were recognized as disease genes while the TUDP study was ongoing. For 40% of cases, pathogenic variants were identified in genes that had never previously been associated with Mendelian diseases.

Conclusion: We report the usefulness of periodic re-evaluation and re-interpretation of negative WES data associated with the systematical implementation of the informatic tools and with the updated biological database. Our approach revealed the molecular bases of a significative portion of previously undiagnosed rare diseases patients within TUDP, which lies within already produced data.

References: Musacchia F, et al. 2018
Zurek B, et al. 2021.

Grants: Telethon Undiagnosed Diseases Program (TUDP, GSP15001).

Conflict of Interest: None declared.

C04.2 Genetic and mechanistic dissection of non-muscle actinopathies caused by ACTB or ACTG1 variants

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Background/Objectives: ACTB and ACTG1 encode highly conserved cytoskeletal non-muscle actins. Some disorders caused by ACTB/ACTG1 variants have been described, but several remain undefined. Underlying disease-mechanisms are poorly understood.

Methods: We performed clinical data analysis from individuals with (likely) pathogenic ACTB/ACTG1 variants and characterized patient cells, mutant proteins, patient-derived iPSC-based models and mutant mice.

Results: Data from 279 patients collected via 110 members of non-muscle actinopathies consortium showed that ACTB loss-of-function (LOF) variants/gene-deletions cause a syndrome with mild neurodevelopmental delay and malformations; ACTG1 LOF variants/gene-deletions increase susceptibility for neurodevelopmental problems; ACTB missense variants (MVs) cause Baraitser-Winter-Cerebrofrontofacial syndrome (ACTB-related BWCFFS) or dystonia-deafness syndrome (single recurrent MV) or a disorder phenotypically overlapping with that caused by LOF ACTB variants; and ACTG1 MVs cause ACTG1-related-BWCFFS or isolated deafness or colobomas. Some pathogenic ACTB/ACTG1 variants still remained phenotypically unclassified.

ACTB protein levels were normal in fibroblasts of patients with ACTB pLOF variants, but ACTA2 was significantly upregulated on both mRNA and protein level, indicating likely dysregulated transcriptional adaptation. However, other ACTB/ACTG1 MVs did not have major impact on overall gene expression. MVs causing 'ACTB-pLOF-phenotype' led to protein degradation during recombinant production suggesting functional haploinsufficiency. Other ACTB/ACTG1 MVs impaired polymerization/depolymerization and/or disturbed actin-myosin interaction when co-polymerised with WT suggesting dominant negative effects. iPSC-derived BWCFFS cortical neuroprogenitors and neurons showed impaired differentiation and migration compared with controls. BWCCFS variants in mice were embryonically lethal.

Conclusion: We delineate eight disorders caused by ACTB/ACTG1 variants (non-muscle actinopathies), define their likely genotype-disorder relationships and demonstrate the diversity of their underlying mechanisms.

References:

Grants:

Conflict of Interest: None declared.

C04.3 ADGRL1 haploinsufficiency causes a variable spectrum of neurodevelopmental disorders in humans and in a mouse model with altered synaptic activity

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Background/Objectives: ADGRL1/latrophilin-1, a well-characterized adhesion G protein-coupled receptor, has been implicated in synaptic development, maturation and activity. However, to date the link between ADGRL1 dysfunction and human disease has not been demonstrated yet.

Methods: Medical evaluation of patients with neurodevelopmental disorders, including mild to moderate intellectual disability, global developmental delay and behavioral disorders followed by comprehensive genetic analysis, in vitro characterization of the identified variants and mouse modelling of *Adglr1* loss of function were utilized in our study.

Results: Here, we describe ten individuals presenting with variable neurodevelopmental features including developmental delay, intellectual disability, attention deficit hyperactivity, autism spectrum disorders and epilepsy, all with heterozygous pathogenic variants in *ADGRL1*. In vitro, our molecular data demonstrate that the pathological functions of the variants found in our cohort are consistent with haploinsufficiency. In vivo, mice carrying heterozygous *Adgrl1* null allele exhibited neurological and developmental abnormalities, overlapping with neurodevelopmental manifestations identified in humans. Ex vivo synaptic preparations displayed altered spontaneous neurotransmitter release.

Conclusion: We dissect the pathophysiological mechanisms leading to synaptic dysfunction in *Adgrl1* mice and provide a detailed characterization of the associated behavioral phenotypes.

Our data demonstrate that *ADGRL1* haploinsufficiency accounts for a spectrum of developmental, neurological and behavioral abnormalities in mice and humans.

References:

Grants:

Conflict of Interest: None declared.

C04.4 Oligogenic inheritance in neural tube defects: major involvment of primary cilia, planar cell polarity and extracellular matrix genes

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Background/Objectives: Neural tube defects (NTDs) are developmental disorders that affect 5 out of 10,000 births resulting in an incomplete closure of the neural tube. Studies in mouse and human suggest an oligogenic inheritance involving environmental and genetic factors.

Methods: We performed exome sequencing on 28 families and 78 patients of French origin with NTDs. We focused on a virtual panel of candidate genes involved in NTDs in human, mouse and in pathways of interest. Oligogenic combinations of selected rare and deleterious variants have been identified based on familial analysis and segregation. In a second phase, conducted in French patients, we searched for an enrichment of these oligogenic combinations in comparison with a control cohort. This analysis has been carried on a second independent American cohort of 248 NTDs patients.

Results: Fifty four percent of the families showed oligogenic combinations in 88 candidate genes. Pathways involved are primary cilia (17%), planar cell polarity (12%), extracellular matrix (ECM) (12%), Wnt/Notch/Sonic hedgehog (SHH) (9%), apoptosis (9%) and folate metabolism (6%). Next, we found a significant enrichment of oligogenic combinations focused on genes selected from the families in the French cohort (p-value=3,78E-11) and in the American cohort (p-value=1,48E-15). Genes significantly involved in both cohorts are *HSP2* (ECM), *FREM2* (ECM), *PCTN* (Primary cilia), *TSC2* (tumor supressor), *CUL7* (Proteic metabolism) and *DISP1* (SHH pathway).

Conclusion: NTDs are complex diseases characterised by a high genetic heterogeneity involving more than one variant (gene). The mentioned pathways have an impact on cells migration and apoptosis which are major mechanisms in neural tube closure.

References:

Grants:

Conflict of Interest: None declared.

C04.5 Genomic studies in analysis of human fetal anomalies

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Background/Objectives: Fetal anomalies affect 3.5% of pregnancies. Approximately 50% of all birth defects cannot be linked to a specific cause. Detailed phenotyping would also be challenging in fetal life. Genomic tests appear to be the most promising way to discover the underlying defects. We aim to integrate genomics and perinatal autopsy in this study.

Methods: We evaluated fetuses/perinatal losses referred for pathological examination. A detailed perinatal autopsy was performed in all after taking an informed consent. Targeted gene sequencing (TS), chromosomal microarray (CMA), exome (ES) and genome sequencing (GS) were carried out in fetuses as feasible.

Results: Six-hundred-and-seventy-eight fetuses were evaluated over thirteen-years with gestational age ranging 10-39 weeks. Seventy fetuses (40 males and 30 females) underwent genetic evaluation (TS-7, CMA-18, ES-52 and GS-5). Genomic analysis is complete in 61 families and diagnoses were established in 50/61 (81.9%). A monogenic etiology was identified in 40 families and chromosomal abnormalities in 10 families. The study also lead to the identification of new disease-gene associations [RSPO2 (MIM#618022); MYLPF (MIM*617378); KIF21A (severe arthrogryposis)] and rare phenotypes [ADGRG6 (MIM#616503); MAP3K7 (MIM#157800); AGRN (lethal arthrogryposis); DOK7 (MIM#618389)]. We also delineate an unrecognized phenotype with agenesis of corpus callosum, bilateral facial clefts, polydactyly of hands and mirror-image polydactyly of feet with single leg bone without an identifiable molecular etiology.

Conclusion: Integration of genomic tests in perinatal evaluation improves diagnosis and counselling for affected families.

References:

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Conflict of Interest: None declared.

C04.6 A novel syndrome caused by the constitutional gain-of-function variant p.Glu1099Lys in NSD2

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Background/Objectives: *NSD2* di-methylates histone H3 at lysine 36 (H3K36me2). The encoding gene is located in the contiguous gene deletion region on chromosome 4p that is critical for Wolf-Hirschhorn syndrome (WHS). Recent descriptions delineated loss-of-function (LoF) variants in *NSD2* with an overlapping, but milder disorder characterized by Russell-Silver syndrome-like features. Here, we describe two individuals carrying the variant p.Glu1099Lys, which has been recurrently identified somatically in leukemia and has a catalytically hyperactivating gain-of-function (GoF) effect.

Methods: Trio exome sequencing with subsequent Sanger sequencing in ectodermal tissues as well as in-depth phenotyping

was performed for both individuals. We compared their phenotypes with individuals with LoF-variants and used omics data from the Cancer Cell Line Encyclopedia (CCLE) to analyse histone modification, DNA-methylation, and RNA-expression.

Results: In both individuals, the heterozygous variant c.3295G>A, p.Glu1099Lys in *NSD2* occurred *de novo* and was confirmed in blood and in independent ectodermal tissues. Both individuals shared a highly similar phenotype including specific dysmorphic features (coarse facial features, square face, large hands with fleshy fingers), organomegaly, and developmental delay. When comparing the symptoms to cohorts from the literature with *NSD2* LoF variants or deletions, we observe wide discrepancies in phenotypes between the groups, thus underlining the opposing pathomechanisms (LoF vs. GoF). We confirm increased H3K36me2 in lines with either GoF variants or *NSD2* duplications. GoF lines show dysregulated gene modules involved in white blood cell activation, cell growth and organ development.

Conclusion: We described a novel specific syndrome of developmental delay due to the heterozygous GoF p.Glu1099Lys variant in *NSD2*.

References:

Grants:

Conflict of Interest: None declared.

C05 POPULATION AND EVOLUTIONARY GENETICS

C05.1 Ancestral genomic contributions to complex traits in contemporary Europeans

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Background/Objectives: The contemporary European genetic makeup formed in the last 8000 years when local Western Hunter-Gatherers mixed with incoming Anatolian Neolithic farmers and Pontic Steppe pastoralists. Previous studies inferred phenotypes in these source populations and investigated how they were influenced by natural selection [1]. However, how ancient populations contributed to present-day phenotypic variation is poorly understood. Here we investigate how the unique tiling of genetic variants inherited from different ancestral components drives the complex traits landscape of contemporary Europeans, and quantify selection patterns associated with these components.

Methods: Using matching individual-level genotype and phenotype data for 27 traits in the Estonian biobank and genotype data directly from the ancient source populations, we quantify the contributions from each ancestry to present-day phenotypic variation in each complex trait. To do so we introduce covA, a measure of the relative similarity between any contemporary individual and the ancestries that contributed to its genetic makeup.

Results: We find substantial differences in ancestry for eye and hair colour, body mass index, waist/hip circumferences and their ratio, height, cholesterol levels, caffeine intake, heart rate and age at menarche. Furthermore, we find evidence for recent positive selection linked to four of these traits and, in addition, sleep patterns and blood pressure.

Conclusion: Our results show that these ancient components were differentiated enough to contribute ancestry-specific signatures to the complex trait variability displayed by contemporary Europeans.

References: [1] Mathieson et al. 2015 (https://doi.org/10.1038/nature16152).

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Conflict of Interest: None declared.

C05.2 Phenome-wide perspective into the role of Finnish Y chromosome variation in complex diseases

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Background/Objectives: The Y chromosome (chrY) is routinely excluded from genetic association studies due to the peculiar biology and analytical challenges specific to chrY. Consequently, potential impacts of chrY genetic variation on complex disease remains largely uncharacterized.

Methods: To broadly investigate the impacts of Y-chromosomal variation on complex disease, we are examining the extensive FinnGen project data for 72,198 Finnish male samples, which have been genotyped on arrays with sufficient number of chrY markers. Currently, we have finalized the analyses of 69 cardiovascular phenotypes and are expecting to complete the association analyses for the remaining 711 endpoints in the coming months. We study the associations both on the Y-chromosomal haplogroup level (including Finnish main haplogroups N1a1, I1, R1a, and R1b) as well as using other available variants on the Y chromosome ($N = 554$ post-QC).

Results: Across the cardiovascular endpoints, we observed four significant ($p < 0.05/69/554 = 1.3e\text{-}6$) risk-increasing associations ($OR \geq 1.38$) between operated calcific aortic valvular stenosis and chrY variants, all of these variants strongly correlating ($R^2 \geq 0.97$) with haplogroup I1. On the main haplogroup-level, we observed no significant results, but detected nominal risk-increasing effects for I1 with myocardial infarction ($OR = 1.04$, $p = 0.01$), which supports previous studies from the UK linking haplogroup I1 with a higher risk for coronary artery disease.

Conclusion: These first results highlight that genetic variants on chrY may contribute to complex disease risk in Finland, yet validation in larger data sets are warranted. Overall, our work serves as the first step towards comprehensive understanding of the role of Y-chromosomal variation in complex diseases.

References:

Grants:

Conflict of Interest: None declared.

C05.3 Disentangling signatures of selection before and after European colonization in Latin Americans

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Background/Objectives: Despite admixture being ubiquitous among human populations, there are few techniques that explicitly account for this admixture when identifying genetic loci facilitating adaptation.

Methods: We describe a novel statistical model, specific to admixed populations, that identifies loci under selection while determining whether the selection occurred post-admixture or prior to admixture in one of the ancestral populations. We assess the performance of our method under realistic demographic settings and compare it to state-of-the-art approaches. We then apply this method to genome-wide data of ~4,000 individuals in five Latin American countries.

Results: We demonstrate our approach has similar or increased power to detect selection relative to state-of-the-art, while also accurately determining whether selection occurred in the ancestral or admixed population. Our selection scan in Latin Americans replicates previous reports of selection, yet also identifies selection evidence at >20 novel signals. These include immune-related genes that indicate responses to pathogen exposures in the Americas both before and after European contact. Further, the strongest overall signal indicates selection in a Native American ancestral group related to present-day Andeans, at a locus implicated in preeclampsia risk, which shows increased incidence in populations living at high elevations.

Conclusion: We present a novel method that identifies adaptive loci in admixed populations, outperforming state-of-the-art in certain scenarios and capable of classifying whether selection occurred before or after admixture. Our analyses provide new insights into the adaptive traits necessary for the early habitation of the Americas and subsequent responses to pathogens corresponding to European contact.

References:

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Conflict of Interest: None declared.

C05.4 Dense sampling of ethnic groups within five African countries reveals extensive historical admixture

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Background/Objectives: While multiple studies have explored genetic signatures of historic empires and migrations in European populations, comparatively fewer have done the same with African genomes. Here we infer admixture events in populations spanning east to west Africa and investigate their links with recorded African history.

Methods: We present analyses of unpublished genetic data comprising >500,000 polymorphic loci typed in ~1360 individuals from ~150 ethnolinguistic groups from Cameroon, Ghana, Nigeria, the Republic of the Congo and Sudan, including >80 groups for which we are not aware of genome-wide data previously being published. We compare inference from multiple methods designed to detect and date putative admixture events.

Results: We highlight strong genetic differences among geographically proximate groups, e.g. living within 20 kilometres. We infer multiple novel signatures of admixture spanning Africa, with inferred dates overlapping across different methods in the majority of cases. We link admixture signals and their associated date estimates to potential underlying historical events. Notable examples include >1200-year-old admixture in northern Cameroon that corresponds with archaeological evidence for an increase in exotic grave goods, intermixing that spans the large trade networks of the early Kanem Empire (700-1400CE) and admixture in coastal Sudan with dates overlapping the Kingdom of Aksum (100-960CE).

Conclusion: Our findings highlight how genetic data can complement historical and archaeological research to create a

powerful framework for understanding the past. Our results emphasize the importance of denser sampling of African regions to recover a more refined picture of history.

References:

Grants:

Conflict of Interest: None declared.

C05.5 Assessment of mitochondrial genome constraint across >50,000 individuals in gnomAD to facilitate variant interpretation

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Background/Objectives: Mitochondria carry a genome (mtDNA) encoding proteins and RNAs. Pathogenic mtDNA variants underlie diverse phenotypes, collectively called mitochondrial diseases. Mitochondrial variant interpretation is challenging, and many variants remain of uncertain significance. Constraint models for the nuclear genome are extremely useful for variant analysis and interpretation. Here we developed the first mitochondrial constraint model and applied it to the gnomAD database with mtDNA variation across 56,434 individuals.

Methods: We used a composite likelihood model and de novo mutation datasets to quantify mutability in trinucleotide contexts. Given selection occurs against the number and heteroplasmy of mtDNA variants, we applied the model to determine the expected sum maximum heteroplasmy of variants in gnomAD. We assess constraint as observed:expected ratios with 90% confidence intervals (CI).

Results: The model accurately predicted the level of neutral variation in gnomAD, establishing its utility (correlation coefficient $R = 1$). The overall synonymous observed:expected ratio was ~1.0 (ratio = 0.99, CI = 0.97-1.01) and consistent across genes (CI upper bounds 0.94-1.17), unlike missense variants which showed lower and variable constraint (ratio = 0.26, CI upper bounds 0.15-0.96). Pathogenic variation in ClinVar was substantially constrained (ratio = 0.12, CI = 0.08-0.17). Analyses across and within genes/loci identified constraint, including in regions overlooked in disease analyses (e.g. rRNA, non-coding elements). Lastly, we demonstrate the utility of this model for variant interpretation, including by showing enrichment of pathogenic variants in areas of regional constraint, and improved in silico prediction.

Conclusion: This work provides new tools for mtDNA analysis and novel insight into which mtDNA regions are most important to function.

References:

Grants:

Conflict of Interest: None declared.

C05.6 The Landscape of the Immune System Over the Last 10,000 Years of Human Evolution

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Background/Objectives: The advent of ancient DNA (aDNA) data has facilitated studies of human adaptation to pathogens. Yet, aDNA studies have only confirmed a few, known immune genes in the recent adaptive history of Europeans (1). Additionally, the extent of negative selection on currently rare, deleterious variants remains an open question in the aDNA field.

Methods: Leveraging a large European database of 2,876 ancient and modern DNA samples, covering a time transect from the beginning of the Neolithic to the present, and using computer simulations together with an Approximate Bayesian Computation model to estimate genome-wide strengths (s) and onsets of positive and negative selection, we consistently explore the evolutionary landscape of the European immune system over the last 10,000 years.

Results: We identify 89 regions evolving under positive selection, enriched in novel immune-related genes. The dissection of the underlying genetic architecture revealed enrichments in missense and regulatory variants, associated to immune-related phenotypes and/or overlapping GWAS-significant variants affecting hematological traits. In particular, we show that, due to this pressure, monocyte and lymphocyte proportions have significantly increased over the last ten millennia. We also show an increased risk of developing inflammatory disorders over time, and highlight the underlying adaptive and pleiotropic role of key immune players, such as IRF1 and FUT2. Last, we experimentally evaluate two IL23R and LBP variants with the highest negative s values and demonstrate significant allele-specific impact on activity or secretion levels.

Conclusion: Our results support a scenario where pathogens contributed predominantly to the recent shaping of European genomes.

References: (1) Mathieson, 2015.

Grants: Pasteur-Roux-Cantarini.

Conflict of Interest: None declared.

C06 COUNSELLING, EDUCATION AND SERVICE DELIVERY

C06.1 Exploring Australian genetic counsellors' perceptions and readiness to deliver behaviour change

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Background/Objectives: Completion of the human genome project promised transformation in health prevention [1], yet there is little evidence that genetic testing leads to changes in health behaviour. Effective behaviour change requires a theory-driven coordinated set of activities (behaviour change techniques)[2]. Genetic counsellors are positioned to facilitate behaviour change. We aimed to explore genetic counsellors' perception and readiness for behaviour change to inform development of an intervention.

Methods: Recruitment was via the Australasian Society of Genetic Counsellors. We conducted five focus groups and one interview over Zoom with 26 genetic counsellors who had varied

training and experience. Verbatim transcripts were analysed using thematic analysis and mapped to the COM-B model [2].

Results: Three client behavioural outcomes of genetic counselling were identified: i) attend recommended appointments, ii) access information about the condition and management and communicate information to other agencies and iii) share accurate information with relevant family members. Influences on clients' behaviour, genetic counsellors' behaviour change strategies and influences on genetic counsellors' behaviour were identified. Some behaviour change techniques were identified, including providing information and encouraging self-management. There were gaps in genetic counsellors'/clients' capabilities, opportunities and motivations to facilitate/change health behaviours.

Conclusion: Although some behaviour change techniques are evident in practice, enhancing awareness and knowledge of behaviour change theories and strategies will assist genetic counsellors to effectively change clients' health behaviour.

References: 1. Shendure et al., (2019) *Cell*, *177*(1), 45-57.
2. Michie, et al., (2011) *Implementation Sci* **6**, 42.

Grants: UTS Early Career Research Capability Development Initiative (CJ and ET).

Conflict of Interest: None declared.

C06.2 The efficacy of online genetic counselling: a meta-analysis

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Background/Objectives: The efficacy of genetic counselling has been demonstrated in several previous systematic reviews and meta-analyses. Traditionally, genetic counselling has been delivered face-to-face but in recent years, in order to reach underserved populations, genetic counselling has increasingly been provided via telehealth platforms. During the Covid-19 pandemic, online genetic counselling has become the main mode of delivery. The aim of the present meta-analysis is to assess the efficacy of online genetic counselling and to identify moderators of its effect.

Methods: An extensive electronic search was conducted investigating the literature published until June 2021. This identified 1175 articles, 23 of which met the inclusion criteria. Effect size parameters and sample sizes for all variables in each study were included.

Results: Online genetic counselling has an overall statistically significant effect size, of small magnitude ($d = 0.126$). Videoconference genetic counselling ($d = 0.354$) has a significantly higher effect than telephone genetic counselling ($d = 0.105$). Pre-test online genetic counselling ($d = 0.264$) has a larger effect size than post-test online genetic counselling ($d = 0.147$). Effect of online genetic counselling is maintained at follow-up assessments ($d = 0.133$).

Conclusion: Results are comparable with other studies exploring online psychosocial interventions. Videoconference genetic counselling appears to be significantly more effective than telephone genetic counselling. This is in line with the experiences reported during the Covid-19 pandemic but clearly this is a novel topic and more research is needed for robust conclusions. Our results support online genetic counselling as a viable and effective option of service delivery to be considered.

References:

Grants:

Conflict of Interest: None declared.

C06.3 Socio-economic costs of rare diseases and the risk of financial hardship (virtual)

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Background/Objectives: To evaluate the burden of RDs in Hong Kong (HK) by estimating societal costs of RDs, investigating risk of financial hardship, and identifying associated potential drivers.

Methods: RD patients or their caregivers were recruited through HK's largest RD patient organisation, Rare Disease Hong Kong, during April-August 2020. Data on service and resource use and out-of-pocket expenditure were collected using CSRI-Ra[1]. Costs were estimated using a prevalence-based, bottom-up approach from a societal perspective. Out-of-pocket expenditure exceeding 10%, 25%, and 40% of household income were used to estimate catastrophic health expenditure (CHE), an official indicator for monitoring financial protection[2]. Multivariate analysis was performed to identify factors associated with overall costs and rate of CHE.

Results: A total of 284 independent participants across 106 unique RDs were recruited. Total RD costs in HK was estimated at $484,256HKD(€54,388)/patient annually. Direct non-healthcare costs were the highest ($193,555HKD/€21,739), followed by direct healthcare ($187,166HKD/€21,021) and indirect costs ($103,535HKD/€11,619). Baseline estimate of CHE (10% threshold) was 36.3%, significantly higher than the global estimate of 12.7%[2]. Multivariate analysis revealed that longer years since diagnosis was negatively associated with total costs ($p = 0.025$) and rate of CHE ($p = 0.002$).

Conclusion: This serves as the first to simultaneously assess societal costs and financial hardship related to RDs. Non-healthcare and indirect costs in the form of informal care support and productivity losses were substantial in this population which warrants careful health- and social-care planning.

References: 1 Chung et al. Scientific Reports(2021). https://doi.org/10.1038/s41598-021-03379-5
2 Wagstaff et al. The Lancet(2018). https://doi.org/10.1016/S2214-109X(17)30429-1.

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Conflict of Interest: Claudia Ching Yan Chung: None declared, Yvette Nga Chung Ng: None declared, Nicole Ying Ting Ng: None declared, Brian Hon Yin Chung Dr Chung serves as a member on the Scientific & Medical Advisory Committee (SMAC) of Rare Disease Hong Kong.

C06.4 Updated guidance on genetic testing in childhood from British Society for Genetic Medicine: practical ethics in the age of genomics

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Background/Objectives: The British Society for Genetic Medicine established a working group to review its guidance on the practical ethics of genetic testing in childhood. The review was timely, given the challenges presented by mainstreaming genomics and the progress in laboratory technology and bioinformatics.

Methods: A multi-disciplinary working group of geneticists, paediatricians, social scientists, philosophers and lawyers

collaborated to prepare the report. A workshop was held in February 2020 to agree a draft structure and the group then worked remotely and iteratively to finalise the report.

Results: The new guidance describes relevant ethical and legal frameworks and provides a clinical section of worked examples highlighting specific ethical issues. These include the importance of balancing the benefits and risks of genetic testing in childhood, and potential challenges of generating variants of uncertain significance and unexpected findings. Expansion of genetic testing to broader panels etc is proving challenging, Genetics specialists will need to support colleagues during the process of 'mainstreaming'.

Proposals for the genomic screening of healthy children need to be weighed carefully before being introduced. While it may be proper to use genetic test results on a child to benefit other family members, undertaking a test with that as the primary goal of testing would usually be inappropriate.

Conclusion: Where this does not disadvantage their medical care, we recommend delaying genetic testing of a child until they can participate in the discussion about it. However, there will be scenarios where testing in childhood at the parent's request is supported.

References:
Grants:
Conflict of Interest: None declared.

C06.5 Preimplantation genetic testing for polygenic conditions (PGT-P): perspectives of healthcare professionals

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Background/Objectives: Recently, preimplantation genetic testing for polygenic conditions (PGT-P) has been developed and introduced commercially. PGT-P aims to screen embryos for the risk of developing polygenic disorders, e.g. diabetes, cancer or schizophrenia. PGT-P raises ethical concerns that must be considered. Currently ethical guidance and regulation regarding PGT-P are lacking.

Methods: We performed a qualitative in-depth interview study with 31 healthcare professionals in the field of reproductive medicine and genetics in European and North-American countries to get insights into their perspectives on the ethics of PGT-P.

Results: Most healthcare professionals were critical about PGT-P and identified concerns about where to draw the line. One issue identified was the scientific uncertainty of applying polygenic risk scores to embryos. Professionals were worried that patients might find PGT-P and particularly risk scores difficult to understand. This could complicate genetic counselling and providing informed consent. Possible use of PGT-P for non-medical traits was seen unethical, preferring use to be limited to serious genetic conditions. The healthcare professionals that were more supportive of PGT-P highlighted the importance of reproductive autonomy and the ability of PGT-P to reduce the occurrence of certain conditions.

Conclusion: The findings make clear that there is currently little support for wider implementation of PGT-P. The identified ethical issues have to be addressed by relevant societies and institutions and guidance has to be provided.

References: -
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Conflict of Interest: None declared.

C06.6 An interactive, online education program to prepare the Australian workforce to incorporate rapid genomics in paediatric critical care

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Background/Objectives: Rapid genomic testing is increasingly becoming standard-of-care for critically ill paediatric patients with rare disease. As part of a national implementation program, we developed, delivered and evaluated an interactive online genomics education program for non-genetic specialists working in neonatal and paediatric critical care, which aimed to increase competence (knowledge, skills and attitudes) and confidence in genomic medicine.

Methods: The education program was co-designed with experts in clinical genetics, critical care, genetic counselling, genomics education and evaluation. Program implementation and outcomes were evaluated quantitatively using surveys at baseline, post-workshop and three-month follow-up, online module learner analytics and quizzes, and workshop polls.

Results: The program comprised four online modules and a virtual, case-based workshop. 270 people registered – intensivists (45%), paediatricians (33%), clinical geneticists (5%), genetic counsellors (6%), nurses (9%), and allied health (2%) – from over 20 sites around Australia. 8% of registrants were from overseas. 175 accessed online materials and 160 attended one of five workshops held in 2021. Gains in knowledge and skills were seen for all professions, for all genomic test result types. Confidence to practise genomics increased (average 50% to 82%). At follow-up, 71% reported performing more genomics-related activities.

Conclusion: An interactive, wholly-online education program can positively impact genomic competence, confidence and practice, and support equitable access to education for geographically-dispersed healthcare workforces. Our sustainable approach addresses current unmet needs for genomics education and the modular design can translate to other national and international settings as use of genomic testing increases.

References:
Grants: Australian Government GHFM76747.
Conflict of Interest: None declared.

C07 METABOLIC AND MITOCHONDRIAL DISEASES

C07.1 Bi-allelic variants in TAMM41 are associated with low muscle cardiolipin levels leading to neonatal mitochondrial disease

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Background/Objectives: Mitochondrial disorders are clinically and genetically heterogeneous. *TAMM41* encodes a mitochondrial protein with CDP-diacylglycerol synthase activity; an essential early step in the biosynthesis of cardiolipin, a mitochondria-specific phospholipid important for many mitochondrial processes. This study aims to validate and characterize the pathogenicity and functional effects of *TAMM41* variants identified in three unrelated paediatric cases of mitochondrial disease.

Methods: WES/WGS (1) identified compound-heterozygous *TAMM41* variants in each proband. Fibroblast/skeletal muscle samples were analysed by SDS/BN-PAGE (1) and lipidomics (2). *TAMM41* variants were modelled in yeast (4)

Results: Shared clinical features included lethargy and hypotonia at birth, developmental delay, myopathy and ptosis. No clear OXPHOS defect was observed in subject fibroblasts, however in skeletal muscle *TAMM41* levels were decreased by ~30% with severe loss of subunits of OXPHOS complexes I-IV. Similarly, lipidomic analysis showed cardiolipin levels were unchanged in subject fibroblasts, but significantly decreased in the skeletal muscle of affected individuals. To further functionally assess *TAMM41* missense variants, equivalent mutations were modelled in yeast. All three mutants failed to rescue the growth defect of the Δtam41 strains on non-fermentable medium compared to wild-type TAM41, confirming the pathogenicity of the variants.

Conclusion: We confirm the pathogenicity of the identified *TAMM41* variants, establish *TAMM41* as a key gene involved in mitochondrial phospholipid biosynthesis/modification and show that its deficiency results in a mitochondrial disorder. Unlike other mitochondrial phospholipid defects causing Senger's (*AGK*) or Barth (*TAFAZZIN*) syndrome, *TAMM41* cases showed no evidence of cardiomyopathy.

References:

(1) PMID:27693233
(2) PMID:31637422
(3) PMID:3323810

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Conflict of Interest: None declared.

C07.2 Bi-allelic LETM1 variants perturb mitochondrial ion homeostasis leading to a clinical spectrum with predominant nervous system involvement

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Background/Objectives: The Leucine zipper-EF-hand containing transmembrane protein 1 (LETM1) gene encodes an inner mitochondrial membrane protein with an osmoregulatory function controlling mitochondrial volume and ion homeostasis [1]. LETM1 is deleted in Wolf-Hirschhorn syndrome, which results from de novo monoallelic deletion of chromosome 4p16.3 [1]. Here we describe the first association of bi-allelic LETM1 variants with a human disease.

Methods: Exome sequencing and international gene-matching efforts were used to identify affected families with bi-allelic pathogenic variants in LETM1. Biochemical and morphological studies on mitochondrial K+ activities, proteins and shape in patient-derived fibroblasts, muscles and in S. cerevisiae as an important model organism for mitochondrial osmotic regulation were performed.

Results: Eighteen affected individuals from eleven unrelated families harboring ultra-rare bi-allelic LETM1 variants and clinical presentations highly suggestive of mitochondrial disease were identified. These manifested as a spectrum of predominantly infantile-onset (14/18, 78%) and variably progressive neurological, metabolic, and dysmorphic symptoms, and multiple organ dysfunction associated with neurodegeneration. The common features included respiratory chain complex deficiencies (100%), global developmental delay (94%), optic atrophy (83%), sensorineural hearing loss (78%), and cerebellar ataxia (78%) followed by epilepsy (67%), spasticity (53%), and myopathy (50%). Our experiments showed defective mitochondrial K+ efflux, swollen mitochondrial matrix structures, and loss of important mitochondrial oxidative phosphorylation protein components.

Conclusion: Our findings highlight the implication of perturbed mitochondrial osmoregulation caused by LETM1 variants in neurological and mitochondrial pathologies.

References: Austin, S., and Nowikovsky, K. (2019). LETM1: Essential for Mitochondrial Biology and Cation Homeostasis? Trends in Biochemical Sciences. 44, 648–658.

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Conflict of Interest: None declared.

C07.3 Transcriptomic analysis reveals dysregulation of different cellular pathways in PMM2-CDG: towards the identification of novel therapeutic targets

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Background/Objectives: PMM2-CDG, the most common glycosylation disorder caused by the deficiency of phosphomannomutase 2 (PMM2), is a clinically heterogeneous disease for which there is no treatment available. Our aim is to elucidate its molecular pathophysiology in order to identify novel biomarkers and possible treatments (pharmacological chaperones and reprofiled drugs) using systems biology approaches.

Methods: RNA-seq was performed to obtain transcriptomic data from control and patient-derived fibroblasts. GO, KEGG and Reactome were used for the functional enrichment analysis. The information generated was integrated into a computational model using TPMS (Therapeutic Performance Mapping System) technology, identifying cellular motives involved in the disease. The most relevant motives were validated through molecular and cellular biology techniques.

Results: Our model established the characterization of the pathophysiology of PMM2-CDG according to 17 cellular motives, among which we decided to validate "Senescence", "Bone regulation", "Cell adhesion and extracellular matrix" and "Response to cytokines". Functional assays confirmed a defect in the cell proliferation process of PMM2 fibroblasts due to an arrest in G0/G1 phase of the cell cycle. They also showed decreased expression of extracellular matrix components (collagen IV and laminin), reduced cell migration rates and increased levels of cyclooxygenase-2, suggesting a dysregulation of cytokine signalling. Additionally, this model could be used to find out approved drugs to treat PMM2-CDG through drug repurposing.

Conclusion: Our results broaden the knowledge of the pathomechanisms of PMM2-CDG, suggesting novel pathways and therapeutic targets that will be validated in the future in the murine model of the disease.

References:

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Conflict of Interest: None declared.

C07.4 Alteration of mitochondrial proteostasis in Costello syndrome

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Background/Objectives: Germline mutations that activate genes in the canonical RAS/MAPK signaling pathway are responsible for rare human developmental disorders known as RASopathies.

Methods: Here, we analyzed the molecular determinants of Costello syndrome (CS) using a mouse model expressing HRASG12S, patient skin fibroblasts, hiPSC-derived human cardiomyocytes, a HRASG12V zebrafish model and human fibroblasts expressing lentiviral constructs carrying HRASG12S or HRASG12A mutations.

Results: The findings revealed alteration of mitochondrial proteostasis and defective oxidative phosphorylation in the heart and skeletal muscle of Costello mice that were also found in the cell models of the disease. The underpinning mechanisms involved the miR-221*-dependent inhibition of AMPKα2 expression and the concomitant alteration of LKB1 activation by mutant forms of HRAS, leading to alteration of mitochondrial turnover and bioenergetics.

Conclusion: Pharmacological rescue of mitochondrial proteostasis restored organelle bioenergetics in HRASG12A/S cell models, reduced heart mass in CS mice and reduced the occurrence of developmental defects in the CS zebrafish model.

References:

Grants:

Conflict of Interest: Didier LACOMBE CHU de Bordeaux.

Université de Bordeaux, Patent on a therapy on Costello syndrome, Sanofi-Genzyme

Amicus, Laetitia Dard INSERM U1211, Patent on a therapy on Costello syndrome, Rossignol Rodrigue INSERM, Patent on a therapy on Costello syndrome.

C07.5 Frequency of MC4R pathway variants in a large US cohort of patients with severe obesity

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Background/Objectives: The melanocortin-4 receptor (MC4R) pathway is critical for regulating energy balance.[1] Genetic variants in this pathway (e.g. *POMC*, *PCSK1*, *LEPR*, *SH2B1*, and *SRC1*) are associated with severe obesity.[1-3] However, the frequency of these variants have not been assessed systematically in a clinically relevant US population.

Methods: We sequenced *POMC*, *PCSK1*, *LEPR*, *SH2B1*, and *SRC1* exons and intron-exon boundaries in 35,276 US individuals with severe obesity (<18 years old, ≥97th percentile body mass index [BMI] for age; ≥18 years old, BMI ≥40 kg/m^2). Included were individuals in the Uncovering Rare Obesity® (URO) diagnostic genetic testing program. This analysis included rare variants classified as pathogenic/likely pathogenic (P/LP) or a variant of uncertain significance (VUS) according to ACMG criteria, and 1 nonrare variant, *PCSK1* p.N221D.

Results: Of those with severe obesity, 10.2% carried ≥1 rare variants in ≥1 of the 5 genes, including 0.7% with a P/LP variant and 9.5% with a VUS variant. Additionally 5.4% carried the *PCSK1* p.N221D variant. Individuals in the URO cohort demonstrated a slightly higher frequency of P/LP and *PCSK1* N221D genotypes, 1.2% and 6.9%, respectively, and 9.8% had VUS genotypes.

Conclusion: In our large US-based cohort of individuals with severe early-onset obesity, 15.6% carried a potentially clinically relevant variant in MC4R pathway genes *POMC*, *PCSK1*, *LEPR*, *SH2B1*, and *SRC1*. Understanding the role of these variants may improve the care of individuals with rare genetic diseases of obesity.

References: 1. Huvenne et al. *Obes Facts*. 2016;9:158-173.
2. Yang et al. *Nat Commun*. 2019;10:1718.
3. Doche et al. *J Clin Invest*. 2012;122:4732-4736.

Grants: N/A.

Conflict of Interest: Ida Moeller Full time employee at the time of data analysis with Rhythm Pharmaceuticals, Inc., Received stock options as an employee of Rhythm Pharmaceuticals, Inc., Patrick Kleyn Full-time employee of Rhythm Pharmaceuticals, Inc., Received stock options as an employee of Rhythm Pharmaceuticals, Inc., Patrick Sleiman: None declared, Courtney Vaccaro: None declared, Hakon Hakonarson: None declared, Samira Bahl Full-time employee of Rhythm Pharmaceuticals, Inc., As an employee of Rhythm Pharmaceuticals, Inc., they received stock options, Tiago Antao Full-time employee of Rhythm Pharmaceuticals, Inc. at the time of data anlysis, As an employee of Rhythm Pharmaceuticals, Inc., they received stock options, Alastair Garfield Full-time employee at Rhythm Pharmaceuticals, Inc. at the time of data analysis, As an employee of Rhythm Pharmaceuticals, Inc., they received stock options.

C07.6 An integrated multiomic approach as an excellent tool for the diagnosis of metabolic diseases: our first 3,720 patients

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Background/Objectives: To present our experience using a multiomic approach, which integrates genetic and biochemical testing as a first-line diagnostic tool for patients with inherited metabolic disorders (IMDs).

Methods: A cohort of 3,720 patients from 62 countries was tested using a panel including 206 genes with single nucleotide and copy number variant (SNV/CNV) detection, followed by semi-automatic variant filtering and reflex biochemical testing (25 assays).

Results: In 1,389 patients (37%), a genetic diagnosis was achieved. Within this cohort, the highest diagnostic yield was obtained for patients from Asia (57.5%, mainly from Pakistan). Overall, 701 pathogenic/likely pathogenic unique SNVs and 40 CNVs were identified. In 620 patients, the result of the biochemical tests guided variant classification and reporting. Top five diagnosed diseases were: Gaucher disease, Niemann-Pick disease type A/B, phenylketonuria, mucopolysaccharidosis type I, and Wilson disease.

Conclusion: We show that integrated genetic and biochemical testing facilitated the decision on clinical relevance of the variants and led to a high diagnostic yield (37%), which is comparable to exome/genome sequencing. More importantly, up to 43% of these patients (n = 610) could benefit from medical treatments (e.g., enzyme replacement therapy). This multiomic approach constitutes a unique and highly effective tool for the genetic diagnosis of IMDs.

References:

Grants:

Conflict of Interest: Ligia Almeida Employees at CENTOGENE GmbH, Catarina Pereira Employee at CENTOGENE GmbH, Ruxandra Aanicai Employee at CENTOGENE GmbH., Sabine Schröder Employee at CENTOGENE GmbH, Tomasz Bochinski Employee at CENTOGENE GmbH, Anett Kaune Employee at CENTOGENE GmbH, Alice Urzi Employee at CENTOGENE GmbH, Tania Spohr Employee at CENTOGENE GmbH, Nikenza Viceconte Employee at CENTOGENE GmbH, Sebastian Oppermann Employee at CENTOGENE GmbH, Mohammed Alasel Employee at CENTOGENE GmbH, Saeedeh Ebadat Employee at CENTOGENE GmbH, Sana Iftikhar Employee at CENTOGENE GmbH, Eresha Jasinge: None declared, Solaf Elsayed: None declared, Hoda Tomoum: None declared, Iman Marzouk: None declared, Anil Jalan: None declared, Agne

Cerkauskaite: None declared, Rimante Cerkauskiene: None declared, Tinatin Tkemaladze: None declared, Muhammad Nadeem Anjum: None declared, Iman Mahmoud: None declared, Fawzia Mossad: None declared, Mona Kamel: None declared, Laila Selim: None declared, Huma Cheema: None declared, Omid Paknia Employee at CENTOGENE GmbH, Claudia Cozma Employee at CENTOGENE GmbH, Carlos Juaristi-Manrique Employee at CENTOGENE GmbH, Pilar Guatibonza Moreno Employee at CENTOGENE GmbH, Tobias Böttcher Employee at CENTOGENE GmbH, Florian Vogel Employee at CENTOGENE GmbH, Jorge Pinto Basto Employee at CENTOGENE GmbH, Aida Bertoli-Avella Employee at CENTOGENE GmbH, Peter Bauer Employee at CENTOGENE GmbH.

C08 NEW FINDINGS FOR OLD NDD GENES

C08.1 Activating RAC1 variants in the switch II region cause a novel developmental syndrome and alter neuronal morphology that can be rescued by targeting CYFIP

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Background/Objectives: RAC1 is a highly conserved Rho GTPase critical for several cellular and developmental processes. We had previously shown that dominant negative *RAC1* variants cause a neurodevelopmental disorder. We hypothesised that activating *RAC1* variants cause a distinct condition.

Methods: We analysed whole exome sequencing and clinical data from patients. We modelled selected variants in vitro and in vivo.

Results: We identified missense variants clustering between residues Q61 and R68 within the switch II region of RAC1 in eight patients with developmental delay, intellectual disability, brain anomalies and cardiovascular defects. Pulldown assays, NIH3T3 fibroblasts spreading assays and staining for activated PAK1/2/3 and WAVE2 demonstrated that these variants increase RAC1 activity in vitro and over-activate downstream signalling targets. Axons of neurons from mutant *Drosophila* embryos were significantly shorter, with an increased density of filopodial protrusions. In vivo, these embryos exhibited axonal organization defects. Mutant Class IV dendritic arborisation neurons showed significant reduction in the total dendritic arbour area, increased branching and failure of self-avoidance. RNAi knockdown of the WAVE regulatory complex component Cyfip significantly rescued morphological defects.

Conclusion: Activating *RAC1* variants within the switch II region cause a novel syndrome characterised by neurodevelopmental problems and susceptibility to congenital heart defects. These variants result in increased downstream signalling and abnormal neuronal morphology. We predict overlapping mechanisms underlying several disorders caused by variants in genes encoding other Rho GTPases, their regulators and downstream effectors. The WAVE regulatory complex/Arp2/3 pathway is a therapeutic target for activating *RAC1* variants and, possibly, for other related disorders.

References:
Grants:
Conflict of Interest: None declared.

C08.2 Mutation-specific pathophysiological mechanisms of AFF3

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Background/Objectives: We previously described the KINSSHIP syndrome, an autosomal dominant disorder associated with *de novo* missense variants in the degron of AFF3. The eighteen affected individuals shared a recognizable pattern of anomalies that included intellectual disability (ID), mesomelic dysplasia and horseshoe kidney. Mouse knock-ins and overexpression in zebrafish suggested a dominant negative mode-of-action and a pathological effect of increasing amount of AFF3.

Methods: Screening of ID cohorts allowed to identify new individuals carrying *AFF3* variants. To assess their impact we profile their transcriptome and engineered animal models.

Results: We identified eleven additional individuals from seven families who carried truncation or deletions rather than missense. They presented with milder phenotypes suggesting an alternative haploinsufficiency mode-of-action. Corroboratingly, mouse knockouts display brain malformations with enlarged lateral ventricles and decreased corpus callosum size, neurological and skeletal anomalies including vertebrae transformation and abnormal skull, whereas zebrafish knockdowns presented with circling behavior and decreased velocity indicative of broad neurological defect. Finally, we reported four unrelated ID patients with homozygous or

compound heterozygous predicted-to-be deleterious missense variants in *AFF3*. Consistent with causativeness, 3D protein modelling of these variants suggested that it cannot be accommodated without affecting the local geometry. Consistent with this hypothesis we found that the three type of variants differently impact cellular transcriptome.

Conclusion: *AFF3* encodes a component of the transcriptional super elongation complex that regulates expression of genes involved in neurogenesis and development. Our data suggest that *AFF3* mutations can result in either recessive, dominant negative or haploinsufficiency phenotypes.

References: Voisin et al., 2021.

Grants:

Conflict of Interest: None declared.

C08.3 Structural variants disrupt a critical regulatory region downstream of FOXG1

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Background/Objectives: The FOXG1 transcription factor is a crucial regulator during embryonic brain development. Pathogenic variants affecting *FOXG1* cause *FOXG1* syndrome, a congenital form of Rett syndrome. Interestingly, 34 reported individuals with *FOXG1* syndrome related features harbor structural variants (SVs) that disrupt the region downstream of *FOXG1*. Yet, the regulatory mechanisms resulting in aberrant *FOXG1* transcription have not been elucidated.

Methods: We used CNV-seq followed by qPCR and Sanger sequencing to map a *de novo* non-coding deletion in a patient with *FOXG1* syndrome. In neural stem cells and neurons, we used UMI-4C to determine *FOXG1* interactions. Using UMI-4C and Hi-C in patient cells, we interrogated possible structural changes in the *FOXG1*-containing topologically associated domain (TAD).

Results: We identified a non-coding deletion in a patient with *FOXG1* syndrome allowing us to narrow down a ~100kb critical regulatory region affected in all patients with SVs 3′ of *FOXG1*. Via UMI-4C, we showed that the *FOXG1* promoter interacts with this region during human neuronal development. Using available epigenomics data, we found multiple regulatory elements in this region, including putative brain enhancers and the distal boundary of the *FOXG1*-containing TAD. Through Hi-C and UMI-4C on patient cells we found that deletion of this region impacts *FOXG1* interactions and TAD structure.

Conclusion: We narrowed down a critical regulatory region downstream of *FOXG1* that is affected in a cohort of *FOXG1* syndrome patients. These results build towards a functional validation of regulatory elements at the *FOXG1* locus, crucial for correct SV interpretation.

References: PMID: 32973355.

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Conflict of Interest: None declared.

C08.4 FBXO11 haploinsufficiency also stems from de novo missense variants and impairs neuronal differentiation and migration in an iPSC-based neuronal model

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Background/Objectives: Recently, we and others identified de novo FBXO11 variants as causative for a variable neurodevelopmental disorder (NDD). We now assembled clinical and mutational information on 23 additional individuals.

Methods: To further characterize the functional consequences of FBXO11 missense variants, we analyzed their effects on protein expression and localization. Furthermore, we generated FBXO11 knockout induced pluripotent stem cells using CRISPR/CAS9 technology and differentiated those cells into neurons. As FBXO11 functions as a nuclear E3-ubiquitin ligase subunit, we hypothesized that target proteins may be involved in transcriptional regulation and performed whole transcriptome analysis on FBXO11 deficient neurons.

Results: We found that the majority of missense variants resulted in subcellular mislocalization and/or reduced FBXO11 protein expression levels. Together with the mutational data our functional results suggest that most missense variants likely lead to a loss of FBXO11 function and thereby highlight haploinsufficiency as the most likely disease mechanism for FBXO11-associated NDDs.

Decreased expression of differentiation genes and increased expression of stemness genes in FBXO11 knockout neurons suggest that neuronal differentiation might be impaired. We confirmed the known stemness factor NANOG to interact with FBXO11 by co-immunoprecipitation. In line with our results from transcriptomic analysis, we found that cell proliferation rates during neuronal differentiation are increased in FBXO11 knockout cells. Additionally, neuronal migration is impaired in the neurosphere assay.

Conclusion: Our data therefore suggest that impaired neural differentiation and migration may be key factors in the pathogenesis of FBXO11-associated NDDs.

References:

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Conflict of Interest: None declared.

C08.5 Clinical and molecular understanding of DYRK1A syndrome, a frequent monogenic form of neurodevelopmental disorder

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Background/Objectives: Haploinsufficiency of the *DYRK1A* gene leads to one of the most frequent monogenic forms of syndromic neurodevelopmental disorders (NDD), the DYRK1A syndrome.

Methods: We refined the molecular and clinical description of this disorder with a cohort of 50 individuals, reported novel symptoms and better delineated the neurobehavioral profile of DYRK1A syndrome. We developed tools to improve interpretation of variants in *DYRK1A*, by combining different clinical, in silico and in vitro approaches. In parallel we used human neural stem cells model (hNSC) to better understand the consequences of loss-of-function mutations

Results: We defined a blood DNA methylation signature specific for loss-of-function *DYRK1A* variants, questioned the pathogenicity of several *de novo* and identified a potential gain-of-function variant. We identified novel protein partners of DYRK1A in hNSC, such as an E3 ubiquitin ligase involved in cell-cycle regulation. We characterized the genes whose expression was altered after transient *DYRK1A* inactivation using siRNA and observed an enrichment of genes encoding transmembrane proteins, components of the extracellular matrix, and involved in the regulation of the cell cycle. In addition, we were able to demonstrate that inactivation of DYRK1A led to a decrease in the proliferation of hNSC, a decrease in the expression of the P21 protein, an important player in the cell cycle, and the activation of ERK pathway.

Conclusion: In conclusion, our study provides a better understanding of the clinical and molecular consequences of the loss-of-function variants responsible for the DYRK1A syndrome, essential to improve the diagnosis and management of patients and to identify potential therapeutic targets

References:

Grants:

Conflict of Interest: None declared.

C08.6 Contribution of non-coding de novo NIPBL variants to Cornelia de Lange syndrome

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Background/Objectives: Pathogenic variants in 6 genes explain ~50% of Cornelia de Lange syndrome (CdLS) cases, *NIPBL* loss-of-function being the main cause. We assessed the contribution of non-coding variants and differential diagnoses to unsolved cases.

Methods: We reannotated gene panel sequencing data of 111 unsolved classic-CdLS patients for *NIPBL* 5'UTR and intronic variants and selected 5 patients for trio-based genome sequencing (GS). We assessed the consequences of variants in a GFP reporter assay, in a lymphoblastoid cell line (LCL) and by RT-PCR and RNAseq from whole blood.

Results: In gene panel data, we identified two *de novo* variants introducing a novel ATG codon in the *NIPBL* 5'UTR, both predicted to create an upstream Open Reading Frame: a novel c.-457_-456delinsAT variant, and a c.-467C>T variant, recently annotated as pathogenic in Clinvar. A GFP reporter assay showed significantly

decreased GFP protein levels with unchanged mRNA levels in a c.-457_-456delinsAT context and in the context of a previously reported c.-94C>T AUG-introducing variant. LCL analysis of the c.-457_-456delinsAT variant carrier showed consistent results with a ~50% NIPBL protein decrease and unchanged mRNA levels.

In GS data, a pathogenic *de novo* variant was observed in a differential diagnosis gene, namely *POU3F3*, *SPEN* and *TAF1*, in 3/5 patients. In the other two, we identified two deep intronic *NIPBL de novo* variants creating a novel splice site (c.869-640G>C and c.5862 + 3487C>T) with RT–PCRs and RNA-Seq showing aberrant transcripts leading to novel frameshift exons.

Conclusion: Non-coding *NIPBL* variants may account for a proportion of unsolved CdLS patients.

References:

Grants:

Conflict of Interest: None declared.

C09 CARDIAC AND NEUROMUSCULAR GENETICS

C09.1 MDFIC mutations cause autosomal recessive Complicated Lymphatic Anomaly (virtual)

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Background/Objectives: Complex lymphatic anomalies (CLAs) are characterised by dysfunction of core collecting lymphatic vessels. These include the thoracic duct and cisterna chyli, and present as chylothorax, chylous ascites and/or lymphoedema. They often result in foetal or perinatal demise. While mutations in RAS/MAPK and PI3K/AKT signalling pathway components have been documented in some patients, the genetic aetiology remains uncharacterised in the majority of cases.

Methods: WES was used to detect mutations in patients with fetal hydrops, chylous ascites, chylothorax and/or lymphedema. A mouse line with a PTC in MDFIC was generated using CRISPR/Cas9, and analysed. MDFIC function was also studied in cell cultures.

Results: We identified recessive mutations in MDFIC in six families. MDFIC encodes the MyoD family inhibitor domain-containing protein. The mouse model revealed that homozygous mutant mice died perinatally, exhibiting chylothorax, the accumulation of lipid rich chyle in the thoracic cavity catalysed by lymphatic vessel rupture. The lymphatic vasculature of homozygous Mdfic mutant mice was profoundly mis-patterned, particularly in the diaphragm and thoracic wall, and exhibited defects in lymphatic vessel valve development. Mechanistically, we determined that MDFIC regulates the activity of GATA2; a key transcription factor important for development and function of the lymphatic vasculature

Conclusion: Our work is the first to reveal a crucial role for MDFIC in CLAs and in the lymphatic vasculature. These discoveries enable more precise differential diagnosis of CLAs. Ultimately, understanding the mechanistic basis of CLAs will facilitate the development of novel therapeutic agents able to effectively treat this devastating disease.

References:

Grants: WELBIO-CR-2015A.

Conflict of Interest: None declared.

C09.2 A new Smad4 mouse model mimicking Myhre Syndrome?

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Background/Objectives: Myhre Syndrome (MS) is a part of acromelic dysplasias, and is characterized by short stature, thick skin, hearing loss, and cardiovascular defects. We previously identified the molecular basis of MS as mutations in *SMAD4* with an autosomal dominant mode of inheritance. Most mutations in *SMAD4* leading to MS are localized at the Ile500 residue. As the co-mediator of the TGFβ and BMP signaling pathways, SMAD4 is essential for the proper development and homeostasis of the whole organism. This project aims to investigate the physio-pathological mechanisms underlying MS.

Methods: To figure out the role of SMAD4 in MS, we generated a new knock-in mouse model by introducing the most frequent mutation found in MS, then analyze the skeletal and cardiovascular phenotypes of the mice.

Results: The mouse model generated *Smad4*$^{Ile500/+}$ is viable and expresses the mutation ubiquitously. The mutated protein Smad4^{Ile500} seems to exhibit a higher halftime life than the wild-type protein. *Smad4*$^{Ile500/+}$ mice present a reduced stature associated with dysregulated extracellular matrix proteins and chondrocyte differentiation within the growth plate. The hearts of mutant mice are shown to be smaller with thicker ventricle walls. Interestingly, homozygous mice *Smad4*$^{Ile500/Ile500}$ exhibit a severer phenotype with a perinatal lethality thought to be linked to cardiovascular defects.

Conclusion: This new mouse model is an original mouse model with a *Smad4* mutation leading to a MS-like phenotype. Our findings suggest that the dysregulation of SMAD4 in MS may lead to a disruption of the extracellular matrix, as other acromelic dysplasias.

References:

Grants: ANR-20-CE14-0002-01

Conflict of Interest: None declared.

C09.3 Heritable pulmonary hypertension: first genotype-phenotype study in TBX4 syndrome

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Background/Objectives: Despite the increasing frequency of *TBX4*-associated pulmonary arterial hypertension (PAH), genotype-phenotype associations are lacking.

Methods: We assembled a multicenter cohort of 137 patients harboring monoallelic *TBX4* variants and assessed the pathogenicity of missense variation (n = 42) using a novel luciferase reporter assay containing T-BOX binding motifs. We sought genotype-phenotype correlations and undertook a comparative analysis with PAH patients with *BMPR2* causal variants (n = 162) or no identified variants in PAH associated genes (n = 741) genotyped via the NIHR BioResource - Rare Diseases (NBR).

Results: Functional assessment of *TBX4* missense variants led to the novel finding of gain-of-function effects associated with an older age at diagnosis of lung disease compared to loss-of-function ($p = 0.038$). Variants located in the T-BOX and nuclear localization domains associated with earlier presentation ($p = 0.005$) and increased incidence of interstitial lung disease ($p = 0.001$). Event-free survival (death or transplantation) was shorter in the T-BOX group ($p = 0.022$). Carriers of *TBX4* variants were diagnosed at a younger age ($p < 0.001$) and had worse baseline lung function ($p = 0.009$) compared to the *BMPR2* and no identified causal variant groups.

Conclusion: We demonstrated that TBX4 syndrome is not strictly the result of haploinsufficiency. The pleiotropic effects of TBX4 in lung disease may be in part explained by the differential effect of pathogenic mutations in critical protein domains.

References: PMID 32409426.

Grants: MLD ED481A-2018/304.

Conflict of Interest: None declared.

C09.4 Unravelling a novel congenital muscular dystrophy

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Background/Objectives: Congenital muscular dystrophies are defined by progressive childhood muscle weakness but encompass a wide phenotypic and genetic spectrum. However, 40% of typical muscular dystrophies do not have yet definitive molecular diagnosis.

Methods: Biological samples taken from the patients included blood, muscle and skin biopsies. Whole exome sequencing for the patients and parents was done followed by Sanger sequencing. Histopathology studies were done with appropriate staining. Primary fibroblasts were cultured from the skin biopsies and were used for immunofluorescent (IF) and protein and RNA extraction. Western blot (WB) for quantification of whole cell extract and subcellular fractionation was done. cDNA from patients and controls RNA was used for qPCR and RT-PCR studies.

Results: We present here six patients from five unrelated families diagnosed with muscular dystrophy frequently associated with cataracts and neurological defects. Skeletal muscle histology showed heterogeneous and atrophic myocytes. Interestingly, whole exome sequencing uncovered novel germline homozygous or compound heterozygous mutations in a key component of importin complex. RNA and protein quantification from patient primary fibroblasts did not establish clear muteins loss-of-function. However, immunofluorescence and western-blot on sub-cellular fractions revealed strong alteration in muteins distribution and other related import/export factors.

Conclusion: Our data unravelled a novel cause of muscular dystrophy associated with nucleo-cytoplasmic trafficking and potential alternative splicing defects. Further studies including in vitro and in vivo modelling are in process to validate our preliminary data.

References:

Grants:

Conflict of Interest: None declared.

C09.5 Interactions between muscle glucose homeostasis and neuromuscular signal transduction – Lessons learned from lack of a muscle-specific long isoform of GFPT1

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Background/Objectives: Glutamine fructose-6-phosphate transferase 1 (GFPT1) is a rate-limiting enzyme of the hexosamine biosynthesis pathway (HBP) to generate UDP-GlcNAc. Mutations in GFPT1 have been reported in congenital myasthenic syndrome. A 54-nucleotide exon 9 of *GFPT1* is specifically included in muscle to make a long isoform of GFPT1 (GFPT1-L), which has a lower enzymatic activity than a short isoform of GFPT1 (GFPT1-S)[1,2]. The aim of this study is to elucidate the splicing regulatory mechanisms and the functional significance of GFPT1-L in skeletal muscle.

Methods: Elucidation of the splicing regulatory mechanism: siRNA screening of RNA-binding proteins (RBPs), MS2- and PP7-mediated tethering of RBPs, and analysis of the spliceosomal complexes. Analysis of the roles of muscle-specific GFPT1-L: knock-out (KO) of muscle-specific *Gfpt1* exon-9, analysis of motor performances, and analysis of glucose metabolisms.

Results: Dissection of the splicing regulatory mechanism of *GFPT1* revealed that RBPs SRSF1, Rbfox1 and 2, and hnRNPs H and F cooperatively regulate the alternative inclusion of exon 9 in *GFPT1* pre-mRNA. Analyzing skeletal muscle phenotype demonstrated that motor performance is compromised in aged *Gfpt1* exon-9 KO mice resulting from simplified NMJ. KO of *Gfpt1* exon 9 activated HBP in skeletal muscle, and suppressed glucose uptake by insulin and muscle glycolysis.

Conclusion: GFPT1-L is required for neuromuscular signal transduction and muscle glucose homeostasis.

References: 1. Niimi M., *et al*. *J Hum Genet*. 2001
2. DeHaven JE., *et al*. *Diabetes*. 2001

Grants: This study was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Conflict of Interest: None declared.

C09.6 Arteriovenous malformations: new genetic and clinical course data in a cohort of 100 patients

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Background/Objectives: Arteriovenous malformations (AVMs) are isolated or syndromic congenital high flow vascular anomalies caused by germline or somatic alterations in different genes, most of them discovered in recent years. Herein we provide new genetic findings and genotype-clinical course correlation in AVMs.

Methods: We have retrospectively reviewed the phenotype, clinical course, and treatment in 100 patients with AVMs. When available, the affected tissue sample was tested by deep NGS (panels and exomes).

Results: Patients were classified into isolated AVMs (69%) —including intramuscular lesions— and syndromic forms (31%) —CM-AVM, PTEN-hamartoma tumor, Parkes Weber—. Pathogenic variants were detected in known AVM genes —67 patients: MAP2K1 (22%), KRAS (17%), HRAS (1%), RASA1 (9%), EPHB4 (2%), BRAF (4%), PTEN (3%)— and in not previously associated genes —PIK3CA (9%), FGFR3 (2%), GJC2 (1%), GNAQ (3%), GNA14 (1%)—. In 7/9 cases with a pathogenic variant in PIK3CA, a concomitant pathogenic variant in MAP2K1, KRAS, PTEN, or RASA1 genes was also detected. Somatic, pathogenic variants in KRAS and MAP2K1 showed a more aggressive progression, whereas AVMs with germline, pathogenic variants in RASA1 showed a milder course without requiring surgical intervention.

Conclusion: We have detected a correlation between the gene affected and the aggressiveness of the lesions. We have also detected pathogenic variants in genes not previously described in AVMs. The presence of concomitant pathogenic variants in two different genes could be showing a mechanism of cumulative defects, which has implications for the genetic diagnosis and for targeted drug treatments.

References:

Grants: Project FIS PI17/00519, ISCIII, FEDER.

Conflict of Interest: None declared.

C10 INTERNAL ORGANS AND IMMUNOLOGY

C10.1 The PALFES study: exome sequencing identified the genetic cause in 40% of 256 pediatric acute liver failure cases without aetiology

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Background/Objectives: Pediatric acute liver failure (PALF) is a rare and life-threatening condition occurring in previously healthy children. In Europe viral infections and inherited metabolic diseases are the main causes. In 50% of PALF cases, the underlying aetiology remains elusive, challenging clinical management, including liver transplantation. Yet, no systematic exome sequencing study (ES) has been performed.

Methods: In the international, multicenter "PALFES study" we recruited 256 patients with indeterminate PALF, 67 were recurrent PALF (RALF), from 18 countries between 2011 and 2021. ES analysis and a systematic clinical and biochemical phenotype study were performed.

Results: ES analysis established a genetic diagnosis in 97 (38%) individuals. Diagnostic yield was particularly high in the first year of life (41%) and in children with RALF (66%). With 37 identified distinct disease genes, the genetic spectrum of PALF is very broad. Defects in NBAS (22%), MPV17 (8%) and DGUOK (7%) occurred most frequently. When categorizing, the most frequent causes were mitochondrial diseases (39%), followed by disorders affecting intracellular trafficking (29%) such as NBAS deficiency and cytosolic aminoacyl tRNA synthetase deficiencies (10%). One-third of patients had a fatal outcome. No phenotypic parameters could be found to differentiate molecularly diagnosed from non-diagnosed cases.

Conclusion: This study elucidates the constantly expanding heterogeneous genetic spectrum of PALF. Mitochondrial diseases constitute the largest group within genetic PALF. A molecular

diagnosis helped to guide patient management. The high proportion of diagnosed cases and potential treatment implications argue for implementation of rapid genome sequencing in routine PALF diagnostics.

References:

Grants: mitoNET, GENOMIT.

Conflict of Interest: Lea Dewi Schlieben: None declared, Dominic Lenz: None declared, Masaru Shimura: None declared, Alyssa Bianzano: None declared, Dmitrii Smirnov: None declared, Robert Kopajtich: None declared, Rüdiger Adam: None declared, Georg-Friedrich Vogel: None declared, Ivo Baric: None declared, Philip Bufler: None declared, Birutė Burnytė: None declared, Pier Luigi Calvo: None declared, Ellen Crushell: None declared, Anibh M. Das: None declared, Felix Distelmaier: None declared, Peter Freisinger: None declared, Nedim Hadzic: None declared, Steffen Hartleif: None declared, Maja Hempel: None declared, Aydan Kansu: None declared, Sonja Kaspar: None declared, Deidre Kelly: None declared, Birgit Knoppke: None declared, Vassiliki Konstantopoulou: None declared, Tatiana Krylova: None declared, Kuster Alice: None declared, Elke Lainka: None declared, Eberhard Lurz: None declared, Hanna Mandel: None declared, Patrick McKiernan: None declared, Dorota Piekutowska-Abramczuk: None declared, Agnès Rötig: None declared, René Santer: None declared, Robert Taylor: None declared, Saskia Wortmann: None declared, Jerry Vockley: None declared, Stefan Kölker: None declared, Georg Friedrich Hoffmann: None declared, Thomas Meitinger: None declared, Kei Murayama: None declared, Christian Staufner C.S. is supported by the Dietmar Hopp Foundation, St. Leon-Rot, Germany (grant number 23011235), Holger Prokisch: None declared.

C10.2 Heterozygous variants in NEK8 kinase domain cause an autosomal dominant ciliopathy (virtual)

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Background/Objectives: *NEK8/NPHP9* encodes a protein that localizes to the primary cilium. Biallelic variants are known to cause severe multiorgan developmental defects including renal cystic dysplasia, with heterozygous carrier parents being asymptomatic.[1] Complementary to this, we propose a dominant-negative effect for specific *NEK8* variants resulting in an autosomal-dominant ciliopathy.

Methods: We performed genetic testing in patients from several medical centers. To explore the consequences of the identified variants we're performing cilia staining assays and replication stress response experiments in both patients' cells and mIMCD3 cells overexpressing the identified variants.

Results: We identified three distinct heterozygous *NEK8* variants in twelve families, all leading to missense alterations in the kinase domain. Interestingly one variant is a recurrent variant we detected in ten unrelated families. All patients have a kidney phenotype that varies from mild focal segmental glomerulosclerosis to prenatal presentation with polycystic kidneys. Most patients have kidney failure needing renal replacement therapy at varying ages of onset. The large symptomatic family and de novo occurrences favor a dominant inheritance mode. Our preliminary results from functional studies show abnormal primary cilia formation in serum-starved cells as well as increased replication stress.

Conclusion: We present the first evidence for a pathogenic effect of heterozygous *NEK8* variants. Remarkably our patients present with a kidney phenotype as compared to multiorgan defects found in patients with biallelic variants. This reveals a new inheritance mode for *NEK8* variants and expands genotype-phenotype correlations for this gene.

References: Grampa et al. Novel NEK8 Mutations Cause Severe Syndromic Renal Cystic Dysplasia through YAP Dysregulation. PLoS Genet 2016;12(3):e1005894.

Grants:

Conflict of Interest: None declared.

C10.3 TSHZ3 is mutated in human CAKUT

Esra Kesdiren[1], *Helge Martens*[1], *Lina Werfel*[1;2], *Imke Hennies*[2], *Robert Geffers*[3], *Jan Hinrich Bräsen*[4], *Zoran Gucev*[5], *Tomáš Seeman*[6], *Velibor Tasic*[5], *Sonja Schmidt*[7], *Frank Brand*[1], *Anne Christians*[1], *Dieter Haffner*[2], *Ruthild G. Weber*[1]

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Background/Objectives: The genetic cause of congenital anomalies of the kidney and urinary tract (CAKUT) remains unexplained in most patients. Because over 180 genes have been associated with CAKUT in mice, we prioritized variants in murine CAKUT-related genes when analyzing whole-exome sequencing data from two siblings with CAKUT, thus identifying a missense variant in the transcription factor gene *TSHZ3* in both.

Methods: The index and 300 further CAKUT families were analyzed by whole-exome or targeted *TSHZ3* sequencing. To explore genotype-phenotype relationships, all patients/nephrectomy specimens were subjected to clinical, radiological, or histological characterization. *TSHZ3* mRNA expression levels were determined in human fetal and adult tissues. Biochemical

analyses are being performed to investigate *TSHZ3* variant-specific effects.

Results: We identified rare heterozygous *TSHZ3* variants predicted to be deleterious in 9/301 (3%) CAKUT families. An N-terminal hotspot region of TSHZ3 was affected significantly more frequently in cases than in gnomAD controls ($p = 0.006$). Hydronephrosis, previously observed in *Tshz3*-null mutant mice, was significantly more frequent in patients with versus without *TSHZ3* variants ($p = 0.010$). CAKUT patients with versus without *TSHZ3* variants were also significantly more likely to have developmental delay ($p = 0.012$) and genital anomalies ($p = 0.008$). *TSHZ3* was highly expressed in fetal human brain, skeletal muscle, and kidney. Co-immunoprecipitation is ongoing to determine whether binding of TSHZ3 mutants affecting the hotspot region to an interaction partner, myocardin, is impaired, as compared to wildtype.

Conclusion: Our findings provide evidence that *TSHZ3* variants in an N-terminal hotspot associate with human CAKUT and specific extrarenal features, i.e. developmental delay and genital anomalies.

References:

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Conflict of Interest: Esra Kesdiren: None declared, Helge Martens Deutsche Forschungsgemeinschaft (MA 9606/1-1), Lina Werfel Else Kröner-Fresenius-Stiftung (grant no. 2018_Kolleg.12, TITUS Kolleg of Hannover Medical School), Imke Hennies: None declared, Robert Geffers: None declared, Jan Hinrich Bräsen: None declared, Zoran Gucev: None declared, Tomáš Seeman: None declared, Velibor Tasic: None declared, Sonja Schmidt: None declared, Frank Brand: None declared, Anne Christians Deutsche Forschungsgemeinschaft (KO5614/2-1), Dieter Haffner: None declared, Ruthild G. Weber: None declared.

C10.4 Development of a new cellular model to understand genotype-phenotype correlations in patients with polycystic kidney disease exploiting CRISPR/Cas9 system

Martina Migliorero[1], *Valeria Bracciamà*[2], *Francesca Arruga*[1], *Nicoletta Vitale*[3], *Monica Sorbini*[1], *Antonio Amoroso*[2], *Tiziana Vaisitti*[1], *Silvia Deaglio*[1;2]

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Background/Objectives: Polycystic kidney disease (PKD) defines a group of monogenic disorders ultimately leading to kidney failure. The single most mutated gene is *PKD1*, for which hundreds of mutations have been described, a significant portion of which is classified as "variant of unknown significance" (VUS). To readily confirm their pathogenicity, a proper experimental model to understand genotype-phenotype correlations for patients carrying VUS is needed.

Methods: As a proof-of-principle, the HEK293T cell line was modified to express an inducible Cas9. Cells were then transfected with a sgRNA targeting exon 15 of *PKD1* to generate knock-out clones, confirmed by Sanger sequencing. Protein expression and functional read-outs were exploited to validate the model.

Results: Six *PKD1*-/- clones were generated, carrying frameshift and nonsense mutations. Protein loss was confirmed, while variability in expression was observed at the transcript level. 3D cultures demonstrated that the wild-type cells organize in a tubular-like manner when grown on matrigel, while knock-out clones form round structures. As expected, KO cells contained higher levels of cAMP and displayed a different tyrosine phosphorylation pattern, confirming the validity of the model.

Conclusion: We generated a new PKD cellular model where we will reproduce some of the variants observed in our cohort of more than 130 patients with genetic diagnosis. With these first experiments we provide proof-of-principle of the feasibility of the approach. We now aim to exploit the homology-directed repair system to generate clones carrying VUS missense variants to assess their phenotypic impact through morphological and functional read-outs.

References:

Grants: Ministry of Education, Progetto strategico di Eccellenza Dipartimentale #D15D1800041000.

Conflict of Interest: None declared.

C10.5 Loss of FOCAD, operating in the SKI mRNA surveillance pathway, is responsible for a syndromic form of pediatric liver cirrhosis

Ricardo Moreno Traspas[1;2], *Tze Shin Teoh*[1;2], *Pui Mun Wong*[1], *Michael Maier*[1], *Kenneth Lay*[1], *Nur Ain Ali*[1], *Austin Larson*[3], *Fuad Al Mutairi*[4], *Nouriya Al-Sanna`a*[5], *Eissa Ali Faqeih*[6], *Huma Cheema*[7], *Juliette Dupont*[8], *Renata Sandoval*[9], *Luiza Ramos*[10], *Peter Bauer*[11], *benjamin cogne*[12], *Aida Bertoli-Avella*[11], *Marie Vincent*[12], *Katta Girisha*[13], *Bruno Reversade*[1;2;14]

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Background/Objectives: Cirrhosis is a chronic, life-threatening disease characterized by fibrotic scarring and inflammation that disrupts liver architecture and function. This hepatic disease is increasingly becoming a major health and economic burden to society, causing more than 1 million annual deaths globally. Despite extensive research, there are still no effective drugs or therapies to combat the disease. The etiology of liver cirrhosis mainly originates from environmental insults, but little has been done to explore the existence of possible underlying genetic causes.

Methods: We identified a total of 14 children from 7 countries presenting with a syndromic form of pediatric liver cirrhosis. By genome/exome sequencing, all cases were found to segregate germline recessive loss-of-function mutations in *FOCAD*, a gene with no previously-reported links to liver biology. Using CRISPR/Cas9 technology, we generated in vitro and in vivo *FOCAD*-knockout biological platforms that helped to delineate the cellular and molecular basis of this congenital liver disease.

Results: Zebrafish lacking *focad* phenocopied the human disease, revealing a signature of altered mRNA degradation processes in the liver. Using patient's primary cells and CRISPR/Cas9-mediated inactivation in human hepatic cell lines, we find that FOCAD deficiency compromises the SKI mRNA surveillance pathway by reducing the levels of the RNA helicase SKIV2L and its cofactor TTC37. FOCAD knockout hepatocytes exhibited lowered albumin expression and signs of persistent injury accompanied by CCL2 overproduction.

Conclusion: Our results reveal the importance of FOCAD in maintaining liver homeostasis and disclose a possible therapeutic intervention point via inhibition of the CCL2/CCR2 signaling axis.

References:

Grants:

Conflict of Interest: None declared.

C10.6 Mapping the effects of human gene knock-outs on immune cells using single cell transcriptomics

Teng Hiang Heng[1;2], *Carla Jones*[1], *Joanna Kaplanis*[1], *David van Heel*[3;4], *Hilary Martin*[1], *Gosia Trynka*[1;5]

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Background/Objectives: Homozygous loss-of-function variants (hom-LoFs), or "naturally-occurring knockouts", provide an opportunity for in vivo studies of potential drug targets. We prioritised participants from East London Genes and Health, a cohort enriched for homozygosity, for a recall-by-genotype to profile the effects of hom-LoFs on immune genes in blood using single-cell RNA-sequencing (scRNA-seq).

Methods: From 5236 exomes, 2713 homozygous, high-confidence predicted LoFs (hom-pLoFs) were called, affecting 2050 genes. Genotype, variant and gene characteristics were explored to reduce spuriously-called variants. To select immune-related genes, we used differential gene expression across tissues and associations to immune traits. We used publicly-available scRNA-seq data from 32 healthy Dutch individuals (1154 cells/individual) to prioritise genes for which the knockout effect on expression would be amenable to our experimental study.

Results: We curated 528 hom-pLoFs, affecting 477 genes. Hom-pLoFs that overlap with regions of homozygosity have higher genotype quality than hom-pLoFs that do not overlap, suggesting they are more likely to be real. Twenty seven genes showed blood-specific expression and were enriched for immune traits associations. Effect size calculations of expression profiles in blood scRNA-seq data identified 22 genes for which we will be powered to detect knockout effects that lead to decreased expression. This resulted in 436 individuals to approach for recall. If analysed before the conference, we will present results from investigating immune disorders in the health records and preliminary scRNAseq-data.

Conclusion: Applying quality-control filters and screening gene expression profiles allowed us to prioritise individuals with hom-LoFs anticipated to have detectable effects in the blood transcriptome.

References: https://www.genesandhealth.org/ https://eqtlgen.org/sc/datasets/1m-scbloodnl.html.

Grants: Wellcome Trust[WT206184].

Conflict of Interest: None declared.

C11 NEW DIAGNOSTICS AND TREATMENTS

C11.1 Discovery and pharmacological treatment of Bachmann-Bupp Syndrome, caused by de novo pathogenic ODC1 variant, in children with developmental delay, alopecia, and hypotonia

Andre Bachmann[1], *Chad Schultz*[1], *Elizabeth VanSickle*[2], *Julianne Michael*[2], *Jeremy Prokop*[1], *Surender Rajasekaran*[2], *Caleb Bupp*[2]

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Background/Objectives: Bachmann-Bupp Syndrome (BABS) is an autosomal dominant genetic disorder caused by heterozygous *de novo* variants in the ornithine decarboxylase 1 (*ODC1*) gene (OMIM #619075). ODC is a rate-limiting enzyme in the polyamine pathway that plays a key role in physiological and developmental processes during embryogenesis, organogenesis, and neoplastic cell growth.

Methods: The first BABS patient was seen at Helen DeVos Children's Hospital and diagnosed by whole exome sequencing (WES). Red blood cells (RBCs) and primary skin fibroblasts were analyzed for ODC protein levels, enzymatic activity, and polyamines (putrescine, spermidine, spermine) using western blot, 14-C radioactive ODC assay, and RP-HPLC, respectively. Computational modeling was performed to depict a 3D structure of ODC and its truncated variants. Under an FDA-approved single patient IND, the patient has been treated with oral ODC inhibitor DFMO (eflornithine) for 28 months (ongoing) and serial blood samples analyzed by LC-MS/MS to monitor the patient's metabolome.

Results: WES revealed a heterozygous *de novo* nonsense mutation in the *ODC1* gene that leads to a premature abrogation of 14-aa residues at the ODC protein C-terminus. Phenotypic manifestations included macrosomia, macrocephaly, developmental delay, alopecia, spasticity, hypotonia, cutaneous vascular malformation, delayed visual maturation, and sensorineural hearing loss. RBCs and primary dermal fibroblasts showed elevated ODC protein, enzyme activity, and putrescine levels compared to healthy controls. Treatment of BABS patient with DFMO reduced N1-acetylputrescine in blood and led to significant clinical improvements.

Conclusion: A total of 12 BABS patients are now known worldwide. These patients benefit from treatment with a repurposed, FDA-approved drug, DFMO/eflornithine.

References:

Grants:

Conflict of Interest: Andre Bachmann Full employment at Michigan State University, 1. R01 – Role: Principal Investigator (30% effort) (MPI, Bachmann, Casero, Bupp)

09/01/22 – 08/31/27 (pending)

NIH/NICHD – Title: "Leveraging Modulation of Polyamine Metabolism for Therapeutic Advantage in Genetic Disorders"

2. SH-MSU-ACF RG101298 – Role: Scientific Director (10% effort)

11/01/18 – 10/31/22

Spectrum Health-MSU Alliance Corporation Funds – Title: "Establishment of a Spectrum Health/Michigan State University International Center for Polyamine Disorders (ICPD)", Donations-A4838F – Role: Principal Investigator

08/01/16 – present

Donation account to Bachmann Lab at MSU – Title: "Bachmann Lab Research", 1. University of Zurich, Institute of Medical Genetics, Zurich, Switzerland. Discovery and treatment of a new syndromic neurodevelopmental disorder in children linked to a gain-of-function ODC1 gene variant. Host. Dr. Anita Rauch, June 22. 2021.

2. Grand Rounds, Henry Ford Health System, Detroit MI. MYC-driven cancers, dependence on polyamines, and treatment with ODC inhibitor DFMO. Host: Dr. Clara Hwang.

, "Methods and compositions to prevent and treat disorders associated with mutations in the ODC1 gene". Inventors: Bachmann AS, Bupp C, Rajasekaran R. Pub. No. U.S. 2020/0215010; Pub. Date July 9, 2020., 2021-present Consultant-Primary Insight Consulting, New York, NY, USA, Chad Schultz Full Time at Michigan State University Department of Pediatrics, Elizabeth VanSickle Full time employment - Spectrum Health, Julianne Michael Spectrum Health, Jeremy Prokop Full time employment – Michigan State University, Gerber Foundation, Surender Rajasekaran Full time employment – Spectrum Health, -Gerber Foundation

-National Institutes of Health Grant # 1R01GM134307-01 $453,660 USD (Year 1). Project titled, "Repurpose Open Data to discover Therapeutics for Understudied Diseases." PI: Chen, B; Co-PI: Rajasekaran, S, Devos Endowed Chair, Pediatric Research, HDVCH, "Methods and compositions to prevent and treat disorders associated with mutations in the ODC1 gene". Inventors: Bachmann AS, Bupp C, Rajasekaran R. Pub. No. U.S. 2020/0215010; Pub. Date July 9, 2020., Caleb Bupp Spectrum Health, Gerber Foundation, Spectrum Health /MSU Alliance funding; R01 pending, DFMO (Eflornithine) provided by Sanofi, "Methods and compositions to prevent and treat disorders associated with mutations in the ODC1 gene". Inventors: Bachmann AS, Bupp C, Rajasekaran R. Pub. No. U.S. 2020/0215010; Pub. Date July 9, 2020.

C11.2 Interventional genomics approaches in a neurodevelopmental syndrome

*Tyler Fortuna[1], Sukhleen Kour[1], Eric Anderson[1], Caroline Ward[1], Om Chauhan[1], Casey O'Brien[1], Dhivyaa Rajasundaram[1], Deepa Rajan[1], **Udai Pandey**[1]*

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Background/Objectives: GEMIN5, an RNA-binding protein, plays an important role in the core assembly of small nuclear Ribonucleoproteins (snRNPs), the building blocks of spliceosome formation.

Methods: We performed whole exome sequencing to identify novel variants among patients with a neurodevelopmental syndrome. We developed patient iPSC and drosophila models for examining the molecular mechanisms and performed an in vivo genetic screen to identify dominant modifiers of disease.

Results: We identified novel autosomal recessive variants among 35 patients presenting with developmental delay, ataxia, motor dysfunction, and cerebellar atrophy using whole-exome sequencing approach. GEMIN5 variants perturb the subcellular distribution, stability, and expression of GEMIN5 protein and its interacting partners in iPSC neurons, indicating a potential loss-of-function mechanism. Interestingly, GEMIN5 mutations disrupt snRNP complex assembly formation in patient iPSC neurons. Molecular determinants of GEMIN5-mediated disease are yet not known.

While doing a genetic screen, we identified SMN, a component of the snRNP complex, as a genetic modifier of GEMIN5 phenotypes in vivo. We found that GEMIN5 levels are upregulated in response to genetic expression of SMN in iPSC neurons and in vivo. Interestingly, treatment of the SMN2 therapeutic antisense oligonucleotide, ISS-N1 (nusinersen), significantly upregulated GEMIN5 protein levels in iPSC neurons, suggesting a functional interaction between both proteins. SMN expression restored the defective snRNP components of the SMN complex in GEMIN5 patient neurons. In addition, we show that SMN expression increases the number of nuclear bodies which are otherwise lost in GEMIN5 patient neurons.

Conclusion: Our findings indicate that SMN is a novel regulator of GEMIN5 expression and neuropathologies.

References:

Grants:

Conflict of Interest: None declared.

C11.3 Thalidomide is an Efficient Treatment for Symptomatic and/or Life-Threatening Arteriovenous Malformations

***Miikka Vikkula**[1], Laurence Boon[2], Liliane Marot[2], Frank Hammer[2], Anne Jeanjean[2], Valerie Dekeuleneer[2], Marleen Tadler[2], Philippe Clapuyt[2], Anne-Christine Bataillle[2], Anne Dompmartin[3]*

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Background/Objectives: Arteriovenous malformations (AVMs) are fast-flow lesions that may be destructive and are the most difficult to treat vascular anomalies. Embolization followed by surgical resection is commonly used; however, complete resection is rarely possible and partial resection often leads to dramatic worsening. Accumulating data implicate germline or somatic mutations that activate the RAS-MAPK signaling pathway to induce angiogenesis.

Methods: We conducted a prospective experimental observational study using Thalidomide, a potent anti-angiogenic inhibitor, in 18 patients with a severely symptomatic AVM refractory to conventional therapies. Thalidomide was given 50 to 200 mg/day for 2 to 52 months.

Results: All patients experienced rapid reduction of pain (VAS from 6-10 to 0-5) (18/18), cessation of bleeding (11/11), and healing of ulcers (6/6). Cardiac failure resolved in all three affected patients. Reduced vascularity on arteriography was observed in two patients. One AVM appeared cured after 19 months of thalidomide and an 8-year follow-up. Eight AVMs were stable after a mean thalidomide cessation of 58 months, and four lesions recurred after 11.5 months. Combined treatment with embolization permitted dose reduction (50 mg/day) in 5 patients with clinical improvement. Five patients continued low dose thalidomide. Grade 3 side-effects were dose-dependent including asthenia ($n = 2$), and erythroderma ($n = 2$).

Conclusion: Thalidomide is efficacious in the management of chronic pain, bleeding, and ulceration of extensive AVMs recalcitrant to conventional therapy.

References:

Grants: FRNS PdR Thema P.C013.20 (to LB).

Conflict of Interest: None declared.

C11.4 Identifying disorder-specific DNA methylation signatures in patients with severe developmental disorders

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[1]Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands; [2]Wellcome Sanger Institute, Human Genetics, Hinxton, United Kingdom.

Background/Objectives: Chromatin-modifying genes (CMGs) are a diverse set of genes whose protein products bind to chromatin and regulate cellular processes including the regulation of proliferation and gene expression. CMGs typically act via the modification of histones. Unsurprisingly given the importance of CMGs in several higher-order cellular functions, deleterious mutations in

over 50 CMGs have been robustly associated with severe developmental disorders (DDs).

Methods: We characterised methylation profiles in blood- and saliva-derived DNA from 442 DD probands with mutations in one of 22 disease-associated CMGs in the Deciphering Developmental Disorders (DDD) study, a UK-wide cohort of 13,600 children with DD. These probands were methylation profiled alongside 34 age, sex, and tissue-matched negative control samples on the Illumina EPIC methylation array.

Results: Using our methylation data as input to a linear correlation model and an elastic net, we established a robust DNA methylation signature in 17 CMGs, indicating that a perturbed methylation phenotype is a general characteristic of CMG disorders. DNA methylation signatures are highly concordant with clinical interpretation of variant pathogenicity in DDD data (sensitivity = 95%, specificity = 97%). Using a likelihood-ratio based classification approach, we show that we can resolve 90% of VUS (130 variants) in 13 selected CMGs. Additionally, we show that DNA methylation signatures outperform gold standard in silico predictors of variant pathogenicity and are robust across classes of genomic variation, diagnostic centres, and tissues.

Conclusion: DNA methylation signatures are scalable biomarkers supporting the diagnosis of CMG disorders.

References:

Grants:

Conflict of Interest: None declared.

C11.5 Effect of whole-genome sequencing on the clinical management of acutely ill infants with suspected genetic disease (virtual)

Alison Coffey[1], *Ian Krantz*[2], *Luca Brunelli*[3;4], *David Dimmock*[5;6], *Marwan Shinawi*[7], *Nora Urraca*[8;9], *Chester Brown*[8;9], *John Belmont*[1], *Julia Ortega*[1], *Keisha Robinson*[1], *Denise Perry*[1], *Subramanian Ajay*[1], *Maren Bennett*[1], *Vani Rajan*[1], *Ryan Taft*[1]

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Background/Objectives: To determine the effect of whole-genome sequencing (WGS) on clinical management in a racially and ethnically diverse, geographically distributed population of acutely ill infants in the US.

Methods: This randomized, time-delayed clinical trial, conducted at 5 US academic medical centers, enrolled from September 11, 2017, to April 30, 2019, with observation to July 2, 2019. Participants included infants admitted to an intensive care unit with suspected genetic disease. Patients were randomized to receive clinical WGS results 15 days (early) or 60 days (delayed) after enrollment, with the observation period extending to 90 days. Usual care was continued throughout the study.

Results: 354 infants were randomized to the early (n = 176) or delayed (n = 178) arms. The mean participant age was 15 days; 201 participants (56.8%) were boys; 19 (5.4%) were Asian; 47 (13.3%) were Black; 250 (70.6%) were White; and 38 (10.7%) were other race. At 60 days, twice as many infants in the early group vs the delayed group received a change of management (COM) and a molecular diagnosis. At 90 days, the delayed group showed a doubling of COM and diagnostic efficacy. The most frequent COMs across the observation window were subspecialty referrals, surgery or other invasive procedures, condition-specific medications, or other supportive alterations in medication. No differences in length of stay or survival were observed.

Conclusion: Access to first-line WGS is associated with an increase in focused clinical management and may reduce health care disparities, enabling diagnostic equity and thus supporting WGS implementation in this population.

References: PMID 34570182.

Grants:

Conflict of Interest: Alison Coffey An employee and shareholder of Illumina Inc during the conduct of the study., Ian Krantz: None declared, Luca Brunelli member of the Illumina Inc speakers' bureau in 2019., David Dimmock receiving previous consulting fees from Audentes, Biomarin, Ichorion, and Complete Genomics; serving on a scientific advisory board for Taysha Gene Therapies; and being an inventor on US patent 8718950B2 assigned to the HudsonAlpha Institute for Biotechnology, Marwan Shinawi: None declared, Nora Urraca: None declared, Chester Brown: None declared, John Belmont full-time employee of Illumina Inc until February 2021 and being a shareholder of Illumina Inc during the conduct of the study., Julia Ortega an employee and shareholder of Illumina Inc during the conduct of the study., Keisha Robinson an employee and shareholder of Illumina Inc during the conduct of the study., Denise Perry an employee and shareholder of Illumina Inc during the conduct of the study, Subramanian Ajay an employee and shareholder of Illumina Inc during the conduct of the study, Maren Bennett an employee and shareholder of Illumina Inc during the conduct of the study, Vani Rajan employee and shareholder of Illumina Inc during the conduct of the study, Ryan Taft receiving compensation for services on the scientific advisory board of Creyon Bio and being an employee and shareholder of Illumina Inc during the conduct of the study.

C11.6 Rapid rare disease diagnosis on a national scale: an integrated multi-omic approach

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Australia; [16]Department of Clinical Genetics, The Canberra Hospital, Canberra, Australia; [17]University of Melbourne, Melbourne, Australia; [18]Murdoch Children's Research Institute, Melbourne, Australia.

Background/Objectives: Whole genome sequencing (WGS) is increasingly deliverable at scale and speed across healthcare systems. However, the improved analytical performance and ever earlier test initiation exacerbate interpretive challenges. Extended bioinformatic analysis and integration of multi-omic approaches hold the promise to optimise diagnostic performance.

Methods: Trio ultra-rapid WGS was performed in a national cohort of 254 critically ill paediatric patients with rare disease, ascertained prospectively between January 2020 and January 2022. Undiagnosed patients underwent research-based RNA sequencing and extended bioinformatic and research analyses. Functional assays and targeted orthogonal tests were employed in selected cases.

Results: Ultra-rapid WGS resulted in a diagnosis in 113 patients (44%), with an average time to clinical report of 2.96 days. Structural and deep intronic variants accounted for 12 of these diagnoses (11%). Of the 142 patients remaining undiagnosed, 17 (12%) were subsequently diagnosed. RNA sequencing identified three diagnoses related to non-coding variants and contributed to classification of five variants. Functional assays secured seven further diagnoses, and evolution of the clinical phenotype resulted in two more. Extended bioinformatic analyses identified one triplet expansion disorder. Four diagnoses were made using orthogonal tests, highlighting limitations of current WGS pipelines in identifying uniparental disomy, variants in regions with poor mappability, and mosaic variants. Further studies are underway in four novel gene candidates.

Conclusion: We demonstrate the integration of multi-omic approaches in a broad clinical cohort of rare disease patients to rapidly optimise WGS diagnostic yield, arguing for increased integration of these approaches into mainstream diagnostic practice.

References:

Grants: Australian Government GHFM76747.

Conflict of Interest: None declared.

C12 GWAS

C12.1 Large scale multi-trait genome-wide association analysis identifies hundreds of glaucoma risk loci

Stuart MacGregor[1], *Xikun Han*[1], *Puya Gharahkhani*[1], *Jue Sheng Ong*[1], *Francesca Pasutto*[2], *Chris Hammond*[3], *Terri Young*[4], *Pirro Hysi*[3], *Alex Hewitt*[5], *Jamie Craig*[6], *Louis Pasquale*[7], *David Mackey*[8], *Janey L. Wiggs*[9;10], *Anthony Khawaja*[11], *Ayellet Segre*[9;10]

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Background/Objectives: Glaucoma, the leading cause of irreversible blindness, is a highly heritable human disease, with previous genome wide association studies (GWAS) identifying over 100 loci for the most common form, open-angle glaucoma. However, much of the heritability of glaucoma remains unexplained and mapping additional loci would advance disease etiology understanding and may inform novel drug targeting.

Methods: We conducted a large-scale multi-trait GWAS combining open angle glaucoma case-control data with two key glaucoma-associated traits: intraocular pressure and optic nerve head damage (quantified as the vertical cup to disc ratio). Taking into account the large size of the multi-ancestry case-control cohorts (29,241 cases and 350,181 controls) and the high correlation between glaucoma and the associated traits, our effective sample size is three times larger than previous GWAS. The novel associations were replicated in a large, independent glaucoma case-control cohort.

Results: We increased the number of independent risk loci to 312, with the vast majority replicating in an independent cohort. Leveraging omics datasets, we are able to identify many potential new drug target genes, including neuroprotection targets likely to act via the optic nerve, a key advance for glaucoma because all existing drugs only target intraocular pressure. We further use Mendelian Randomization and genetic correlation-based approaches to identify novel links to other complex traits, including immune dysfunction diseases such as multiple sclerosis and systemic lupus erythematosus.

Conclusion: Our study has dramatically improved the number of loci associated with open angle glaucoma and uncovers many potential new therapeutic targets.

References:

Grants:

Conflict of Interest: Stuart MacGregor Australian National Health and Medical Research Council grants 1150144 and 1116360, Co-founder of and holds stock in Seonix Bio Pty Ltd., Xikun Han: None declared, Puya Gharahkhani: None declared, Jue Sheng Ong: None declared, Francesca Pasutto: None declared, Chris Hammond: None declared, Terri Young Research to Prevent Blindness, Inc., Centennial Scholars Research Fund, Pirro Hysi: None declared, Alex Hewitt Australian National Health and Medical Research Council grants 1150144 and 1116360, Co-founder of and holds stock in Seonix Bio Pty Ltd., Jamie Craig Australian National Health and Medical Research Council grants 1150144 and 1116360, Co-founder of and holds stock in Seonix Bio Pty Ltd., Louis Pasquale R01 EY032559 and R01 EY015473, LRP is a consultant to Eyenovia, Twenty Twenty and Skye Bioscience., David Mackey Australian National Health and Medical Research Council grants 1150144 and 1116360, Janey L. Wiggs NIH/NEI EY022305, Anthony Khawaja: None declared, Ayellet Segre NIH/NEI R01 EY031424-01.

C12.2 Estimating age-specific genetic effects for age-at-onset phenotypes

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Background/Objectives: A common assumption in genome-wide association analyses is that the SNP effects remain constant throughout the lifespan. Although this assumption is natural for many traits, it is more questionable for time-to-event traits where the onset hazard changes due to SNPs having a different impact across lifespan. Disregarding the potential age-specific marker

effects can therefore lead to decreased power and incomplete description of the genetic architecture.

Methods: We firstly propose a joint Bayesian model for estimating age-specific marker effects on age-at-diagnosis traits with changes in effect sizes captured by modelling the variance and correlation of different age-specific genetic liabilities. Furthermore, the model partitions markers between genomic annotations and estimates respective age-specific genetic variances and correlations. We expand the results with a marginal time-varying Cox proportional hazards model to obtain age-specific marginal SNP effects.

Results: We apply our model on age-at-menopause data in the UK Biobank, replicating the associations in the Estonian Biobank. In contrast to the constant effect assumption, our joint model discovers hundreds of genomic regions for which age-specific genetic correlation is non-positive suggesting evidence for independent or balancing age-specific mechanisms. Moreover, the marginal association analysis finds 9% of genome-wide significant SNPs exhibit altering effect size and five novel SNPs have effects specific to a single period.

Conclusion: Our comparisons enable a more accurate description of the survival-related SNP effects providing novel insight into the genetic architecture of large-scale time-to-event data and improved statistical power for traits that exhibit a change in effect sizes across the lifespan.

References:

Grants: SNSF Eccellenza Grant to MRR (PCEGP3-181181).

Conflict of Interest: None declared.

C12.3 Genetic architecture of longitudinal obesity trajectories in primary care electronic health records

Samvida Venkatesh[1;2], *Habib Ganjgahi*[2;3], *Duncan Palmer*[2;4], *George Nicholson*[3], *Christoffer Nellaker*[2;4], *Chris Holmes*[3;5], *Cecilia Lindgren*[2;4;6]

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Background/Objectives: Genome-wide association studies (GWASs) for obesity (body mass index (BMI)) have identified hundreds of associated loci. However, cross-sectional outcomes do not capture the biology of weight change over adult lifespan.

Methods: Here, we integrate UKBiobank-linked genotyping with primary care electronic health records (GP-EHRs) in ~250,000 individuals with >2.8 million observations of BMI and weight to discover genetic associations with obesity trajectories. We model obesity change with mixed effects models with random slopes for time and, crucially, capture non-linear effects of time with natural cubic splines. We perform GWAS on components of obesity trajectories, including intercept, slope, and cluster identity defined by mixture model clustering of spline coefficients.

Results: Three independent variants are significantly associated with weight change per year adjusted for baseline weight: rs814573 ($\beta = 0.058$ per SD increase in weight per year, $P = 2.8E\text{-}39$), rs811041 ($\beta = 0.022$, $P = 3.1E\text{-}09$), and rs1596181 ($\beta = -0.018$, $P = 4.5E\text{-}08$), each of which reside in known cholesterol loci. 98 independent variants are associated with modelled intercepts of BMI, 6 of which represent novel loci for BMI or other obesity-related phenotypes. Incorporating longitudinal information offers increased power to detect associations. We observe a 14% boost in effective sample size over that expected from cross-sectional outcomes alone. Further, by accounting for longitudinal information, we are able to classify individuals by disease risk, and characterise genetic loci associated with non-linear obesity trajectories.

Conclusion: This is, to our knowledge, the largest study of its kind, demonstrating the potential of leveraging GP-EHRs to provide insight into the complex genetics of longitudinal traits.

References:

Grants:

Conflict of Interest: Samvida Venkatesh: None declared, Habib Ganjgahi: None declared, Duncan Palmer: None declared, George Nicholson: None declared, Christoffer Nellaker: None declared, Chris Holmes: None declared, Cecilia Lindgren Research grants from Bayer AG and Novo Nordisk., Partner who works at Vertex.

C12.4 Disentangling the aetiological pathways between body mass index and site-specific cancer risk using tissue-partitioned Mendelian randomization

Genevieve Leyden[1;2], *Michael Greenwood*[1], *David Murphy*[1], *George Davey Smith*[2], *Tom G. Richardson*[2;3]

[1]*University of Bristol, Bristol Medical School: Translational Health Sciences, Bristol, United Kingdom;* [2]*University of Bristol, MRC Integrative Epidemiology Unit, Bristol, United Kingdom;* [3]*Novo Nordisk Research Centre, Oxford, United Kingdom.*

Background/Objectives: Body mass index (BMI) influences risk of various site-specific cancers, although dissecting the sub-components of this heterogenous lifestyle factor responsible for driving cancer risk has proven difficult to establish. In this study, we have leveraged tissue-specific gene expression to separate and estimate the independent effects of distinct phenotypes underlying BMI on risk of 6 site-specific cancers.

Methods: We recently developed methodology (Leyden et al., 2022) to leverage BMI-associated variants as instrumental variables within a multivariable Mendelian randomization (MR) framework weighted by their evidence of genetic colocalization with subcutaneous adipose- and brain-tissue derived gene expression. Here, we extend this approach to a two-sample setting to harness findings from large-scale consortia.

Results: Our results provide evidence that brain-tissue colocalizing variants are predominantly responsible for driving the genetically predicted effect of BMI on lung cancer (OR:1.17; 95%-CI = 1.01-1.36; P = 0.03). Similar findings were identified when analysing cigarettes per day as an outcome (Beta=0.44; 95%-CI = 0.26-0.61; P = 1.62×10-6), suggesting that neurobiological pathways may underlie the relationship between BMI and increased lung cancer risk. Our findings also suggest that adipose-tissue colocalizing variants predominantly drive the effect of BMI and increased risk for endometrial cancer (OR:1.71; 95%-CI = 1.07-2.74; P = 0.02) highlighting the putatively important role of adipogenesis in the aetiology of this outcome.

Conclusion: Our novel extension to multivariable MR provides valuable insight into the divergent underlying pathways between BMI and risk of site-specific cancers. Conducting this approach in a two-sample setting has wide potential applicability to disentangle mechanisms between adiposity and a spectrum of disease outcomes.

References: https://doi.org/10.1016/j.ajhg.2021.12.013.

Grants: BHF: FS/17/60/33474, MRC: MC_UU_00011/1.

Conflict of Interest: Genevieve Leyden: None declared, Michael Greenwood: None declared, David Murphy: None declared, George Davey Smith: None declared, Tom G. Richardson TGR is employed part-time by Novo Nordisk outside of this work.

C12.5 Phenome-wide association study of clonal haematopoiesis somatic mutations in 422,456 UK Biobank participants

Jonathan Mitchell[1], *Fengyuan Hu*[1], *Quanli Wang*[1], *Keren Carss*[1], *Andrew Harper*[1], *Slavé Petrovski*[1]

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Background/Objectives: Clonal haematopoiesis (CH) is a common, age-related process underpinned by somatic mutations in genes (~80% in DNMT3A, TET2, and ASXL1) that regulate haematopoietic stem cell self-renewal and differentiation. CH is a well-established risk factor for haematological malignancy and is increasingly recognised as a cause of common non-oncological disease that encompass prominent inflammatory features, such as atherosclerosis and chronic obstructive pulmonary disease. To further appreciate gene-phenotype associations attributable to CH we analysed exome sequencing data from 422,456 UK Biobank participants.

Methods: Mutect2 somatic variant calling was run on a virtual panel of genes known to be drivers of CH and putative somatic mutations identified based on gene specific variant effects. We performed a gene-level collapsing analysis-based phenome-wide association study (PheWAS) of the detectable somatic mutations with 15,709 binary and 1,446 quantitative phenotypes. This analysis was restricted to 391,734 individuals of European ancestry and was adjusted for age, sex, smoking and BMI.

Results: Across 19 genes we identified 23,060 putative somatic variants in 21,241 individuals. The PheWAS showed 124 statistically significant ($P < 5 \times 10^{-8}$) binary trait associations, of which 39 (31%) were not cancer-related, and 19 associations with quantitative traits. Of the studied genes, most associations were with *TET2* (n = 64).

Conclusion: We have analysed sequencing data in the UK Biobank for CH mutations and validated and expanded upon their known trait associations. Our work demonstrates the broad clinical impact of CH and future analysis will investigate germline genetic predisposition to CH.

References:

Grants:

Conflict of Interest: Jonathan Mitchell AstraZeneca, AstraZeneca, Fengyuan Hu AstraZeneca, AstraZeneca, Quanli Wang AstraZeneca, AstraZeneca, Keren Carss AstraZeneca, AstraZeneca, Andrew Harper AstraZeneca, AstraZeneca, Slavé Petrovski AstraZeneca, AstraZeneca.

C12.6 Effect of sex and age on disease prediction with polygenic scores in INTERVENE

Brooke Wolford[1], *Bradley Jermy*[2], *Tuomo Hartonen*[2], *Kristi Läll*[3], *Zhiyu Yang*[2], *Remo Monti*[4], *Stavroula Kanoni*[5], *Ying Wang*[6;7], *Kristina Zguro*[8], *Henrike Heyne*[2;4;9], *Julian Wanner*[2;4], *Omar Youssef*[10;11], *FinnGen Consortium*[2], *Alicia Martin*[7], *David van Heel*[5], *Kristian Hveem*[1], *Reedik Mägi*[3], *Aarno Palotie*[2;7], *Nina Mars*[2], *Andrea Ganna*[2;7], *Samuli Ripatti*[2;7;12]

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Background/Objectives: The INTERnational consortium of integratiVE geNomics prEdiction (INTERVENE) project includes 7 biobanks with harmonized genetic data and electronic health records (EHR) from 1.3 million biobank participants in Europe and abroad. In this research, we aim to understand how interactions of polygenic scores (PGS) with sex and heterogeneous effects of PGS across age strata may influence clinical screening recommendations.

Methods: We selected 39 heritable disease (e.g., coronary heart disease, asthma, gout, and prostate cancer) with a significant contribution to global burden of disease and available GWAS summary statistics or published PGS. We evaluated the heterogeneity of PGS prediction across sex and age strata using multivariable logistic regression.

Results: Of 25 disease PGSs tested in INTERVENE biobanks, we identified a significant interaction of PGS and sex in the Estonian Biobank for gout ($OR_{interaction}$=1.09, p-value =1×10^{-3};OR_{Male} = 1.61 [95% CI 1.55-1.67] versus OR_{Female} = 1.48 [95% CI 1.42-1.54]) which replicated with consistent directions of effect in FinnGen and UK Biobank. The prostate cancer PGS association decreases linearly over the lifespan from age 40 onwards across biobanks, with the greatest difference seen in FinnGen ($OR_{Age\ 40\text{-}50}$ = 3.16 [95%CI 2.56-3.91] versus $OR_{Age\ 70\text{-}80}$ = 1.87 [95%CI 1.78-1.96]). These analyses are now being replicated in additional biobanks and across other clinically relevant strata.

Conclusion: The INTERVENE project is exploring factors such as age and sex which impact the clinical utility of disease prediction with PGS. These insights, in combination with longitudinal clinical data from the EHR, will inform the development and deployment of PGS.

References:

Grants: Horizon 2020 (101016775).

Conflict of Interest: None declared.

C13 PATIENT VIEWS ON CLINICAL GENETICS

C13.1 What is the impact of BRCA1/2 status on young women's reproduction and relationships after predictive testing? An Australian case-control study

Laura Forrest[1;2], *Rowan Forbes Shepherd*[1;3], *Louise Keogh*[4], *Lucinda Salmon*[5], *Linda Warwick*[6], *Jo Burke*[7], *Rebecca D'Souza*[8], *Rachel Williams*[9;10], *Catherine Beard*[11], *Tim Spelman*[12;13], *Paul James*[1;2]

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Background/Objectives: The psychosocial implications for young women of living with a *BRCA1/2* pathogenic variant (PV) are thematically well-evidenced but not well quantified. The aim of this study is to investigate the impact of *BRCA1/2* status on women's reproduction and partnering.

Methods: Data were collected using an online survey with a case-control design from June 2019 to May 2021. Eligible participants were invited from eight Australian clinical genetics services, recruiting women aged 18—40 years who had predictive *BRCA1/2* testing, received either a positive or negative result, and were unaffected by cancer. Outcomes included childbearing, relationship status, intimacy, and adaptation. Descriptive and inferential statistics were used; p values <0.05 were considered statistically significant.

Results: 579 women participated (62.0% *BRCA1/2* positive; 38.0% *BRCA1/2* negative). More women who were *BRCA1/2* positive had children compared to those who tested negative (49.0% *c.f.*, 40.5%; $p = 0.045$). No other demographic differences were observed. Multivariate regression analyses determined that women's *BRCA1/2* status did not predict whether they had children ($p = 0.109$), whether they were partnered ($p = 0.375$), their experience of intimacy ($p = 0.877$), or adaptation to their genetic status ($p = 0.475$). Subgroup analyses examining outcomes for women with a *BRCA1/2* PV indicated that increasing age was negatively associated with adaptation (β co-eff 0.05, 95% CI -0.08—-0.03, $p < 0.001$), whereas uptake of risk-reducing mastectomy was positively associated (β co-eff 0.80, 95% CI 0.53—1.07, $p < 0.001$).

Conclusion: Living with a *BRCA1/2* PV does not detrimentally impact young women's childbearing, partnering, or adaptation. These findings contribute to the evidence-base to inform long-term follow-up for women after predictive *BRCA1/2* testing.

References:

Grants:

Conflict of Interest: None declared.

C13.2 Making the right choice. How unaffected women carrying BRCA1/BRCA2 germline pathogenic variants decide for prophylactic mastectomy to reduce cancer risk

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Background/Objectives: For women at very high risk of breast cancer, prophylactic surgery is an alternative to intensive surveillance to preserve their health. Risk-reducing mastectomy (RRM) strongly reduces the risk of breast cancer but may generate health and psychosocial issues. The choice between intensive surveillance and RRM appears therefore challenging and highly personal.

Methods: This grounded theory study explores the decision-making process for RRM of unaffected women carrying *BRCA1/BRCA2* germline pathogenic variants. Data is acquired from two Swiss datasets of biographical qualitative interviews performed in these women. The first set of data was collected between 2011 and 2014 and included narratives of 32 women; the second set was collected between 2019 and 2021 and included narratives of 14 women.

Results: The data shows that RRM decision is influenced by several factors, including women's self-identity and moral values, prevention norms, risk perception based on lay theories, stage in the life course, and experiences with intensive surveillance and relationship with physicians. All these factors interact with each other, sometimes creating contradictions that makes decision-making more difficult. To navigate in this context of uncertainty, women progressively build their RRM decision by engaging in a three-step process: thinking of RRM as an obligation, de-dramatizing RRM and building consensus around it.

Conclusion: The decision to undergo RRM is more the result of a complex interaction between the woman and her context than an intimate and private choice. Health professionals should be aware of this decision-making process and help women to govern it.

References: -

Grants: Swiss Cancer League KLS-4294-08-2017.

Conflict of Interest: None declared.

C13.3 A cross-country comparison of women's perspectives on non-invasive prenatal testing in Belgium and the Netherlands

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Background/Objectives: Belgium and the Netherlands are among the few countries in the world to offer non-invasive prenatal testing as a first-tier screening test to all pregnant women. An informed and autonomous decision is considered important when deciding to participate in prenatal screening for aneuploidy. Despite the similarities, counselling modalities and uptake rates between both countries differ. We assessed the differences in perspectives of pregnant women who opted for prenatal screening with NIPT.

Methods: A cross-country comparison study between the Netherlands and Belgium, using a questionnaire. The questionnaire was developed for the TRIDENT-2 study and assessed informed choice (MMIC), and personal and societal perspectives on Down syndrome.

Results: A total of 1031 women having NIPT participated in the survey study; 444 women from Belgium (B) and 587 women from the Netherlands (NL). Differences between Belgian and Dutch women were shown for the level of informed choice (58.8%(B) vs. 82.6%(NL)), intention to terminate in case of confirmed Down syndrome (61.9%(B) vs. 50.5%(NL)) and how the disorder was perceived in terms of severity (80.9%(B) vs. 64.3% (NL)). More Belgian women indicated that they believed parents are judged

for having a child with Down syndrome, compared to the Dutch women (42.3% vs. 16.3%). Also, Belgian women were less positive about the care and support for children with Down syndrome, as compared to their Dutch counterparts (22.5% vs. 62%).

Conclusion: This study indicates that counseling modalities and societal and cultural aspects may impact women's perspectives.

References:

Grants: -

Conflict of Interest: None declared.

C13.4 GeneEQUAL: Inclusive research to gain insights into the knowledge, perspectives, and experiences of people with intellectual disability about genomic healthcare

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Background/Objectives: Little is known about the genomic health care experiences of people with intellectual disability from their perspective. This is a key gap in the delivery of accessible and equitable genomic healthcare.

Methods: Informed by our systematic literature review[1], an interview schedule was co-designed to explore the knowledge, perspectives, and experiences of people with mild to moderate intellectual disability with genomic healthcare in the state of NSW, Australia. Inclusive research practices were used, e.g., Easy Read consent forms, and a co-researcher with intellectual disability is a member of the research team. Emerging themes are iteratively discussed with a multi-stakeholder Advisory Committee, including health service and government representatives.

Results: Key areas of concern affecting people with intellectual disability include: (i) referrals to 'help' a relative, rather than to empower clients; (ii) exclusion from the genetic consent process; (iii) lack of information on how their diagnosis affects health surveillance and management; (iv) failure to recognise the emotional impact of genomics, adding to existing stigma and trauma.

Conclusion: Targeted genomic health literacy for people with intellectual disability is needed in schools and primary healthcare. Genetic health professions require upskilling in the provision of trauma-informed and respectful care, including appropriate specialist mental health referrals. Easy Read genomic counselling and condition-specific management toolkits must be co-designed to improve accessibility of genomic health services.

References: 1 Strnadová I, et al. The opinions and experiences of people with intellectual disability regarding genetic testing and genetic medicine: A systematic review. Genetics in Medicine. 2021.

Grants: Ministry of Health, NSW Grant 8576.

Conflict of Interest: None declared.

C13.5 Patient perspectives on the offer of information letters from healthcare to relatives at risk for hereditary cancer: a qualitative study

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Background/Objectives: Hereditary cancer risk information to at-risk relatives (ARRs) is patient-mediated in most countries. DIRECT, a Swedish multicenter randomized controlled trial, evaluates if information letters from healthcare to ARRs affects the proportion of relatives reached, as compared with patient-mediated disclosure (control). This paper describes DIRECT participants' perception of communicating risk with ARRs.

Methods: We conducted 17 in-depth interviews during 2021 (n = 10 intervention, n = 7 control). The interviews were recorded, transcribed, and analyzed with an inductive qualitative approach inspired by grounded theory.

Results: Many patients perceived risk disclosure as important and both groups disclosed information to close relatives themselves. Some considered it their obligation also to tell relatives they did not have any social connection to, whereas others limited their responsibility to only include close relatives. Sharing responsibilities and discussing hardship within the family were facilitators, while lack of closeness, energy or understanding hindered reaching ARRs. No patients offered disclosure assistance reported integrity-related issues, and the majority accepted information letters to all ARRs. Letters were not crucial for informing close relatives, but some patients considered assisted disclosure valuable for relatives they were not in contact with.

Conclusion: Patients' view on their duty to inform relatives varied, making it unpredictable which relatives would be informed. This underlines the need for more tailored approaches to risk disclosure. In families with weak social bonds, the healthcare-assisted offer could be an option to reach more ARRs.

References:

Grants: Swedish Research Council for Health, Work life and Welfare (FORTE) grant 2018-00964, Cancer research foundation grant 2020-1107.

Conflict of Interest: None declared.

C13.6 Experiences of pregnant women with genome-wide non-invasive prenatal testing in a national screening program

***Karuna van der Meij**[1], Mireille Bekker[2], Linda Martin[3], Janneke Gitsels - van der Wal[3], elsbeth van vliet-lackotzki[4], Merryn Macville[5], Marjan Weiss[6], Robert-Jan Galjaard[7], erik sistermans[1], Lidewij Henneman[1], dutch nipt consortium[1]*

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Background/Objectives: Pregnant women's perspectives should be included in the dialogue on the expanding offers of non-invasive prenatal testing (NIPT), especially now that technological possibilities are rapidly increasing. This study evaluated women's experiences with the offer of genome-wide (GW) first-tier NIPT and their opinion on the future scope of NIPT.

Methods: A nationwide pre-and post-test questionnaire was completed by 473 pregnant women who chose NIPT within the Dutch TRIDENT-2 study. The questionnaires assessed satisfaction, reasons for choosing targeted (only trisomies 21, 18 and 13) or GW-NIPT (including a report on findings on other autosomes), general anxiety, pregnancy-related anxiety, and opinion on the future scope of NIPT.

Results: The majority of respondents (90.4%) were glad to have been offered the choice between GW-NIPT and targeted NIPT; 76.5% (362/473) chose GW-NIPT. Main reasons to choose GW-NIPT were 'wanting as much information as possible' (38.6%) and 'to be prepared for everything' (23.8%). Main reasons to choose targeted

NIPT were 'avoiding uncertain results' (33.7%) and 'not wanting to unnecessarily worry' (32.6%). No differences were found in anxiety levels between women choosing for GW- or targeted NIPT. Most respondents (93%-77%) were favourable towards a future screening offer aimed at screening lethal disorders, neurodevelopmental disorders, disorders treatable in pregnancy and physical disabilities, regardless of their choice for GW or targeted NIPT.

Conclusion: The results from this study can inform the dialogue surrounding the expansion of NIPT, contribute to the development of national and professional guidelines and improve information provision for pregnant women.

References:

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Conflict of Interest: Karuna van der Meij employed by grant from Netherlands Organization for Health Research and Development (ZonMw, grant no. 543002001). TRIDENT-2 study, Mireille Bekker: None declared, Linda Martin: None declared, Janneke Gitsels - van der Wal: None declared, elsbeth van vliet-lackotzki: None declared, Merryn Macville Collaborator TRIDENT-2 study: Netherlands Organization for Health Research and Development (ZonMw, grant no. 543002001)., Marjan Weiss: None declared, Robert-Jan Galjaard Collaborator TRIDENT-2 study: Netherlands Organization for Health Research and Development (ZonMw, grant no. 543002001)., erik sistermans Collaborator TRIDENT-2 study: Netherlands Organization for Health Research and Development (ZonMw, grant no. 543002001)., Lidewij Henneman PI, Netherlands Organization for Health Research and Development (ZonMw, grant no. 543002001)., dutch nipt consortium Collaborator TRIDENT-2 study: Netherlands Organization for Health Research and Development (ZonMw, grant no. 543002001).

C14 LATE BREAKING ABSTRACTS

C14.1 Progressive liver, kidney and heart degeneration in adults affected by TULP3 mutations

Elisabeth Ott[1], John Devane[1], Eric G. Olinger[2], Daniel Epting[1], Eva Decker[3], Anja Friedrich[3], Nadine Bachmann[3], Gina Renschler[3], Tobias Eisenberger[3], Genomics England Research Consortium[4], Inga Gruenewald[5], Martin Konrad[6], Jens König[6], Bernhard Schlevogt[7], John Sayer[8], ***Carsten Bergmann****[1;3]*

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Background/Objectives: Organ fibrosis is an endpoint of many diseases. Ciliopathies usually start early in life and represent a considerable disease burden in paediatric patients.

Methods: We performed NGS, clinical, imaging and histopathological analysis involving eight unrelated families. Mechanistic studies were conducted using patient liver, heart and kidney biopsies, a vertebrate model and patient cells.

Results: We detected biallelic deleterious variants in TULP3, a critical adapter protein for ciliary trafficking, in 15 patients who presented with progressive degenerative disease in different organs including fibrocystic kidney disease, liver fibrosis and hypertrophic cardiomyopathy. Liver biopsies revealed a distinct fibrotic pattern not in line with ductal plate malformation usually seen in paediatric ciliopathies and myocardial fibrosis followed an atypical pattern reminiscent of systemic disease with cardiac involvement. We recapitulated the human phenotype in zebrafish as a vertebrate model and confirmed disruption of ciliary cargo composition in patient-derived primary cells. Additionally, we validated a novel interaction between TULP3 and the nuclear deacetylase SIRT1, with roles in DNA damage repair and fibrosis. Increased levels of DNA damage were also seen in patient cells. Patient-cell based transcriptomic studies highlighted the upregulation of profibrotic pathways with gene clusters for hypertrophic cardiomyopathy, WNT and TGFß signalling.

Conclusion: These findings identify a novel monogenic cause for progressive degenerative disease of major organs in which patients benefit from early detection and improved clinical management. Elucidation of mechanisms crucial for well-balancing DNA-damage repair and tissue maintenance will help guiding novel therapeutic avenues for this and similar genetic and non-genomic diseases.

References:

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Conflict of Interest: Elisabeth Ott The authors declare no competing interests., John Devane The authors declare no competing interests., Eric G. Olinger The authors declare no competing interests., Daniel Epting The authors declare no competing interests., Eva Decker Full time employment at Medizinische Genetik Mainz.The authors declare no competing interests., Anja Friedrich Full time employment at Medizinische Genetik Mainz.The authors declare no competing interests., Nadine Bachmann Full time employment at Medizinische Genetik Mainz.The authors declare no competing interests., Gina Renschler Full time employment at Medizinische Genetik Mainz.The authors declare no competing interests., Tobias Eisenberger Full time employment at Medizinische Genetik Mainz.The authors declare no competing interests., Genomics England Research Consortium: None declared, Inga Gruenewald The authors declare no competing interests., Martin Konrad The authors declare no competing interests., Jens König The authors declare no competing interests., Bernhard Schlevogt The authors declare no competing interests., John Sayer The authors declare no competing interests., Carsten Bergmann The authors declare no competing interests.

Carsten Bergmann holds a part-time faculty appointment at the University of Freiburg in addition to the Medizinische Genetik Mainz and his employment with the Limbach Group for which he heads and manages Limbach Genetics GmbH., His research lab receives support from the Deutsche Forschungsgemeinschaft (DFG) (BE 3910/8-1, BE 3910/9-1 and Collaborative Research Center SFB 1453) and the Federal Ministry of Education and Research (BMBF, 01GM1903I and 01GM1903G).

C14.2 Random glucose GWAS trans-ethnic meta-analysis in almost half a million individuals provides insights into diabetes pathophysiology, complications and treatment stratification

Vasiliki Lagou[1], Longda Jiang[2], Anna Ulrich[3], Liudmila Zudina[3], Zhanna Balkhiyarova[3], Alessia Faggian[3], Jared Maina[4], Giuseppe Deganutti[5], Ayse Demirkan[3], Christopher Reynolds[5], Marika Kaakinen[3], Ben Jones[6], ***Inga Prokopenko****[3;4], the MAGIC investigators[7]*

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Kingdom; [7]Meta-Analysis of Glucose and Insulin-related Traits Consortium, https://magicinvestigators.org, United Kingdom.

Background/Objectives: Conventional measurements of fasting/postprandial blood glucose levels investigated in genome-wide association studies (GWAS) cannot capture the effects of DNA variability on "around the clock" glucoregulatory processes. We performed GWAS meta-analysis of glucose measurements under non-standardised conditions (random glucose; RG) in 493,036 individuals of diverse ethnicities and without diabetes, enabling powerful locus discovery and innovative pathophysiological observations.

Methods: We dissected associations (additive genetic model) between HRC-imputed DNA variants and RG, adjusted for age/sex/population structure, time since last meal (where available) in 17 studies, including UK Biobank. We investigated RG genetic (LD score regression/PRSs/hierarchical clustering) and causal (MR-Base) relationships with other phenotypes, and gene expression (metaXscan, DEPICT).

Results: We discovered 142 RG loci (185 distinct signals), including 84 novel signals for glycaemia, 14 with sex-dimorphic effects, 9 identified through trans-ethnic analysis and 25 low/rare frequency signals. Regulatory, glycosylation, and metagenomic annotations highlight ileum and colon tissues, indicating an underappreciated role of gastrointestinal tract in the control of blood glucose. Functional follow-up and molecular dynamics simulations of lower frequency coding variants in *GLP1R*, a type 2 diabetes (T2D) treatment target, reveal that optimal selection of GLP-1R agonist therapy in the clinic will benefit from a tailored genetic stratification. We provide novel compelling evidence from Mendelian randomisation, that lung function is modulated by blood glucose levels ($\beta_{MR\text{-}RG} = -0.61$, $P = 3.5 \times 10^{-4}$; $\beta_{MR\text{-}T2D} = -0.062$, $P = 1.42 \times 10^{-21}$), and settle the longstanding controversy that pulmonary dysfunction is a diabetes complication.

Conclusion: Our investigation yields wide-ranging insights into the biology of glucose regulation, diabetes complications and pathways for treatment stratification.

References:

Grants: H2020-SC1-HBC-28-2019-LONGITOOLS, WCRF-2017/1641, Diabetes UK(BDA number:20/0006307), PreciDIAB(ANR-18-IBHU-0001).

Conflict of Interest: None declared.

C14.3 Recurrent inversion polymorphisms in humans associate with genetic instability and genomic disorders

Wolfram Höps[1], *David Porubsky*[2], *Hufsah Ashraf*[3], *PingHsun Hsieh*[2], *Bernardo Rodriguez-Martin*[1], *Feyza Yilmaz*[4], *Jana Ebler*[3], *Pille Hallast*[4], *Flavia Maggiolini*[5;6], *William Harvey*[2], *Barbara Henning*[2], *Peter Audano*[4], *David Gordon*[2;7], *Peter Ebert*[3], *Patrick Hasenfeld*[1], *Eva Benito*[1], *Qihui Zhu*[4], *Charles Lee*[4], *Francesca Antonacci*[5], *Matthias Steinrücken*[8;9], *Christine Beck*[4;10], *Ashley Sanders*[11;12;13], *Tobias Marschall*[3], *Evan Eichler*[2;7], *Jan Korbel*[1;14]

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Background/Objectives: Inversions remain an understudied class of genetic variation, whose discovery has been challenging due to the frequent presence of large segmental duplications (SDs) at their flanks. Likewise, although some inversions can recurrently toggle between a direct and inverted state, the frequency of inversion recurrence and its association with genomic disorders has remained largely unexplored.

Methods: Within the framework of the Human Genome Structural Variation Consortium, we integrated multiple genomic technologies, including *Strand-Seq, Bionano* optical mapping and long read assemblies, to accurately detect inversions in a haplotype-resolved manner. Recurrent inversions were distinguished from 'single' events using complementary population genetics approaches.

Results: We discover 729 inversion loci genotyped across 41 diverse human genomes. Approximately 85% of inversions <2 kbp form by twin-priming during L1 retrotransposition. Balanced inversions show an excess of common variants, and 72% are flanked by SDs or retrotransposons. We present evidence for inversion recurrence in 40 loci, encompassing 0.6% of the human genome, and with a bias towards sex-chromosomes. In addition, our analysis associates inversion recurrence with predisposition to chromosomal instability and genomic disorders, including microdeletions at the disease relevant 3q29, 15q13.3 and Williams syndrome regions.

Conclusion: A comprehensive analysis of inversions in human genomes has unveiled widespread inversion recurrence and establishes a link to genetic instability and disease.

References:

Grants: National Institutes of Health (U24HG007497, U01HG010973, R01HG002385, R01HG010169), German Ministry for R&D (BMBF 031L0184), German Research Foundation (DFG 391137747), German Human Genome-Phenome Archive (DFG (NFDI 1/1)).

Conflict of Interest: None declared.

C14.4 Genetic basis of right and left ventricular heart shape

Richard Burns[1], *Julia Ramírez*[2], *William Young*[2], *Catrin Sohrabi*[2], *Nay Aung*[3;4], *Steffen Petersen*[3;4;5;6], *Alistair Young*[1], *Patricia Munroe*[2;4]

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Background/Objectives: Heart shape is a cardiovascular trait that captures variation in cardiac structure and is poorly represented by traditional phenotypes, demonstrating stronger relationships with cardiac disease risk factors (e.g., wall thickness, concentricity) and disease, and its genetic basis has not been studied.

Methods: Automated analysis of cardiovascular magnetic resonance (CMR) images was performed in 45,683 participants in the UK Biobank to construct a heart shape atlas from right and left ventricular end-diastolic surface mesh models. The first ten principal components (PCs) of the atlas were defined as phenotypes, accounting for 82.5% of the total shape variance. We performed genome-wide association studies (GWAS) and identified 43 loci across the ten PCs.

Results: Thirteen loci have not previously been reported with any ventricular structure, function, electrocardiogram (ECG) or cardiac disease traits. Bioinformatics analyses collated 63 candidate genes from eQTL, Hi-C and S-PREDIXCAN analyses alongside

literature review. For PC4 which is associated with right ventricular conicity, we discovered 8 loci (5 are novel). Two candidate genes at these novel loci are TSPAN12 and TBX18 and MGI knockout model models demonstrate blood vessel abnormalities. Pathway analysis in g:Profiler including all candidate genes indicates significant enrichment in heart development, contraction and functional regulation process GO terms (p = 2.04E-9, 7.73E-8, 7.85E-8). Heritability estimates of the PCs ranged 8.5-36.3%, and lead/independent secondary variants identified in this study explain 0.8 – 3.9% of their variance.

Conclusion: By characterising the genetics of heart shape, we have identified new candidate genes and explore the biological pathways implicated in defining cardiac shape.

References:

Grants:

Conflict of Interest: None declared.

C14.5 Noninvasive Prentatal Test results indicative of maternal malignancies: a nationwide genetic and clinical follow-up study

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Background/Objectives: Noninvasive prenatal testing (NIPT) for fetal aneuploidy screening using cell-free DNA derived from maternal plasma can incidentally raise suspicion for cancer. We detail malignancy suspicious–NIPT cases, describe clinical characteristics, chromosomal aberrations, and diagnostic routing of the patients with a confirmed malignancy.

Methods: We retrospectively included patients with a malignancy suspicious-NIPT referred for tumor diagnostics between April 2017 and April 2020 from a Dutch nationwide NIPT implementation study, TRIDENT-2. NIPT profiles from patients with confirmed malignancies were reviewed, and the pattern of chromosomal aberrations related to tumor type was analyzed. We evaluated the diagnostic contribution of clinical and genetic examinations.

Results: Malignancy suspicious–NIPT results were reported in 0.03% after genome-wide NIPT, and malignancies confirmed in 16 patients (16/48, 33.3%). Multiple chromosomal aberrations were seen in 23 of 48 patients with genome-wide NIPT, 16 patients (16/23, 69.6%) had a malignancy. Different tumor types and stages were diagnosed, predominantly hematologic malignancies. NIPT data showed recurrent gains and losses in primary mediastinal B-cell lymphomas and classic Hodgkin lymphomas. Magnetic resonance imaging and computed tomography were most informative in diagnosing the malignancy.

Conclusion: In 231,896 pregnant women, a low percentage (0.02%) of NIPT results were assessed as indicative of a maternal malignancy. However, when multiple chromosomal aberrations were found, the risk of a confirmed malignancy was considerably high. Extensive oncologic examination may be guided by tumor-specific hallmarks in the NIPT profile and the clinical lessons learned from this study.

References:

Grants: The TRIDENT-2 study is supported by the Netherlands Organization for Health Research and Development (ZonMw, No. 543002001).

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Uncompensated relationships: Illumina (Inst).

C14.6 Machine learning-based detection of immune-mediated diseases from genome-wide cell-free DNA sequencing datasets

Huiwen Che[1], *Tatjana Jatsenko*[1], *Lore Lannoo*[2], *Kate Stanley*[1], *Luc Dehaspe*[2], *Leen Van Coillie*[2], *Nathalie Brison*[2], *Ilse Parijs*[2], *Kris Van Den Bogaert*[1;2], *Koenraad Devriendt*[1;2], *Sabien Severi*[2], *Ellen De Langhe*[2], *Séverine Vermeire*[2], *Bram Verstockt*[2], *Kristel Van Calsteren*[2], *Joris Vermeesch*[1;2]

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Background/Objectives: The early detection of tissue and organ damage associated with immune-mediated diseases (IMD), including autoimmune diseases (AID) and inflammatory bowel diseases (IBD), has been identified as key to improving long-term survival in the general population. In addition, AID are known risk factors for pregnancy. However, biomarkers to prospectively identify and stratify IMD patients are lacking.

Methods: Here we developed a generic approach, coined GIP*Xplore* that uses unsupervised clustering and supervised machine learning to investigate genome-wide signatures of cell-free DNA (cfDNA) profiles from both obstetric and general populations. IMD cases were identified from a population of 81,611 noninvasive prenatal screening (NIPS) profiles and a cohort of 161 non-pregnant individuals were evaluated.

Results: We demonstrated that pregnant women with IMD have higher odds, 60-fold and 9-fold increase with lupus and IBD, respectively, of receiving inconclusive NIPS results. Unsupervised clustering of plasma cfDNA profiles showed similar disease-associated patterns in both pregnant and non-pregnant patients. A machine learning model incorporating such profile

patterns detected 70% and 50% of patients with AID in these obstetric and general cohorts, respectively, at 95% specificity.

Conclusion: Maternal IMD is a risk factor for inconclusive NIPS results. The ability to detect IMD patterns from the cfDNA profiles has the potential to stratify at risk pregnancies during routine NIPS and latent IMD in the general population.

References:

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Conflict of Interest: Huiwen Che: None declared, Tatjana Jatsenko Agentschap Innoveren en Ondernemen (VLAIO; Flanders Innovation & Entreprenership grant HBC.2018.2108), Lore Lannoo Clinical board for research and education Funding of the University Hospitals Leuven mandate funding., Kate Stanley EU H2020-MSCA-ITN-EJD-MATER (EU fund NUMBER 813707), Luc Dehaspe Patent application pending on 'Method for analyze cell-free nucleic acids'., Leen Van Coillie: None declared, Nathalie Brison: None declared, Ilse Parijs: None declared, Kris Van Den Bogaert: None declared, Koenraad Devriendt: None declared, Sabien Severi: None declared, Ellen De Langhe: None declared, Séverine Vermeire SV receive research grants from AbbVie, J&J, Galapagos, MSD, Pfizer, and Takeda., SV Receive speaker fees from AbbVie, Falk, Ferring, Hospira, MSD, Pfizer, Takeda, and Tillotts, Consultant for AbbVie, Avaxia, Celgene, Ferring, Galapagos, Genentech/Roche, Gilead, Robarts Clinical Trials, Hospira, Janssen, MSD, Mundipharma, Pfizer, Prodigest, Prometheus, Second Genome, Shire, and Takeda, Bram Verstockt BV is supported by Clinical Research Fund (KOOR) from the University Hospitals Leuven. BV is financially supported for research from Pfizer., Lecture fees from AbbVie, Biogen, Chiesi, Falk, Ferring, Galapagos, Janssen, MSD, Pfizer, R-Biopharm, Takeda, and Truvion., BV receives consultancy fees from Applied Strategic, Atheneum, Bristol Myers Squibb, Guidepoint, Ipsos, Janssen, Progenity, Sandoz, Sosei Heptares, and Takeda., Kristel Van Calsteren University Hospitals Leuven advanced project funding (RT 1065)., Joris Vermeesch Research Foundation-Flanders (FWO-Vlaanderen) (G080217N to JV); EU H2020-MSCA-ITN-EJD-MATER (EU fund NUMBER 813707) to JV; FWO-SBO (S003422) to JV; KU Leuven funding (C14/18/092), Patent application pending on 'Method for analyze cell-free nucleic acids'.

C16 PRENATAL GENETICS

C16.1 Non-invasive prenatal diagnosis of monogenic diseases by enhanced relative haplotype dosage analysis

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Background/Objectives: Non-invasive prenatal diagnosis for single-gene disorders (SGD-NIPD) has been widely adopted by patients, but is mostly limited to paternal or *de novo* mutation exclusion. However, it is still challenging to infer the inheritance of maternal allele from cell free DNA analysis. Relied on the imbalance of maternal haplotypes in cfDNA, relative haplotype dosage analysis has been employed to handle this challenge[1]. Although RHDO has been shown to be reliable[2-4], robust statistical error control and explicit characterization of critical parameters for analysis quality assessment are not thoroughly addressed for implementation into routine care.

Methods: We propose here an enhanced RHDO (eRHDO) procedure adjusted for any SGD-NIPD, regardless of the mode of transmission and the type of molecular variant, characterized by a tight control of the statistical error. We defined precise and standardized, yet easy-to-implement data input requirements as well as quality score calculation to assess the robustness of our approach. Validation was performed on 82 cases from 74 families, carriers for *CFTR*, *NF1*, *DMD* or *F9* mutations.

Results: Construction of the parental haplotypes and detection of the inherited parental mutations were successfully achieved 77/77(100%) conclusive cases, and the use of quality scores alerted us to the inadequate quality of 5/82(6%) cases, thus limiting the risk of unreliable results.

Conclusion: Our new procedure for eRHDO, designed to fulfill the quality requirements of diagnostic laboratories, has been shown to be 100% specific and 94% sensitive, and is now suitable for implementation into clinical service.

References:

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[3]ParksM,PrenatDiagn,2016
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Grants: R13188KK(2013), RC2013500852(2012), RC2016050 1598(2016), RC20200502668(2020), AFM AO2019-N°19832(2019).

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Vaincre la Mucoviscidose
RC2013500852 (2012) ~25,000 €
RC20160501598 (2016) ~16,000 €
RC20200502668 (2020) ~20,000 €
Association Française contre les Myopathies
Project AFM AO2019-N°19832 (2019), ~100,000 €.

C16.2 Multicentric longitudinal performance monitoring of different non-invasive prenatal screening technologies used in Belgium

***Armelle Duquenne**[1;2], Axel Marichal[3], Machteld Baetens[4], Bettina Blaumeiser[5], Nathalie Brison[2], Marie Bruneau[6], Saskia Bulk[7], Bert Callewaert[4], Sandra Coppens[6], Michelle Deberg[7], Anne De Leener[1], Marjan Derademaeker[5], Julie Desir[3], Koenraad Devriendt[2], Annelies Fieuw[8], Jean Stéphane Gatot[7], Mauricette Jamar[7], Katrien Janssens[5], Sandra Janssens[4], Colombine Meunier[3], Ilse Parijs[2], Bruno Pichon[6], Sonia Rombout[3], Ileen Slegers[8], Yves Sznajer[1], Olivier Vanakker[4], Kim Van Berkel[8], Leen Van Coillie[2], Ann Van Den Bogaert[8], Isabelle Vandernoot[6], Kris Van Den Bogaert[2], Joris Vermeesch[2]*

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Background/Objectives: Belgium was the first country to fully reimburse the noninvasive prenatal screening (NIPS) as a nationwide first-tier screening test to all pregnant women. Different commercial and in-house developed NIPS technologies are being used. Although the accuracies (sensitivity, specificity, positive predictive value, negative predictive value) of the commercial tests are provided by the companies, multicentric longitudinal studies to monitor and compare performance of those methods are lacking.

Methods: Since all invasive prenatal genetic testing following positive NIPS are analyzed at the Belgian genetic centers, we are uniquely positioned to determine the performance of different NIPS technologies. From all invasive genetic tests done following a positive NIPS in a clinical laboratory between 01/01/2020 and 01/05/2021, the PPVs were compared per technologies.

Results: For 303 positive NIPS; 134, 37 and 24 were respectively indicative of trisomy 21, 18 and 13. For trisomy21, the actual PPVs for VeriSeq®(Illumina), Harmony®(Roche) and Vanadis®(Perkin-Elmer) were respectively 69%, 91% and 65%, significantly lower than the 95%, 98% and 94% advertised. The PPV from the 8 genetic centers using a Laboratory Developed Test (LDT) was 92% (1).

Conclusion: This difference in PPV has a significant impact on pregnant women and the health care system. In Belgium there are about 120000 pregnancies/year. For example, with a population incidence for trisomy21 of 0.3%, a PPV of 69% versus 92% corresponds to a yearly increase of unnecessary invasive tests from 28 to 112. Our study underscores the value of LDT to improve prenatal health care.

References: 1. Van Den Bogaert K *et al* Genet Med. 2021.

Grants:

Conflict of Interest: None declared.

C16.3 Methylome analysis of cfDNA to predict preeclampsia presymptomatically

Marie De Borre[1], *Huiwen Che*[1], *Qian Yu*[1], *Lore Lannoo*[2], *Leen Van Coillie*[3], *Joachim Van Keirsbilck*[4], *Wilfried Gyselaers*[5], *Dreesen Pauline*[6], *Jeroen Breckpot*[3], *Koenraad Devriendt*[3], *Joris Vermeesch*[3], *Kristel Van Calsteren*[2], *Bernard Thienpont*[1]

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Background/Objectives: Preeclampsia is a leading cause of intrauterine growth retardation, premature birth, and low birth weight. Early preeclampsia risk assessment is of the utmost importance for pregnant women, as it can minimize adverse perinatal events by enabling both closer monitoring and early pharmacological intervention. However, first trimester screening models that are solely based on maternal demographic characteristics and medical history typically have a low detection rate and are inadequate for effective prediction.

Methods: Here, we assessed if methylation differences in the plasma-derived cell-free DNA (cfDNA) between case and control groups are indicative of a risk to develop preeclampsia. We developed a method to measure cfDNA methylation at 34,735 selected regions of interest using target-enrichment bisulfite sequencing. We next compared cfDNA from expectant mothers around 12 weeks of gestation that will go on to develop preeclampsia with matching controls, as well as cfDNA obtained at the moment of preeclampsia diagnosis.

Results: In both sample sets, differences in DNA methylation changes between control and preeclamptic pregnancies were detected. These changes enable classification of patients (n = 44) and controls (n = 27) at time of diagnosis (area under the receiver operating characteristic (AUROC) curve of 0.95), and crucially, also early in pregnancy (< 15 weeks), when patients (n = 96) could be differentiated from controls (n = 92) (AUROC of 0.83).

Conclusion: cfDNA methylome analysis can contribute to early preeclampsia risk assessment, in a time window where prophylactic therapy can still be initiated, which can result in better pregnancy management.

References:

Grants: Research Foundation – Flanders G0C7519N, 1524119N, S003422N.

Conflict of Interest: Marie De Borre A patent has been applied for that covers some of the inventions described here., Huiwen Che: None declared, Qian Yu: None declared, Lore Lannoo: None declared, Leen Van Coillie: None declared, Joachim Van Keirsbilck: None declared, Wilfried Gyselaers: None declared, Dreesen Pauline: None declared, Jeroen Breckpot: None declared, Koenraad Devriendt: None declared, Joris Vermeesch: None declared, Kristel Van Calsteren A patent has been applied for that covers some of the inventions described here., Bernard Thienpont A patent has been applied for that covers some of the inventions described here.

C16.4 Homozygosity at BCHE in the fetus as a risk factor for preeclampsia during pregnancy

Laura Balagué[1], *Alejandro Cáceres*[1;2;3], *Natàlia Carreras*[1], *Carlos Ruiz Arenas*[4;5;6], *Sandra Andrusaityte*[7], *Angel Carracedo*[8;9], *Maribel Casas*[1;2;10], *Leda Chatzi*[11], *Regina Grazuleviciene*[7], *Kristine Gutzkow*[12], *Johanna Lepeule*[13], *Lea Maitre*[1;2;10], *Mark Nieuwenhuijsen*[1;2;10], *Remy Slama*[13], *Nikos Stratakis*[1], *Cathrine Thomsen*[12], *Jose Urquiza*[1;2;10], *John Wright*[14], *Tiffany Yang*[14], *Geòrgia Escaramís*[2;15;16], *Mariona Bustamante*[1;2;10;17], *Martine Vrijheid*[1;2;10], *Luis Pérez-Jurado*[4;5;6;18], *Juan Ramón González*[1;2;19]

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for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain; [18]Genetics Service, Hospital del Mar, Barcelona, Spain; [19]Universitat Autònoma de Barcelona, Department of Mathematics, Bellaterra, Spain.

Background/Objectives: Runs of Homozygosity (ROH) are genomic regions with identical-by-descent haplotypes inherited from each parent. ROH have enabled the localization of recessive Mendelian diseases-associated genes and have been widely linked to complex diseases. We aimed to study ROH patterns in children and assess their relationship with their phenotype and exposome.

Methods: We analysed whole-genome shared ROH(>0.1Mb in size) in 1,382 children of 6 European populations from the Human Early Life Exposome (HELIX) project. We assessed the relationship of recurrent ROH regions with 88 phenotypes and 298 exposures.

Results: We detected ROH segments distributed along the chromosomes in >70% of the children. Some subpopulations presented specific distribution patterns of recurrent ROH regions. We found 7 associations with reproductive and neurological traits and 34 with exposures with FDR < 0.05. We uncovered a significant association between maternal preeclampsia and ROH in the butyrylcholinesterase (BCHE) gene region of the unborn child (FDR = 0.025), a locus which is also correlated with particulate matter (PM) absorbance ratio during pregnancy (FDR = 0.01).

Conclusion: Shared ROHs significantly contribute to several complex traits and exposure effects. Homozygosity at BCHE (likely hypomorphic alleles) in the fetus and placenta is a risk factor for preeclampsia in the pregnancy, probably by facilitating PM absorbance and toxicity. Our data are consistent with previously established risk for preeclampsia following exposure to PM in Jewish women and with low butyrylcholinesterase (Bche) levels during pregnancy.

References: Clark et al, Nat.Commun. (2019)
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Daniel et al, Environ Int. (2021).

Grants: FP7/2007-206/308333(HELIX);H2020-EU.3.1.2./874583 (ATHLETE);ISCIII(PI17/01225,PI17/01935).

Conflict of Interest: None declared.

C16.5 Consideration of urine miRNomes of pregnant women as potential biomarkers for placental function

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Background/Objectives: Placental biomarkers reflect its functional 'health' and may predict a risk to pregnancy complications. Altered levels of placental microRNAs are implicated in pre-eclampsia[1] and other pregnancy pathologies. To evaluate their potential as non-invasive biomarkers, we investigated for the first time miRNomes of maternal urine compared to placental and serum samples of the same pregnant women.

Methods: We analyzed 3rd trimester placental (279-286 gestational days, g.d.), serum (217-230 g.d.) and urine samples (228-230 g.d.) of three healthy pregnant women. MiRNome sequencing was carried out by the commercial service provider (Qiagen Genomic Service; Hilden, Germany). Bioinformatic data analysis was performed in R, and miRNA set enrichment analysis in TAM 2.0[2].

Results: Placental tissue was significantly enriched for microRNAs (n = 1,174 miRNAs; p < 0.00001) compared to body-fluids (serum, n = 341; urine, n = 193). Equivalent expression in serum and urine was measured for only 44 microRNAs, potentially suitable as non-invasive urinary biomarkers. In total, 25.3% of placenta-specific microRNAs were identified in maternal circulation (C19MC cluster, n = 13; C14MC, n = 27), but only 5.7% are detectable in urine (C19MC, n = 8; C14MC, n = 1). Pathway enrichment analysis indicated that circulating C19MC and C14MC miRNAs modify transcripts of genes implicated in regulating cell cycle, apoptosis and immune response ($p < 1.5\times10^{-9}$).

Conclusion: Urine is a valid source to monitor the expression of a subset of placenta-specific microRNAs to evaluate their potential as biomarkers for placental (dys)function and pregnancy pathologies.

References: 1 Inno et al (2021) Front Cell Dev Biol 9:697947
2 Li, Han, Wan et al (2018) NAR 46: W180–W185.

Grants: PRG1021 (Estonian Research Agency).

Conflict of Interest: None declared.

C16.6 High diagnostic yield using whole genome sequencing and an in silico gene panel of 281 genes associated with non-immune hydrops fetalis in a clinical setting

Eini Westenius[1;2], *Ellika Sahlin*[1;2], *Peter Conner*[3], *Anna Lindstrand*[1;2], *Erik Iwarsson*[1;2]

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Background/Objectives: Due to the wide range of underlying genetic etiologies of non-immune hydrops fetalis (NIHF), a comprehensive approach is essential in the evaluation of the cases. Whole genome sequencing (WGS) could be a valuable tool in the diagnosis of NIHF, as it enables simultaneous detection of different genetic variants across the complete genome.

To the best of our knowledge, this is the first study to investigate the diagnostic yield of WGS in prenatally diagnosed NIHF cases.

Methods: Twenty-four prenatally diagnosed cases of NIHF, negative for trisomies and copy number variants, were retrospectively analyzed with WGS and an in silico gene panel of 281 genes associated with hydrops fetalis.

Results: A molecular diagnosis was achieved in 50% (12/24) of the cases. Pathogenic or likely pathogenic variants were identified in seven genes: HRAS (n = 5), RIT1 (n = 2), FOXP3 (n = 1), GLB1 (n = 1), MAP2K1 (n = 1), PTPN11 (n = 1) and RASA1 (n = 1).

Conclusion: We demonstrate a diagnostic yield of 50% with clinical WGS in NIHF using a gene panel of 281 genes. A notable contribution of RASopathy genes (HRAS, MAP2K1, PTPN11, RASA1, RIT1) is in line with results from previous studies. Taking into consideration that chromosome aberrations were previously excluded in our cohort, a detection rate of up to 75% can be possible in prenatally diagnosed cases of NIHF when WGS analysis includes calling of chromosome aberrations.

References: -

Grants: Stockholm County Council (EW,AL,EI), Karolinska Institutet (EI), Sällsyntafonden (EW,EI), Swedish Research Council (AL), the Swedish Brain Foundation (AL).

Conflict of Interest: None declared.

C17 CLINICAL IMPACT OF MOLECULAR TUMOR PROFILING

C17.1 Whole-genome and transcriptome sequencing of 1063 colorectal cancers reveals novel drivers and prognostic subgroups

Luís Nunes[1], *Fuqiang Li*[2], *Klara Hammarström*[1], *Meizhen Wu*[2], *Emma Lundin*[1], *Tian Luo*[2], *Artur Mezheyeuski*[1], *Ingrid Ljuslinder*[3], *Lucy Mathot*[1], *Nicole Yacoub*[1], *Anna Löfgren-Burström*[3], *Carl Zingmark*[3],

Per-Henrik Edqvist[1], Inês Neves[1], Unnur Gudnadottir[1], Erik Osterman[1], Anna-Maria Dénes[1], Chatarina Larsson[1], Frederik Ponten[1], Richard Palmqvist[3], Kui Wu[2], Cong Lin[2], Mathias Uhlén[4;5], Bengt Glimelius[1], Tobias Sjöblom[1]

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Background/Objectives: Colorectal cancer (CRC) harbours considerable genetic and transcriptional heterogeneity leading to different outcomes and responses to individualized therapies. Our aim was to collect and incorporate genomic and clinical patient data of a large population-based cohort to identify prognostic and predictive biomarkers.

Methods: Fresh frozen CRC tumour and patient-matched normal tissue were subject to whole-genome and transcriptome sequencing. Data analyses defined somatic mutations, copy number variants, mutational signatures and RNA expression landscapes.

Results: Complete sequencing was possible for 1,063 tumours of which 26% were in the rectum while 47% and 27% were in the right- and left-colon, respectively. Metastatic CRC tumours corresponded to 12% of the cohort. Older patients, late-stage and high-grade tumours had worst overall survival, but tumour location did not impact survival. Microsatellite instability was detected in 17% of the cases but was not prognostic. Of the 77 significantly mutated driver genes, APC (74%), TP53 (60%) and KRAS (44%) were most frequently mutated whereas SMAD4 (53%), PTEN (26%) and CDKN2A (14%) were most frequently targets of copy number variations. Through unsupervised clustering of gene expression data, we identified 5 subgroups and validated them to be prognostic also in external data. Overall 27 single and 8 doublet base substitutions and 11 small indels mutational signatures were identified.

Conclusion: The results include novel driver genes as well new prognostic RNA subgroups in CRC, similar to the consensus molecular subtypes. This cohort is the largest whole-genome and transcriptome analysis of CRC, and will be important for advancing the understanding of the genomic basis of CRC.

References:

Grants:

Conflict of Interest: Luís Nunes: None declared, Fuqiang Li: None declared, Klara Hammarström: None declared, Meizhen Wu: None declared, Emma Lundin: None declared, Tian Luo: None declared, Artur Mezheyeuski: None declared, Ingrid Ljuslinder: None declared, Lucy Mathot: None declared, Nicole Yacoub: None declared, Anna Löfgren-Burström: None declared, Carl Zingmark: None declared, Per-Henrik Edqvist: None declared, Inês Neves: None declared, Unnur Gudnadottir: None declared, Erik Osterman: None declared, Anna-Maria Dénes: None declared, Chatarina Larsson: None declared, Frederik Ponten: None declared, Richard Palmqvist: None declared, Kui Wu: None declared, Cong Lin: None declared, Mathias Uhlén: None declared, Bengt Glimelius: None declared, Tobias Sjöblom Oncodia AB.

C17.2 Discovery of early clonal gene fusions in malignant pleural mesothelioma (MPM) by matched multiregional tumour exome and transcriptome sequencing

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Background/Objectives: MPM is a pleural tumour associated with asbestos exposure, with a long latency period (up to 40 years) between asbestos exposure and disease presentation. Our aim was to identify gene fusions that occurred early in the evolutionary history of the tumour.

Methods: We conducted multiregional whole exome sequencing (WES) of 106 samples for 20 patients, as part of the MEDUSA study (Zhang et al., 2021). We identified gene fusion events, and filtered for tumour-specific events using the exome sequence from the matched blood. Gene fusions observed across all regional samples of a particular tumour were validated by Sanger sequencing of the breakpoint. Fusion transcripts were identified from tumour RNAseq and compared with gene fusions identified at the DNA level.

Results: We found 24 truncal fusion events, three of which were novel (FMO9P-OR2W5, GBA3 and SP9). Tumour suppressor genes involved included BAP1, MTAP, and LRP1B, a potential oncogenic fusion CACNA1D-ERC2, and other truncal events such as in-frame PARD3B-NT5DC2 and out of frame STAB2-NT5DC2 fusions. Transcriptome sequencing confirmed a subset of gene fusions were transcribed.

Conclusion: Our findings suggest that gene fusions events occur early in mesothelioma evolution with marked heterogeneity across the cohort, as no recurrent truncal fusions event were found. Some truncal fusion events involve genes previously shown to be mutated in MPM and are likely to be drivers of cancer progression.

References: Zhang M, et al. (2021) Nat Commun. 12(1):1751.

Grants: University of Leicester College of Life Sciences PhD studentship to MJ

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C17.3 Integrative genomic and transcriptomic analysis of refractory metastatic cancers

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Background/Objectives: Metastatic cancer refractory to treatment has a dismal prognosis. While genetic mechanisms of primary tumors and, to a lesser extent, of metastatic cancers have been studied on large cohorts, metastatic tumors refractory to systemic treatment are not yet sufficiently characterized. Molecular profiling of such tumors might help predict patients' survival time and guide treatment decisions.

Methods: Here we present META-PRISM, a pan-cancer cohort of 1031 metastatic tumors resistant to at least one systemic therapy, for which whole exome ($n = 571$) and/or transcriptome sequencing ($n = 947$) was performed. Genetic and transcriptomic

markers were used alongside standard clinical markers in multivariate survival models.

Results: Different kinds of genomic instability were observed in META-PRISM: whole genome duplications (54% of the cohort), copy number alterations (median 26% of the genome) and mutational burden (median 3 M/Mb), all of which were significantly enriched as compared to tumor type-matched primary tumors. Driver genes with enriched somatic alterations compared to primary tumors included: CDKN2A, PTEN, EGFR, CCND1, MYC. Genes associated with shorter overall survival in META-PRISM were: TP53, CDKN2A, KRAS, RB1, SMARCA4, KMT2D. Immune-depleted types of the tumor microenvironment (TME) were enriched in META-PRISM and were associated with poor survival. We identified genomic markers of resistance for standard of care and investigational treatments in 7.5% and 39% of META-PRISM, respectively.

Conclusion: Integrating molecular markers to standard clinical markers adds important prognostic value that helps better characterize the risk profile of each patient.

References:

Grants: This work is supported by the French national center for precision medicine PRISM.

Conflict of Interest: None declared.

C17.4 Evaluating the spatiotemporal intratumour heterogeneity captured by tumour and plasma profiling in colorectal cancer patients with liver metastasis

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Background/Objectives: Despite the establishment of primary prevention, colorectal cancer (CRC) remains the second leading cause of cancer-related death in western countries, with over 50% of patients developing liver metastasis (LM)[1]. To assess the molecular profile underlying drug resistance and relapse rates of CRC, we evaluated the spatiotemporal intratumor heterogeneity (ITH) between primary and matched metastatic tumours. To further investigate ITH, we coupled tumour profiling with circulating tumour DNA sequencing using liquid biopsies.

Methods: A total of 122 multi-regional tumour and perioperative liquid biopsies from 18 patients were subjected to targeted next-generation sequencing and bioinformatics analysis to determine the molecular profile of each specimen.

Results: From multiregional tumour analysis, the proportion of patients with ITH were 53% in primary CRC and 56% in patients with LM. 35% of patients with LM harboured de novo mutations indicating the spatiotemporal tumour evolution. Furthermore, in 25% of patients we identified de novo mutation in their plasma samples that were undetectable in both CRC and LM tumour samples, highlighting the advantage of liquid biopsy in capturing ITH. Targetable mutations were identified in 17 patients who can benefit from approved drugs.

Conclusion: Our proof-of-concept study provides evidence on the clinical benefit in determining ITH during tumour evolution and metastasis, and the superiority of liquid biopsy in determining sub-clonal driver events that can be crucial in therapy selection or re-evaluation.

References: 1. Siegel, R.L., Miller, K.D., Goding, S.A., Fedewa, S.A., Butterly, L.F., et al. (2020). Colorectal cancer statistics, 2020. CA Cancer J Clin 70, 145-164.

Grants:

Conflict of Interest: Marilena Elpidorou full time employment, ioannis kyrochristos: None declared, Alexia Eliades employment full time, Achilleas Achilleos full time, Charalambos Loizides full time, Christos Lemesios full time, Kyriakos Tsangaras full time, Irene Hadjidemetriou full time, Georgios K Glantzounis: None declared, Anna Goussia: None declared, Marios Ioannides full time, George Koumbaris full time, Michalis Mitsis: None declared, Philippos Patsalis full time, Dimitrios Roukos: None declared.

C17.5 Genetic ancestry inference from tumor profiling data of 100,000 cancer patients

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Background/Objectives: The incidence and mortality of cancer vary widely across race and ethnicity due to a variety of factors. Cancer research has underrepresented non-White patients, thereby limiting our understanding of cancer biology in these populations. Given the missingness of race/ethnicity annotations in real-word (RW) data, inferring genetic ancestry from tumor sequencing can provide a more accurate substrate to investigate such disparities in this setting.

Methods: We inferred genetic ancestry from 100,000 de-identified records from cancer patients who underwent tumor genomic profiling with the Tempus xT next-generation sequencing assay (targeting 648 genes). We used ancestry informative markers overlapping assay capture regions to infer continental ancestry proportions: Africa, Amerindian, Europe, East Asia, and South Asia. Recognizing the complexity of ancestry and race relationships, we also imputed race/ethnicity categories using admixture thresholds based on literature.

Results: While most patients in our dataset are of European descent (72%), our RW cohort includes proportionally 4.7 and 3.8-fold more patients with substantial (>50%) African and Amerindian ancestry, correspondingly, compared with TCGA. We observed higher percentages of African ancestry patients with prostate, breast, and colorectal cancer (1.8-3.1%) and Amerindian ancestry patients with colorectal cancer (2.4%) compared to the overall cohort-level distributions ($p < 0.05$). Using imputation on subjects lacking race/ethnicity labels, we identified 60% and 121% more patients as likely Black and Hispanic/Latino, respectively.

Conclusion: Our results show that genetic ancestry inference from tumor profiling data can partially compensate for the missingness of race/ethnicity in RW data and allow research on biological race differences in cancer etiology and outcomes.

References:

Grants:

Conflict of Interest: Francisco De La Vega Tempus Labs, Inc., Tempus Labs, Inc., Brooke Rhead Tempus Labs, Inc., Tempus Labs, Inc., Yannick Pouliot Tempus Labs, Inc., Tempus Labs, Inc., Sean Irvine Real Time Genomics, Ltd., Justin Guinney Tempus Labs, Inc., Tempus Labs, Inc.

C17.6 Genome-wide DNA methylation profiling and identification of potential pan-cancer and tumor-specific biomarkers

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Background/Objectives: DNA methylation alterations have already been linked to cancer, and their usefulness for therapy and diagnosis has encouraged research into the human epigenome. Several biomarker studies have focused on identifying cancer types individually, yet common cancer and multi-cancer markers are still underexplored. This study aimed to profile genome-wide CpG sites and identify both pan-cancer and type-specific cancer detection biomarkers, using TCGA data.

Methods: Following initial differential methylation analysis, binary logistic regression was used to identify combinations of CpG sites as pan-cancer markers. For type-specific biomarkers, a 3-step feature selection approach, involving reliefF and K-means clustering, was implemented to select the most informative CpG sites and integrate them in the final classifier model.

Results: In total, 1,991 pan-cancer and between 75 and 1,803 cancer-specific differentially methylated CpG sites were discovered. Differentially methylated blocks and regions were also discovered for the first time on such a large-scale. Through a three-step computational approach, a combination of four pan-cancer CpG markers was identified from these sites and externally validated (AUC = 0.90), maintaining comparable performance across tumor stages. Additionally, 20 tumor-specific CpG markers were identified and made up the final type-specific prediction model, which could accurately differentiate tumor types (AUC = 0.87-0.99).

Conclusion: Our study highlights the power of the methylome as a rich source of cancer biomarkers, and the signatures we identified provide a new resource for understanding cancer mechanisms on the wider genomic scale with strong applicability in the context of new minimally invasive cancer detection assays.

References:

Grants: Fellowship of the Research Foundation – Flanders (FWO; 11B5220N).

Conflict of Interest: None declared.

C18 MACHINE LEARNING, BIOINFORMATICS AND BIOSTATISTICS

C18.1 LT-Free: a novel method for leveraging family history in genetic association studies of arbitrarily complex diseases

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Background/Objectives: Rich phenotypes collected in biobanks have provided opportunities to improve genetic association study power via alternative analysis methods (1). For example, the LT-FH method leveraged family history data to estimate genetic liability for a given trait, thereby improving study power (2). However, this method imposes assumptions about underlying trait genetic architectures and requires external estimates of heritability and generational prevalence.

Methods: Here we introduce LT-Free as a freer and interpretable model that does not require external information and does not assume any genetic architecture. Briefly, LT-Free generates a latent phenotype for each combination of case-control status, sex, and family history data observed in the data. LT-Free fits these latent phenotypes to optimize linear regression test statistics at SNPs known to be associated with the target phenotype.

Results: In simulated data, LT-Free matches or outperforms LT-FH across a range of simulated genetic architectures and family histories. Interestingly, the phenotype values optimized by LT-Free provide insights into underlying genetics including sex-biased disease, parental effects, and assortative mating. We then apply LT-Free to 12 diseases in the UKBB and similarly meet or exceed the performance of LT-FH. In the cases where LT-Free outperforms LT-FH (hypertension $p = 0.007$, depression $p = 0.05$, and heart disease $p = 0.02$) we observe evidence of different latent phenotypes estimated across sexes and parents.

Conclusion: Family history data is a powerful way to improve genetic association studies. By modeling this data with more general models, we can both improve study power and gain insights into genetics architectures.

References: 1. https://doi.org/10.1038/ng.3975
2. https://doi.org/10.1038/s41588-020-0613-6.

Grants:

Conflict of Interest: None declared.

C18.2 A novel Parent-of-Origin inference method for biobanks

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Background/Objectives: Studies on Parent-of-Origin (PofO) effects, in which identical genetic variations have different phenotypic effects depending on their PofO, are largely limited: researchers usually rely on parental genomes or known genealogies. However, these are rarely available in biobanks, preventing the study of PofO effects on a large number of phenotypes and samples.

Methods: We present a novel probabilistic approach to infer the PofO that does not require prior knowledge of genealogy. Our model (i) identifies surrogate parents using identity-by-descent (IBD) sharing between individuals, (ii) uses a Hidden Markov Model to model a specific haplotype from its close relatives, (iii) leverage IBD sharing on chromosome X for male individuals to assign parental origin.

Results: Using the UK Biobank dataset, we inferred the PofO for ~25,000 samples, representing a five-time increase compared to classical approaches on this dataset, with an estimated ~0.5% of errors in our PofO assignment. We validated our approach by replicating known PofO effects across four studies and demonstrated the increase of power provided by our large sample size.

We scanned hundreds of phenotypes for PofO effects and provided all summary statistics in a user-friendly publicly available database.

Conclusion: Our approach leverages the inter-individual relatedness in biobanks to infer the PofO, outpassing the traditional use of parental genomes or genealogy. Applied on many biobanks, this would enable the largest meta-analysis on PofO effects.

References: Hofmeister *et al*., bioRxiv 2021; Kong *et al*., Nature 2009.

Grants: SNSF-PP00P3_176977

Conflict of Interest: None declared.

C18.3 Quantifying the role of transcript levels in mediating DNA methylation effects on complex traits and diseases

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Background/Objectives: While many genetic factors controlling DNA methylation (DNAm) and complex traits are shared, mechanisms linking DNAm to phenotypes are not yet well characterized. A key mediator of this relationship is believed to be gene expression.

Methods: Here, we estimated the proportion of DNAm-to-trait causal effects that is mediated through the cis-regulation of transcript levels in a three-sample multivariable Mendelian randomization framework (3S-MVMR), using both methylomic- and transcriptomic quantitative trait loci (mQTLs and eQTLs, respectively) as instruments.

Results: Applying our method on a genome-wide scale to GWAS summary statistics of 50 complex traits and diseases (Naverage>320,000) and QTL data from the GoDMC (cis-mQTL, N = 32,851) and eQTLGen (cis-eQTL, N = 31,684) consortia, we found that at least 37.8% (95% CI: [36.0%-39.5%]) of the DNAm-to-trait effects were mediated through (typically multiple) transcripts in the *cis*-region. We ascertained the robustness of this estimated mediation proportion by conducting conditional F-statistics and heterogeneity tests tailored to the detection of weak instruments and pleiotropy in MVMR settings. Results revealed several regulatory mechanisms, such as DNAm at cg10385390 (chr1:8'022'505) increasing the risk of inflammatory bowel disease by reducing *PARK7* whose gene product has anti-oxidant and anti-inflammatory characteristics. Additionally, DNAm at cg09070378 (chr1:161'183'762) was found to decrease the risk of asthma by reducing FCER1G expression, a gene part of the KEGG pathway for asthma.

Conclusion: The proposed integrative framework can be extended to other omics layers to identify causal molecular chains, providing a powerful tool to map and interpret GWAS signals.

References:

Grants: Swiss National Science Foundation (310030_189147).

Conflict of Interest: None declared.

C18.4 A machine-learning approach for disease prediction can reduce misclassification and improve GWAS and polygenic scores

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Background/Objectives: GWAS assume there is a correct classification of subjects into cases and controls. Nonetheless, misclassification is expected, especially in biobanks that rely on administrative health data. Accounting for misclassification and for diseases liability can improve GWAS discovery and development of more predictive polygenic scores.

Methods: To address these limitations, we implemented a gradient boosted classifier (XGBoost) that we used to predict 6 diseases (type 2 diabetes, ischemic stroke, coronary heart disease, Alzheimer, Dementia and Breast cancer) by integrating information on 3828 diagnoses, 499 medications, socio-economic data and other health information. Instead of binary disease labels, the model outputs a continuous liability measure which was used to perform GWAS. The analyses were conducted on N = 317687 individuals from the FinnGen study (n = 356,077).

Results: In a preliminary classification using ischemic stroke, with only diagnoses and medication usage, we could already identify three GW-significant loci that were not found in GWAS that used the original case control classification. All three loci are known to be associated with stroke based on results from the largest stroke GWAS meta-analysis to date. Next we will also test other applications of the model, such as including different diseases or improving the polygenic risk score (PRS).

Conclusion: Our method extends the capabilities of GWAS by taking into account potential misclassifications and using the disease liability of individuals who have not yet been diagnosed. The possibility to go beyond a case-control model for GWAS analyses opens new venues to improve variant discovery and development of more predictive polygenic scores.

References:

Grants:

Conflict of Interest: None declared.

C18.5 Systematic analysis and prediction of genes associated with disorders on chromosome X

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Background/Objectives: Disease gene discovery on chromosome (chr) X is challenging owing to its unique modes of inheritance. We aimed at providing an inventory of disorder genes on chrX and predict genes that remain to be associated with human disease.

Methods: We undertook a systematic analysis of human chrX genes and compared the proportion and characteristics of associated disorders to those on autosomes. We analyzed gene constraints, exon and promoter conservation, expression and the existence of paralogues to highlight genes sharing features with known chrX disorder genes. In parallel, we trained a neural network to distinguish disease-associated from dispensable genes and used it to predict genes that remain to be associated with human disease.

Results: We observe a higher proportion of disorder-associated genes and an enrichment of genes involved in cognition, language, and seizures on chrX compared to autosomes. We report 127 genes not yet associated with a disorder sharing one or more attributes with the 205 disorder genes known on chrX. Using a neural network, we classify 235 genes, including 121 of the 127 genes, as having high probability of being associated with disorder. We provide evidence of an excess of variants in predicted genes in existing databases. Furthermore, we report damaging variants in CDK16 and TRPC5 in patients with intellectual disability or autism spectrum disorders.

Conclusion: This study predicts large-scale gene-disease associations that could be used for prioritization of X-linked pathogenic variants.

References: This study is available on medRxiv (MEDRXIV/2022/270779).

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Conflict of Interest: None declared.

C18.6 Meta-analysis fine-mapping is often significantly miscalibrated at single-variant resolution

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Background/Objectives: While meta-analysis is commonly used to combine multiple GWAS, its fine-mapping is challenging due to heterogeneous characteristics across cohorts (*e.g.*, phenotyping, genotyping or imputation). Here, we developed a new method to mitigate potential issues in meta-analysis fine-mapping.

Methods: We developed a summary statistics-based QC method, SLALOM (suspicious loci analysis of meta-analysis summary statistics), that identifies suspicious loci for meta-analysis fine-mapping by detecting outliers in associations based on local LD structure. We applied SLALOM to i) 14 traits from the Global Biobank Meta-analysis Initiative (GBMI)[1] and ii) 483 summary statistics from the GWAS Catalog to evaluate its performance.

Results: Out of 422 loci, SLALOM predicted 285 loci (68%) were suspicious for fine-mapping. We demonstrated that the predicted suspicious loci were significantly depleted for having likely causal variants as a lead PIP variant, such as nonsynonymous variants (2.8x), high-PIP GWAS (5.4x) and cis-eQTL (5.2x) fine-mapped variants from previous studies[2,3]. Having observed widespread suspicious loci in the GWAS Catalog too, we found the issues were primarily due to sample size imbalance across variants, which was caused by different genotyping and imputation quality.

Conclusion: We found meta-analysis fine-mapping is often miscalibrated at single-variant resolution and do not recommend it without careful considerations or further methodological development.

References: 1. Zhou et al. medRxiv. (2021).
2. Kanai et al. medRxiv. (2021).
3. Ulirsch et al. in prep.

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Conflict of Interest: Masahiro Kanai: None declared, Roy Elzur: None declared, Mark Daly M.J.D. is a founder of Maze Therapeutics., Hilary Finucane: None declared.

C19 NEW GENE DEFECTS AND PATHWAYS IN SYNDROMIC CONDITIONS

C19.1 FOSL2 truncating variants in the last exon cause a new neurodevelopmental disorder with scalp and enamel defects: description of 10 patients

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Background/Objectives: Description of a new recognizable syndrome, including ten individuals with pathogenic heterozygous de novo truncating variants in the last exon of the FOSL2 gene.

Methods: Through a multi-centric collaboration, we described clinical features and single nucleotide variants of FOSL2, investigated their immune phenotype and dental specificities, and explored escape of nonsense-mediated mRNA decay by mRNA analysis.

Results: Individuals shared a striking similar phenotype including growth retardation, localized scalp aplasia with/without skull defects, and enamel defects confirm by three-dimensional analysis. Other features were neurodevelopmental disorder, with/without intellectual deficiency, and congenital cataracts.

FOSL2 (FOS Like 2, AP-1 Transcription Factor Subunit) is a member of the Fos gene family and part of the AP1 transcription factor complex, interacting with cJUN and other proteins.

Mice overexpressing Fosl2 show a systemic inflammatory phenotype. Immunological characterisation realised in three patients with FOSL2 variant did not confirm this phenotype in human.

We performed functional studies to assess that FOSL2 variants do not undergo nonsense mediated RNA decay and result in increased stability of the AP1 complex. We compared osteoblast differentiation in retrotransduced cells with pathogenic variants. Zebrafish models are in progress.

Conclusion: We propose a role for FOSL2 in human pathology, as a differential diagnosis for atypic Adams-Oliver Syndrome.

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Bozec, A. et al. Fra-2/AP-1 controls bone formation by regulating osteoblast differentiation and collagen production. J Cell Biol(2010).

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C19.2 Biallelic variants in SLC35B2 cause a novel chondrodysplasia with hypomyelinating leukodystrophy

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Background/Objectives: Sulfated proteoglycans are essential in skeletal and brain development. Recently, pathogenic variants in genes encoding proteins involved in the proteoglycan biosynthesis have been identified in a range of chondrodysplasia associated with intellectual disability. Nevertheless, several patients remain with unidentified molecular basis. This study aimed to contribute to the deciphering of new molecular bases in patients with chondrodysplasia and neuro-developmental disease.

Methods: Exome sequencing was performed to identify pathogenic variants in patients presenting with chondrodysplasia and intellectual disability. The pathogenic effects of the potentially causative variants were analyzed by functional studies, using qPCR, western blot, immunostaining and HPLC.

Results: We identified homozygous variants (c.1218_1220del and c.1224_1225del) in the SLC35B2 gene in two patients with pre- and postnatal growth retardation, scoliosis, severe motor and intellectual disabilities and hypomyelinating leukodystrophy. SLC35B2 encodes a member of the solute carrier family located in the Golgi apparatus membrane and implicated in proteoglycan sulfation. By functional analyses, we showed that the variants affect SLC35B2 mRNA expression and protein subcellular localization leading to a functional impairment of the protein. Consistent with those results, we detected proteoglycan sulfation impairment in SLC35B2 patient fibroblasts and serum.

Conclusion: Our data support that SLC35B2 functional impairment causes a novel syndromic chondrodysplasia with hypomyelinating leukodystrophy, most likely through a proteoglycan sulfation defect. This is the first time that SLC35B2 variants are associated with bone and brain development in human.

References:

Grants:

Conflict of Interest: None declared.

C19.3 Clinical, genetic, epidemiologic and functional delineation of TSPEAR-related Autosomal Recessive Ectodermal Dysplasia 14

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Background/Objectives: Bi-allelic *TSPEAR* variants were recently identified to cause Autosomal Recessive Ectodermal Dysplasia (ARED) 14. However, the mutation spectrum, clinical features and underlying mechanism for this disorder are poorly understood. Human TSPEAR is a protein of unknown structure and function.

Methods: We performed clinical, genetic, epidemiologic and functional characterization.

Results: We identified 12 new individuals with ARED14. Dental phenotypes (conical-shaped teeth and hypodontia) were present in all individuals, but other features were variable. Pathogenic *TSPEAR* missense variants were located in the EAR domains. Linkage analysis in 100KGP data led to identification of a founder *TSPEAR* variant (p.Asp639Asn) in White Europeans with a common ancestor estimated to be ~11,160 years ago via mutational and recombination clock analyses. *TSPEAR* gene carrier rate was found to be ~1/140 using gnomAD data in Non-Finnish European population. CRISPR-Cas9-mediated zebrafish *tspeara/tspearb* double-knockout resulted in fewer and abnormally shaped teeth. Phylogenetic and AlphaFold structural analyses showed drosophila *Closca* to be a likely homolog of *TSPEAR*. Based on known functions of Closca, we hypothesised TSPEAR to participate in primary enamel knot dependent signaling. Using mouse scRNA-Seq data, we demonstrated highly restricted expression of *Tspear* in enamel knot of developing tooth.

Conclusion: This work will improve diagnosis and management of individuals with ARED14. We identify a founder *TSPEAR* mutation and show that ARED14 is one of the commonest AREDs. We also demonstrate functional conservation of TSPEAR and for the first time uncover its biological function.

References:

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C19.4 Biallelic variants in CRIPT cause Rothmund-Thomson syndrome and genome instability with excessive cellular senescence

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Background/Objectives: Rothmund-Thomson syndrome (RTS) is characterized by poikiloderma, sparse hair, small stature, skeletal defects, cancer, cataracts, and other features resembling premature aging. *RECQL4* and *ANAPC1* are disease genes associated with RTS in over 75% of cases, but in the remaining patients no causative genetic alterations have been detected. We describe two individuals clinically diagnosed with RTS both with homozygous variants in the *CRIPT* gene (OMIM#615789).

Methods: *CRIPT* patients (including three previously published) were systematically compared to RTS. Senescence markers, sensitivity to chemotoxic agents, and mitotic progression in live-cell imaging were analyzed in patient-derived fibroblasts and compared to *RECQL4*-deficient cells.

Results: All five *CRIPT* patients fulfilled diagnostic criteria for RTS and additionally had severe developmental delay which is not a commonly described finding in RTS. Using computational gestalt analysis of patient photographs, *CRIPT* individuals showed greatest phenotypic overlap with RTS individuals. Histologic analysis of skin biopsies revealed poikiloderma and increased expression of senescence markers (p53/p16/p21). Knockdown of *CRIPT* in fibroblasts resulted in significantly upregulated ß-galactosidase activity. *RECQL4*- and *CRIPT*-deficient fibroblasts showed no chromosomal aberrations, normal mitotic progression, unaltered numbers of mitotic errors and appropriate cell cycle arrest after nocodazole treatment. In a viability screen, both cell types were sensitive to the chemotoxic agent potassium bromate.

Conclusion: *CRIPT* is reported as a third causative gene in the RTS-spectrum of disorders associated with a more severe neurological phenotype. At the cellular level, *RECQL4*- and *CRIPT*-deficient cells both displayed excessive senescence and impaired genome maintenance, suggesting shared mechanisms leading to the clinical phenotypes.

References:

Grants:

Conflict of Interest: None declared.

C19.5 Pathogenic variants in the paired-related homeobox 1 (PRRX1) gene are associated with craniosynostosis

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Background/Objectives: Craniosynostosis, the premature fusion of the cranial sutures, affects 1 in every 2000 live births. Although a specific genetic cause can be identified in ~25% of cases, additional genetic factors are suspected. We describe 13 patients (from 11 families) with craniosynostosis, who harbour rare missense or frameshifting variants in the paired-related homeobox 1 (PRRX1) gene. Functional studies have previously implicated PRRX1 in craniofacial development, including specific expression of murine Prrx1 in the pre-osteogenic cells of the cranial sutures.

Methods: Genome/targeted sequencing, clinical assessment, cell transfection.

Results: Trio-based genome sequencing of 10 families with a sporadically affected child with bicoronal/multisuture craniosynostosis, identified two with undocumented heterozygous variants in PRRX1. Targeted re-sequencing of PRRX1 in 631 undiagnosed patients with craniosynostosis identified 4 further individuals with rare heterozygous variants predicting either premature truncation or missense substitution of the homeodomain, a 20-fold enrichment compared to gnomAD data from ~125,000 individuals. By collaboration we identified four further homeodomain missense variants and a 61.5 kb partial deletion of PRRX1. Multi-suture synostosis was present in 50% of patients, with the coronal and sagittal sutures commonly affected. Variants were de novo in two cases but in the majority, the variant segregated in unaffected family members ($n = 9$), demonstrating frequent non-penetrance. By transfection of fluorescently labelled PRRX1 constructs, we demonstrated that missense variants within the PRRX1 homeodomain cause abnormal nuclear localisation.

Conclusion: This work supports a key role for PRRX1 in cranial suture development and shows that variants affecting the homeodomain of PRRX1 are a novel and frequent cause of craniosynostosis.

References:

Grants: MRC DTP.

Conflict of Interest: None declared.

C19.6 Differential effects of v-ATPase subunit a2 loss vs. specific inhibition on N- and O-glycosylation, neuronal migration, Golgi trafficking and pH regulation as the basis of autosomal recessive cutis laxa type 2A

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Background/Objectives: Autosomal recessive cutis laxa type 2A (ARCL2A) is a congenital disorder of glycosylation (CDG) characterized by lax and wrinkly skin, developmental delay and abnormal neuronal migration in the cortex. ARCL2A is caused by mutations in *ATP6V0A2* encoding the subunit a2 of vacuolar ATPase (v-ATPase), which is mainly localized at the Golgi and early endosomal compartments. This v-ATPase subunit is directly involved in proton transport, but an additional function in vesicle fusion was discussed. The pathophysiological contributions of impaired N- and O-glycosylation remain to be determined.

Methods: We generated a total knock-out (*Atp6v0a2-/-*) and a knock-in mouse model (*Atp6v0a2RQ/RQ*) in which only proton transport is specifically blocked. We characterized brain and skin in these mouse models using mass spectrometry, immunofluorescence and immunoblotting. Mouse embryonic fibroblasts (MEF) were used for functional in vitro analyses.

Results: We found elongated decorin glycan chains and enhanced N-glycan fucosylation in MEF. This was more pronounced in *Atp6v0a2-/-* and correlated with extended Golgi retention and reduced recruitment of Gopc to Golgi membranes. In contrast, overmigration of cortical neurons was stronger in Atp6v0a2RQ/RQ and correlated with reduced O-glycosylation of alpha-dystroglycan. Golgi pH measurements revealed higher values in *Atp6v0a2RQ/RQ* than in *Atp6v0a2-/-* while the opposite was true for Golgi trafficking.

Conclusion: O-glycosylation defects are crucial for the neuronal migration defect in ARCL2A. While the Golgi trafficking delay was stronger in *Atp6v0a2-/-*, pH dysregulation was more pronounced in *Atp6v0a2RQ/RQ*. Although this underpins the suggested role of subunit a in vesicle function, the dysregulation of Golgi pH clearly predominates in the pathomechanism of ARCL2A.

References:

Grants:

Conflict of Interest: None declared.

C20 NON-CODING GENOME VARIATION IN MENDELIAN DISEASES

C20.1 The impact of germline inversions in the rare-disease arm of the 100,000 Genomes Project

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Background/Objectives: Genetic testing with microarrays, MLPA and PCR-Sanger is ineffective at detecting chromosomal inversions. The role of inversions in rare-disease is therefore likely underappreciated.

Methods: We used SVRare[1], to prioritise rare inversions in 71,408 individuals from the rare disease arm of the 100K Genomes Project. Inversions were clustered using an 80% overlap threshold.

Results: Focussing on 333 genes known to cause or predispose to disease via haploinsufficiency, we identified 44 likely pathogenic inversions which have been fed back to clinicians. Several well-known gene-phenotype correlations were observed which include a *de novo* inversion disrupting *PHEX* in a patient suspected to have hypophosphataemic rickets. An inversion disrupting *EXT2* was detected in a parent-child duo with multiple exostoses. A *de novo* inversion where the proximal/distal breakpoints disrupt *EP300* and *TCF20* likely results in a unique blended phenotype. The 2nd reported family with Kantaputra mesomelic dysplasia (described clinically in 2004[2]) harboured a *de novo* 23kb inversion of the *HOXD* gene cluster. Inversions identified in patients with familial cancer predisposition syndromes include an inversion disrupting *RB1* in a patient with retinoblastoma and two patients with an identical inversion involving exons 2-6 of *MSH2*. The latter cases were shown to share a 3Mb haplotype, confirming previous speculation that this may represent a founder mutation[3].

Conclusion: Our work highlights the significant role that inversions play in many disease areas and the importance of combining appropriate SV calling software with robust tools for prioritisation.

References: 1. www.medrxiv.org/content/10.1101/2021.10.15.21265069v1, 2. PMID: 15211647, 3. PMID: 26498247.

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Conflict of Interest: None declared.

C20.2 Mapping the 3D genome of the human retina and its role in retinal disease

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Background/Objectives: Genome-wide cis-regulatory elements (CREs) coordinate retinal development by meticulously controlling gene expression. Yet a map of the retinal 3D genome, required to link CREs to their target genes, is still missing. Therefore, we mapped the 3D genome of adult human retina to (1) assess the structure and conservation of topologically associating domains (TADs) at retinal disease loci and (2) determine how these are affected by pathogenic structural variants.

Methods: We used in situ Hi-C to map genome-wide interactions in neural retina and retinal pigment epithelium from adult donor eyes (n = 3), as well as patient fibroblasts with dominant cone dystrophy due to a duplication at the *IRXB* cluster (n = 2).

Results: Comparing TAD structure in Hi-C maps from retina versus clinically accessible tissues (e.g. fibroblasts, lymphoblastoid cells; public Hi-C data), we found that retinal tissues displayed distinct TAD structures at several retinal disease loci. This has important implications for the usability of Hi-C on clinically accessible tissues. For example, using Hi-C on patient fibroblasts, we determined that a duplication at the ultraconserved *IRXB* locus resulted in the formation of a neoTAD containing *IRX5*. However, comparison to retinal TAD boundaries implied that a different neoTAD would be formed in retina, possibly with distinct effects on gene expression.

Conclusion: We have generated the first 3D genome maps of human retina and identified both conserved and retina-specific 3D topologies. The latter potentially limit the usability of Hi-C on accessible tissues for the interpretation of structural variation in (retinal) disease.

References:

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C20.3 Quantifying the contribution of near-coding variation to rare disease

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Background/Objectives: Genomic regions adjacent to the protein coding sequence, such as untranslated regions (UTRs) and gene promoters (collectively termed 'near coding' regions), play a role in transcriptional and post-transcriptional gene regulation. Variants that disrupt these elements have been shown to cause severe disease phenotypes. However, they are not routinely included in clinical diagnostic pipelines. The extent of 'missed' near-coding diagnoses that may be identified by expanding coding specific pipelines is currently unknown.

Methods: Here, we analysed near coding de novo variants in 11,371 rare disease trios in the Genomics England 100,000

Genomes dataset. We selected variants within near coding regions of genes flagged as potentially involved in each proband's phenotype. Each variant was annotated using Ensembl VEP alongside the UTRannotator plugin, and external data sources such as ClinVar, GnomAD, and GenCC.

Results: We found 406 potentially deleterious rare de novo near-coding variants in patients without an existing coding diagnosis. 54 within 5′ UTR exons; 304 within 3′ UTR exons; 3 within 5′ splice regions (with a SpliceAI score >0.5); and 45 within the core promoter (200 bps directly upstream of the transcription start site). Previously identified disease-causing variants in both PAX6 and MEF2C were identified within this candidate variant set, alongside several novel diagnostic candidates. These include a 5′UTR splice donor variant in SETD5, and a 5′UTR variant that creates an upstream start-codon out-of-frame with the coding sequence of SLC2A1.

Conclusion: Here, we demonstrate that incorporating near-coding regions into existing diagnostic pipelines, will uncover additional diagnoses that are missed when using a coding-centric approach.

References:
Grants:
Conflict of Interest: None declared.

C20.4 Identification of the active enhancer landscape in Neural Stem Cells by ChIP-STARR-seq and validation using zebrafish

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Background/Objectives: Transcriptional enhancers play a crucial role in the regulation of cell-type specific gene expression and their alteration can drive human disease. Widespread assessment of such non-coding sequences in a clinical setting is currently lacking, as functional annotation of enhancers and the determination of the consequences of variants herein are still challenging to perform.

Methods: Here, we implemented the massively parallel reporter assay ChIP-STARR-seq to characterize the functional enhancer landscape in human neural stem cells (NSCs), as a model system for early stages of human brain development. The generated enhancer activity maps were used to analyse non-coding variants encountered in patient whole genome sequencing data. Several enhancer candidates were tested in vivo by reporter assays using zebrafish as animal model.

Results: ChIP-STARR-seq experiments focussing on the regions bound by the transcription factors YY1 and SOX2 and the histone modifications H3K27ac and H3K4me1 in NSCs identified enhancers belonging to different activity classes that are linked to genes involved in transcription and nervous system development. Increased enhancer activity is associated with increased expression and likelihood of loss-of-function intolerance of the enhancer target gene. Functional validation of multiple enhancers in zebrafish larvae showed enhancer activity during early developmental stages providing further evidence of the findings and allowed to assess the impact of genetic variants in those sequences encountered in patients.

Conclusion: Together, our extensive genome-wide assessment of enhancers in human NSCs expands the growing knowledge on regulatory sequences in humans and testing them using zebrafish will help interpreting variants detected in clinical whole genome sequencing.

References:
Grants:
Conflict of Interest: None declared.

C20.5 A systematic analysis of splicing variants identifies new diagnoses in the 100,000 Genomes Project

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Background/Objectives: Variants which disrupt splicing are an important cause of genetic disease. One in four splice-disrupting de novo variants which cause developmental disorders lie outside of the essential splice sites. However, these are difficult to interpret clinically.

Methods: We examined the landscape of splicing variants in whole-genome sequencing data from 38,688 individuals in the 100,000 Genomes Project. We functionally characterised likely diagnostic variants with RNA studies to make new diagnoses in unsolved cases. We also calculate measures of selective constraint acting at near-splice positions and splicing branchpoints genome-wide in 26,660 unaffected parents in the 100,000 Genomes Project.

Results: From 258 de novo splicing variants in known rare disease genes, 84 were already considered to be diagnostic. We identified 35 new likely diagnoses in probands with an unsolved rare disease. We used phenotype matching and RNA studies to confirm a new diagnosis for six individuals to date.

We show that near-splice regions and deep-intronic splicing branchpoints are highly constrained by purifying selection. These loci harbour damaging non-coding variants which are amenable to systematic analysis in sequencing data.

Conclusion: Our approach identifies novel diagnostic and likely diagnostic variants at scale, improving the diagnostic yields of existing sequencing data. Non-canonical splicing positions and splicing branchpoints harbour damaging variants which cause genetic diseases. Overall, we demonstrate the clinical value of examining non-canonical splicing variants in individuals with unsolved rare diseases.

References:
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Conflict of Interest: None declared.

C20.6 22q11.2 rearrangements caused by NAHR and PATRR-mediated pathways

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Background/Objectives: The 22q11.2 deletion syndrome (22q11.2DS) is with an incidence of 1 in 3000 live births about ten-fold more frequent than any other genomic disorder. The rearrangement is thought to be caused by non-allelic homologous recombination (NAHR) between low copy repeats on chromosome 22 (LCR22-A until -H). Due to their genetic complexity, the full sequence nor the breakpoints have been charted.

Methods: Cell lines were established from patients and parents with an LCR22-ADdel (n = 15), LCR22-ABdel (n = 5), LCR22-ACdel (n = 3), LCR22-BDdel (n = 1), and LCR22-CDdel (n = 1). Fiber-FISH was applied to determine the parent-of-origin and to map the rearrangement at subunit level. Guided by the fiber-FISH data, Cas9-targeted, ultra-long-read, or CRISPR-targeted-ultra-long-read Nanopore sequencing was performed to pinpoint the breakpoint at nucleotide level.

Results: The rearrangement locus was identified in all duos, ranging between 20kb-160kb. Specific composition patterns in the rearranged LCR22s were observed for some duos. By sequencing these duos, the exact breakpoint was located in a palindromic AT-rich repeat (PATRR). In other sequenced duos, the breakpoints were mapped in or in close vicinity to repeat elements.

Conclusion: For the first time, LCR22s were systematically sequenced and 22q11.2DS breakpoints could be mapped at nucleotide level. The rearrangement position varies amongst 22q11.2DS patients. In a subset, the breakpoint is located in a PATRR, which are double strand break hotspots. Hence, the 22q11.2DS rearrangements are not only caused by NAHR but also by PATRR-mediated breakage repair. PATRRs likely increase the region's vulnerability to rearrangements which can explain why 22q11.2DS is the most common microdeletion.

References:

Grants: FWO-GOA2622N, Delacroix.

Conflict of Interest: None declared.

C21 DIFFERENT STRATEGIES TO UNRAVEL THE GENETIC BASIS OF NDDS

C21.1 The impact of rare coding genetic variation on adult cognitive function

Chia-Yen Chen[1], *Ruoyu Tian*[1], *Tian Ge*[2;3], *Max Lam*[3;4], *Sanchez-Andrade Gabriela*[5], *Tarjinder Singh*[6], *Jimmy Liu*[1], *Mark Sanderson*[5], *Christine Rowley*[5], *Holly Ironfield*[5], *Terry Fang*[1], *Mark Daly*[6;7], *Aarno Palotie*[3;7], *Ellen A. Tsai*[1], *Hailiang Huang*[3], *Matthew Hurles*[5], *Sebastian Gerety*[5], *Todd Lencz*[4], ***Heiko Runz***[1]

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Background/Objectives: Compelling evidence suggests that cognitive function in adults is strongly influenced by genetics. Cognitive function is often severely impaired in single-gene disorders. Conversely, over a thousand common genetic loci have been linked to cognitive function or proxy phenotypes through genome-wide association studies (GWAS). However, the role of rare protein-coding variants for cognitive function in the normal population and whether rare and common genetic risk for reduced cognitive function intersect has thus far remained largely unexplored.

Methods: Exome sequencing and genotyping of 454,787 UK Biobank participants with replication.

Results: We demonstrate that a higher exome-wide burden of damaging missense and protein-truncating variants (PTVs) as ascertained from UK Biobank participants is associated with reduced cognitive function parameters in adults. We identify eight genes in which PTV-burden individually showed exome-wide significant damaging effects on cognitive function and replicate this association in two independent cohorts. We further show that adult cognitive function shares rare genetic architecture with neurodevelopmental disorders. For one of the cognitive function genes, *KDM5B*, we demonstrate in mice and in humans that reduced adult cognitive function at the population level can be part of a clinical spectrum in which neurocognitive performance depends on the genetic dose of a single gene. Finally, we provide evidence that rare and common variant-based polygenic risk contribute additively to cognitive function.

Conclusion: Our findings uncover a contribution of rare protein-coding genetic variation to cognitive function and highlight that the spectrum of cognitive function in the normal adult population is influenced by the action of single genes.

References:

Grants:

Conflict of Interest: Chia-Yen Chen Full-time employee at Biogen, Ruoyu Tian: None declared, Tian Ge: None declared, Max Lam: None declared, Sanchez-Andrade Gabriela: None declared, Tarjinder Singh: None declared, Jimmy Liu Full-time employee at GSK, Mark Sanderson: None declared, Christine Rowley: None declared, Holly Ironfield: None declared, Terry Fang Full-time employee at Biogen, Mark Daly: None declared, Aarno Palotie: None declared, Ellen A. Tsai Full-time employee at Biogen, Hailiang Huang: None declared, Matthew Hurles: None declared, Sebastian Gerety: None declared, Todd Lencz: None declared, Heiko Runz Full-time employee at Biogen.

C21.2 Damaging rare coding variants in constrained genes are associated with reduced generalised intelligence in the UK Biobank

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Background/Objectives: Rare coding variants (RCVs) in constrained genes have been shown to confer risk for psychiatric and neurodevelopmental disorders. However, the degree to which they impact general cognitive ability in people without these disorders has not been fully investigated. We analysed sequencing data from 200,625 UK Biobank individuals to investigate how

constrained RCVs impact cognitive ability in people without neuropsychiatric disorders.

Methods: VCF files were processed, quality-controlled and annotated using Hail. The number of damaging variants occurring in loss-of-function intolerant (LoFi) genes, defined as those with pLI-score≥0.9, was quantified for each sample.

A measure of generalised intelligence score, *g*, was defined by the first principal component to emerge from a principal component analysis of 4 cognitive tests (numeric memory, reaction time, trail making test B, and pairs matching) available for 32,184 individuals. Association between RCV burden and *g* was evaluated using a linear regression model.

Results: Singleton protein-truncating variants in LoFi genes were associated with lower *g* (beta = -0.015, $p = 1.91\times10^{-10}$), as were singleton damaging missense variants (beta = -0.053, $p = 3.96\times10^{-4}$). Singleton synonymous variants in LoFi genes, RCVs of any type in LoF tolerant genes, and more common RCVs of any type were not associated with cognition. Findings were consistent between *g* and the individual cognitive tests which formed it.

Conclusion: The types of RCVs that confer risk to neuropsychiatric disorders also cause cognitive deficits in people without these conditions. This association was only found for ultra-rare variants, suggesting variants impacting cognition are under strong selective constraint.

References:

Grants: Wellcome Trust Integrative Neuroscience PhD studentship. UKRI FLF MR/T018712/1.

Conflict of Interest: None declared.

C21.3 Routine transcriptome sequencing improves diagnosis for neurodevelopmental disorders by identifying pathogenic effects of non-coding, putatively benign and missed variants

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Background/Objectives: A molecular diagnosis is key for predicting outcome, treatment and genetic counseling options in neurodevelopmental disorders (NDD). However, in about half of NDD cases routine DNA-based diagnostics fail to establish a molecular diagnosis. Transcriptome analysis (RNA-seq) improves the diagnostic yield for some groups of diseases, but has not been applied to NDD and in a routine diagnostic setting.

Methods: Here, we explored the diagnostic potential of RNA-seq in a cohort of 96 undiagnosed individuals including 67 undiagnosed NDD subjects. We created a user-friendly web-application to analyze RNA-seq data from single individuals' cultured skin fibroblasts for gene, exonic and intronic expression outliers, based on modified OUTRIDER Z-scores. Candidate pathogenic events were complemented/matched with genomic data and, if needed, confirmed with additional functional assays.

Results: We identified pathogenic small genomic deletions, mono-allelic expression, deep intronic variants resulting in pseudo-exon insertion, but also exonic synonymous variants or predicted "benign" nonsynonymous variants with deleterious effects on transcription. This approach increased the diagnostic yield for NDD by 12%. Identified pitfalls during transcriptome analysis include splice abnormalities in putative disease genes caused by benign polymorphisms and/or absence of expression of the responsible gene in the tissue of choice. This was misleading in one case and could have led to the wrong diagnosis in the absence of appropriate phenotyping.

Conclusion: Nonetheless, our results demonstrate the utility of RNA-seq in molecular diagnostics and stress the importance of multidisciplinary consultation. In particular, the approach is useful for the identification and interpretation of unexpected pathogenic changes in mRNA processing and expression in NDD.

References:

Grants:

Conflict of Interest: None declared.

C21.4 Brain transcriptomic profiling reveals common alterations across neurodegenerative and psychiatric disorders

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Background/Objectives: Neurodegenerative and neuropsychiatric disorders (ND-NPs) are multifactorial, polygenic, and complex behavioral phenotypes caused by brain abnormalities. Large-scale collaborative efforts have tried to identify the genetic architecture of these conditions. However, the specific and shared underlying molecular pathobiology of brain illnesses is not clear.

Methods: Here, we examine transcriptome-wide characterization of eight conditions, using a total of 2,633 post-mortem brain samples from patients with Alzheimer's disease (AD), Parkinson's disease (PD), Progressive Supranuclear Palsy (PSP), Pathological Aging (PA), Autism Spectrum Disorder (ASD), Schizophrenia (Scz), Major Depressive Disorder (MDD), and Bipolar Disorder (BP)–in comparison with 2,078 brain samples from matched control subjects.

Results: Similar transcriptome alterations were observed between NDs and NPs with the top correlations obtained between Scz-BP, ASD-PD, AD-PD, and Scz-ASD. Region-specific comparisons also revealed shared transcriptome alterations in frontal and temporal lobes across NPs and NDs. Co-expression network analysis identified coordinated dysregulations of cell-type-specific modules across NDs and NPs.

Conclusion: This study provides a transcriptomic framework to understand the molecular alterations of NPs and NDs through their shared- and specific gene expression in the brain.

References:

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Conflict of Interest: None declared.

C21.5 Somatic mosaicism in infantile spasms with brain malformations

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Background/Objectives: Focal brain malformations commonly cause of infantile spasms (IS) but the genetic basis of this disorder remains poorly understood. This study aimed to investigate the genetic landscape of IS with focal brain malformations using resected brain tissue.

Methods: Histopathologic review and genomic testing were performed in 59 individuals with IS and a focal brain malformation following epilepsy neurosurgery for seizure control at the Royal Children's Hospital, Melbourne. Blood or brain tissue was analysed using 200x (n = 3) or 400x depth WES (n = 32), or targeted panel sequencing (n = 24). Variants were filtered using GATK & MuTect variant caller pipelines for germline and somatic candidate variants. Single nuclei RNA-seq analysis (snRNA-seq) was performed on resected brain to understand pathomechanism in cases caused by variants in *SLC35A2*.

Results: Germline putative pathogenic variants were identified in 24/59 (41%) individuals, in *TSC2* (x17), *TSC1* (x1), *CDKL5* (x1), *DEPDC5* (x1), *PIK3CA* (x1), *COL4A1* (x1), *SCN8A* (x1) and *NPRL3* (x1) genes. Putative pathogenic brain somatic variants were identified in 19/59 (32%) cases, in *SLC35A2* (x9), *AKT3* (x2), *TSC2* (x2), *MTOR* (x2), *PIK3CA* (x1), *OFD1* (x1) *TSC1* (x1) and *DEPDC5* (x1). Histopathologic review identified all 9 individuals with *SLC35A2* variants as mild malformation of cortical development with oligodendroglial hypoplasia in epilepsy (MOGHE). Preliminary snRNA-seq data suggests oligodendrocyte progenitor cells and astrocytes exhibit aberrant expression profiles in lesional tissues and present potential molecular determinants for epileptogenicity.

Conclusion: The genetic landscape of IS with brain malformations other than *TSC2* comprises predominantly brain somatic mutations. Somatic variants in SLC35A2 were a major cause of malformative IS.

References:

Grants:

Conflict of Interest: None declared.

C21.6 Dissecting the autism-associated 16p11.2 locus identifies multiple drivers in brain phenotypes and unveils a new role for the major vault protein

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Background/Objectives: Using mouse genetic studies, we set out to identify which of the 30 genes causes brain size and other NeuroAnatomical Phenotypes (NAPs) at the autism-associated 16p11.2 locus, independently in male and female.

Methods: To assess NAPs, we developed or acquired through collaboration single-gene heterozygous knockout mice, representing 20 unique genes of the 16p11.2 locus. For the remaining 10 genes, the germline transmission of the mutation failed despite multiple attempts or no mouse model was available during the course of the study.

Results: Here we show that multiple genes mapping to this region regulate brain size in contrast to previous studies, with female significantly less affected. Major Vault Protein (MVP), the main component of the vault organelle, is a highly conserved protein found in higher and lower eukaryotic cells, yet its function is not understood. While we find MVP expression highly specific to the limbic system, *Mvp* stood out as the top driver of NAPs, regulating the morphology of neurons, postnatally and specifically in male. Finally, we demonstrate that the double hemideletion *Mvp*::*Mapk3* rescues NAPs and alters behavioral performances, suggesting that MVP and ERK share the same pathway.

Conclusion: Our results highlight that sex-specific neuroanatomical mechanisms must be considered in neurological disorders such as autism and provide the first evidence for the involvement of the vault organelle in the regulation of the mammalian brain shape.

References: https://www.biorxiv.org/content/10.1101/2022.01.23.477432v1.full.

Grants: SNSF Ambizione and ANR PDOC grants.

Conflict of Interest: None declared.

C22 INNOVATION IN GENETIC RISK ASSESSMENT AND DIAGNOSTICS

C22.1 Solo-RNA sequencing of blood combined with trio-genome sequencing decipher molecular diagnostic of Neurodevelopmental Disorders

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Genetics and Reproductive Biology, Reims, France; [15]University Hospitals Pitié Salpêtrière - Charles Foix, Clinical Genetics Unit, Paris, France.

Background/Objectives: Short fragment Genome Sequencing (GS) is becoming the gold standard for molecular diagnosis of genetic neurodevelopmental disorders (NDD). Total RNA-sequencing (RNAseq) can complement GS with a quantitative and qualitative analysis, making it possible to explore the transcriptional consequences of variants identified by GS with a possible additional diagnostic yield ranging from 7.5% to 36%, depending on the tissue and the disease.

We applied a combined strategy with trio-GS (tGS) and blood solo-RNASeq to evaluate the diagnostic gain of RNASeq, in patients with NDD.

Methods: 61 patients with NDD without diagnosis after panels or exome sequencing were included. Following 3 strategies: tGS, tGS+sRNAseq and solo-GS + sRNAseq, data were analyzed with double interpretation, in blind to the other strategies. The results of each strategy were compared independently

Results: 53/61 patients (87%) benefited from all 3 strategies. The molecular cause was identified in 6/53 patients (11%), 5/6 of whom were retained by the 3 strategies. In the 6th patient, only the tGS+sRNAseq strategy identified a causal deep intronic variant leading to activation of a cryptic splice site. Variants of uncertain signification were identified in 21/53 patients (40%). For 12/21 (57%) candidate variants retained by the tGS strategy alone, sRNAseq ruled out an effect on splicing, giving no argument for their causality.

Conclusion: The additional contribution of blood-based RNA-seq seems to be limited in patients with NDD (1/53; 2%). Nevertheless, it appears of great utility for the interpretation of intronic variants (12/53; 23%), which represent the majority of variants identified by GS.

References:

Grants:

Conflict of Interest: None declared.

C22.2 Evaluating scientific and clinical factors affecting genomic diagnoses in a large paediatric cohort

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Background/Objectives: Paediatric disorders include a range of highly genetically heterogeneous conditions that are amenable to genome-wide diagnostic approaches, but variant interpretation can change over time.

Methods: The Deciphering Developmental Disorders (DDD) Study recruited >33,500 individuals from families with severe developmental disorders from 24 centres around the UK and Ireland. We collected detailed standardised phenotype data and performed whole-exome sequencing and microarray analysis to investigate novel genetic causes. We developed an iterative clinical variant analysis and reporting pipeline and communicated candidate variants back to individual clinicians for interpretation.

Results: To date, 19,283 sequence and structural variants have been identified in DDD probands and reported to referring clinicians. Most candidate diagnoses were identified using a >2000 gene panel, which we updated by ~100 genes/year through literature curation and cohort-wide enrichment analyses. We estimate that 33-40% of DDD probands are currently diagnosed, based on clinical interpretation augmented by automated Bayesian variant classification using ACMG guidelines. Being in a parent-offspring trio has the largest impact on the chance of being diagnosed (OR = 4.7). Probands who were extremely premature, (OR = 0.4), had in-utero exposure to anti-epileptics (OR = 0.4) or whose mothers had maternal diabetes (OR = 0.5) are less likely to be diagnosed, as are those of African ancestry.

Conclusion: Our results show the benefit of interrogating all variant types across known and novel disease genes with multiple modes of inheritances, coupled with automated and repeated rounds of variant analysis and interpretation, to maximise diagnostic yield.

References:

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Conflict of Interest: Caroline Wright: None declared, Ruth Eberhardt: None declared, Patrick Campbell: None declared, Kaitlin Samocha: None declared, Kartik Chundru: None declared, Daniel Perrett: None declared, Simon Brent: None declared, Julia Foreman: None declared, Rachel Hobson: None declared, Hilary Martin: None declared, Matthew Hurles Scientific Director of Congenica, David FitzPatrick: None declared, Helen Firth: None declared.

C22.3 Genetic assessments of breast cancer risk that do not account for polygenic background are incomplete and lead to incorrect preventative strategies

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Background/Objectives: Breast cancer is the most common cancer among women and is a leading cause of cancer mortality worldwide. There is a significant genetic component to breast cancer risk which is the result of both rare pathogenic mutations and common genome-wide variation. However, the penetrance of pathogenic mutations varies widely and their frequency is low. Polygenic risk scores, which aggregate the effect of hundreds to millions of common genome-wide variants offer a way to further understand the contribution of genetics to disease risk.

Methods: Here we describe a new PRS for breast cancer and jointly analyse genome-wide and whole exome sequence data from 221,479 women to understand how rare and common variation affect lifetime breast cancer risk.

Results: We show that PRS strongly modulates the penetrance of mutations in 8 breast cancer susceptibility genes. For example, lifetime risk in *BRCA1* carriers with low polygenic risk is almost one third that of carriers with high PRS. Adding family history of breast cancer provides additional stratification on the potential outcome of disease in carriers of rare mutations. PRS also identifies a significant fraction of the population at equivalent risk to carriers of moderate impact pathogenic variants and who are an order of magnitude more common at a population level.

Conclusion: These results have important implications for breast cancer risk mitigation strategies, indicating that the genetic risk of breast cancer is determined by both monogenic mutation and polygenic background. Assessments of genetic risk for breast cancer risk that do not consider polygenic background are imprecise and unreliable.

References:

Grants:

Conflict of Interest: George Busby Allelica Inc, Allelica Inc, Paul Craig Allelica Inc, Paolo Di Domenico Allelica Inc, Allelica Inc, Alessandro Bolli Allelica Inc, Jen Kintzle Allelica Inc, Giordano Bottà Allelica Inc, Allelica Inc.

C22.4 Integration of clinical AI into health care of patients with rare diseases: experiences in the German national diagnostic framework for molecular testing beyond the exome

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Background/Objectives: A novel structured diagnostic concept employing multidisciplinary expertise at centers for rare diseases (CRDs) that has been established at German university hospitals in recent years, was evaluated in a prospective study (TRANSLATE-NAMSE).

Methods: Between January 2018 and December 2020, 5652 patients were enrolled in the study and were comprehensively assessed by multidisciplinary teams at ten CRDs. Exome sequencing was initiated for 282 adult and 1283 pediatric patients and complemented by additional molecular tests if indicated.

A subcohort of 210 individuals was analyzed with the artificial intelligence-based PEDIA protocol, which integrates next-generation phenotyping results on medical photographs from DeepGestalt and GestaltMatcher. Highly suggestive clinical diagnoses that could not be confirmed by exome sequencing alone were subjected to genome sequencing, full-length transcriptome sequencing, or analysis of the proteome and methylome.

Results: Conclusive diagnoses were established in 494 individuals, covering 400 diagnostic-grade genes, suggesting that ultra-rare disorders were enriched in this cohort. In addition, we describe 56 novel gene-phenotype associations, mainly in individuals with neurodevelopmental delay.

With this phenotype-driven testing strategy, we identified small genomic deletions, protein reductions, and epigenetic patterns that could establish the diagnosis (examples will be given for KANSL1, KMT2D, MDH2). Phenotype analysis was also supportive in the delineation of molecular dual diagnoses.

Conclusion: With the entire cohort data, we developed a tool to predict the diagnostic yield from the clinical features of a patient if advanced molecular testing strategies are applied. By this means, clinical AI can be used for efficient staged diagnostics, particularly if sequencing yields only variants of unknown significance.

References:

Grants:

Conflict of Interest: None declared.

C22.5 Integration of RNA-seq functional evidence into the ACMG/AMP variant interpretation framework

Dmitrii Smirnov[1;2], *Lea Dewi Schlieben*[1;2], *Fatemeh Peymani*[1;2], *Riccardo Berutti*[1;2], *Vicente Yépez*[1;3], *Julien Gagneur*[3;4], *Holger Prokisch*[1;2]

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Background/Objectives: Over the last five years, RNA sequencing (RNA-seq) has been established as an effective complementary approach to DNA sequencing in molecular diagnostics of individuals with Mendelian disorders. It provides diagnosis in 16% of WES unsolved cases. Still, there is no formal consensus on assessing the strength of pathogenicity of RNA phenotypes (aberrant expression, splicing and monoallelic expression). Odds of pathogenicity (OddsPath)[1] calculation was introduced to determine the strength of pathogenicity of functional readouts (PS3) in the ACMG/AMP framework.

Methods: Aberrant RNA phenotypes were called using DROP [2] in a cohort of 303 individuals with Mendelian disorders [3]. A total of 394 pathogenic and 723 benign variants were extracted to calculate the OddsPath for each aberrant RNA phenotype.

Results: We evaluated quality metrics and sample size as robustness factors of clinical RNA-seq and determined thresholds for three RNA phenotypes qualifying strong evidence of pathogenicity (PS3). Based on these results we outlined guidelines for clinical interpretation of RNA-seq data and proposed an extension to ACMG/AMP framework. For example, aberrant expression with z-score < -2 provides strong evidence of pathogenicity for rare non-coding variants in the corresponding gene.

Conclusion: This study recommends guidelines on how to integrate functional RNA-seq data into the ACMG/AMP variant interpretation framework.

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Conflict of Interest: None declared.

C22.6 Decreased retinal vascular complexity is an early biomarker of myocardial infarction supported by a shared genetic control

Ana Villaplana Velasco[1], *Justin Engelmann*[1], *Konrad Rawlik*[1], *Oriol Canela-Xandri*[1], *Claire Tochel*[1], *Frida Lona Durazo*[2], *Muthu Mookiah*[3], *Alexander Doney*[3], *Esteban Parra*[2], *Emanuele Trucco*[3], *Tom MacGillivray*[1], *Kristiina Rannikmae*[1], *Albert Tenesa*[1], *Erola Pairo-Castineira*[1], *Miguel Bernabeu*[1;4]

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Background/Objectives: There is increasing evidence that retinal vascular complexity (measured as fractal dimension, Df) might offer insights into the progression of coronary artery disease (CAD) before traditional biomarkers can be detected. This association could be partly explained by a common genetic basis; however, the genetic component of Df is poorly understood. We present a comprehensive genetic study aimed to elucidate Df genetic component and to analyse its relationship with CAD.

Methods: We obtained Df from retinal fundus images and genotyping information from ~38,000 white-British participants in the UK Biobank. We performed a genome-wide association study, functional and enrichment analyses to characterise Df genetic basis and its association with CAD.

Results: We discovered 9 loci associated with Df, previously reported in retinal tortuosity, and CAD studies. Significant

negative genetic correlation estimates endorse the inverse relationship between Df, CAD, and myocardial infarction (MI), one of CAD fatal outcomes. This strong association motivated us to developing a MI predictive model combining clinical information, Df, a CAD polygenic risk score and a random forest algorithm. Internal cross validation evidenced a considerable improvement in the area under the curve (AUC) of our predictive model (AUC = 0.770) when comparing with an established risk model, SCORE,(AUC = 0.719).

Conclusion: Our findings shed new light on the genetic basis of Df, unveiling a common control with CAD, and highlights the benefits of its application in individualised MI risk prediction.

References:

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C24 MOLECULAR MECHANISMS IN CANCER

C24.1 WGS of Basal Cell Carcinoma of skin reveals distinct mutational landscape and the role of HIPPO-YAP pathway in disease progression

Sergey Nikolaev[1], *Andrey Yurchenko*[2], *Fatemeh Rajabi*[2], *Konstantin Gunbin*[2], *Oltin-Tiberiu Pop*[3], *Ismael Padioleau*[2], *Laurent Parmentier*[4], *Denis Salomon*[5], *Lukas Flatz*[4]

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Background/Objectives: Basal Cell Carcinoma (BCC) is the commonest type of skin cancer which has not been yet studied by whole genome sequencing (WGS).

Methods: We have assembled the largest to date BCC cohort representing major histological subtypes for genetic analysis by WGS (89), WES (310) and RNAseq (360).

Results: Mutational profile in BCC is due to UV-light exposure, however it differs from melanoma by high fraction of COSMIC signature SBS7b mutations. We explained it by high contribution to mutagenesis in BCC of GC-rich early replicating regions, which in BCC are characterized by more condensed and difficult to repair chromatin. We report that loss of *TP53* is the first event in BCC and precedes the loss of *PTCH1*. Moreover, we deduce from 17p copy neutral LOH regions that loss of *TP53* results in a dramatic increase of mutation rates in BCC. In line with that BCC with TP53 mutations have 2.5-fold higher mutation burden than BCC with wt-*TP53*. We discovered novel BCC driver genes outside the Hh pathway. 65% of BCC harbor mutations in HIPPO-YAP and Contact Inhibition of Proliferation pathways: (*FAT1*(30%), *NF2*(9%), *ARHGAP35*(18%), *CREBBP*(21%), *PTPN14*(21%), *LATS1*(9%)). We validated the role of these genes in BCC cell line by siRNA screening followed by migration and proliferation assays. Promoter mutations of *TERT* were identified in 48% of BCC and resulted in its overexpression.

Conclusion: High-risk morpheaform BCCs were different from low-risk BCCs by activation of HIPPO-YAP pathway and fibrotic tumor microenvironment. BCC with intrinsic resistance to vismodegib demonstrated a hyperactivation of HIPPO-YAP pathway.

References: -

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Conflict of Interest: Sergey Nikolaev ARC foundation, Andrey Yurchenko: None declared, Fatemeh Rajabi: None declared, Konstantin Gunbin: None declared, Oltin-Tiberiu Pop: None declared, Ismael Padioleau: None declared, Laurent Parmentier: None declared, Denis Salomon: None declared, Lukas Flatz: None declared.

C24.2 Functional classification of nearly all possible SNVs in VHL using Saturation Genome Editing

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Background/Objectives: Germline mutations in *VHL* predispose individuals to numerous tumors, including renal cell carcinoma (RCC), hemangioblastoma, and pheochromocytoma. In ClinVar, over 600 "variants of uncertain significance" (VUS) have been reported in *VHL*, illustrating the challenge of accurate variant classification. Here, we aim to improve diagnosis of VHL syndrome and advance our molecular understanding of why specific variants cause distinct tumors via systematic characterization of variants with high-throughput assays.

Methods: Saturation Genome Editing (SGE) uses CRISPR/Cas9 to assay the functional effects of hundreds of endogenously expressed variants per experiment. We applied a highly-optimized SGE protocol to engineer all possible *VHL* single nucleotide variants (SNVs) in human HAP1 cells. Next-generation sequencing was used to quantify the depletion of SNVs causing loss-of-function (LoF) and subsequent cell death, and variants' effects on transcript abundance were measured using targeted RNA-sequencing. Several large databases of observed variants were used to associate functional effects with clinical phenotypes.

Results: To date, >1,500 *VHL* SNVs have been confidently scored. The assay reproducibly identifies nonsense, missense, and splice variants causing LoF and discriminates RCC-predisposing SNVs with >95% accuracy. Among VUS assayed, 10.3% resulted in LoF, and an additional 104 SNVs absent from ClinVar were deemed LoF. Integrative analysis of functional data, VHL structure, and clinical phenotypes reveals numerous molecular insights.

Conclusion: SGE data for *VHL* will be directly valuable for improving clinical variant interpretation. Moreover, integrative analyses of *VHL* variants encompassing functional data can advance our molecular understanding of tumor predisposition across tissues.

References:

Grants:

Conflict of Interest: None declared.

C24.3 Alterations at the CDH1/CDH3 loci regulatory architecture as a mechanism for E- to P-cadherin switch and gastric cancer distant metastases

Celina São José[1;2;3], *carla pereira*[4], *Marta Ferreira*[1;5], *ana andré*[1], *Rita Matos*[1;6], *Fátima Carneiro*[1;2], *Carla Oliveira*[1;2]

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Background/Objectives: *CDH1*/E-cadherin is a cell-cell adhesion and invasion suppressor molecule. Loss of E-cadherin is classically associated with adherens junctions disassembly, epithelial tumour progression and epithelial to mesenchymal transition. Normal epithelial tissues lack *CDH3*/P-cadherin, but its expression increases in tumours and rescues adherens junctions in distant organs metastases. Herein, we propose that E- to P-cadherin switch contributes to distant metastases in epithelial tumours and searched for the underlying mechanism.

Methods: RNAseq and clinico-pathological features from 43 GC patients were used to analyze E- to P-cadherin switch. *CDH1* 5'-exons were CRISPR/cas9 deleted from gastric cancer (GC) cell lines. Edited clones were profiled with transcriptomics and proteomics; and chromatin was accessed by ATAC-seq and 4C-seq with a *CDH1* promoter viewpoint to evaluate the cadherin switch.

Results: In 24/43 (57%) GC, *CDH3* expression was increased 2-fold in comparison to *CDH1* (*CDH1*- to *CDH3*-switch). In these 24, were included the only 9 cases presenting distant metastases in this cohort. To explore the switch mechanism, we depleted *CDH1* expression and found *CDH3*/P-cadherin upregulation at RNA and protein levels. This was accompanied by an increase in the number of *CDH1* promoter 4C-interactions with open chromatin regions at the *CDH3* locus, absent from normal stomach epithelium (control).

Conclusion: We found a potential regulatory network, involving chromatin interactions between the *CDH1* promoter and the *CDH3* locus, that may support the E- to P-cadherin switch occurring in over 50% of GC cases. As all metastatic GC from our series presented this switch, this may enclose the missing mechanism triggering GC metastases to distant organs.

References:

Grants:

Conflict of Interest: None declared.

C24.4 How protein expression links somatic mutations to tumor phenotypes in chronic lymphocytic leukemia

Fabienne Meier-Abt[1;2;3], *Junyan Lu*[4;5], *Ester Cannizzaro*[2], *Marcel F. Pohly*[2], *Sandra Kummer*[2], *Sibylle Pfammatter*[6], *Laura Kunz*[6], *Ben C. Collins*[7], *Ferran Nadeu*[8;9], *Kwang Seok Lee*[10], *Peng Xue*[3], *Myriam Gwerder*[2], *Michael Roiss*[2], *Jennifer Hüllein*[10], *Sebastian Scheinost*[10], *Sascha Dietrich*[5;11], *Elias Campo*[8;12;13], *Wolfgang Huber*[4;5], *Ruedi Aebersold*[3], *Thorsten Zenz*[2]

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Background/Objectives: Many functional consequences of somatic mutations on tumor phenotypes in chronic lymphocytic leukemia (CLL) are unknown. This is partly due to missing information on global protein expression profiles in CLL.

Methods: We determined the proteome of 117 CLL patient samples with data-independent acquisition mass spectrometry. Proteomic results were integrated with genomic, transcriptomic, ex-vivo drug response and clinical outcome data for the same patients.

Results: We identified trisomy 12 and IGHV mutational status as main disease drivers influencing protein abundance in CLL (1055 and 542 differentially expressed proteins, FDR = 5%). Evaluating gene-RNA-protein relations for the disease drivers trisomy 12 and trisomy 19, we detected different degrees of gene dosage effects. More protein abundance buffering with upregulated RNA and unchanged protein levels was observed for trisomy 19 compared with trisomy 12 CLL ($P = 0.0002$, Kolmogorov–Smirnov test). Proteins known to be part of stable protein complexes demonstrated higher levels of buffering than others, suggesting that formation of protein complexes helps maintain the stoichiometric balance of proteins. Enriched amongst non-buffered proteins in trisomy 12 CLL were members of the PI3K-AKT-MTOR pathway, implicating this pathway in the tumorigenic function of trisomy 12.

We identified STAT2 protein expression to be linked with the response to kinase inhibitors. STAT2 was upregulated in IGHV-unmutated, trisomy 12 CLL and required for apoptosis-preventing interferon-α signaling in CLL.

Conclusion: Our study elucidates fundamental principles governing gene dosage effects and protein expression in CLL. It identifies STAT2 protein expression as determinant of drug response in CLL.

References:

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Conflict of Interest: Fabienne Meier-Abt: None declared, Junyan Lu: None declared, Ester Cannizzaro: None declared, Marcel F. Pohly: None declared, Sandra Kummer: None declared, Sibylle Pfammatter: None declared, Laura Kunz: None declared, Ben C. Collins: None declared, Ferran Nadeu: None declared, Kwang Seok Lee: None declared, Peng Xue: None declared, Myriam Gwerder: None declared, Michael Roiss: None declared, Jennifer Hüllein: None declared, Sebastian Scheinost: None declared, Sascha Dietrich: None declared, Elias Campo: None declared, Wolfgang Huber: None declared, Ruedi Aebersold R.A. holds shares of Biognosys AG which operates in the proteomics field. The remaining authors declare no competing financial interests., Thorsten Zenz: None declared.

C24.5 Single cell transcriptomics of Pituitary Neuro-Endocrine Tumors (PitNETs)

Maxime Brunner[1;2], *Federico Santoni*[1;2], *Jenny Meylan-Merlini*[1], *Mahmoud Messerer*[2;3], *Roy Daniel*[2;3], *Stefano La Rosa*[2;4]

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Background/Objectives: The pituitary gland, a master controller of hormones production and secretion, is a main component of the endocrine system. Unlike neoplastic formation in other organs, Pituitary Neuroendocrine Tumors (PitNETs) are almost exclusively benign adenomas. PitNETs have been extensively characterized by DNA sequencing, bulk RNA and DNA methylation in order to link those features with tumor invasiveness, prognosis and possible relapses.

Methods: Here, we present the single cell transcriptome analysis on 7 independent tumors for a total of ~38000 single cells: a bi-hormonal (GH-PRL) tumor, two corticotropic (*POMC*) macroadenoma and 4 non-secreting adenomas.

Results: Characterization of all tumors showed heterogeneous cell populations; on top of tumorigenic cells, structural cells (endothelial and fibroblasts) and immune cells can be detected. In the two most invasive tumors we identified an unexpected small population of proliferative cells (MKI67+, TOPA2+, BIRC5+, PBK +). Intriguingly, BIRC5 and PBK, two genes already known to be implicated in different type of carcinomas, were highly expressed in this cluster, suggesting a possible link between PitNETs and other types of tumors of various origin. In two non-secreting adenomas we identified a group of cells expressing autophagy related genes, supporting the recent hypothesis that autophagy is an important mechanism for tumor homeostasis. Moreover, we identified in all tumors a cluster of specialized cells expressing mitochondrial genes and a cluster expressing ribosomal proteins, suggesting a recurrent structural configuration to optimize energy balance and transcriptional activity.

Conclusion: Our results give a new perspective on the comprehension of the structural composition and the dynamic progression of pituitary tumors.

References:

Grants:

Conflict of Interest: None declared.

C24.6 Investigating breast cancer cellular complexity and cell communication by single-cell transcriptomics of tumor organoids

*Alice Chiodi[1], Valentina Nale[1], Cinzia Cocola[1], Eleonora Piscitelli[1], Edoardo Abeni[1], Ingrid Cifola[1], Francesca A. Cupaioli[1], Theodoros Karnavas[2], Angelos Gritzapis[3], Ioannis Missitzis[4], Martin Götte[5], Mira Palizban[5], Burkhard Greve[6], Giovanni Bertalot[7], Alessandra Mezzelani[1], Luciano Milanesi[1], Rolland A. Reinbold[1], Ileana Zucchi[1], **Paride Pelucchi**[1], Ettore Mosca[1]*

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Background/Objectives: Organoids are a valuable model for cancer research as they recapitulate cellular heterogeneity, tissue architecture and some environmental conditions of the tumor (such as hypoxia and lack of nutrient in the core). To study this complex model, the Single Cell Analysis (SCA) approach for transcriptomic profiling has become mandatory. Moreover, SCA opens the possibility to study how different cell types interact and contribute to the tumor progression, thus allowing inferences about the communication mechanisms among the different populations.

Methods: Organoids were obtained from four breast tumor samples by mechanic/enzymatic tissue dissociation, filtration and grown in specific matrix that allows the formation of 3D structures recapitulating the parental organ. Organoids were then dissociated into single cells and processed for SCA using 10X Genomics technology. SC data analysis: CellRanger, Seurat, scMuffin. Ligand-receptor interactions (LRI) data: Omnipath. Cell-cell communication (CCC) analysis: Ulisse.

Results: SCA approach allowed to resolve the complex heterogeneity of breast cancer organoids, identifying different cell populations representing all the mammary gland lineages and different cell states among them. The copy number variant inference implemented in scMuffin allowed to discern tumor from microenvironment cells. Ulisse was used with LR and LRI data to identify significant CCC between cell population and states, also highlighting the interactions between normal and tumor cells.

Conclusion: Our novel developed tool, Ulisse, proved to be a valuable approach to disentangle the interactions among the different cell types in a complex context such as organoids cultures. In this work we identified specific and relevant cross-talks between tumor populations and the microenvironment.

References:

Grants:

Conflict of Interest: None declared.

C25 REPRODUCTIVE GENETICS

C25.1 Large-scale analyses of X-chromosomal variants in 2354 patients with spermatogenic failure reveals recurrently affected novel candidate genes

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Background/Objectives: Although the evolutionary history of the X chromosome indicates its specialization in male fitness, its role in spermatogenesis has been largely unexplored. Currently only three genes reach high enough evidence for gene-disease relationship (GDR) to be proposed for a diagnostic gene panel in non-obstructive azoospermia (NOA)[1]. We aimed to provide the analysis of all X-linked protein coding genes in the largest group of NOA/cryptozoospermic men screened so far.

Methods: X-chromosome data of 2,354 NOA/cryptozoospermic men from four independent cohorts were analyzed and compared with data of normozoospermic controls and gnomAD. We searched for variants in: i) known NOA genes; ii) recurrently mutated genes not previously linked to spermatogenic failure. We performed RNAi-mediated knockdown experiments of RBBP7 orthologue in *Drosophila*.

Results: We identified: i) 83 predicted pathogenic variants in 17 X-linked genes previously described in NOA; ii) novel recurrently mutated genes, among them 21 (N = 70 variants) strongly associated and 34 (N = 123 variants) moderately associated with spermatogenesis. We validated and upgraded the GDR for 17 known NOA genes and among the 21 strong novel candidates, three reached sufficiently high GDR for diagnostic setting. The most frequently mutated novel gene was *RBBP7*: our functional experiments showed that the loss of its orthologue caused a dramatic reduction of germ cells in *Drosophila*.

Conclusion: Thanks to the joint efforts of four centers, we provide the most comprehensive data on the X-chromosome in relationship with NOA, reducing the gap in our understanding of X-linked genetic causes of spermatogenic failure.

References: 1. PMID:34498060

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Conflict of Interest: None declared.

C25.2 Exome sequencing of azoospermic and severe oligozoospermic men identifies novel recessive monogenic causes of human male infertility

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Background/Objectives: Infertility affects 7% of men in developed societies and approximately 70% of these cases remain unexplained. Genetics plays a role in severe forms of male infertility, however, using current clinical approaches it only explains 4% of cases. We combined gene discovery in a unique patient-parent cohort with replication in a large cohort of infertile patients and fertile fathers to look for recessive causes of male infertility.

Methods: We performed exome-sequencing in 186 men diagnosed with non-obstructive azoospermia or severe oligozoospermia (< 5 million sperm cells/ml) and their fertile parents. Potentially pathogenic variants were identified by filtering for rare variants (gnomAD AF < 0.01) classified as pathogenic or unclear by the ACMG guidelines. Genes affected by these variants were prioritized according to their likely role in male fertility. A replication analysis of candidate genes was performed in a cohort of infertile men (n = 1,580) and fertile men (n = 5,784).

Results: We identified 50 potential pathogenic variants (20 homozygous, 30 compound heterozygous) affecting 49 protein-encoding genes with a potential role in male fertility. Our replication analysis revealed a significant enrichment of predicted pathogenic homozygous mutations in 5 of these 49 genes (AATF, FKBP6, M1AP, TEX14 and TOPAZ1, burden test p < 0.05) in infertile compared to fertile men. Interestingly, while M1AP and TEX14 are known male infertility genes, the other three genes are novel male infertility candidate genes.

Conclusion: This study shows the power of patient-parent trio-based exome-sequencing combined with a large replication cohort for the identification of both known and novel recessive causes of male infertility.

References:

Grants:

Conflict of Interest: None declared.

C25.3 Scrutinizing the human TEX genes in the context of human male infertility

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Background/Objectives: The most severe form of male infertility is azoospermia, assumed to be frequently of genetic origin. Variants in *TEX11*, *TEX14*, and *TEX15* are well-established causes of azoospermia and *TEX13B*, *TEX13C*, and *TEX39B* were also described in this context. Overall, there are 47 human genes, called "*TEX*" (testis expressed) genes, which we aimed to analyse systematically.

Methods: We screened the exome sequencing data of 1,305 men from our MERGE study for rare (minor allele frequency, MAF < 1% in gnomAD database) variants in all human *TEX* genes. Exome data of a cohort of >5,700 proven fathers served as control cohort. We generated *Drosophila melanogaster* knockdown models of 10 available orthologous *TEX* genes using the GAL4/-UAS system, performed phylogenetic analysis of the *TEX* genes across multiple species, and analysed single-cell RNA sequencing data sets to determine the expression of these genes.

Results: Hemizygous loss-of-function (LoF) variants in *TEX13B, TEX13C* and *TEX39A* were detected in infertile men as well as in controls. A hemizygous inframe insertion in *TEX39B* was found in two infertile men but not in controls. Knockdowns of the orthologues of *TEX2, 9, 10, 13, 27, 28, 30, 42, 261, 292* in *Drosophila* resulted in normal reproductive phenotypes.

Conclusion: Based on our findings, we refute previous findings that LoF variants in *TEX13B, TEX13C* and *TEX39A* are monogenic causes for male infertility. In contrast, we significantly strengthen evidence for *TEX39B* as a candidate gene for male infertility.

References:

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Conflict of Interest: None declared.

C25.4 Bi-allelic variants in genes related to piRNA biogenesis are a major cause for male infertility

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Background/Objectives: Non-obstructive azoospermia (NOA) represents the most severe form of male infertility with no sperm found in the ejaculate due to spermatogenic failure. Heterogeneous genetic causes for NOA are suspected, but only few disease genes have been identified yet.

Piwi-interacting RNAs (piRNAs) are a specific type of small non-coding RNAs. They play a crucial role during spermatogenesis being essential for protecting the germ cell genome from retrotransposons. Knock-out of genes involved in piRNA biogenesis leads to male infertility in mice. However, in infertile men only variants in *PNLDC1* were described in the context of impaired piRNA biogenesis so far.

Methods: Exome sequencing data of >1600 infertile men was screened for bi-allelic high-impact variants (loss-of-function and missense variants with CADD-score >20) in 45 piRNA-pathway related candidate genes. Functional analyses to unravel the impact of variants on testicular phenotype and piRNA biogenesis are ongoing.

Results: Eight men with bi-allelic loss-of-function variants and 10 men with homozygous high-impact missense variants in nine different candidate genes (*GTSF1*, *GPAT2*, *PIWIL1*, *MOV10L1*, *MAEL*, *PLD6*, *DDX4*, *TDRD1*, *TDRD12*) were identified. All men were azoospermic and those that underwent testicular biopsy had spermatogenic arrest phenotypes similar to those described for knock-out mice of respective orthologous genes.

Conclusion: Impaired function of proteins involved in piRNA biogenesis leads to non-obstructive azoospermia in humans. Identified novel disease genes broaden the spectrum of genetic causes for male infertility.

References:

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Conflict of Interest: None declared.

C25.5 Single-cell evaluation of DNA damage in offspring after prenatal exposure to chemotherapy

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Background/Objectives: Although chemotherapy during pregnancy is considered relatively safe, genotoxic chemotherapeutics can cross the placenta and could lead to DNA damage in the newborn.

Methods: Cord blood mononuclear cells (CBMCs) of (i) pregnant breast cancer patients treated with epirubicin, cyclophosphamide and/or paclitaxel (BrCa+EC/T, $n = 3$), (ii) non-treated control pregnant breast cancer cases (BrCaCo, $n = 4$), (iii) pregnant Hodgkin lymphoma patients treated with doxorubicin, bleomycin, vinblastine and dacarbazine (HL + ABVD, $n = 4$) and (iv) healthy pregnant women (HPr; $n = 10$) were subjected to cytokinesis-block micronucleus analysis and whole-genome sequencing (WGS) of clonally expanded hematopoietic stem cells to map genetic damage via micronucleus frequency (MNF), single nucleotide variants (SNVs) and small indels respectively.

Results: MNF was significantly increased in CBMCs from BrCa +EC/T (2,75%), HL + ABVD (3,36%) and BrCaCo (1,89%) patients compared to HPr cases (0,72%; $p < 0.01$), suggesting chromosomal instability being associated with prenatal chemotherapy exposure and/or a genetic or oncological underlying factor. WGS revealed a significant increase in somatic SNVs ($p = 0.04$) and indels ($p = 0.0001$) in all treated cases versus HPr ($n = 3$). Both EC/T and ABVD treatment associated with C>T and C>A substitutions, whereas ABVD treatment also showed T>C conversions. The EC/T- and ABVD-linked mutation patterns presented similarities with the SBS COSMIC platinum signature and DNA mismatch repair signatures, respectively.

Conclusion: Prenatal chemotherapy exposure is linked to increased MNF, SNVs and indels in CBMCs. Further larger cohort investigations are ongoing to confirm these findings and define the exact mutational signature.

References:

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Conflict of Interest: None declared.

C25.6 An Israeli expanded preconception screening panel – findings and insights

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Background/Objectives: To analyse the efficacy and effectiveness of targeted mutation-based Israeli expanded carrier-screening panel (IECSP).

Methods: A curated mutation-based IECSP tailored to the unique diverse Israeli population (CarrierScan® ThermoFisher) was offered at two tertiary hospitals and one major laboratory. The panel includes 1487 variants in 357 genes. Individuals' ethnicity was self-reported.

Results: During an 18-month period, July 2020-December 2021, 10,115 ethnically-mixed Israeli samples were analysed. Of these 6036 (59.7%) were couples and 4079 (40.3%) were singles. The common genes in carriers were *GJB2/6* (allele frequency) (1:22) followed by *CFTR* (1:28), *GBA* (1:34) *TYR* (1:39) *PAH* (1:50) *SMN1* (1:52) and *HEXA* (1:56).

Of 3018 couples tested, 753 (24.9%) had no findings; in 1464 (48.5%) only one partner was a carrier; and in 733 couples (24.3%) both were carriers of different disease. Remarkably, some couples were at risk for as many as five diseases. Seventy nine (2.6%) were at-risk cases (both partners being carriers of the same autosomal recessive condition or a female carrying an X-linked disease). Of these, 48.1%% would not have been detected utilizing the ethnically-based screening test currently provided by the Ministry

of Health and local HMOs. These include variants in *GBA, TYR, PAH and GJB2/GJB6*

Conclusion: This is the first screening test tailored specifically for the diverse Israeli population, including minority groups. A unified Israeli panel can identify couples at risk and aid reproductive decision making. Hopefully in the future such a panel will be provided by the National Health System to the entire population

References:

Grants:

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C26 NEW GENES IN MULTIPLE CONGENITAL ANOMALIES

C26.1 FOXI3 pathogenic variants cause one form of craniofacial microsomia

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Background/Objectives: Craniofacial microsomia (CFM also known as Goldenhar syndrome), is a craniofacial developmental disorder of variable expressivity and severity with a recognizable set of abnormalities. These birth defects are associated with structures derived from the first and second branchial arches, can occur unilaterally and include ear dysplasia, microtia, preauricular tags and pits, facial asymmetry and other malformations. The inheritance pattern is controversial, and the molecular etiology of this syndrome is largely unknown.

Methods: Various genomic methods (whole exome and whole genome sequence with short and long read technologies, Sanger sequencing, optical mapping technologies), computational analysis, cellular experiments, and knock-in mice were used to study the molecular basis of CFM cases.

Results: 670 patients from unrelated pedigrees of European and Chinese ancestry with microtia or CFM, were investigated. We identified 20 likely pathogenic variants in 23 probands in FOXI3 (3.4%). Biochemical experiments in HEK293 cells, and 3 knock-in mouse models support the involvement of FOXI3 in CFM. Our findings indicate autosomal dominant inheritance with reduced penetrance, and/or autosomal recessive inheritance. The phenotypic expression of the FOXI3 variants is variable. We present suggestive evidence that common variation in the FOXI3 allele in trans with the pathogenic variant could modify the phenotypic severity and penetrance.

Conclusion: Craniofacial microsomia is a genetically heterogeneous disorder of variable severity, one form of which is due to FOXI3 likely pathogenic variants. Rare and common variants in compound heteroyzgosity could explain the penetrance issue in the majority of cases. This work provides new insights into the molecular mechanism underlying CFM.

References:

Grants:

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C26.2 Bi-allelic loss-of-function variants in RABGAP1 cause a novel neurodevelopmental syndrome

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Background/Objectives: Rab GTPase-Activating protein 1 (RABGAP1) is a GTPase activating protein implicated in a variety of cellular processes including mitosis, cell migration, vesicular trafficking and intracellular lysosomal positioning, important for mTOR signaling. There are no known Mendelian diseases described to date caused by variants in *RABGAP1*.

Methods: Through GeneMatcher, we identified five patients internationally with homozygous variants in *RABGAP1* found on whole exome sequencing (WES) and inherited from consanguineous, heterozygous parents. We established lymphoblastoid cells lines derived from an affected individual and performed RNA sequencing and functional studies. *RABGAP1* knock-out mice were generated and underwent phenotyping.

Results: We report three bi-allelic variants in *RABGAP1* in five affected individuals from three unrelated families presenting with a spectrum of neurodevelopmental abnormalities including global developmental delay/intellectual disability, microcephaly, bilateral sensorineural hearing loss and seizures, as well as overlapping dysmorphic features. Brain imaging revealed anomalies including delayed myelination, white matter volume loss, ventriculomegaly and thinning of the corpus callosum. *RABGAP1* knock-out mice also demonstrated microcephaly and similar brain abnormalities, including thinning of the corpus callosum and ventriculomegaly. Transcriptomic analysis performed with patient cells revealed downregulation of mTOR signaling. Furthermore, decreased levels of both phospho-AKT and phospho-S6 and abnormal positioning of lysosomes and early endosomes were seen, consistent with loss of RABGAP1 function.

Conclusion: Collectively, our results provide evidence of a novel neurodevelopmental syndrome caused by bi-allelic loss-of-function variants in *RABGAP1*.

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C26.3 Biallelic DAW1 variants cause a motile ciliopathy characterized by laterality defects and subtle ciliary beating abnormalities

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Background/Objectives: The clinical spectrum of motile ciliopathies includes laterality defects, hydrocephalus and infertility, as well as primary ciliary dyskinesia (PCD) when impaired mucociliary clearance results in otosinopulmonary disease. Importantly, ~30% of PCD patients currently lack a genetic diagnosis.

Methods: We performed clinical and genetic studies (exome sequencing, segregation studies) in four individuals from two unrelated families to investigate the cause of a motile ciliopathy disorder comprising laterality defects and mild oto-sinopulmonary disease. Alongside this, we performed immunofluorescence, particle tracking and ciliary motion high-speed video microscopy studies of patient cilia, as well as gene expression studies during murine development, biochemical investigations to define mutant protein stability, and in vivo gene variant modelling studies in zebrafish.

Results: We identified biallelic DAW1 variants as the cause of disease. In early mouse embryos, we show that Daw1 expression is limited to distal, motile ciliated cells of the node, consistent with a role in left-right patterning. Daw1 mutant zebrafish exhibit reduced cilia motility and left-right patterning defects including cardiac looping abnormalities. Importantly, these defects were rescued by wild type but not mutant daw1 gene expression. Additionally, pathogenic DAW1 missense variants display reduced protein stability, while DAW1 loss of function is associated with distal type 2 outer dynein arm assembly defects involving axoneme respiratory cilia proteins, explaining reduced cilia-induced fluid flow in particle tracking experiments.

Conclusion: We define biallelic DAW1 variants as causative of human motile ciliopathy and determine that the disease mechanism involves motile cilia dysfunction, explaining the ciliary beating defects observed in patients.

References:

Grants: MRC.

Conflict of Interest: None declared.

C26.4 OTX2 duplications: a recurrent cause of oculo-auriculo-vertebral-spectrum

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Background/Objectives: Oculo-Auriculo-Vertebral Spectrum (OAVS) is the second most common cause of head and neck malformation in children after orofacial clefts. OAVS is clinically heterogeneous and characterized by a broad range of clinical features including ear anomalies with or without hearing loss, hemifacial microsomia, orofacial clefts, ocular defects and vertebral abnormalities. Various genetic causes are associated with OAVS and Copy Number Variations represent a recurrent cause of OAVS, but the responsible gene often remains elusive. In the present study, we described six probands and three relatives with OAVS carrying a 14q22.3 microduplication only involving *OTX2* gene.

Methods: All patients had chromosomal micro-array analysis and clinical examination according to the local investigation protocol. We subsequently studied the effects of *OTX2* overexpression in a zebrafish model.

Results: We defined a 406-kb minimal common region that only overlaps with the *OTX2* gene. Head and face defects with a predominance of ear malformations were present in 100% of patients. The variability in expressivity was significant, ranging from simple chondromas to severe microtia, even between intrafamilial cases. Heterologous overexpression of *OTX2* in zebrafish embryos showed significant effects on early development with alterations in craniofacial development.

Conclusion: Our results indicated that proper *OTX2* dosage seems to be critical for the normal development of the first and second branchial arches. Overall, we demonstrated that *OTX2* genomic duplications are responsible for OAVS marked by auricular malformations of variable severity.

References:

Grants:

Conflict of Interest: None declared.

C26.5 Dual molecular effects of constitutional SF3B2 variants cause a novel dominant spliceosomopathy displaying retinitis pigmentosa or developmental skeletal anomalies

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Background/Objectives: Genetic defects in core components of the spliceosome cause a spectrum of phenotypes known as spliceosomopathies, of which isolated inherited retinal diseases (IRD) and syndromic developmental anomalies represent well known phenotypic groups. Here, we identified constitutional genetic defects in the spliceosomal *SF3B2* gene leading to divergent phenotypes from the spliceosomopathy groups.

Methods: Exome and genome sequencing were performed in IRD and developmental anomaly cohorts, followed by transcriptome analysis of autosomal dominant retinitis pigmentosa (adRP) patients' lymphocytes and targeted morpholino (Mo) *Sf3b2* knockdown in *Xenopus tropicalis*.

Results: We showed that distinct missense variants affecting residue Tyr806 lead to isolated adRP in four individuals from two families, while presumptive loss-of-function variants lead to syndromic developmental phenotypes characterized by craniofacial and skeletal defects in five individuals from three families. Transcriptome analysis revealed a dysregulation of downstream genes involved in RNA splicing and RNA processing. Targeted Mo knockdown showed developmental craniofacial anomalies in *Xenopus*. Rescue experiments using both human and *Xenopus* wild type (WT) or mutant RNA restored the Mo-mediated defects almost to normal, however neither WT nor mutant *sf3b2* overexpression displayed any discernible effect in the embryonic stages. These findings support gain-of-function as the mechanism driving the postnatal adRP phenotype and haploinsufficiency underlying the developmental *SF3B2* phenotype.

Conclusion: This study reveals a dual role for *SF3B2* both during early development and in postnatal life, expanding the spliceosomopathy spectrum in human. Moreover, we put forward *SF3B2* as the first spliceosomal gene outside of the U4/U6-U5 tri-snRNP complex causing a retinopathy.

References: /

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Conflict of Interest: None declared.

C26.6 Recurrent de novo missense variants across multiple histone H4 genes underlie a neurodevelopmental syndrome

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Background/Objectives: Chromatin is essentially an array of nucleosomes, each of which consists of the DNA double-stranded fiber wrapped around a histone octamer. This organization supports cellular processes like DNA replication, DNA transcription and DNA repair in all eukaryotes. Human histone H4 is encoded by fourteen canonical histone H4 genes, all differing at the nucleotide level but encoding an invariant protein.

Methods: Subjects were identified by whole exome sequencing and matchmaking. Modeling in zebrafish was performed by mRNA injections in fertilized eggs. Early embryonic effects were evaluated 28 hours post fertilization.

Results: Here we present a cohort of 29 subjects with *de novo* missense variants in six H4 genes (*H4C3*, *H4C4, H4C5, H4C6, H4C9* and *H4C11*). All individuals present with neurodevelopmental features of intellectual disability and motor and/or gross developmental delay, while non-neurological features are more variable. Ten amino acids are affected, six of which recurrently, and are all located within the H4 core or C-terminal tail. These variants cluster to specific regions of the core H4 globular domain, where protein-protein interactions occur with either other histone subunits or histone chaperones. Functional consequences of the identified variants were evaluated in zebrafish embryos, which displayed abnormal general development, defective head organs and reduced body axis length, providing compelling evidence for the causality of the reported disorder(s).

Conclusion: While multiple developmental syndromes have been linked to chromatin-associated factors, missense-bearing histone variants (e.g. H3 oncohistones) are only recently emerging as a major cause of pathogenicity. Our findings establish a broader involvement of H4 variants in developmental syndromes.

References:

Grants:

Conflict of Interest: None declared.

C27 NEW APPROACHES AND LARGE DATASETS TO UNRAVEL HUMAN TRAITS

C27.1 Multi-ancestry meta-analysis of genome-wide association studies of lung function and variant-to-gene-mapping undercover hundreds of novel implicated genes and loci

Abril Izquierdo[1], *Nick Shrine*[1], *Jing Chen*[1], *Anna Guyatt*[1], *Richard Packer*[1], *Chiara Batini*[1], *Karsten Suhre*[2], *Alfred Pozarickij*[3], *Robin G Walters*[3;4], *Stephanie London*[5], *Andrew Morris*[6], *Louise Wain*[1;7], *Ian P. Hall*[8], *Martin D Tobin*[1;7]

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Background/Objectives: Lung function is a complex polygenic trait underlying chronic obstructive pulmonary disease (COPD), for which ~300 genetic signals have been reported, the majority within non-coding regions, making it challenging to dissect disease mechanisms. We conducted the largest and most ancestrally diverse meta-analysis to improve power for detection of complex underlying genetic variants and used a systematic strategy to identify and prioritise putative causal genes and biological pathways.

Methods: We performed a meta-analysis of lung function traits: forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio and peak expiratory flow (PEF) in 580,869 individuals across European, African, East and South Asian and American ancestries. We integrated evidence from gene and protein expression colocalisation, exome sequencing, epigenomic marks, and respiratory-pertinent Mendelian disease and mouse-knockout genes to prioritise putative causal genes.

Results: We identified 1,020 independently associated signals and highlighted 424 causal putative genes implicated by two or more lines of evidence. Among these findings, we implicated putative genes for which the most likely causal variant was a rare predicted pathogenic variant, including *ABCA3*, involved in surfactant production. Additionally, we reported novel causal genes with diverse roles in smooth muscle function (*FGFR1*, *GATA5*, *STIM1*), tissue organisation (*ADAMTS10*), alveolar and epithelial function and inflammation and immune response to infection (*CLDN18*).

Conclusion: Integration of a multi-ancestry meta-analysis yielded superior statistical power, boosted the number of lung function signals, and identified causal putative genes. Our findings yield novel insights into the genetics and mechanisms of lung function and COPD.

References:

Grants:

Conflict of Interest: Abril Izquierdo Full, Nick Shrine Full, Jing Chen Full, Anna Guyatt Full, Richard Packer Full, Chiara Batini Full, Karsten Suhre Full, Alfred Pozarickij Full, Robin G Walters Full, Stephanie London Full, Andrew Morris Full, Louise Wain Full, Ian P. Hall Full, Martin D Tobin Full.

C27.2 Non-additive outlier effects of gene expression on anthropometric traits

Théo Dupuis[1], *Ambra Sartori*[2], *Aline Réal*[2], *David Davtian*[1], *Ewan Pearson*[1], *Emmanouil Dermitzakis*[2], *Ana Viñuela*[3], *Andrew Brown*[1]

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Background/Objectives: It is well known that rare variants can cause extreme phenotypes at the transcript level and on disease risk. However, consequences of extreme expression caused by combinations of common variants are mostly unknown. Methods linking multiple variants to intermediate phenotypes like TWAS focus on their additive contribution, ignoring threshold effects and compensatory biological processes. We investigated whether combinations of variants could cause expression outliers with epistatic consequences on downstream phenotypes. As multiple variants must act consistently to cause extreme expression, linkage contamination and pleiotropy are less likely to generate false positives and genes identified that way are more likely to be causal.

Methods: We used eQTLs from DIRECT and GTEx v8 to calculate predicted expression in 44 tissues for 405,719 individuals (UK Biobank). For every gene across all tissues, we defined low (LEE) and high extreme expression (HEE) as individuals whose predicted

expression levels fell in the bottom percentile or the top one and tested both for trait differences with the rest of the population for 5 traits: blood pressure, BMI, grip strength, height, waist-hip ratio.

Results: We found 7, 44, 30, 585 and 32 significant associations with these traits respectively (FDR = 0.05), testing 54,000 LEEs and 53,000 HEEs. In most cases, genes with these non-additive associations between extreme expression and phenotype also showed significant additive correlations (584/689).

Conclusion: In these cases, extreme expression generally attenuates rather than exacerbates the contribution of the additive component, suggesting that biological processes such as buffering, or pathway substitution can compensate for extremes of expression.

References:

Grants:

Conflict of Interest: Théo Dupuis: None declared, Ambra Sartori: None declared, Aline Réal: None declared, David Davtian: None declared, Ewan Pearson: None declared, Emmanouil Dermitzakis Worked at the University of Geneva when involved in this project but currently employed by GlaxoSmithKline., Ana Viñuela: None declared, Andrew Brown: None declared.

C27.3 Optical genome mapping for repeat expansion disorder testing

Kornelia Neveling[1], *Erik-Jan Kamsteeg*[1], *Maartje Pennings*[1], *Ronald van Beek*[1], *Alexander Hoischen*[1]

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Background/Objectives: Short tandem repeat (STR) expansions are often unstable and can be associated with genetic disorders. Being able to accurately detect the total length of expansion is important. Current diagnostic assays (e.g. repeat-primed PCR-based tests, Southern blotting) are unable to precisely determine long repeat expansions and/or require set-up for each locus separately. Sequencing based assays have not yet replaced these diagnostics assays. Here we show that using optical genome mapping (OGM), the length of repeat expansions across the genome can be revealed efficiently.

Methods: We performed OGM for 90 samples with known clinically relevant repeat expansions for 4 loci (*FMR1, DMPK, CNBP, RFC1*). Using different algorithms measuring the exact label distances flanking the repeats of interest, we can estimate the mean and variance of the repeat sizes. A histogram of repeat sizes and a Gaussian mixture model can be used to identify the zygosity of the repeat expansion region.

Results: All known repeat expansions were detected, and allelic differences were obvious – either between wildtype and expanded alleles, or two expanded alleles for recessive cases. An apparent strength of OGM was the more accurate length measurement for very long repeat expansion alleles. We also identified somatic repeat instability for several repeat expansions. Whether absolute repeat size or somatic (in)stability have prognostic value is currently investigated.

Conclusion: OGM provides an efficient method to identity repeat lengths, across multiple loci simultaneously. With long intact molecules spanning repeats even kilobases in size, absolute repeat lengths and somatic instability can be detected with high confidence.

References:

Grants:

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C27.4 Shared structural variant mutagenesis mechanisms in cancer and the constitutional genome

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Background/Objectives: Genomic disorders are a class of disease resulting from structural variation (SV) of the constitutional genome. Within cancer, genome evolution due to somatic mutagenesis may also result in the formation of SVs causative for a clinical diagnosis.

Methods: Utilizing multiomics approaches with two Mendelian disease cohorts, *MECP2* Duplication Syndrome (MDS) and Potocki-Lupski Syndrome (PTLS), we investigated the shared SV mutagenesis mechanisms between the constitutional and cancer genome.

Results: Analysing 154 PTLS families/probands, we identified a cluster of individuals with amplifications of a 2.3Mb 17p11.2 genomic interval which maps to the isochromosome 17q cancer breakpoint; the most common abnormality in human neoplasia. This structurally polymorphic region is hypothesized to result in a rolling-circle mechanism causing amplifications in PTLS and isochromosome formation in cancer. In characterizing the duplication-triplication/inverted-duplication (DUP-TRP/INV-DUP) or '*Carvalho Structure*', at Xq28 associated with MDS, we identified four distinct structural haplotypes generated by two template switches. Analysis by the Pan Cancer Genome Consortium found evidence for the *Carvalho Structure* as one of the 12 most common cancer signatures.

Conclusion: Studying SVs in Mendelian disease elucidated insights into cancer genome evolution. We found that 1) inherent structural features can lead to SV formation in the germline and 2) patterns dictating SV formation are shared biological processes that can lead to the same mutation types including the *Carvalho Structure*. Deciphering the mechanisms for SV mutagenesis provides insight to genome evolution which drives our understanding of all human disease: Mendelizing traits, genomic disorders, chromosomal syndromes, and cancer.

References:

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Conflict of Interest: Christopher Grochowski: None declared, James Lupski U01 HG011758, R01 GM106373, R35 NS105078, 23andMe, Regeneron Genetics Center, Co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, genomic disorders, eye diseases and bacterial genomic fingerprinting.

C27.5 Manually curated annotation of uORFs in the OMIM genes reveals pathogenic variants in 5`UTRs

Alexandra Filatova[1], *Ivan Reveguk*[2], *Maria Piatkova*[3;4], *Daria Bessonova*[4], *Olga Kuziakova*[4], *Victoria Demakova*[4], *Alexander Romanishin*[4;5], *Veniamin Fishman*[6;7], *Yerzhan Imanmalik*[6], *Nikolay Chekanov*[6], *Rostislav Skitchenko*[8], *Yury Barbitoff*[9;10;11], *Olga Kardymon*[6], *Mikhail Skoblov*[12]

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Background/Objectives: To date, the interpretation of disease-causing variants routinely focuses on the coding part of genes. Several previous studies have shown that variants in the 5`UTR can lead to Mendelian disorders through disruption of upstream open reading frames (uORF). However, the detection and interpretation of such variants in clinical genetic testing still remains a challenge.

Methods: Using various types of publicly available (Ribo-seq, mRNA-seq, CAGE data, previously predicted uORFs) and self-obtained data (Kozak scores) we performed a manual annotation of upstream translation initiation sites (TIS) in ~3'600 OMIM genes. Experimental validation was conducted using dual luciferase assay. Two software tools were created.

Results: We annotated and characterized ~5 thousand upstream TISs in 1835 OMIM genes. The major group of these TISs were related to uORFs. We compared our annotation with the previous studies and provided a list of "high confidence" uORFs. Besides, we created a tool to evaluate the effects of nucleotide variants located in uORFs. This allowed us to reveal the variants from HGMD/ClinVar that disrupt uORFs and thereby could lead to Mendelian disorders. In addition, we experimentally analysed predicted uORFs and mutations in several genes. We also observed that the distribution of uORFs-affecting variants differs between pathogenic variants and population variants from gnomAD. Finally, drawing on manually curated data, we developed a machine-learning algorithm that allows us to predict the TISs in other human genes.

Conclusion: Thus, we attempted to obtain qualitative annotation of uORFs in the OMIM genes, which may be useful in interpreting the patient-derived variants in 5`UTRs.

References:

Grants:

Conflict of Interest: None declared.

C27.6 Objective 3D facial phenotyping in Cri-du-Chat Syndrome

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Background/Objectives: Cri-du-Chat Syndrome (CdCS) is caused by chromosome 5p deletions. The phenotypic spectrum is broad and multiple critical regions have been defined. We report five patients from three families with a small 2.7Mb 5p15.33-15.32 deletion overlapping with a proposed critical region for facial dysmorphism. We objectively assess the facial phenotype of these individuals by 3D imaging and use 3D facial images of a large cohort of individuals with CdCS as a reference. We aim to evaluate the involvement of the 5p15.33-15.32 region in facial dysmorphism in CdCS.

Methods: We modelled the 3D facial shape of our patients ($n = 5$), a large cohort of individuals with CdCS ($n = 97$) from a dataset with various genetic disorders ($n = 3313$) and controls ($n = 40$) using dense surface registration. To objectively model shape variation, we calculated facial signatures using craniofacial growth curves. We applied principal component analysis and cosine-based distance metrics to these signatures to compare the facial phenotype of our patients with the reference CdCS group, with other genetic disorders and with controls.

Results: All 5p15.33-15.32 deletion carriers clustered away from CdCS patients. The cosine distances to the average CdCS phenotype independently indicated that facial features in both groups are distinct (range: 0.77-1.30). The within-group variance for our patients was high, objectively supporting the clinical observation of a heterogeneous facial phenotype in these patients.

Conclusion: Facial dysmorphism in patients with a small 5p15.33-15.32 deletion is distinct from the facial dysmorphism in a large cohort of CdCS patients. We present 3D facial phenotyping as a tool to objectively study genotype-phenotype correlations.

References:

Grants:

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C28 NOVEL NEUROGENETIC DISORDERS

C28.1 PIK3C2B germline heterozygous mutations cause defective lipid signaling and focal epilepsy

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Background/Objectives: Loss-of function (LoF) mutations in mTOR (mammalian target of rapamycin) pathway genes play an important role in the pathogenesis of focal epilepsy. However, the genetic aetiology of many focal epilepsies remains unknown.

Methods: To identify novel epilepsy genes, we performed whole exome sequencing in 37 trios with sporadic focal epilepsy, and targeted resequencing of candidate genes in larger cohorts of focal epilepsy probands. To confirm variant pathogenicity and elucidate disease mechanisms, we performed in vitro and in vivo functional studies.

Results: In 6 patients we identified ultra-rare germline heterozygous variants in *PIK3C2B* gene, clustering at the C-terminal end of the encoded protein. PI3K-C2b is a class II phosphatidylinositol 3-kinase, recently shown to be a repressor of mTOR pathway. Using in vitro functional studies, we demonstrated that the identified variants act as LoF alleles, leading to impaired synthesis of phosphatidylinositol 3,4-bisphosphate, and mTOR pathway hyperactivation. LoF transgenic mice models showed that mutant Pik3c2b alleles caused dose-dependent neuronal hyperexcitability and increased susceptibility to seizures. Acute mTOR pathway inhibition with everolimus prevented experimentally induced seizures and lowered neuronal excitability in brain slices, providing a potential therapeutic option for a selective group of patients with focal epilepsy.

Conclusion: Our findings widen the spectrum of mTOR-related epilepsies, revealing a novel role for class II PI3K-mediated lipid signaling in regulating neuronal excitability.

References:

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C28.2 CAPRIN1 haploinsufficiency causes an autosomal dominant neurodevelopmental disorder with defects in hiPSCs-derived neurons and an identifiable episignature in patients

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Background/Objectives: *CAPRIN1* gene was recently considered a candidate in autism spectrum disorders (ASD). The encoded Cell cycle Associated PRoteIN 1 (CAPRIN1) is a cytoplasmic ubiquitous phosphoprotein tightly linked with cellular proliferation. In neurons, it regulates the transport/translation of mRNAs of proteins involved in synaptic plasticity and interacts with proteins implicated in neurodevelopment, including FMRP and CYFIP1.

Methods: *CAPRIN1*$^{+/-}$ hiPSCs were generated by CRISPR/Cas9 mutagenesis and differentiated into neuronal progenitors and cortical neurons. *CAPRIN1* specific methylation profile was investigated on patients' DNA.

Results: We identified twelve patients heterozygotes for a loss-of-function variant in *CAPRIN1*, presented with language impairment/speech delay (100%), Intellectual Disability (83%), ADHD (82%), and ASD (67%). In patient-derived cells, monoallelic expression of the *CAPRIN1* wild-type allele and subsequent reduction of transcript and protein, compatible with a half-dose, were demonstrated. *CAPRIN1*$^{+/-}$ hiPSCs-derived neurons showed decreased processes length, overall disruption of the neuronal structure, and an enhanced neuronal death. We observed an increased global translation and oxidative stress, together with an impaired calcium signalling, which may directly impact neuronal networks development and function. In line with the *Caprin*$^{+/-}$ mouse model, Microelectrode Arrays measurements of *CAPRIN1*$^{+/-}$ neuronal activity showed lower spike rates and bursts, with an overall reduced activity. Additional transcriptome analysis of immature and mature neurons is ongoing. Interestingly, a *CAPRIN1* distinguishing episignature was identified in DNA from the patients.

Conclusion: Functional impairment of *CAPRIN1*$^{+/-}$ hiPSCs-derived neurons showcases that *CAPRIN1* haploinsufficiency causes a novel autosomal dominant neurodevelopmental disorder in patients where an identifiable episignature is also established.

References:

Grants:

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C28.3 De novo variants in the PABP-domain of PABPC1 lead to developmental delay

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Background/Objectives: To investigate the role of *PABPC1* in developmental delay (DD)

Methods: Children were examined by Geneticists and Pediatricians. Variants were identified using exome sequencing and standard downstream bioinformatics pipelines. We performed in silico molecular modelling and co-immunoprecipitation to test if the variants impact the interaction between PABPC1 and PAIP2. We performed in utero electroporation of mouse embryo brains to enlighten the function of PABPC1.

Results: We describe four probands with an overlapping phenotype of developmental delay, expressive speech delay and autistic features and heterozygous *de novo* variants that cluster in the PABP domain of PABPC1. Further symptoms are seizures and behavioral disorders. Molecular modeling predicted that the variants are pathogenic and would lead to decreased binding affinity to mRNA metabolism-related proteins, such as PAIP2. Co-immunoprecipitation confirmed this as it demonstrated a significant weakening of the interaction between mutant PABPC1 and PAIP2. Electroporation of mouse embryo brains showed that *Pabpc1* knockdown decreases the proliferation of neural progenitor cells. The wild type Pabpc1 could rescue this disturbance, while three of the four variants did not.

Conclusion: Pathogenic variants in the PABP-domain lead to DD, possibly due to interfering with the translation initiation and subsequently an impaired neurogenesis in cortical development.

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C28.4 CLEC16A mislocalization and impaired interactions with retromer complex components underly a severe recessive neurodevelopmental disorder

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Background/Objectives: *CLEC16A* is a C-type lectin (CLEC) transmembrane protein that recognizes and guides antigens to the cell surface and has been localized to the late endosomes of antigen presenting cells. CLEC16A functions as E3 ubiquitin ligase which prevents autophagy and promotes mitophagy. GWAS studies have associated CLEC16A SNVs to autoimmune disorders like multiple sclerosis and type-1 diabetes. However its role in physiological development is unexplored.

Methods: We identified bi-allelic loss-of-function variants in *CLEC16A*, in siblings from unrelated families, with a severe neurodevelopmental disorder, progressive microcephaly, brain atrophy, corpus callosum dysgenesis, growth delay, hypotonia and early demise. We studied the cellular CLEC16A properties in vitro and in zebrafish embryos.

Results: Exogenous expression in HEK293T cells shows that CLEC16A prominently localizes to early endosomes, while the protein bearing a human C-terminal deletion loses it physiological localization. Proteomics of the CLEC16A interactome shows binding to the retromer heterotrimer components VPS35, VPS26, and to TRIM27, an interaction which is partially lost for the C-terminal truncated protein. Targeted knock-down of *Clec16a* by CRISPR-Cas9 in zebrafish embryos resulted in the accumulation of acidic/autophagolysosome compartments and abnormal staining of mitochondria, both in neuronal and microglial lineages, a phenotype rescued with WT but not with mutant mRNA.

Conclusion: This study reveals a constitutional function for CLEC16A during human brain development. Retromer is a crucial component of the endosomal network, mediates retrograde transport to the trans-Golgi network and plasma membrane, and regulates autophagy and mitophagy. The interaction between CLEC16A and retromer shows the importance of (retromer dependent) endosomal trafficking during brain development.

References:

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C28.5 Variants in the zinc transporter TMEM163 cause a hypomyelinating leukodystrophy

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Background/Objectives: Hypomyelinating leukodystrophies (HLDs) comprise a subclass of genetic disorders with deficient myelination of the central nervous system (CNS) white matter. Genomic testing has significantly improved the diagnosis and discovery of these disorders which are predominantly monogenic in nature.

Methods: Clinical evaluation followed by genomic testing was performed in five individuals from four families. Families 1, 2 and 4 underwent trio exome sequencing (ES) while family 3 underwent singleton ES. The effect of the variants on zinc efflux was determined in vitro using HEK293 cells electroporated with patient-specific mCherry-TMEM163 plasmids. Further, effect of the variants on oligodendrocytes was evaluated with respect to expression of key myelin genes, cell morphology and viability using oli-neu cells electroporated with patient-specific mCherry-TMEM163 plasmids.

Results: The clinical presentation of the subjects resembled Pelizaeus-Merzbacher disease with congenital nystagmus, hypotonia, delayed global development and neuroimaging findings suggestive of significant and diffuse hypomyelination. Genomic testing identified three distinct heterozygous missense variants in TMEM163. TMEM163 is highly expressed in the CNS, particularly in newly myelinating oligodendrocytes and was recently revealed to function as a zinc efflux transporter. Functional in vitro analysis of the mutant protein demonstrated significant impairment in the ability to efflux zinc out of the cell. Expression of the mutant proteins in an oligodendroglial cell line resulted in substantially reduced expression of key myelin genes, reduced branching and increased cell death.

Conclusion: Heterozygous variants in TMEM163 cause a HLD and uncover a novel role for zinc homeostasis in oligodendrocyte development and myelin formation.

References:

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C28.6 Recessive PRDM13 mutations cause severe brainstem dysfunction with perinatal lethality, cerebellar hypoplasia and disrupt Purkinje cell differentiation

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Background/Objectives: Pontocerebellar hypoplasias (PCH) are congenital disorders characterized by hypoplasia or early atrophy of the cerebellum and brainstem, leading to a very limited motor and cognitive development. Although over 20 genes have been shown to be mutated in PCH, a large proportion of patients remains undiagnosed.

Methods: We describe four families with children presenting with severe neonatal brainstem dysfunction, and pronounced deficits in cognitive and motor development, associated with four different bi-allelic mutations in *PRDM13*, including homozygous truncating variants in the most severely affected cases.

Results: Patients MRI and foetopathological examination revealed a PCH-like phenotype, associated with major hypoplasia of inferior olive nuclei and dysplasia of the dentate nucleus. Notably, histopathological examinations highlighted a sparse and disorganized Purkinje cell layer in the cerebellum. *PRDM13* encodes a transcriptional repressor known to be critical for neuronal subtypes specification in the mouse retina and spinal cord, but had not been implicated, so far, in hindbrain development. snRNAseq data mining and in situ hybridization in human, show that *PRDM13* is expressed at early stages in the progenitors of the cerebellar ventricular zone, which gives rise to cerebellar GABAergic neurons, including Purkinje cells. We also show that loss-of-function of *prdm13* in zebrafish leads to a reduction in Purkinje cells numbers and a complete absence of the inferior olive nuclei.

Conclusion: Altogether our data identified biallelic mutations in *PRDM13* as causing a new olivopontocerebellar hypoplasia syndrome, and suggest that early deregulations of the transcriptional control of neuronal fate specification could contribute to a significant number of cases.

References:

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C29 EYE GENETICS

C29.1 The contribution of common regulatory and protein-coding TYR variants in the genetic architecture of albinism

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Background/Objectives: There is abundant evidence supporting the view that rare genetic diseases are caused by rare, high-impact variants in individual genes. However, for most rare disorders, it is not possible to identify such pathogenic changes in every affected individual, leaving significant diagnostic/knowledge gaps. Here, we used two large-scale cohorts and performed case-control analyses aiming to elucidate the contribution of common variants in the genetic architecture of an archetypal rare disorder, albinism.

Methods: We studied a cohort of 1,313 individuals with albinism (most identified through the database of the University Hospital of Bordeaux Genetics Laboratory). A "control" cohort of 29,497 unrelated individuals from the Genomics England 100,000 Genomes Project dataset was also analysed. We focused on *TYR*, the gene encoding tyrosinase, and used computational variant prediction tools and regression analysis to investigate the contribution of common regulatory and protein-coding variants.

Results: Computational analysis identified three functionally-relevant *TYR* variants of interest: c.-301C>T; c.575C>A (p.Ser192-Tyr); and c.1205G>A (p.Arg402Gln). Case-control analysis revealed that homozygosity for two haplotypes that involve these three common variants, confers a high risk of albinism (OR>77 for c.[-301C;575A;1205A] and OR>19 for c.[-301C;575C;1205A]). Taking these findings into consideration increased the diagnostic yield of genetic testing in an albinism cohort from 57% to 76%.

Conclusion: Our observations have important implications for the design of genetic laboratory pipelines for albinism. These results are likely to be relevant to the understanding of other rare disorders, and locus-wide, haplotype-based approaches are expected to narrow the diagnostic gap for significant numbers of affected individuals

References:

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C29.2 Identification and characterization of a novel retina-specific lncRNA upstream ABCA4 with a potential role in ABCA4-associated inherited retinal disease

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Background/Objectives: Inherited retinal diseases (IRDs) are a major cause of blindness. While mutations in coding regions account for 60% of IRDs, non-coding variants can explain missing heritability. A major knowledge-gap lies in the role of long non-coding RNAs (lncRNAs), highly tissue-specific molecules that regulate gene

expression. Here we identified a novel retina-specific lncRNA upstream *ABCA4*, the gene implicated in Stargardt disease.

Methods: Expression specificity was determined by re-analysis of short-read (GTEx, adult human retina, retinal organoids) and single-cell RNAseq data. Nanopore long-read sequencing and single-molecule RNA in situ hybridisation (RNAScope/BaseScope) were performed on adult human retina. Chromatin interaction profiles were generated to evaluate interaction with the *ABCA4* promoter. Genomic variation was evaluated in smMIPs data of the *ABCA4* locus in 1,054 Stargardt cases.

Results: Analysis of ~7,400 transcriptomes of 54 tissues and 152 transcriptomes of adult human retinas revealed a novel retina-specific lncRNA upstream *ABCA4*. Long-read sequencing identified two isoforms with expression demonstrated in the outer nuclear layer. The lncRNA is transcribed from an enhancer interacting with the *ABCA4* promoter, suggesting a *cis*-acting effect. We identified 4 heterozygous novel copy number variants overlapping the lncRNA, representing potential pathogenic/modifying alleles. Perturbation studies in human retinal explants are ongoing to further elucidate its function.

Conclusion: We identified and characterized a novel retina-specific lncRNA, potentially implicated in *ABCA4*-retinopathy. This study provides novel insight into the role of this lncRNA - an unexplored class of molecules in the retina field - in gene regulation and IRD pathogenesis, which may entail therapeutic perspectives.

References:

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Conflict of Interest: None declared.

C29.3 Whole genome sequencing for USH2A-associated disease reveals several treatable pathogenic deep-intronic variants

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Background/Objectives: A significant portion of Usher syndrome type 2 (USH2) and autosomal recessive retinitis pigmentosa (arRP) cases remain genetically unsolved because only one, or no causative variants are detected in the *USH2A* exons. We assessed USH2 and arRP cases with a mono-allelic *USH2A* variant using whole genome sequencing (WGS) and developed an antisense oligonucleotide (AON)-based therapeutic strategy for a subset of the identified variants.

Methods: One-hundred cases were screened using WGS to detect missed *USH2A* exonic, deep-intronic, structural, and regulatory variants, and variants in other genes associated with arRP and USH2. Minigene splice assays were performed for variants with a predicted effect on splicing. For variants that were confirmed to cause pseudoexon inclusion, we evaluated the effect of AONs in a minigene splice assay and in patient-derived photoreceptor-precursor cells.

Results: Fifty-one cases were (likely) solved as we identified novel splice variants, structural variants or bi-allelic variants in arRP or USH2-associated genes. Twelve *USH2A* variants showed a deleterious effect in the minigene splice assays. Four of these variants resulted in the inclusion of a pseudoexon, which was reversed upon delivery of AONs.

Conclusion: Through our study, 51/100 cases received a (likely) completed genetic diagnosis. This highlights that WGS is a powerful approach to genetically explain cases that remain mono-allelic after standard screening of *USH2A* exons and flanking intronic sequences. We identified four novel deep-intronic *USH2A* variants and we confirmed that AONs are a valuable tool to correct splicing defects.

References:

Grants: Velux Stiftung

Conflict of Interest: None declared.

C29.4 Patient-derived Retinal Organoid as a Disease Model for Adult-Onset Retinitis Pigmentosa 1

Patricia Berber[1], *Andrea Milenkovic*[1], *Christina Kiel*[1], *Lisa Michaelis*[1], *Tobias Strunz*[1], *Caroline Brandl*[2], *Bernhard Weber*[1;3]

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Background/Objectives: Retinal organoids (ROs) are three-dimensional cell cultures derived from human induced pluripotent stem cells, which are unique to study inherited retinal dystrophies such as retinitis pigmentosa (RP). Recent data suggest that in many aspects ROs closely resemble the fetal retina making the modelling of adolescent- or adult-onset retinopathies challenging.

Methods: ROs were differentiated from three healthy donors and one adult-onset RP patient harboring an autosomal dominant mutation in the Retinitis Pigmentosa 1 gene (adRP1, OMIM #603937). ROs were analyzed after 0.5, 1, 1.5 and 2 years in culture via immunocytochemistry ($n = 34$), RNA-sequencing ($n = 47$) and methylation analysis ($n = 38$).

Results: ROs from each timepoint revealed a positive developmental state of a trilayered retinal structure, with rod and cone photoreceptors, horizontal, amacrine, ganglion and Mueller cells. Surprisingly, ganglion cell markers were detectable even after 2 years. In general, the expression of photoreceptor markers decreased over time, although individual photoreceptors showed improved retinoschisin 1 (RS1) expression at the inner segment membrane. In 1.5-year-old adRP1 ROs we found a lower expression of seven rod markers including rhodopsin (RHO, $p = 1.99$ x10-6) and neural retina leucine zipper (NRL, $p = 2.49$ x10-27) when compared to controls. The adRP1 ROs also tended to have fewer cone rod homeobox positive photoreceptors (CRX, $p = 0.053$).

Conclusion: Characterizing 1, 1.5 and 2-year-old ROs proved most insightful to establish their long-term development and to show their suitability to model adult-onset adRP1.

References:

Grants: Supported in part by a grant from the German Research Foundation to BHW (WE1259/32-1).

Conflict of Interest: None declared.

C29.5 Genetic deletion of Sarm1 confers functional protection in an in vivo mouse model of retinal ganglion cell degeneration

Laura Finnegan[1], *Naomi Chadderton*[1], *Paul Kenna*[2], *Arpad Palfi*[1], *Michael Carty*[3], *Andrew Bowie*[3], *Sophia Millington-Ward*[1], *G. Jane Farrar*[1]

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Background/Objectives: The optic nerve, comprised of axons of retinal ganglion cells (RGCs), delivers signal from the retina to the brain. Therapeutic strategies to limit axon degeneration are of potential value for optic neuropathies, where degeneration of RGCs and their axons underlies loss of visual function. Here we explore potential therapeutic benefit afforded by ablation of SARM1, a pro-degenerative NADase, in a mouse model of RGC degeneration.

Methods: *Sarm1+/+* and *Sarm1-/-* mice were injected intravitreally with rotenone, a complex I inhibitor. Optokinetic responses were measured 2 and 4 months after insult. Whole-mounted retinas were stained for RGC marker BRN3A. Optic nerves were cryosectioned and stained for NF200, enabling axon quantification.

Results: Rotenone-insulted *Sarm1-/-* mice had significantly higher optokinetic responses relative to *Sarm1+/+* controls at 2 and 4 months post-injection. This protection was observed in both males and females. *Sarm1-/-* retinas retained more RGCs than controls, with a trend towards more even distribution of remaining cells across the retina. Axons in *Sarm1-/-* optic nerves were protected from rotenone insult, with significantly higher axon numbers compared to controls.

Conclusion: SARM1 ablation protected against rotenone-induced degeneration of RGCs and optic nerves. This was accompanied by functional benefit, with preservation of spatial vision in rotenone-insulted knockout mice. Importantly, this protection was sustained over time, suggesting that *Sarm1* ablation may provide long-lasting protection against optic nerve degeneration.

References:

Grants: Irish Research Council (GOIPG/2017/1631), Science Foundation Ireland (16/IA/4452; 16/IA/4376), Health Research Board of Ireland (HRAPOR-2015-1140), Fighting Blindness Ireland-Health Research Board of Ireland-Health Research Charities Ireland (MRCG-2012-4 and MRCG-2016-14).

Conflict of Interest: None declared.

C29.6 Implication of a non-coding variation of FOXE3 in an individual displaying a complex microphthalmia

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Background/Objectives: *FOXE3* encodes a transcription factor which expression is extremely conserved throughout species that use vision. It plays a role mainly in the development of the lens. Biallelic truncating mutations in this gene have been reported to cause complex microphthalmia with lens, anterior segment and retinal anomalies. We report the identification of a heterozygous *FOXE3* nonsense mutation in an individual displaying a complex microphthalmia with cataract, consistent with the involvement of this transcription factor. Familial segregation analysis revealed transmission of the change by her asymptomatic father. Further screening of the *FOXE3* regulatory sequences in search of a second disease allele, identified a previously unreported sequence variation in a highly conserved region known to regulate *Foxe3* expression in mouse. This variation was inherited from the asymptomatic mother, thus fitting with a recessive inheritance.

Methods: Functional studies, including Luciferase assay, combined DNA pull-down assay and mass spectrometry and gene silencing using siRNA studies were consistent with a functional effect of the variant. We generated mouse lines carrying the non-coding variant and a nonsense mutation in homozygosity (KI/KI and KO/KO, respectively), we mated to generate compound heterozygous animals (KI/KO).

Results: Analysis showed a spectrum of eye growth, cornea, anterior segment and lens abnormalities of increasing severity in KI/KI, KI/KO and KO/KO mice, respectively. These anomalies, absent in wildtype and single heterozygous counterparts are reminiscent of human phenotypes.

Conclusion: This work expands the mutational landscape of *FOXE3*-associated ocular developmental defects and underline the importance to search for variants in *CIS* regulatory elements in unsolved cases.

References:

Grants: Retina France 2019

Conflict of Interest: None declare.

CONCURRENT SYMPOSIAS01 REPRODUCTIVE CARRIER SCREENING IN 2022

S01.3 Reproductive carrier screening in the Netherlands

Lidewij Henneman

In the Netherlands, carrier screening for the general population is not implemented in national healthcare, besides local initiatives. Moreover there are no private providers offering carrier testing. Yet, there has been a long history of research studies on the feasibility and desirability of preconception carrier screening, such as pilot studies for cystic fibrosis, hemoglobinopathies and expanded screening panels. Research includes ethical aspects and the perspectives of stakeholders to assess acceptability..

A national working group on preconception carrier screening with affiliates from all clinical genetics centers aims to align the screening offers. In 2020, the guideline "Preconception Carrier Testing for High-Risk Populations" was launched as a first step, although national implementation is still challenging. In 2022, the Minister of Health informed the House of Representatives on the societal support for population-based preconception carrier screening, requesting advice from the Health Council of the Netherlands on next steps based on current evidence.

Conflict of Interest: None declared.

S02 METHODS FOR ADMIXED POPULATIONS

S02.1 Ancestry deconvolution and polygenic scores

Luca Pagani

Efforts to represent the full spectrum of human genetic diversity in GWAS studies are pivotal for a comprehensive understanding of the biology of complex traits. As progresses are being made at a steady pace towards that extent, the path to apply genetic risk scores onto individuals of genetically mixed origin is instead yet to be fully traced. Here I will review recent approaches that leverage on the unique ancestry tiling of recently admixed individuals to produce ancestry specific polygenic risk scores, and highlight further challenges and opportunities that the focus on mixed individuals may offer to the field..

Conflict of Interest: None declared.

S02.2 Methods of including admixed individuals in association studies

Elizabeth Atkinson

Genetic studies offer a promising basis for understanding pathophysiology and identifying new molecular targets for medicine. Recent landmark papers have made major strides in our understanding of the genetic architecture of complex disorders, which may lead to characterization of actionable drug targets. However, these studies are based overwhelmingly on subjects of European ancestry, and their results may not fully generalize to other populations. Due to the paucity of methodological and computational approaches that account for their additional genomic complexity, admixed populations are systematically excluded from statistical genomic studies. Admixed populations, including African American and Latinx individuals, make up more than a third of the US populace, yet these groups face severe disparities in health research and treatment due to being so underrepresented. To reap full and equitable benefits from existing mixed ancestry cohorts and ongoing large-scale data collection efforts, there is an urgent unmet need for the development of tools facilitating the well-calibrated study of complex psychiatric traits in admixed peoples alongside the recruitment of more diverse study participants.

We recently released a novel local-ancestry aware GWAS model, *Tractor*, which corrects for fine-scale population structure at the genotype level, better localizes GWAS signal, identifies ancestry-specific loci that would have been missed with standard procedures, and produces ancestry-specific effect size estimates and *p* values. Building off of this work, we are developing new computational and statistical strategies for ancestry informed gene discovery and polygenic risk prediction that will improve the clinical interpretability of GWAS findings. Here, we describe our new methods related to ancestry-informed gene discovery, including a novel statistical method for rare variant association studies in admixed cohorts, the creation of ancestry-specific polygenic risk scores (PRS) that are more accurate in their prediction for diverse populations, and a strategy leveraging the naturally varying haplotype structure in admixed cohorts to better understand gene-gene interactions at clinically meaningful loci. We benchmark these new computational tools using the large and diverse whole genome sequences in the All of Us Project Dataset.

Ancestry informed GWAS for admixed populations will aid in the identification of new risk loci through improved effect estimation precision, discovery power, and fine-mapping, leading to a more complete representation of genetic risk for complex psychiatric traits across the allele frequency spectrum. This work fills a gap in existing resources and will improve our understanding of complex diseases across understudied admixed populations. While the inclusion of diverse participants in gene discovery efforts directly improves understanding for these individuals, diverse cohorts also offer the opportunity to expand and accelerate gene discovery findings relevant for individuals of all ancestries.

Conflict of Interest: None declared.

S03 PERSONALIZED CARE IN BREAST CANCER PATIENTS

S03.2 Personalising breast cancer risk prediction for prevention and early detection

Antonis C. Antoniou

Personalising breast cancer risk prediction for prevention and early detection

Antonis C. Antoniou

Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, U.K..

Much more reliable and powerful breast cancer risk prediction be achieved by combining data on all genetic, lifestyle and hormonal risk factors for the disease. We have recently enabled multifactorial breast cancer risk-assessment through the CanRisk tool (www.canrisk.org) which allows healthcare professionals to obtain personalised cancer risks easily. The presentation will review the CanRisk development process, the challenges in combining the effects of rare pathogenic variants in known susceptibility genes, polygenic risk scores, questionnaire-based risk factors, mammographic density and family history into multifactorial cancer risk prediction algorithms; and will review the efforts to assess the clinical validity of the predicted risks in large independent studies. The presentation will finally discuss ongoing efforts for the implementation of multifactorial cancer risk assessment in routine clinical practice for enabling cancer risk stratification and the better targeting of early detection and prevention approaches to those most likely to benefit.

Conflict of Interest: None declared.

S04 HOW DO WE DELIVER GENOMICS EDUCATION FOR ALL?

S04.2 Ensuring best practice in genomics education and evaluation

Clara Gaff

Quality, evidence-based education on a large scale is required to build a healthcare workforce that can practice genomic medicine competently. Australian provided an opportunity to support best practice education and evaluation as part of a national translational research program in genomic medicine. We mapped existing genomics education activity, finding that education was primarily developed and delivered by genetics experts without formal education qualifications or experience. Few programs were informed by the needs of the target audiences, sufficiently funded or evaluated to determine their effectiveness.

We therefore developed a suite of tools to support genomics education providers to plan, develop, deliver, evaluate and consistently report effective education. Each tool was informed by the literature and developed using consensus methodologies with an international panel of experts. The tools are designed to

be easily adopted by genetic/genomic experts and help those who are not familiar with education or evaluation theory. Several have already been endorsed or adopted by organisations internationally.

The tools include: a program logic model and template; surveys to assess health professionals' current genomic practice, attitudes and educational needs; an evaluation framework to enable more rigorous evaluation with clear outcomes and measures; and reporting standards to provide consistent descriptions of education programs and their evaluation to build an evidence base for effective genomics education. These tools will be described, using contemporary examples of their application to underpin high-quality genomics education with evidence and translate research into practice.

Conflict of Interest: None declared.

S05 CHROMOTHRIPSIS AND COMPLEX REARRANGEMENTS

S05.1 Chromothripsis mechanisms along the landscape of diverse constitutional chromosome rearrangements

Maria Clara Bonaglia

By whole-genome approach, including whole-genome sequencing and genotyping of trios, we found that many of the *de novo* unbalanced translocations (37 cases) and de novo non-recurrent small supernumerary marker chromosomes (sSMC, 12 cases) were the final product of two-step mechanism triggered by chromosome non-disjunction at maternal meiosis followed by postzygotic anaphase lagging of the supernumerary chromosome present in a trisomic zygote.

This trisomic zygote can be modified by a chromothripsis event that removes the supernumerary chromosome either totally or partially. In the latter case, three types of de novo unbalanced structural abnormalities can be formed: (i) unbalanced translocations where at least the telomeric region of the supernumerary chromosome stick to the distal portion of another chromosome (Bonaglia et al. 2018 https://doi.org/10.1007/s00439-018-1941-9), (ii) insertional translocations where non-telomeric and sometimes non-contiguous portions of the supernumerary chromosome are inserted inside another chromosome (Kato et al. 2017 Cytogenet Genome Res https://doi.org/10.1159/00048 1586), and (iii) small supernumerary marker chromosomes which are most frequently derived by interstitial non-contiguous portions of the supernumerary chromothripsed chromosome (Kurtas et al. 2019, https://doi.org/10.1002/humu.23683).

Among the 37 cases of de novo unbalanced translocations, we detected that the duplicated translocated portion was of maternal origin in more than half of the de novo unbalanced translocations, with 25% of them showing two maternal and one paternal haplotypes. Thus, non-disjunction at maternal meiosis I, followed by trisomy rescue of at least the distal region of the supernumerary maternal chromosome through chromotripsis-mediated event, underlies a major fraction of *de novo* unbalanced translocations. An increased maternal age documented in all cases not only supports this but also an underlying trisomy rescue following a maternal meiotic-II non-disjunction in cases in which the maternal duplication has the same haplotype (Bonaglia et al, 2018). A similar two-step mechanism might also account for at least some unbalanced insertional translocations in which two different maternal alleles together with the paternal one had been detected within the duplicated region (Kato et al, 2017). The parental origin of sSMC was paternal in half of the cases whereas the two normal homologs were in maternal hetero/isodisomy, directly demonstrating the initial event of maternal meiosis non-disjunction. In the remaining half, the sSMC was of maternal origin with the two corresponding homologous chromosomes being of biparental origin. In all cases, maternal age was increased (Kurtas et al, 2019).

According to our data, this two-step mechanism underlying the formation of most non-recurrent de novo structural anomalies identifies a link between numerical and structural chromosomal anomalies and highlights how frequently some unbalanced de novo translocations, insertions, and non-recurrent sSMCs may be the final result of a mechanism initiated by a trisomy, passing through the elimination of the supernumerary chromosome by anaphase lagging and subsequent chromothripsis. In these cases, the abnormal phenotype is due not only to abnormal dosage of deleted and duplicated dosage-sensitive genes but also to topologically associating domains breakage, and fusion-genes formation (consequent to chromothripsis). Furthermore, in unbalanced rearrangements originating from partial trisomy rescue, the following hetero/isodisomy for the remaining two chromosomes might generate further pathogenicity. The final and important implication of our data is that there is no risk of recurrence in the following pregnancies for any of the *de novo* unbalanced abnormalities discussed here.

Conflict of Interest: None declare.

S05.2 Identification of complex genomic rearrangements structures in disease

Claudia Carvalho

Complex genomic rearrangements (CGRs) are defined as structural variants (SVs) harboring more than one breakpoint junction and/or structures made up of more than one SV in cis. While CGRs containing ≥ 5 breakpoints, i.e. chromoanagenesis events, are extremely rare in congenital disorders, CGRs containing 3 to 4 breakpoints and forming at least 2 breakpoint junctions, are important contributors to genetic diseases. Neurodevelopmental disorders primarily caused by SVs may consist of 20% to 30% of CGRs, many of which have a recurrent pattern generated *de novo* in probands that contribute to change severity of the clinical phenotype. To investigate the genomic architecture of CGRs, parental origin and contribution to clinical variability in disease, we are studying a large cohort of patients with a copy-number gain spanning Xq28 varying from 64 kb to 16 Mb, N = 115. Whole genome sequencing including Illumina short-reads, PacBio HiFi, ONT as well as Bionano optical genome mapping have been applied to investigate probands and parental genomes. Combined pipeline analysis revealed CGRs in 32% of the cohort, a majority constituted by inverted triplications (54%) and terminal duplications (27%). Remarkably, large inversions (few kbs to Mb) are formed concomitantly with the copy number gain in almost all CGRs, 70% of them generated by X chromosome inverted repeat pairs sharing > 99% of nucleotide identity. These data indicate that DNA repair mechanisms using ectopic homology are responsible for formation of majority of CGRs affecting the Xq28 region. In summary, SV-mediated genomic disorder provides a unique opportunity to identify CGRs, investigate their genomic architecture, mechanism of origin and disease contribution.

Conflict of Interest: None declared.

S06 DISORDERS OF LYSOSOMAL BIOGENESIS AND AUTOPHAGY

S06.1 Disorders of the HOPS complex

Fredrik Sterky

The recent year has seen a number of reports on disease-causing variants linked to the homotypic fusion and vacuole protein

sorting (HOPS) complex – a multisubunit tethering complex that mediate fusion between lysosomal or late endosomal vesicles. Fusion of early endosomes is instead orchestrated by the related class C core vacuole/endosome tethering (CORVET) complex. Both complexes share core subunits VPS11, VPS16, VPS18 and VPS33A, while HOPS also contains VPS39 and VPS41, and CORVET VPS3 and VPS8. Disorders of HOPS/CORVET complexes show distinct but overlapping features resembling other intracellular trafficking disorders as well as lysosomal storage disorders such as the mucopolysaccharidosis (MPS).

We identified a homozygous intronic variant in the gene encoding *VPS16* in two independent patients that presented with progressive psychomotor regression, leukoencephalopathy, neutropenia, coarse facial features and dysostosis multiplex. The variant impaired normal splicing of the transcript and caused an ~85% reduction of VPS16 protein levels, assessed in patient-derived fibroblasts. Other HOPS/CORVET subunits were similarly reduced, but restored upon re-expression of VPS16. Patient-derived cells displayed accumulations of autophagosomes and lysosomal compartments, and defects in endosomal transferrin trafficking. A Zebrafish model showed similar accumulation of lysosomes and autophagosomes in the brain, and reduced myelination.

Our findings demonstrate that recessive variants in *VPS16* lead to a multisystemic disorder by causing a quantitative loss of HOPS/CORVET complexes below a certain threshold. This disorder resembles the previously described "MPS plus" syndrome caused by a missense variant in *VPS33A*, the direct binding partner of VPS16. How these findings relate to recent reports linking heterozygous variants in *VPS16* to primary dystonia will be discussed.

Conflict of Interest: None declared.

S06.3 mTORC1 hyperactivity in Birt-Hogg-Dubé syndrome

Chiara Di Malta

The Birt-Hogg-Dube' (BHD) syndrome is a hamartoma syndrome associated with increased risk of developing kidney cancer and is due to loss of function germline mutations in the gene encoding folliculin (FLCN). The mTOR complex 1 (mTORC1) signaling pathway is a key regulator of cell proliferation and growth and is activated at the lysosome thanks to the RagGTPases (Rags), heterodimers consisting of RagA/B bound to RagC/D. Cell biology studies identified FLCN as a positive regulator of mTORC1 signalling, by serving as a GTPase activating protein (GAP) for RagC/D. However, clinical evidence show that renal tumors associated with BHD syndrome display a paradoxical hyper-activation of mTORC1 signaling, which in turn fuels tumor cell proliferation and growth. The paradoxical hyperactivation of mTORC1 signaling under FLCN deficiency has been understood only recently. Transcription factors (TFs) belonging to the MiT/TFE family, in particular TFEB and TFE3, are known oncogenes responsible for translocation Renal Cell Carcinoma (tRCC), an aggressive type of tumour that accounts for about 20% of all pediatric cases of renal cell carcinoma. These TFs regulate the same target genes and their activity is inhibited by the kinase complex mTORC1, which phosphorylates them at conserved serine residues preventing their nuclear translocation. Our studies demonstrated the existence of a feedback loop in which TFEB and TFE3 in turn regulate mTORC1 activity by inducing the expression of RagC/D. We found that TFEB and TFE3 are constitutively nuclear and active in cellular and murine models of BHD syndrome, and this promotes mTORC1 hyper-activation, which fuels kidney cystogenesis and tumorigenesis associated with BHD syndrome. Strikingly, genetic depletion of TFEB corrects mTORC1 hyper-activation, completely rescues kidney cystogenesis, and restores normal kidney function and the lifespan of kidney specific Flcn KO mice. In addition, our latest data demonstrate that depletion of TFEB, or the one of TFE3, completely inhibits the growth of a BHD-patient-derived tumor cell line in vivo, thus suggesting that both TFs are key drivers of tumour growth in BHD syndrome. Our findings demonstrate the relevance of constitutive activation of TFEB and TFE3 in the growth of kidney tumors associated with BHD syndrome and encourage future studies exploiting TFEB/TFE3 inhibitors as a therapy for these tumors.

Conflict of Interest: None declared.

S07 CELL LINEAGES AND ORGANOIDS

S07.2 Modelling human blastocysts by reprogramming fibroblasts

Jose Polo

In 2007 Shinya Yamanaka demonstrated that human fibroblasts can be reverted back to a pluripotent state by the forced expression of four transcription factors; OCT4, SOX2, KLF4 and cMYC (OSKM). These so called induced pluripotent stem cells (iPSCs), like embryonic stem cells (ESCs) derived from the epiblast of blastocysts, can give rise to any cell types of the body. Furthermore, iPSCs carry the promise of personalized regenerative medicine and hold tremendous potential for applications such as cell replacements therapeutics, disease modelling and in vitro drug screening. However, the molecular mechanisms of these cellular transitions into primed or naive human-induced pluripotency remained poorly understood. To address this, we reconstructed the molecular reprogramming trajectories using single cell transcriptomics. This revealed that reprogramming into primed and naive human pluripotency follows diverging and distinct trajectories into the pluripotent states. The integration of regulatory element usage with transcriptomics unveiled an unexpected role of trophectoderm (TE) lineage-associated transcription factors as well as a subpopulation of cells that transiently upregulated a TE-like signature during reprogramming. We demonstrated that this TE state could be stabilised by changing the culture condition, allowing the derivation of induced Trophoblast Stem Cells (iTSCs). Further inspection of this cell cultures revealed also the upregulation of a primitive endoderm like signature in some of the cells. Unexpectedly, when all these cells are allowed to contact each other in a 3D culture, they self-organised giving rise to blastocyst-like structures which we have called iBlastoids. iBlastoids are capable of modelling in vitro, many molecular, morphological and functional aspects of the blastocyst as well as stage of implantation

Conflict of Interest: None declared.

S09 MUTATION SIGNATURES

S09.2 Mutational signatures in cancer

Fran Supek

Genomic analyses have revealed mutational footprints associated with DNA maintenance gone awry, or with mutagen exposures. Because cancer therapeutics often target DNA synthesis or repair, we asked if mutational signatures make useful markers of drug sensitivity. We detect mutational signatures in cancer cell line exomes (where matched healthy tissues are not available) by adjusting for the confounding germline mutation spectra across ancestries. We identify robust associations between various mutational signatures and drug activity across cancer cell lines;

these are as numerous as associations with established genetic markers such as driver gene alterations. Signatures of prior exposures to DNA damaging agents – including chemotherapy – tend to associate with drug resistance, while signatures of deficiencies in DNA repair tend to predict sensitivity towards particular therapeutics. Replication analyses across independent drug and CRISPR genetic screening data sets reveal hundreds of robust associations, which are provided as a resource for drug repurposing guided by mutational signature markers. (see Levatić et al., 2022, Nature Communications [in press])

Somatic mutations are an inevitable component of ageing and the most important cause of cancer. The rates and types of somatic mutation vary across individuals, but relatively few inherited influences on mutation processes are known. We performed a gene-based rare variant association study with diverse mutational processes, using human cancer genomes from over 11,000 individuals of European ancestry. By combining burden and variance tests, we identify 207 associations involving 15 somatic mutational phenotypes and 42 genes that replicated in an independent data set at a FDR of 1%. We associate rare inherited deleterious variants in genes such as MSH3, EXO1, SETD2, and MTOR with two phenotypically different forms of DNA mismatch repair deficiency, and variants in genes such as EXO1, PAXIP1, RIF1, and WRN with deficiency in homologous recombination repair. In addition, we identify associations with other mutational processes, such as APEX1 with APOBEC-signature mutagenesis. Many of the genes interact with each other and with known mutator genes within cellular sub-networks. Considered collectively, damaging variants in the identified genes are prevalent in the population. We suggest that rare germline variation in diverse genes commonly impacts mutational processes in somatic cells. (see Vali-Pour et al., 2022, Nature Communications [in press])

Conflict of Interest: None declared.

S10 LEFT/RIGHT PATTERNING/HETEROTAXY

S10.2 Myosin1 proteins as evolutionarily conserved regulators of animal Left-Right asymmetry

Maximilian Fürthauer

Myosin1 proteins as evolutionarily conserved regulators of animal Left-Right asymmetry

Thomas Juan, Akshai Janardhana Kurup, Charles Géminard, Jean-Baptiste Coutelis, Delphine Cerezo, Sophie Polès, Stéphane Noselli & Maximilian Fürthauer.

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The genetic pathways governing antero-posterior and dorso-ventral patterning have been extensively conserved throughout evolution. In contrast, the mechanisms controlling Left-Right (LR) asymmetry in different phyla appear strikingly different. While numerous vertebrates - including humans - use the beating of motile cilia to create directional symmetry-breaking fluid flows, birds or reptiles do not appear to harbor any motile cilia in their Left-Right Organizer (LRO). Even more strikingly, species such as the fruitfly *Drosophila* establish LR asymmetry in the complete absence of any cilia.

In an attempt to identify a unifying mechanism for the establishment of animal LR asymmetry, we are using the zebrafish as a vertebrate model organism to study the function of unconventional non-muscle type 1 Myosins, which have been identified as master regulators of *Drosophila* LR asymmetry. The zebrafish LRO is an epithelial vesicle that is decorated on its inside by motile cilia, the beating of which creates a symmetry-breaking fluid flow. Our work shows that while *Drosophila myosin1D (myo1D)* controls LR asymmetry in the complete absence of any cilia, its zebrafish homologue is required for the formation and function of a ciliated LRO. During the chiral morphogenesis of the *Drosophila* hindgut, Myo1D interacts with Planar Cell Polarity (PCP) proteins to promote chiral tissue torsion. We show that zebrafish *myo1d* interacts with the PCP gene *vangl2* to control LRO cilia orientation and thereby shape a productive symmetry-breaking fluid flow. Our work identifies *myo1d* as an evolutionarily conserved regulator of animal LR asymmetry. Importantly, studies from other labs have provided additional evidence that *myo1d* is also required for LR asymmetry in frogs (Tingler et al.) and potentially even humans (Alsafwani et al.)

In addition to Myo1D, the zebrafish genome encodes the closely related protein Myo1G as well as two Myo1C proteins that can act as Myo1D/G antagonists. By dissecting the function of different Myosin1 proteins in zebrafish development, we have obtained evidence that Myo1 proteins act not only to control motile cilia orientation, but also exert additional novel functions in LR asymmetry.

Conflict of Interest: None declared.

S11 PEOPLING THE WORLD: ONE ARCHIPELAGO AT A TIME

S11.1 Population history and biological adaptation in Oceania (virtual)

Lluis Quintana-murci

Pacific islanders primarily descend from two ancestral groups, who can be related to the out-of-Africa dispersal ~50,000 ya and the Austronesian expansion ~5,000 ya, the most recent human expansion into empty territories. However, the detailed genomic history of Oceanians remains largely uncharacterised. Using high-coverage genomes and genome-wide SNP data from different populations from Near and Remote Oceania, we find that the ancestors of Near Oceanians underwent a strong bottleneck before the settlement of the region, and separated ~20,000-40,000 years ago. Using Approximate Bayesian Computation, we show that the Austronesian expansion — thought to start from Taiwan ~5,000 years ago — was not followed by an immediate, single admixture event with Near Oceanians, but involved recurrent episodes of genetic interactions. Focusing on archaic introgression, we find that while the levels of Neanderthal ancestry are relatively homogeneous across populations (~2.2 to 2.9%), those of Denisovan ancestry vary markedly (0 to ~3.1%). Our analyses also reveal differences in the nature of the Denisovan heritage among Pacific groups, suggesting independent admixture events with highly-structured populations of archaic hominins. Furthermore, while Neanderthals facilitated modern human adaptation related to multiple phenotypes, Denisovan introgression was primarily beneficial for immune-related functions. We also report evidence of selective sweeps and polygenic adaptation associated with pathogen exposure and lipid metabolism in the Pacific. Finally, focusing on the Vanuatu archipelago, we observe fine-scale genetic structure that mirrors admixture, geographical barriers and sociocultural practices. For example, our analyses detect Polynesian ancestry arriving between 600 and 1,000 years ago to southern Vanuatu, and show that Polynesian ancestry is not restricted to islands where Polynesian languages are spoken today, suggesting an extensive network of cultural and genetic exchanges between Vanuatu islands. Collectively, our analyses provide new insight into the interactions between demographic and cultural factors in the Pacific, and increase our understanding of mechanisms of biological adaptation to island environments.

Conflict of Interest: None declared.

S12 THE GENETICS OF OMICS AND BEYOND

S12.3 Deciphering the genomic aetiology of osteoarthritis

Eleftheria Zeggini

Osteoarthritis is one of the leading causes of disability and pain worldwide, with over 300 million people affected. Currently no curative treatments are available. A detailed understanding of disease aetiopathology and novel drug targets are therefore urgently needed.

In this talk, I will give an overview of how we have used translational genomics approaches to enhance our understanding of the genetic aetiology of osteoarthritis, shed novel biological insights, and provide a stepping stone for translating genetic associations into osteoarthritis drug development.

Conflict of Interest: None declared.

S13 UNSTABLE HERITABLE GENOMIC VARIANTS AND CANCER DEVELOPMENT

S13.1 Triggers of recurrent genomic amplification

Hisashi Tanaka

The human genome contains hundreds of tandemly-duplicated segments (segmental duplications, low copy repeats) that are structurally diverse and are insufficiently represented in the reference genome. Structural diversity in the human population suggests that these segments may be intrinsically unstable in the germline; however, the mechanisms underlying the instability and the roles in disease etiology, such as carcinogenesis, remain critical areas of investigation. Tandemly-duplicated segments can be subject to intra-strand DNA base pairing and the formation of DNA secondary structures. DNA secondary structures impede the movement of DNA replication machinery, resulting in stalled replication forks and DNA breaks. Therefore, such segments can be fragile. To test the idea, we have used cytogenetic and genomic approaches to study the fragility and aberrantly structured DNA coming from tandemly duplicated segments in somatic and germline cells. We found that a 500 kb block of tandemly-duplicated segments on 17q12-21, called KRTAP_region_1, is fragile and recurrently demarcates the amplicon of the *ERBB2* (HER2) oncogene by triggering large inverted duplications. We also found that segmental duplications are a predominant source of extrachromosomal circular DNA (eccDNA) in human sperm. We propose that duplicated segments in human genomes are fragile and are major sources of aberrantly structured DNA, including inverted duplications and eccDNA.

Conflict of Interest: None declare.

S13.3 Germline predisposing duplications in myeloid neoplasms

Isabelle Plo

Isabelle Plo, Jean Pegliasco, Pierre Hirsch, Graciela Rabadan Moraes, Christophe Marzac, William Vainchenker, François Delhommeau, Jean-Baptiste Micol and Christine Bellanné-Chantelot.

Myeloid malignancies include three major groups of diseases: Acute Myeloid Leukemia (AML), Myelodysplasia (MDS) and Myeloproliferative Neoplasms (MPN). These clonal disorders arise from the transformation of hematopoietic stem cells (HSC), except AML that can also occur from transformation of more committed progenitors. They are rare mostly sporadic but rare familial forms of predisposition syndromes exist. While familial AML/MDS families are heterogeneous being related to 65 predisposing genes, the etiologies of familial MPN remain mostly unknown. We and others have identified three distinct germline copy number variations (CNVs) of the 14q32 chromosomal region in families with a broad spectrum of myeloid malignancies including MPN, MDS and AML. These CNVs consist of heterozygous duplications varying from 700 kb to 1.8 Mb and partially overlapping. The pedigrees linked to the 14q32 germline CNVs have in common autosomal dominant inheritance, a high penetrance of the predisposition locus, and an intra-familial phenotypic heterogeneity. Initially, we identified one of these germline CNVs, a 700-kb head-to-tail tandem duplication including 6 genes (TCL1A, ATG2B, GSKIP, BDKRB1, BDKRB2, AK7), in 2 large families originating from the French West Indies. Here we will deal with: i) the clinical and molecular description of these patients in order to characterize the natural history of hematological malignancies, ii) the function of the CNV on hematopoiesis.

In one hand, we analyzed 12 asymptomatic carriers and 52 patients from 6 families by targeted sequencing of 41 genes commonly mutated in myeloid malignancies. We found that 75% of healthy carriers displayed early clonal hematopoiesis mainly driven by TET2 mutations. Molecular landscapes of patients revealed two distinct routes of clonal expansion and leukemogenesis: i) one characterized by the clonal dominance of MPN-driver events associated with TET2 mutations in half of cases and mutations affecting splicing and/or the RAS pathway in one-third of cases, leading to the early development of MPN with a high risk of transformation (50% after 10 years), ii) The other one distinguished by a genomic landscape specific to post-MDS AML without MPN drivers.

On the other hand, we investigated the mechanism of this 14q32 CNV. Among the 6 genes, *ATG2B* and *GSKIP* were expressed in hematopoiesis and overexpressed in patients' cells. We have demonstrated using IPSC and primary cells of patients, that ATG2B and GSKIP, stimulates hematopoietic progenitor cells, including megakaryocyte progenitors by increasing their sensitivity to TPO. Moreover, these genes cooperate with signaling mutations and epigenetic mutations. We have also developed a knock in mouse model mimicking the CNV and have investigated the role of the predisposition on hematopoiesis. We have found that the CNV was able to cooperate with JAK2V617F at the level of HSC to enhance the MPN phenotype, suggesting that it changes the fitness of JAK2V617F HSC.

In conclusion, etiology of family studies has profound and general implications in understanding the predisposition to MPN, MDS and AML and has an immediate relevance to clinical prognosis and diagnosis.

Conflict of Interest: None declared.

S14 PREDICTIVE GENETIC COUNSELLING FOR NEURODEGENERATIVE CONDITIONS

S14.1 Overview of degenerative dementia genetics and predictive biomarkers

Paola Mandich

Purpose of the lecture: to give a practical approach to early diagnosis of most common young onset neurocognitive disorders.

The early diagnosis of inherited dementia has significant repercussions on the patient and his family and, sometimes, on therapy. Therefore, any effort has to be done to improve early diagnosis of these pathologies, still today hardly recognized at an early stage.

The lecture will be focused on those neurodegenerative disorders in which the cognitive deficit is the onset symptom or is the prevailing symptom (e.g. AD, FTD, small vessel diseases, prion diseases, HD and HD like).

MRI, which is often the first test to which the patient with cognitive impairment undergoes, and functional imaging sometimes allow specific diagnosis based on suggestive patterns, at other times they indicate which subsequent tests should be carried out (e.g. biomarkers or genetic testing).

The role of genetics has completely changed. The NGS approach is much more powerful and faster, less expensive and laborious. However, it requires careful and expert analysis, which must be integrated with clinical features and biomarkers.

The main diagnostic workflow will be discussed and the synergic role of advanced techniques of imaging and genetics, implemented with the use of available biomarkers, will be enhanced.

Conflict of Interest: None declare.

S15 GENETIC ARCHITECTURE OF THE HUMAN FACE

S15.1 From DNA to face: Genetics of human faces

Peter Claes

The human face develops according to a biological 'script' written in our genetic code and played out through complex biological processes. The human face is therefore a powerful window to the underlying genetic architecture and prior development of an individual. Facial dysmorphism, in clinical genetics indicates when something has gone wrong in development. Facial resemblance, in genetic epidemiology e.g., reflects shared genetics between individuals following kinship and the rules of inheritance. I.e., facial shape is largely influenced by genes and accurate characterization of facial shape, referred to as facial phenotyping, is important to study the role of genetic factors in determining individual health and disease in families and populations. It can provide insight into human evolutionary processes, facilitate surgical planning and outcome assessment, aid in forensic investigations and support variant interpretation and prioritization as part of syndrome diagnosis in clinical genetics among other things. Traditional, facial phenotyping using anthropometric measurements (e.g. linear distances or angles), generally measured between specific points or landmarks on a set of 2D or 3D facial images have recently been supplanted by more advanced phenotypic descriptions that can capture the complexity of the 3D facial shape. In a first instance, I provide an overview of data-driven facial phenotyping as input to genome-wide association scans on normal range facial variation. Broadly speaking successful and emerging strategies can be divided into supervised and unsupervised learning paradigms. In a second instance, I briefly discuss the current knowledge on the genetics of human faces today, successes obtained so far and challenges ahead going from DNA to face.

Conflict of Interest: None declare.

S15.3 Genetics and DNA prediction of human appearance with applications

Manfred Kayser

During the last 15 years, striking progress has been made in the genetic understanding of (non-pathogenic) human appearance, including facial traits, mostly through genome-wide association studies (GWASs). In contrast to earlier expectations (such as for eye color), we now know that all human appearance traits are polygenetic by nature, determined by many genes with large numbers of identified genes for the few appearance traits for which large-sized GWASs were done already (such as 124 for hair color). Genetic trait prediction is one way to express the practical relevance of GWAS-derived associated DNA variants. Moreover, if high-enough prediction accuracy is achieved, inferring (non-pathogenic) appearance information from genetic data can be applied in different areas of science and society. Although fairly accurate appearance prediction from DNA is available for some eye, hair, and skin color categories (such as for blue and brown eye color with AUC of 0.95), which therefore is already applied in practice, this is currently not possible for the face due to the relatively limited genetic knowledge available for the face to date. For some other appearance traits such as freckles, eyebrow color, head hair shape, male pattern hair loss, and extremely tall stature, first genetic prediction models were introduced, but do not achieve the high prediction accuracies known for some eye, hair, and skin color categories. In this talk, I will summarize the current knowledge on the genetic basis and genetic prediction of (non-pathogenic) human appearance traits. Furthermore, I will exemplify how genetic prediction of (non-pathogenic) appearance is practically applied to answer open questions in forensics, anthropology, and human history.

Conflict of Interest: None declared.

S18 THE IMPORTANCE OF SOMATIC VARIATION

S18.3 Clonal hematopoiesis and risks for cardiovascular and severe infectious diseases

Pradeep Natarajan

Analyses of blood DNA have uncovered the presence of somatic variants indicative of clonal hematopoiesis. With increasing age, an increasing proportion of asymptomatic individuals without hematologic malignancy have clonally-expanded somatic variants. Clonal hematopoiesis of indeterminate potential (CHIP) and mosaic chromosomal alterations (mCA), two forms of clonal hematopoiesis detected from somatic variants, are associated strongly with future myeloid and lymphoid cancers, respectively. Recent studies have now uncovered distinct influences on age-related non-oncologic conditions by clonal hematopoiesis type. Clear distinctions include the associations of CHIP with cardiovascular disease and mCAs with severe infectious disease. Such observations inform novel genomic models of aging for both non-oncologic conditions.

Conflict of Interest: None declared.

S19 POLYGENIC SCORES: FROM METHODS TO APPLICATIONS

S19.3 Moving polygenic scores from research to the clinic

Cathryn Lewis

Moving polygenic scores from research to the clinic

Cathryn M Lewis

Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London.

Polygenic scores are widely applied in genetic research studies, for example to show prediction within traits, to assess overlap of genetic contributions, or for stratification of research participants. However the real value of polygenic scores would be implementation within clinical services. This symposium talk will consider possible applications of polygenic scores and barriers to their implementation. Covering breast cancer, cardiovascular disease and psychiatric disorders, the symposium will show the strength of current prediction, potential target areas for implementing

polygenic scores, and interventions available for those identified at high polygenic risk. Opportunities include assessing how polygenic scores moderate the penetrance of rare pathogenic variants, and the potential for individual level risk prediction to motivate behavioural and life-style changes that are relevant to the whole population. Barriers to be discussed include ensuring applicability to all ancestries, the modest predictive value attained for many polygenic scores, and the assessment of clinical utility.

Conflict of Interest: None declared.

S20 CROSS-CULTURAL COMMUNICATION AND COUNSELLING

S20.2 The lessons learned from the role of religion and spirituality on the lived experience of Muslim patients with genetic disorders

Khadijah Bakur

Background: Genetic services are rapidly growing in many Islamic countries, leading to more diagnoses. Because Muslim patients almost integrate religion throughout their lives, it has become vital to understand the role of Islam on their coping and decision-making in the context of genetic counselling. This could provide patients with the most appropriate services that do not contradict their religious beliefs, hence contributes to better outcomes. This study explored the role of religion in the experiences of Saudi patients with long QT syndrome (LQTS).

Methods: The study employed semi-structured interviews with 13 patients who had Long QT syndrome or were carriers of Jervell and Lange-Nielsen syndrome to explore the role of Islam in their perceptions of the cause of diagnosis, coping strategies and decision-making. The participants were recruited from two Saudi Arabian cardio-genetic centres and investigated via interpretative phenomenological analysis.

Results: Data analysis produced number of superordinate themes: 1) Common belief and idiosyncratic interpretation; 2) Using religion to justify positive reframing of current illnesses; 3) Interplay between belief in medicine and in religion. From patients' perspective, the main factors influencing perceptions of the cause of diagnosis, coping and decision-making were the idiosyncratic interpretations of religious beliefs and rulings and the availability and understanding of medical information.

Conclusion: This research provided evidence that providing patients with clear medical information could alter their perceptions of the cause of diagnosis, which could contribute to better outcomes. Religious beliefs help reduce cognitive dissonance by casting wise decision-making as a religious duty.

Conflict of Interest: None declared.

S21 TRANSLATIONAL GENETICS OF BONE

S21.1 Genetics and Therapy of Osteogenesis Imperfecta

Nick Bishop

Osteogenesis imperfecta is a complex disease primarily affecting the connective tissues although effects in other systems also now emerging.

The majority of cases are due to mutations in the type one collagen genes but changes in at least a further 20 genes are now known to result in the OI phenotype.

Treatment approaches are symptomatic and focused on increasing bone mass as a means to reduce fracture risk; anti-resorptive bisphosphonates are the most widely used drugs, and positive results in terms of vertebral reshaping and reduction in fracture risk are apparent in children, less so in adults. Anti-fracture efficacy overall is regarded as "equivocal". New treatments are emerging to increase bone mass through increasing bone formation as opposed to reducing resorption. No bespoke or targeted therapies have yet emerged as clinically applicable, although targeting of autophagy and pro-inflammatory pathways have been reported recently. Cell-based interventions have had equivocal impact. The prospect of gene therapy remains a distant one at present.

Conflict of Interest: None declare.

S21.2 New therapies targeting FGFR3-related skeletal disorders: changing the rules of the game

Ravi Savarirayan[1]

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The management of skeletal dysplasia (inherited disorders of bone and cartilage) has been predominantly symptomatic and reactive, like many other genetic conditions. This status quo is now being challenged by the promise of precision therapies, underpinned by advances in the understanding of disease pathogenesis in animal models, that alter the natural history of these disorders, and offer patients new options for better health.

To assess the safety and clinical efficacy of these new potential disruptive treatments, we have been leading clinical trials in children with the most common form of human dwarfism, achondroplasia.

This talk will summarise the current state of play with these human clinical trials, and will focus on newly approved and potential therapies for children with achondroplasia and related FGFR3 skeletal disorders, including c-natriuretic peptide analogues, tyrosine kinase inhibitors, and soluble *FGFR3*, as examples of this exciting new paradigm.

Conflict of Interest: None declared.

S23 NON-CODING RNAS IN BIOLOGY AND DISEASE

S23.2 Non-coding RNAs in genetic disease and as target of therapy

Sandro Banfi

Sandro Banfi

Dipartimento di Medicina di Precisione, University of Campania "Luigi Vanvitelli" and Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy..

Non-coding transcripts (ncRNA) represent functional RNA molecules that do not encode for proteins. It is now widely accepted that ncRNAs are endowed with a previously unrecognized role in many pathophysiological processes, mainly through regulation of gene expression. Among ncRNAs, microRNAs (miRNAs) represent the best characterized subgroup thus far. They are short ncRNAs that control fundamental biological processes by targeting networks of functionally correlated genes. Our laboratory is interested in unraveling the contribution of miRNAs to retinal development and function. Towards this goal, we first aimed at reconstructing the architecture of the human retina miRNome by Next Generation Sequencing procedures. In parallel, we started a characterization of the functional role of several miRNAs, including miR-204 and miR-181a/b, in the retina both in physiological and pathological conditions. We uncovered the first example of an Inherited Retinal Disease (IRD) caused by a point mutation in a miRNA (namely miR-204). Finally, due to their pervasive control of many

pathophysiological processes and to their easy manipulation, miRNAs may represent ideal gene/mutation-independent therapeutic tools for genetic disease, including IRDs. Therefore, we aimed at identifying miRNAs putatively able to exert a protective effect on IRD progression. We found that the expression modulation in the retina of several miRNAs, via Adeno Associated Viral (AAV)-mediated delivery, slows down photoreceptor cell death and improves visual function in different in vivo IRD models. We believe that this work may pave the way towards the implementation of gene-independent therapeutic strategies for IRDs that can be used as alternative or in complementation to gene-based approaches.

Conflict of Interest: None declared.

S25 MULTIOMICS FOR DIAGNOSTICS

S25.2 Epigenomics for stratification of Cancer and Covid

Manel Esteller

Josep Carreras Leukaemia Research Institute (IJC).

For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flagship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy case of MGMT hypermethylation in the prediction of alkylating drug response to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with anticancer drugs, including immunotherapy. Beyond cancer, DNA methylation is starting to be recognized as playing a major role in infectious diseases, and in this regard, the present lecture will also address the epigenomic component of COVID-19.

Conflict of Interest: None declared.

S26 FEDERATION OF GENOMIC MEDICINE DATABASES

S26.2 Federation of genomic medicine databases: A bioinformatics perspective

Melissa Cline

When these containers analyze personal data and generate aggregated, anonymized results, the results can often be shared externally even when the input data cannot, for sharing knowledge while safeguarding the privacy of the participants. Existing applications of this approach include clinical variant interpretation and pediatric oncology. The Global Alliance for Genomics and Health is fostering the development of federated methods through development of data standards, cloud workflow technologies, and policy guidance, and several GA4GH driver projects are leveraging federated analysis for responsible international data sharing.

Conflict of Interest: None declare.

S26.3 Federation of genomic medicine databases: A modern archivist perspective

Jordi Rambla

Genomic medicine databases choose to join federations for two main purposes 1) to control the access to the data of donors from a given jurisdiction and 2) to leverage the knowledge distributed among databases by joining them in a common space. Modern archivists try to get both benefits at once. This result could be achieved by applying modern tools for data sharing that facilitate the federation while preserving the control on the sharing of the data. Global Alliance for Genomics and Health (GA4GH) Beacon v2 protocol is one of such tools.

Conflict of Interest: None declared.

EDUCATIONAL SESSIONSE02 GENETICS, 200 YEARS AFTER THE BIRTHS OF MENDEL AND GALTON

E02.2 From Mendel's legacy to Mendelian disorders

Sarka Pospisilova[1,2,3]

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Gregor Johann Mendel - a multitalented scientist, considered the "father of modern genetics", was born in 1822 and the bicentennial of his birth in July 2022 provides a unique opportunity to remember the legacy of this great scientist. Mendel made the key discoveries in the Augustinian Abbey in Brno, Czech Republic, thanks to his interdisciplinary knowledge of plant biology, mathematics and statistics. His fundamental experimental work on a hybridization of about 30,000 pea plants led to a formulation of the principles of heredity. The Mendelian laws, published in 1865, described for the first time the presence of two versions of each element (gene) in a dominant or recessive form that is inherited from both parental organisms into the filial generation (known as the laws of segregation, independent assortment, and dominance of alleles). The disorders with Mendelian inheritance, named also monogenic disorders, appear as a result of a single-gene mutation present in one or both alleles.

Currently, 200 years from Mendel's birth and 138 years from his death, there persisted an uncertainty about the place of his grave. Therefore, on the occasion of his anniversary, the scientists, in close collaboration with representatives of the Order of Augustinians, initiated a project on an archeological research of the Augustinian tomb followed by anthropological and genetic research of the found remains. Very exciting novel findings on Gregor Johann Mendel personality have been discovered and, symbolically, the genome of the father of genetics has been isolated. In addition, the geneticists analyzed whether Mendel was a carrier of any disease with Mendel's mode of inheritance, also called Mendelian disorders. This project and obtained novel findings could further highlight the Mendel's crucial discoveries

and legacy, but also attract society's attention to genetics and science.

Acknowledgement: To all, who initiated and realized the interdisciplinary archeological, anthropological and genetic research project of G. J. Mendel.

Conflict of Interest: None declared.

E04 ESHG-Y: FILLING THE GAPS BY PUBLISHING NEGATIVE RESULTS IN GENETICS

E04.2 Why don't researchers publish "negative" results in genetics? and Panel Discussion

Virginia Arechavala-Gomeza

As researchers, we strive to obtain answers to questions following the scientific method: we have a hypothesis, and we use some experiments that could provide us with a simple answer to our question: is my hypothesis right or wrong? And we will be wrong, many times. However, publications are biased towards results that confirm the proposed hypothesis.

The pressure to publish and the metrics used in the evaluation of researchers are one of the possible reasons behind this bias, but there are things we could do (but we are not doing) to try to avoid this problem. Why are we not publishing so-called negative results?

Conflict of Interest: None declared.

E05 MOSAICISM

E05.1 Chromosomal mosaicism in preimplantation and prenatal diagnosis

Francesca Romana Grati

Chief Genomics Officer at R&D Menarini Biomarkers Singapore.

Abstract

The finding of a chromosomal mosaicism is one the most complex situation when it is detected both in preimplantation and in prenatal diagnosis. Due to the variability of the distribution of the abnormal cell line in the different embryo-fetal tissues, each situation needs to be assessed on a case-by-case basis and counseled accordingly. In most of the cases, above all for chromosomal mosaicisms for rare autosomal trisomies, the prediction of genotype-phenotype correlations has to be based on tenuous information (single case reports or small cohorts) that may not be entirely applicable.

During this workshop a deep dive into chromosomal mosaicisms will be presented with a particular focus on the clinical impact of finding a chromosomal mosaicism at 5 key diagnostic time points: 1) Embryo biopsy; 2) Chorionic Villus Sampling, 3) Amniocentesis, 4) Non-invasive prenatal testing (NIPT) using cell free DNA (cfDNA) and 5) Non-invasive prenatal testing (NIPT) using isolated circulating trophoblasts in maternal blood.

Conflict of Interest: None declared.

E06 GENETIC DISCRIMINATION: SURVEYING THE ETHICO-LEGAL LANDSCAPE

E06.1 Genetic discrimination: International ethico-legal perspectives and the Genetic Discrimination Observatory

Yann Joly

The risk of discrimination based on genetic characteristics that is intended to infringe or has the effect of infringing human rights, fundamental freedoms and human dignity remains a major challenge to participation in genetics research and uptake of genetics in the clinical context. Many countries currently share this problem and finding a solution will require more than jurisdiction specific uncoordinated laws. In 2020, an international consortium of researchers and other stakeholders, the Genetic Discrimination Observatory (GDO), was organised to document and address instances of genetic discrimination around the world.

This presentation will cover genetic discrimination, its incidence and impact, as well as, the program set-up by the GDO to address this pervasive legal, social and ethical issue. The objectives, activities and future plans of the GDO will also be discussed.

Conflict of Interest: None declared.

E07 PROGRESS OF IMPUTATION

E07.2 Haplotype phase inference

Brian Browning

A haplotype is a sequence of alleles that is inherited from the same parent. Statistical haplotype phasing is the inference of haplotypes from inter-marker correlation. The accuracy of statistical phasing is usually measured in parent-offspring trios because true haplotypes in the offspring can be inferred from parental genotypes and Mendelian inheritance constraints. Phasing accuracy is usually measured in terms of the switch error rate, which is the proportion of pairs of consecutive heterozygous genotypes that are incorrectly phased. However, genotype error inflates the switch error rate observed in trio offspring. Recent work has shown that it is possible to estimate the true switch error rate, which can be much smaller than the observed switch error rate in large-scale data. In the UK Biobank White British trio offspring, we find that the observed switch error rate is 2.4 times larger than the estimated true switch error rate.

Conflict of Interest: None declared.

E09 PHARMACOGENOMICS FOR PERSONALIZED DRUG TREATMENT

E09.2 Pharmacogenomics knowledge for personalized medicine

Michelle Whirl-Carrillo

Clinical implementation of pharmacogenomics is critical to achieving the goal of personalized medicine. Before clinicians can incorporate a patient's genotype into their decisions of which medication or dosage to prescribe, supporting evidence must reach the threshold of clinical actionability. Determination of whether pharmacogenomic evidence meets this threshold depends on comprehensive review of knowledge including gene-drug and variant-drug associations, drug pharmacokinetics and pharmacodynamics, and genetic variation of important pharmacogenes. Publicly available pharmacogenomic knowledge resources enable comprehensive evidence review by providing curated and integrated information from sources including peer-reviewed literature, clinical trials, regulatory-agency approved drug labels and primary data from pharmacogenomic studies. This talk will discuss knowledge resources that support clinical implementation of pharmacogenomics for personalized medicine.

Conflict of Interest: None declared.

E11 NEW TREATMENTS FOR CONGENITAL DISORDERS

E11.2 Derepression of imprinted gene alleles as a new approach to treat imprinting disorders

Ulrich Zechner

Current treatment strategies of imprinting disorders mainly focus on the alleviation of some of the symptoms like behavioural and endocrine manifestations. There are no treatments specifically for clinical signs like intellectual disability which is an overarching finding associated with several imprinting disorders such as Angelman syndrome (AS) and Birk-Barel syndrome (BIBARS). Imprinting disorders with loss or mutation of the active copy of an imprinted gene offer unique possibilities of causal therapeutic intervention by derepression of the other silent allele. Novel derepression-based therapeutic approaches using epigenetic drugs, antisense-oligonucleotides (ASOs) or epigenome editing, their preclinical application in mouse models of AS and BIBARS as well as recently started first-in-humans studies with Angelman-ASOs will be presented.

Conflict of Interest: None declared.

E12 INHERITED METABOLIC DISORDERS WITH ACUTE PRESENTATIONS

E12.1 Update on the diagnosis and treatment of hyperammonaemias

Johannes Häberle

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Hyperammonaemia is the hallmark of several disorders of variable etiology and can occur in inherited as well as in acquired disorders. It should thus be regarded as an unspecific laboratory sign, and is defined as a plasma ammonia level > 50 µmol/L (and > 100 µmol/L in newborns). Hyperammonaemia should always be regarded as an emergency; possible causes include an increased production of ammonia (e.g. in intestinal bacterial overgrowth, neurogenic bladder) or a diminished detoxification (e.g. in decreased urea cycle flux, blood bypass of the liver or insufficient action of glutamine synthetase). Often, endogenous protein catabolism triggers an increase of ammonia such as during infections or due to any other energy deficit. Detoxification of ammonia takes mainly place in periportal hepatocytes as this is the part of the liver lobule with highest urea cycle expression. A defect of any of the involved enzymes or transporters and also inhibition of the urea cycle through metabolites or by substrate deficiencies can affect ammonia detoxification.

In addition, various other situations such as organic acidemias, fatty acid oxidation defects, some rare diseases including pyrroline-5-carboxylate synthetase deficiency, the hyperammonaemia-hyperinsulinism syndrome, and the defect of carbonic anhydrase type VA as well as some drugs (e.g. valproic acid, cyclophosphamide) can cause hyperammonaemia. While the aforementioned conditions lead to a secondary impairment of urea cycle function, which might manifest at any age, patients with a primary urea cycle disorder are at risk for developing irreversible hyperammonaemic brain edema during their entire life.

The main prognostic factors are, irrespective of the underlying cause, the duration of the hyperammonaemic coma and the extent of ammonia accumulation.

Thus, early recognition of hyperammonaemia and initiation of specific treatment are of utmost importance. In particular, there is need for a high awareness amongst neonatologists treating sick newborns considered initially as bacterial sepsis cases. As well, neurologists or adult physicians confronted with an unusual and unexplained change in the neurological status of a patient should include hyperammonaemia early in their differential diagnosis and work-up.

This lecture will briefly discuss the biochemical background of primary and secondary hyperammonaemias, will give an overview on the various underlying disorders including their genetic backgrounds, will describe current diagnostic strategies and will summarize the present therapeutic management with a short outlook into future therapies.

Conflict of Interest: None declared.

E14 PRECISION MEDICINE IN THE DIGITAL HEALTH ERA

E14.1 Looking beyond the lamppost: the "genome-first" approach to disease prevalence, penetrance, and phenotype

Douglas Stewart

The traditional approach to evaluate an individual with a rare disease phenotype has included single-gene testing for those who meet certain clinical criteria. This "phenotype-first" approach may be subject to ascertainment bias towards the most highly penetrant clinical manifestations, miss non-penetrant cases, miss rare or unknown disorder manifestations, or over-estimate disease severity. The availability of large-scale, population-based exome-sequenced cohorts now allows for the "genome-first" strategy, which permits geneticists to look well beyond the light cast by the lamppost of our limited knowledge of phenotype. This approach has detected a higher-than-expected prevalence of pathogenic germline variation in a variety of disorders, suggesting that these variants might cause unidentified or less severe clinical phenotypes. The genome-first approach has potential to reduce ascertainment bias, identify a more complete phenotypic spectrum, and permit more precise gene and/or variant penetrance estimates. This session will illustrate the challenges, opportunities, and "lessons learned" of the genome-first approach, with specific examples from recent work in a variety of monogenic disorders.

Conflict of Interest: None declare.