ABSTRACTS COLLECTION

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

PLENARY SESSIONS

PL1 Opening Plenary

PL1.2 The COVID-19 host genetics initiative - an international, open science effort to identify genetic risk factors for COVID19 severity and susceptibility

Andrea Ganna

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The genetic makeup of an individual contributes to susceptibility and response to infectious viruses. While environmental, clinical and social factors play a role in exposure to SARS-CoV-2 and COVID-19 disease severity, host genetics may also be important. Identifying host-specific genetic factors indicate biological mechanisms of therapeutic relevance and clarify causal relationships of modifiable environmental risk factors for SARS-CoV-2 infection and outcomes. We formed a global network of researchers to investigate the role of human genetics in SARS-COV-2 infection and COVID-19 severity. We describe the results of three genome-wide association meta-analyses comprising 49,562 COVID-19 patients from 46 studies across 19 countries worldwide. We reported 15 genome-wide significant loci that are associated with SARS-CoV-2 infection or severe manifestations of COVID-19. Several of these loci correspond to previously documented associations to lung or autoimmune and inflammatory diseases. They also represent potentially actionable mechanisms in response to infection. We further identified smoking and body mass index as causal risk factors for severe COVID-19. The identification of novel host genetic factors associated with COVID-19, with unprecedented speed, was enabled by prioritization of shared resources and analytical frameworks. This working model of international collaboration a blue-print for future genetic discoveries in the event of pandemics or for any complex human disease.

A. Ganna: None.

PL2 What's New? Highlight Session

PL2.1 Clinical implementation of RNA sequencing for Mendelian disease diagnostics

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Introduction: Whole exome (WES) and whole genome sequencing remain unsuccessful in providing a firm diagnosis for about half of the individuals with a suspected Mendelian disorder. RNA sequencing (RNA-seq), which directly probes gene expression defects, has emerged as a promising complementary tool increasing the diagnosis rate.

Materials and Methods: We performed RNA-seq on fibroblasts from 305 individuals affected with a mitochondrial disease who had previously undergone WES, which represents one of the largest RNA-seq compendium for rare disease diagnostics. To detect the genetic cause, we prioritize genes harbouring aberrant expression (using OUTRIDER), aberrant splicing (using FRASER), or mono-allelic expression of a rare allele, compiled with our recently developed computational workflow DROP, which is further extended with an RNA-seq based variant calling module.

Results: We provided a diagnosis for 16% (33 out of 205) of WES unsolved cases. By associating transcriptome aberrations with rare variants, we found a major role of nonsense-mediated decay in underexpression outliers and of coding and intronic variants in splicing outliers. We also assessed the importance of RNA source material and showed that the majority of OMIM disease genes are expressed in clinically accessible tissues, with the highest number in fibroblasts. Finally, we have gathered a reference dataset containing gene- and junction- level counts that can be integrated as controls for studies with small sample sizes.

Conclusion: Our study demonstrates the routine implementation of RNA-seq as a complementary tool to DNA sequencing for the diagnosis of rare diseases, paving the path towards more comprehensive OMICS-based diagnostics.

V. Yepez: None. **M. Gusic:** None. **J. Gagneur:** None. **H. Prokisch:** None.

PL2.2 Local gene co-expression measurements in single-cells highlight inter-individual specificity

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Nearby genes are often expressed as a group. Recent studies highlight the existence of regulatory domains (e.g. groups of enhancers) orchestrating the organised expression of nearby genes (Delaneau et al. 2019 Science 364(6439)). Yet, the prevalence and genetic control of local gene co-expression are far from being understood. By leveraging gene expression measurements across 49 human tissues and hundreds of individuals, we found local gene co-expression to be highly prevalent, occurring in 13% to 53% genes per tissue. Notably, we identified >30.000 expression quantitative trait loci (eQTLs) which associate with co-expressed gene pairs and often overlap enhancer regions. Due to affecting several genes, these eQTLs are more often associated with multiple human traits than eQTLs associated with only one gene. Next, to understand how the observed local gene co-expression and its regulation manifests at the single-cell level, we analysed a dataset of single-cell RNA-seq across 60 genotyped individuals in a homogeneous cell type (iPSC). By taking advantage of co-expression measurements for >50 cells per individual, we identified 100-550 locally co-expressed gene pairs per individual. Interestingly, while many co-expressed gene pairs are specific to certain individuals, we discovered that those present across >50% of the individuals often participate in the same biological pathway (OR = 16.5, P-value = 1e-9). Finally, in a bid to comprehend disease comorbidity and provide functional interpretation of QTL and GWAS findings, we identify eQTLs affecting co-expressed gene pairs in individuals suffering from various disease comorbidities in the UK Biobank. Grant: Marie Sklodowska-Curie nº885998

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PL2.3 A cross-disorder dosage sensitivity map of the human genome

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Rare copy number variants (rCNVs) contribute to a broad spectrum of human diseases. Most disease-association studies of rCNVs have focused on haploinsufficiency caused by deletions, while our understanding of triplosensitivity caused by duplications remains rudimentary. We meta-analyzed rCNVs from 753,994 individuals across 30 primarily neurological disease phenotypes to discover 114 rCNV-disease associations at 52 distinct loci surpassing genome-wide significance ($P = 3.72\text{x}10^{-6}$), 42% of which involve duplications. Further, we statistically fine-mapped 38 novel triplosensitive disease genes (*e.g.*, *GMEB2*), including three known haploinsufficient genes that we now reveal as bidirectionally dosage sensitive (*e.g.*, *ANKRD11*). We found that disease-associated rCNV segments were enriched for genes constrained against damaging coding variation and identified likely dominant driver genes for about one-third (32%) of rCNV segments based on *de novo* mutations from exome sequencing studies of developmental disorders. However, most of the rCNVs showing genome-wide significant association were incompletely penetrant (mean odds ratio=11.6) and we also identified two examples of noncoding disease-associated rCNVs (*e.g.*, intronic *CADM2* deletions). Finally, we developed a statistical model to predict dosage sensitivity for all genes, which defined 3,006 haploinsufficient and 295 triplosensitive genes where the effect sizes of rCNVs were comparable to deletions of genes constrained against truncating mutations. These dosage sensitivity scores classified disease genes across molecular mechanisms, prioritized pathogenic *de novo* rCNVs in children with autism, and revealed features that distinguished haploinsufficient and triplosensitive genes. Collectively, the cross-disorder rCNV maps and metrics derived here set the foundation for future studies of dosage sensitivity throughout the human genome.

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PL2.4 Biallelic *ATG7* variants impair autophagy leading to neurological disease

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Autophagy is an essential developmental and homeostatic process, driving the endolysosomal degradation of protein aggregates, organelles and pathogens. Dysfunctional autophagy has been implicated in complex human diseases, yet congenital autophagy disorders remain exceedingly rare. Using whole exome sequencing we identified pathogenic, biallelic variants in *ATG7*, encoding the principal driver of autophagy, in twelve patients from five families. These patients display complex neurodevelopmental disorders distinguished by selective neurological, neuromuscular and endocrine dysfunction. Contrasting conditional *Atg7* deletion in mice which causes perinatal lethality, patients survive into adulthood despite loss of ATG7. Fibroblasts from each affected family displayed diminished autophagy, and expression of mutated *ATG7* failed to rescue autophagy-deficient model systems, supporting the pathogenicity of these variants. ATG7-deficient patient muscle revealed myopathic and inflammatory changes, together with subsarcolemmal P62 accumulation. Despite loss of ATG7, autophagic structures were readily detected in patient fibroblasts and muscle, suggesting that ATG7-independent autophagosome biogenesis pathways are sufficient to maintain basal autophagic degradation in human cells. Our study provides the first clinical, genetic and mechanistic demonstration that mutated *ATG7* leads to neurodevelopmental disease in humans, who can survive for decades with defective canonical autophagy. Importantly, two patients with undetectable ATG7 display a relatively mild phenotype, revealing that human life is compatible with the absence of a nonredundant core autophagy gene, thereby challenging current perceptions regarding the relationship between autophagy and human health and disease.

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PL2.5 The first human importin-β-related disorder: syndromic thoracic aortic aneurysm caused by bi-allelic loss-of-function variants in *IPO8*

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Introduction: *IPO8* encodes importin-8, a ubiquitously expressed nuclear transport receptor belonging to the importin-β protein family that translocates cargo molecules such as proteins, RNAs and ribonucleoprotein complexes to the nucleus in a RanGTP-dependent manner. Important cargoes of importin-8 are suggested to be TGF-β signaling components such as SMAD1-4.

Materials and methods: Exome or genome sequencing are used to identify variants in *IPO8*. A C57Bl/6N *Ipo8* knock-out mouse model is phenotypically characterized and studied using transthoracic echocardiography, immunohistological investigation, compliance assays to study biomechanical properties of the ascending aorta and RT-qPCR assays.

Results: Four bi-allelic loss-of-function variants in *IPO8* were discovered in unrelated families causing a novel syndromic form of thoracic aortic aneurysm (TAA) with clinical overlap with Loeys-Dietz and Shprintzen-Goldberg syndrome. Patients are young children clinically characterized by early-onset TAA, motor developmental delay, craniofacial dysmorphic features and connective tissue findings. The *Ipo8* knock-out mouse model showed a TAA development from 8-12weeks onwards in both sexes, but most prominently shows ascending aorta dilatation with a propensity for dissection in males. Immunohistological investigation and RT-qPCR assays of the aortic wall revealed elastic fiber

disorganization and fragmentation and nuclear pSmad2 accumulation, respectively a decreased *Smad6/7* and increased *Mmp2* expression, reinforcing a role for dysregulation of the TGF-β signaling pathway in TAA development.

Conclusions: We report the first importin-β-related human disease caused by bi-allelic loss-of-function variants in *IPO8* presenting a novel aortopathy syndrome.

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PL2.6 Epilepsy polygenic risk scores in > 269k individuals with and without epilepsy

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Epilepsy affects approximately 1% of individuals worldwide. Making an epilepsy diagnosis is often difficult with estimates that up to 25% of epilepsy could initially be misdiagnosed. The SNP heritability of genetic generalized epilepsy is high (32%) and it has recently been shown that individuals with epilepsy also have elevated epilepsy polygenic risk scores (PRS). However, systematic investigation of PRS for distinct epilepsy diagnoses has so far been lacking. Here, we studied epilepsy PRS in detailed longitudinal electronic health records of > 269k Finns including ICD codes and drug purchases of over 50 years. Our dataset included 9660 individuals with epilepsy related diagnoses. We could confirm an elevated PRS for generalized epilepsy (PRS_{gen}) in individuals with generalized epilepsy. This was particularly high for adolescent myoclonic epilepsy, which could be because it represented the largest diagnosis group of the GWAS that was used to construct the PRS. We also made multiple surprising discoveries e.g. one ICD diagnosis G40.1 defined as a focal epilepsy had an elevated PRS_{gen} but no increased PRS for focal epilepsy (PRS_{focal}). We further found that over half of individuals with specific diagnoses of generalized or focal epilepsy were initially diagnosed with unclear convulsions (R56.8) or unclear epilepsy (G40.9). Their PRS_{gen} and PRS_{focal} was significantly higher than of those individuals who had only one unclear seizure event and did not later receive an epilepsy diagnosis.These results indicate a future potential for epilepsy PRS to help in predicting progression to epilepsy in clinical practice.

H.O. Heyne: None. **R. Kälviäinen:** None. **M.J. Daly:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Maize Therapeutics.

PL4 Mendel Award Lecture

PL4.1 Mendel Lecture- Cell-free DNA in Plasma: Coming in Different Sizes and Shapes

Yuk M. D. Lo

The Chinese University of Hong Kong, Hong Kong, China.

My group first reported the presence of cell-free fetal DNA in maternal plasma in 1997. This phenomenon has contributed to the development of non-invasive prenatal testing (NIPT). Circulating DNA in plasma exists in the form of short DNA fragments. Circulating fetal DNA molecules are generally shorter than those derived from the mother. Recently, I have been particularly interested in the fragmentation process of such DNA molecules, now referred to as fragmentomics. Through the use of mouse models in which different nuclease genes have been inactivated, different steps of the fragmentation process has been worked out. Nucleases that participate in this process include DNASE1L3, DNASE1 and DNA fragmentation factor beta (DFFB). The end motif sequences of circulating DNA molecules carry cleavage signatures from these nucleases. My group has also demonstrated that circulating DNA molecules consist of a predominance of molecules carrying jagged ends. More recently, circular DNA species have been found in plasma. Interestingly, the sizes and topology of circulating DNA species have been found to be correlated to their tissues of origin. Apart from shedding light on the mechanism of cell-free DNA generation, these observations may also contribute towards the development of novel biomarkers for prenatal and cancer liquid biopsies.

Y.M.D. Lo: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Grail. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Grail, Take2, DRA Limited, Illumina, Sequenom, Xcelom. F. Consultant/ Advisory Board; Significant; Grail, Decheng Capital.

CONCURRENT SYMPOSIA

S01 Machine learning methods for prioritizing genetic variants

S01.2 Leveraging public epigenomic datasets to examine the role of regulatory variation in complex traits

Sushmita Roy

University of Wisconsin-Madison, Madison, WI, USA.

Many variants identified by genome-wide association studies (GWAS) occur in non-coding sequences. Understanding the impact of such variants on complex phenotypes is a significant challenge. This is because such regulatory variants can affect the expression of gene hundreds of kilobases away, and the target genes themselves are part of a larger unknown biological pathway. High-throughput Chromosome Conformation Capture (3C) technologies that measure the three-dimensional proximity of genomic loci at high resolution (e.g. 5kb) can be used to link genes to regulatory variants over long distances, but they exist only for a handful of model cell lines due to high sequencing costs. We have developed a computational framework for predicting such data, comprising (1) a Random-Forest based regression model to predict *in-silico* contact counts of pairs of genomic loci, and (2) a multi-task subnetwork identification approach to identify potential pathways targeted by a set of variants from a GWAS study. Our count prediction approach takes as input one-dimensional signals such as histone modifications, chromatin accessibility and accessible sequence motifs of architectural proteins such as CTCF and predicts the contact count for pairs of regions. We trained our approach on five high-resolution Hi-C datasets and applied the trained models to 55 human cell lines and tissues from the Roadmap Epigenomics

project using both measured and imputed features. Our predictions, when aggregated to a lower resolution (e.g. 40kb), accurately recapitulate the measured low-resolution contact count, as well as identify larger units of 3D chromosome organization. Significant interactions from our predicted counts are associated with increased gene expression and enriched for interactions from ChIA-PET experiments. We used our compendium of significant interactions to predict target genes of regulatory SNPs associated with traits from the NHGRI GWAS catalog. We applied our multi-task graph clustering approach to identify subsets of cell types associated with a phenotype of interest, e.g., autism spectrum disorder (ASD) and schizophrenia. We found that the subnetworks are enriched for processes that are relevant to the phenotype of interest (e.g. SNPs associated with ASD and schizophrenia are enriched for cognition, associative and visual learning and regulation of nerve impulse in fetal brain and fetal spinal cord tissue). Taken together, our analysis pipeline offers a powerful resource to study three-dimensional genome organization in a large number of cell lines and interpret the role of regulatory variation in complex phenotypes.

S. Roy: None.

S02 Spatial omics

S02.1 Imaging the Accessible Genome at Nanometer Scale

Zhe J. Liu

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To image active chromatin at nanometer scale in situ, we developed 3D ATAC-PALM that integrates the assay for transposase-accessible chromatin (ATAC), PALM super-resolution imaging and lattice light-sheet microscopy. Multiplexed with oligopaint DNA-FISH, RNA-FISH and protein fluorescence, 3D ATAC-PALM connected microscopy and genomic data, revealing spatially segregated accessible chromatin domains (ACDs) that enclose active chromatin and transcribed genes. Using these methods to analyze genetically perturbed cells, we identify the BET family scaffold protein BRD2 as a key factor responsible for compartmentalization of the accessible genome. Specifically, BRD2 mixes and compacts active compartments in the absence of Cohesin. This activity is independent of transcription but requires BRD2 to recognize acetylated nucleosomes through its double bromodomain. We also show that BRD2 safeguards compartmental boundaries by preventing intermingling between active and inactive chromatin. Notably, genome organization mediated by BRD2 is antagonized on one hand by Cohesin and on the other by the BET homolog protein BRD4, both of which inhibit BRD2 binding to chromatin. Polymer simulation of the data supports a BRD2-Cohesin 'tug-of-war' model of nuclear topology, where genome compartmentalization results from a competition between loop extrusion and chromatin state-specific affinity interactions.

Z.J. Liu: None.

S02.2 Spatially resolved gene expression

Joakim Lundeberg

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Advances in the field of transcriptomics have deepened our understanding of tissue organization by integrating global gene expression in a spatial context. Among these approaches, in situ capturing technologies produce whole-transcriptome spatial gene expression by tagging spatial barcodes to transcripts after poly(A)-capture. Current protocols for in situ capturing, using barcoded surfaces, are based on short-read sequencing and fresh frozen samples. In this presentation we will provide insights to new developments expanding the analysis into FFPE material as well as spatial full length information. We provide examples of how new data types leads to an improved understanding of biological systems in health an disease and provide an additional layer of information to Cell Atlas initiatives.

J. Lundeberg: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; 10X Genomics Inc. F. Consultant/ Advisory Board; Significant; 10X Genomics Inc.

S03 Transposons

S03.2 Retrotransposition in brain: does LINE activity in the central nervous system matter?

Jose L. Garcia-Perez

HGU-MRC, Institute of Genetics and Molecular Medicine, Edinburgh, United Kingdom.

Transposable Elements (TEs) are stretches of DNA that can move within genomes. As pieces of mobile DNA, TE activity can change the genome of any species. Indeed, germline TE activity over evolution has significantly shaped the structure and function of the genome of all living organisms, including humans. Currently, active TEs in our germline genome (termed LINEs for Long Interspersed Elements) continue to generate genomic variability among humans. As a result, LINE activity continues to impact the functioning and regulation of our genome. As a type of selfish DNA, LINE activity is prominent in cellular niches that transmit genetic information to the next generation (i.e., germ cells and during early human embryogenesis). However, due to its mutagenic potential, the activity of LINE elements is tightly regulated, ensuring an equilibrium with the host. My laboratory aims to understand how active LINEs are regulated at the molecular level, and to infer the impact of their activity in relevant cellular models and model of disease. Surprisingly, it was recently demonstrated that active LINEs also move in neuronal cells of our brain (and of model organisms). These discoveries were unexpected, and suggest that the same mechanisms used to evolve germline genomes can act in our brain, but without leaving heritable traces. An emerging hypothesis suggests that TEs affect brain functionality, and my lab is using zebrafish to dissect how active TEs affect the vertebrate brain.

J.L. Garcia-Perez: None.

S03.3 Activation of transposons in neurological disorders

Johan Jakobsson

Lund University, Lund, Sweden.

The underlying cause for most neurological disorders is poorly understood and current treatments are largely ineffective. New ideas and concepts are therefore vitally important for future research in this area. In this talk I will discuss the concept that dysregulation of transposable elements (TEs) contributes to the appearance and pathology of neurodevelopmental and neurodegenerative disorders. Despite TEs making up at least half of the human genome, they are vastly understudied in relation to brain disorders. However, recent advances in sequencing and gene editing are now starting to unravel the pathological role of TEs.

Aberrant activation of TEs has been found in many neurological disorders and the resulting pathogenic effects, which includes alterations of gene expression, neuroinflammation, and direct neurotoxicity, are currently being investigated. In summary, our work is based on the idea that an increased understanding of the relationship between TEs and pathological processes in the brain will result in novel diagnostic tool and therapeutic approaches for brain disorders.

J. Jakobsson: None.

S04 Impact of GDPR on genomic data sharing

S04.1 How to transfer genomic data internationally in compliance with the GDPR

Heidi Beate Bentzen

University of Oslo, Oslo, Norway.

Legal challenges currently impede personal data transfers to outside the European Economic Area (EEA), gravely affecting medical research globally. Ironically, it is even more difficult to transfer genomic data from the EEA to a public research institution such as the U.S. National Institutes of Health than to a private company. Though if the U.S. company in question is a cloud provider, difficulties abound. Following Brexit, data transfers to the United Kingdom face an uncertain future. It will be explained exactly what the legal issues are that are causing these challenges for the genetics field.

Most importantly, how you currently and in the future *can* transfer genomic data to non-EEA collaborators in compliance with the European Union General Data Protection Regulation (GDPR), will be covered in this presentation. The answer depends on the country and entity to which you intend to transfer data, and the purpose for the data transfer. Legal, technical, and organizational solutions will be suggested based on extensive work conducted to facilitate international collaborations.

H. Bentzen: None.

S04.3 1+ Million Genomes Initiative and the GDPR

Regina Becker

ELIXIR-Luxembourg, University of Luxembourg, Esch-sur-Alzete, Luxembourg.

The 1+ Million Genome Initiative was initiated at the European Digital Day in 2018 by a core group of 13 EU Member States. By now, 24 European countries signed the joint declaration to make more than 1 million genomes with accompanying phenotypic data available cross-border for research and healthcare purposes. The aim is namely to "*provide cross-border, data-driven health and care solutions to benefit citizens of the Union*". Scope of the activities to achieve this goal are research for a better understanding of diseases, secondary use of data for healthcare applications and policy development with the aim to provide better healthcare to a collective of patients.

The ambitious goal is to go beyond a general availability of genomes for healthcare and research and create a virtual European genome cohort across the participating countries. This implies a much stronger harmonisation of procedures and a joint governance to allow research, policy and healthcare users feasible and straight-forward access procedures. However, such harmonisation will have to face the differences in legislation across Europe, most notably in the implementation of the General Data Protection Regulation (GDPR). While being a Regulation, the GDPR foresees a number of opening clauses and scope for EU Member State legislation. This will affect the 1+ Million Genome initiative in particular as the processing of health and genetic data based on Art. 9(4) GDPR and the processing of personal data for scientific research in general based on Art. 89(1) GDPR are subject to Member State legislation. In addition, different practices on a preferred or even legally prescribed legal basis for the processing influences the overall processing considerations. The applicability of national provisions needs to be investigated, which depends largely but not entirely on the assignment of responsibilities as controller under the GDPR. Additional challenges are provided by divergent interpretations of the GDPR, which leads to further differences in national or local interpretations and implementations of the GDPR. The consequences such differences will be reviewed and potential approaches to find solutions for a European implementation of the 1+ Million Genome initiative discussed.

R. Becker: None.

S05 Endogenous and exogenous mutagenesis in cancer

S05.2 Oncometabolites, DNA repair, and Cancer

Peter Glazer

Yale University, New Haven, CT, USA.

Abnormally elevated levels of the metabolites, 2-hydroxyglutarate (2HG), succinate, and fumarate, can occur in human malignancies due to somatic mutations in the isocitrate dehydrogenase-1/2 (IDH1/2) genes or germline mutations in the fumarate hydratase (FH) and succinate dehydrogenase (SDH) genes, respectively. These structurally related metabolites inhibit alpha-ketoglutarate-dependent enzymes, including dioxygenases that modify chromatin. Mutations in IDH1 and IDH2 are found most frequently in gliomas and acute myelogenous leukemias, along with cholangiocarcinomas, chondrosarcomas, and melanomas. Inherited mutations in FH and SDH are linked to the familial cancer predisposition syndromes, Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) and Hereditary Paraganglioma and Pheochromocytoma (SDH PGL/PCC), respectively. Our prior work has made an unexpected connection between elevated levels of these metabolites and DNA repair by showing that they suppress the pathway of homology-dependent repair (HDR) and confer an exquisite sensitivity to poly (ADP-ribose) polymerase (PARP) inhibitors that is being tested in clinical trials. In this presentation, we will discuss our recent work elucidating the mechanistic basis for this suppression of DNA repair. Rather than acting indirectly through epigenetic regulation of gene expression, we find that these metabolites act directly by inhibiting HDR factor recruitment to DNA double-strand breaks (DSBs). We have determined that oncometabolite-mediated inhibition of the lysine demethylase, KDM4B, results in aberrant hypermethylation of histone 3 lysine 9 (H3K9) at loci surrounding DNA double-strand breaks, which effectively prevents a local H3K9 trimethylation signal that is essential for the proper execution of HDR at the break. As a consequence, the recruitment of Tip60 and ATM, two proximal HDR factors, is significantly impaired at DNA breaks, with reduced end-resection and diminished recruitment of downstream repair factors. These findings provide a mechanistic basis for oncometabolite-induced HDR suppression and may guide effective strategies to exploit these defects for a therapeutic gain.

P. Glazer: E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Cybrexa Therapeutics, Gennao Bio. F. Consultant/Advisory Board; Modest; pHLIP Inc.

S05.3 Mutational signatures of environmental agents and chemotherapeutics in human cellular models

Jill E. Kucab

King's College London, London, United Kingdom.

Whole-genome sequencing (WGS) of human tumours has revealed a multitude of distinct mutational signatures present in cancers, some of which are linked to environmental exposures. Many environmental carcinogens, such as UV light and chemicals in tobacco smoke, can damage DNA and induce specific mutation patterns. Characterising these patterns experimentally allows us to better understand the mutations observed in human cancer. We previously examined whole-genome mutational signatures in human induced pluripotent stem cells (hIPSCs) treated with 79 agents, including known or suspected carcinogens and chemotherapeutics. Forty-one agents induced characteristic single base substitution signatures, some of which were highly similar to signatures found in human tumours. To expand upon our previous work, we are now using organoids derived from normal human epithelial tissues, including the gastrointestinal tract, pancreas and kidney, to determine patterns of chemically-induced mutations by WGS. We performed a pilot study using a bottleneck sequencing approach (NanoSeq) on DNA from hiPSCs and gastric organoids treated with the mutagenic phytochemical aristolochic acid I and the tobacco carcinogen benzo(a)pyrene. NanoSeq enables error-free, sensitive detection of subclonal mutations by combining molecular barcoding with a dilution step prior to library amplification, eliminating the need to isolate single cell clones. Using NanoSeq we were able to identify mutagen-induced signatures consistent with those previously found using a conventional WGS platform, validating our approach. We are now using this model to evaluate the mutational signatures of additional environmental carcinogens and chemotherapeutics in organoids, which will subsequently be compared to those found in patients exposed to these agents.

J.E. Kucab: None.

S06 Comparative genomics across species and populations

S06.1 Rapid evolution of genome regulation in mammals

*Masa Roller[1], Ericca Stamper[2], Paul Flicek[1], **Duncan T. Odom**[3,2]*

[1]EMBL - European Bioinformatic Institute, Hinxton, United Kingdom, [2]Cancer Research UK - Cambridge Institute, Cambridge, United Kingdom, [3]DKFZ & University of Heidelberg, Heidelberg, Germany.

My laboratory has investigated the mechanisms driving regulatory evolution across tissues and species. In this presentation, I will describe how plastic regulatory networks and even genome architecture is across the mammalian clade, with a focus on our recent work where we experimentally mapped promoters, enhancers, and gene expression in liver, brain, muscle, and testis from ten diverse mammals. The regulatory landscape around genes included both tissue-shared and tissue-specific regulatory regions, where tissue-specific promoters and enhancers evolved most rapidly. Genomic regions switching between promoters and enhancers were more common across species, and less common across tissues within a single species. LINE L1s were associated with tissue-specific regulatory regions, whereas more ancient LINE L2s were associated with tissue-shared regulatory regions and with those switching between promoter and enhancer signatures across species. Our analyses of the tissue-specificity and evolutionary stability among promoters and enhancers revealed how specific repeat families helped shape the dynamic regulatory patterns of mammalian tissues.

M. Roller: None. **E. Stamper:** None. **P. Flicek:** None. **D.T. Odom:** None.

S07 Mind the gap: Translating genomic advances into clinical care

S07.1 The art, science and practice of implementing genomics in clinical care

Stephanie Best[1,2]

[1]Australian Institute of Health Innovation, Macquarie University, Sydney, Australia, [2]Australian Genomics, Murdoch Childrens Research Institute, Australia.

Continual progress in genetic discoveries, alongside growing evidence of clinical utility, brings ever increasing calls for implementation of genomic testing in day to day clinical settings. Implementation science is acknowledged to support implementation of evidence-based practices into front line care, taking in a range of perspectives from patients and clinicians through to policy makers. However, clinical genomics is a complex intervention and health care is a complex adaptive system so using implementation science requires an element of science, art and, of course, practice. This presentation will explore the use of implementation science in clinical genomics, drawing on examples from the Australian health care system.

S. Best: None.

S07.3 Humanities and social sciences informing the implementation of genomics

Catherine Lejeune[1], *Charley Robert-Viard[1], Anne-Laure Soilly[2], Hamza Achit[3], Francis Guillemin[3], Valérie Seror[4]*

[1]Inserm CIC-EC 1432, Dijon, France, [2]DRCI CHU Dijon, Dijon, France, [3]Inserm CIC-EC 1433, Nancy, France, [4]IRD, APHM, SSA, VITROME, Marseille, France.

Context. Genomic sequencing has seen an increase in use in most developed countries, as illustrated by the implementation of national programs. For optimized routine use, data must be collected regarding stakeholders' viewpoints and the risks and advantages for individuals (for patients, families and health professionals) but also on a collective level (health system, payers). To explore these trade-offs, multidisciplinary studies are particularly useful. This type of approach takes equally into account the medical, economic, organizational, sociological, and psychological dimensions of genomics. None of these fields are considered as exclusive or having priority over the others.

Material and Methods: The DEFIDIAG project is one of the pilot studies of the French Plan for Genomic Medicine 2025. It illustrates the complementarity of various methodologies from the field of humanities and social sciences (HSS). DEFIDIAG is a prospective national multicenter study in which patients -children or adults with intellectual disability (ID) of unknown etiology - are their own controls. The main goal of the study is to compare the diagnostic performance (*via* the percentage of genetic causal diagnoses identified in ID patients) of trio whole genome sequencing analysis (WGS_T) compared to the French reference strategy (CGH-array, X-fra and genes panel). In addition to this clinical goal, a cost-effectiveness evaluation is planned as well as two impact studies: the first will estimate the cost of the diagnosis odyssey among previously investigated patients that could be avoided,

whereas the second one aims to assess the frequency and nature of changes in medical, medico-social and psychological follow-up in the first year after WGS results are disclosed. A qualitative study based on face-to-face interviews among parents will also explore their expectations, their emotional adjustment to the test results, and their perception of the future.

Conclusion: DEFIDIAG is a major opportunity for all branches of the scientific community to contribute their skills. This study is expected to participate to the implementation of genomic sequencing in France, while providing all the information needed to optimize the decision-making process, financing and how to best support patients and their families.

C. Lejeune: None. **C. Robert-Viard:** None. **A. Soilly:** None. **H. Achit:** None. **F. Guillemin:** None. **V. Seror:** None.

S08 Single cell genomics in cancer

S08.2 Early cancer development from a single-cell perspective: limitations, challenges and opportunities

Renee Beekman

CRG, Barcelona, Spain.

Due to the evolving single-cell technologies, the opportunities to study biological processes at the single-cell level are expanding rapidly. Those opportunities comprise among others, the identification of rare cell populations, detection of rare events and mapping heterogeneity in cell populations. At the same time, these technologies have their limitations, due for example to poor sensitivity, lack of information of multiple layers at the single-cell level, as well as the limited opportunities for the analysis of clonal expansion in non-engineered systems, such as human primary samples. I will address these advantages and disadvantages in the context of studying early cancer development.

Tumor formation is the result of stepwise accumulation of genetic and epigenetic lesions. In our lab, we specifically study the process of Non-Hodgkin lymphoma formation, which is a tumor arising from normal B lymphocytes. I will explain how we aim to study this process at the single-cell level using different layers of information: single-cell transcriptomics, chromatin accessibility and DNA methylation. I will address how increased sensitivity can be achieved and/or how studying heterogeneity of signatures rather than analysis of single genes or genomic regions can circumvent this limiting factor and lead to meaningful results. Furthermore, I aim to address which technologies are currently available to integrate different layers of information, focusing on the layers mentioned above, together with somatic mutations and cell surface marker expression.

Overall, in my talk I will present an overview of the opportunities we see and the challenges we face to study oncogenesis from the single-cell perspective and the solutions that single-cell technology and data-analysis tool development provide to better understand this complex process.

R. Beekman: None.

S09 Biobanks in under-represented populations

S09.3 Ethics and inclusivity when working with Indigenous populations

Emma Kowal

Deakin University, Melbourne, Australia.

As precision medicine increasingly becomes part of routine health care, many countries are concerned about equity. It is well recognized that our existing health systems are less accessible, appropriate and effective for many disadvantaged populations. The transformation of our health systems by precision medicine provides an opportunity to address these disparities and enable the health benefits of precision medicine to be fairly distributed between population groups. Currently, the lack of participation in genomic research among non-European populations means that precision medicine is more likely to increase rather than narrow health disparities (Popejoy and Fullerton 2016), although various initiatives seek to change this (Bentley, Callier and Rotimi 2017). Indigenous/First Nations groups are the least represented in genomic research, and often suffer the most health disadvantage, particularly within settler colonial societies including the United States, Canada, Australia and New Zealand. Scholarship, commentary and guidelines produced by Indigenous scholars over the last decade or so make the following points: 1) Indigenous people experience ongoing dispossession, oppression and discrimination; 2) Indigenous people seek the restoration sovereignty over their lands, and sovereignty over their cultural and intellectual property; 3) Many Indigenous people have had negative experiences with scientific and medical research - and genetic research in particular - where they have been harmed rather than benefited from their participation; 4) Efforts to include Indigenous people in genomic research need to be controlled by Indigenous people themselves. These principles have important implications for establishing a research project and research questions; ethical review; negotiating consent; ownership, control and access to data; benefit sharing arrangements; and acknowledging contributions in publications. Some examples of Indigenous-led genomic research will be discussed.

E. Kowal: None.

S10 Gene-based therapy for inherited liver diseases

S10.1 AAV gene therapy for glycogen storage diseases

Dwight D. Koeberl

Duke University School of Medicine, Durham, NC, USA.

Glycogen storage disease (GSD) is caused by the deficiency of specific enzymes involved in the storage and retrieval of glucose, including glucose-6-phosphatase (G6Pase) in GSD type Ia. G6Pase deficiency in GSD Ia affects primarily liver and kidney, and causes severe hypoglycemia and related biochemical abnormalities. We developed gene therapy with adeno-associated virus (AAV) vectors to deliver G6Pase. AAV vectors were pseudotyped with novel AAV serotypes, such as AAV8, and demonstrated enhanced tropism for the liver. We demonstrated efficacy through reductions in glycogen storage and correction of associated biomarkers in G6Pase-knockout mice and GSD Ia dogs. The biochemical abnormalities in these models of GSD Ia provided robust endpoints for demonstrating efficacy following AAV vector administration, including the correction of hypoglycemia and hyperlipidemia, lactic aciduria, G6Pase deficiency, and glycogen accumulations in the liver and kidney. We demonstrated that an AAV vector encoding human G6Pase reversed these biochemical abnormalities and prolonged long-term survival in mice and dogs with GSD Ia. We have identified abnormalities of autophagy that drive the pathobiology of GSD Ia, and showed that new small molecule therapies to reverse these abnormalities. Currently we are evaluating small molecule therapy and AAV vector mediated genome editing for GSD Ia.

GSD type II (Pompe disease) results from the deficiency of acid alpha-glucosidase (GAA) and causes primarily muscle disease. Enzyme replacement therapy (ERT) has become available for several lysosomal storage disorders over the past two decades;

however, the limitations of ERT have become increasingly evident, especially in Pompe disease. Pompe disease is characterized by the massive accumulation of lysosomal glycogen in striated muscle with an accompanying disruption of cellular functions. While ERT has prolonged the survival of infantile-onset Pompe disease patients, no curative therapy is available. Important limitations of ERT include the formation of anti-GAA antibodies, and poor uptake of GAA in muscle. We demonstrated that AAV vector-mediated gene therapy will likely overcome limitations of ERT, including formation of anti-GAA antibodies and the need for frequent infusions. Currently we are evaluating liver depot gene therapy in a clinical trial enrolling adults with late-onset Pompe disease.

D.D. Koeberl: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Sangamo Therapeutics, Inc., Pharming, Askbio, Moderna. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Takeda, Moderna, Askbio. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Askbio. F. Consultant/Advisory Board; Modest; Moderna, Askbio.

S10.2 mRNA replacement therapy for inborn errors of liver metabolism

Paolo Martini

Moderna Inc., Cambridge, MA, USA.

mRNA therapy for inborn error of liver metabolismPaolo G.V. Martini, PhD.

Messenger RNA, as a therapeutic agent, is becoming a promising paradigm to address several different diseases including infectious diseases with the now approved vaccines for Covid 19 as well as demonstrated in several animal models of diseases, in other therapeutic areas such as oncology, cardiovascular, rare genetic liver metabolic disorders. While in vaccines, the immune recognition of exogenously injected mRNA seems to be an advantage, in rare genetic and metabolic disorders, modification of the mRNA are needed to render it less responsive to the immune system. At Moderna, our efforts have been focused on specific modification of the mRNA to bypass the immune system and to favor translation of an active protein to replace the defective one. Moreover, lipid nanoparticles, used to protect and deliver mRNA in-vivo, have been modified for maximum efficiency in transducing the target organs. mRNA also offers the flexibility of combination of different mRNA sequences simultaneously, which will translate in-vivo, multiple proteins creating, in some instances, very stable complexes. We will discuss mRNA as therapeutic for Propionic acidemia which highlight the combination of 2 mRNA subunits in a single drug to translate a PCCA-PCCB hexadodecamer complex with significant impact on the phenotype of an animal model of disease

P. Martini: A. Employment (full or part-time); Significant; Moderna Inc.

S11 Prevention, detection and therapy in cancer

S11.3 Therapeutic vulnerabilities from epigenetic alterations in cancer

Nada Jabado

McGill University and the Research Institute of the McGill University Health Centre, Montreal, QC, Canada.

High grade gliomas in children and young adults are a failure of normal development. As such, they have a paucity of targets for therapy, and several remain intractable, especially at relapse or when metastatic. Previous data we acquired suggest that the hotspot histone mutations we identified lead to stalled development, which is at the root of their oncogenic processes. Importantly, there is exquisite temporo-spatial specificity of several of the drivers identified in these cancers, indicating a major role of altered developmental processes and the impact of the tumour microenvironment in their genesis and progression. We will review how oncohistone-mediated tumourigenesis is promoted through the maintenance of early progenitor states at the expense of differentiation in defined cellular and developmental contexts. We will describe the effects on the epigenome and how co-option of major signaling pathways involved in brain development fuel tumour formation and can be possibly targeted at the bedside.

N. Jabado: None.

S12 Functional annotation of genomic variation

S12.1 DNA methylation episignatures in Mendelian neurodevelopmental disorders

Bekim Sadikovic

Wester University, London, ON, Canada.

One functional consequence of genetic defects in patients with hereditary neurodevelopmental disorders is the disruption of genomic DNA methylation. There is a rapidly growing number of genetic disorders that exhibit DNA methylation "episignatures" or "EpiSigns" as highly sensitive and specific DNA methylation biomarkers. In this talk I describe how EpiSigns can be used for diagnosis of patients with a broadening range of neurodevelopmental genetic conditions, as well as effective functional tool for resolving ambiguous genetic test findings or clinical phenotypes.

B. Sadikovic: None.

S13 Beauty of gametogenesis

S13.1 New insights into aneuploidy in mammalian embryos

Melina Schuh

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

The Schuh lab studies meiosis in mammalian oocytes. In her presentation, Melina will summarize her lab's recent research on the spindle in mammalian oocytes. Her lab found that human oocyte spindles are surprisingly unstable, and identified functions for actin and a liquid-like meiotic spindle domain for spindle assembly in mammalian oocytes. In the main part of her talk, she will present recent work from her lab that sheds light on the origin of high aneuploidy rates in mammalian embryos. The vast majority of human embryos are aneuploid. Aneuploidy frequently arises during the early mitotic divisions of the embryo, but the origin of this remains elusive. Using bovine embryos as a model for human embryos, we identify an error-prone mechanism of parental genome unification which often results in aneuploidy. Surprisingly, genome unification initiates hours before breakdown of the two pronuclei that encapsulate the parental genomes. While still within intact pronuclei, the parental genomes polarize towards each other, in a process driven by centrosomes, dynein, and microtubules. The maternal and paternal chromosomes eventually cluster at the pronuclear interface, in direct proximity to each other. Parental genome clustering often fails however,

leading to severe chromosome segregation errors, incompatible with healthy embryo development. Nucleoli, which associate with chromatin, also cluster at the pronuclear interface in human zygotes. Defects in nucleolar clustering correlate with failure in human embryo development, suggesting a conserved mechanism.

M. Schuh: None.

S14 Genome architecture

S14.1 Unraveling the sequence of the centromere

Karen H. Miga[1], *T 2. T. Centromere Satellite Working Group*[2], *T 2. T. Consortium*[3]

[1]*UCSC Genomics Institute, Santa Cruz, CA, USA,* [2]*https://sites.google.com/ucsc.edu/t2tworkinggroup/who-we-are/centromere-satellite-working-group, Bethesda, MD, USA,* [3]*https://sites.google.com/ucsc.edu/t2tworkinggroup, Bethesda, MD, USA.*

Despite their essential role in ensuring proper chromosome segregation during cell division, the millions of bases that define endogenous human centromeres remain absent from most reference assemblies and are detached from high-resolution sequence-based studies aiming to understand their function. Here we present a highly accurate genetic and epigenetic reference of all human centromeric regions, representing 188.2 Mb, or 6.1% of the CHM13 genome, and the first comprehensive characterization of peri/centromeric satellite arrays, novel transposable elements, and centromere-associated genes. In doing so, we are able to reveal new patterns of satellite array organization, homogenization, and complex inversions. Centromeric sequences are expected to vary in repeat composition and copy number between individuals in the population. To study the extent of this variation, we report an initial variation map of centromeric regions, including array-specific structural variants, repeat copy number estimates, and centromere-spanning linkage maps. This high-resolution genetic reference provides an unparalleled opportunity to study sites of transcription, spatial organization, and sequences that are coincident with centromere formation, defined as the interface with centromere-specific chromatin (CENP-A, B, C). In total, this work offers the first high-resolution genomic and epigenetic study of all human centromeric regions, and it establishes a foundation for future studies of centromere genomic evolution, structure, and function.

K.H. Miga: None. **T.2.T. Centromere Satellite Working Group:** None. **T.2.T. Consortium:** None.

S14.2 De novo assembly of human genomes

Adam Ameur

SciLifeLab, Uppsala University, Uppsala, Sweden.

Due to advances in long-read sequencing, de novo assembly of high-quality human genomes has gone from being a huge technical challenge to a routine exercise. From the resulting assemblies, we gain new insights about the genomic architecture in an individual human DNA sample without being restricted to a reference sequence. On a population scale, we assembled the genomes of two human individuals representing the Swedish population and demonstrated that the resulting de novo assemblies can be used to improve the analysis of Swedish 1000-genomes reference dataset. On an individual level, the latest long-read technologies have enabled us to assemble near-complete personal genome sequences at a relatively modest cost, something that opens up for new applications in clinical diagnostics and precision medicine. Most recently, we demonstrated the first de novo genome assemblies of single human cells. To achieve this, we constructed a new workflow for whole genome amplification and coupled this with downstream long-read sequencing. This method was applied to CD8+ T-cells from a human donor, and resulted in an assembly size of up to 600Mb with 12.8% of human genes completely resolved. Further, we were able to assemble complete mitochondrial genome sequences and even to identify mitochondrial heteroplasmy between T cells originating from different clones. In conclusion, we demonstrate that long-read genome assembly can be performed not only at a species, population, or individual-level, but also for single human cells.

A. Ameur: None.

S14.3 Genetic architecture of autism

Evan Eichler

University of Washington & HHMI, Seattle, WA, USA.

The last decade has witnessed tremendous advances in sequencing technology to understand the full spectrum of human genetic variation and its relationship to disorders associated with mental health. The talk will present the laboratory's most recent work based on sequencing and analysis of over 60,000 families with autism and developmental delay. The talk will focus on the impact of rare, high-impact variants to our understanding of the genetic architecture of neurodevelopmental delay. I will show how forms of both inherited and sporadic mutations have helped to identify new genes associated with both syndromic and non-syndromic forms of autism. The available data suggest that multiple rare, privately inherited genetic variants contribute significantly to autism disease risk. This oligogenic architecture provides an alternate paradigm distinct from monogenic and polygenic models of disease for characterizing more complex genetic forms of these disorders and argues for a more complete analysis of the genetic variants present in families.

E. Eichler: None.

S15 Cells competing cells - mosaicism and cancer

S15.2 Mechanisms of cell competition in pluripotent stem cells

Miguel Torres*, Jose A. Valverde-López, Lin Li-Bao*

Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.

Cell Competition promotes the active elimination of otherwise viable cells when confronted with cells with a fitness advantage. Cell Competition is envisioned as a conserved and widespread cell quality control mechanism that eliminates less fit or misplaced cells during embryonic development and tissue homeostasis. During tumour progression, depending on the context, cell competition may protect the organism by eliminating cells with oncogenic mutations, or promote the expansion of the oncogenic cells by elimination of the surrounding normal cells. Mammalian pluripotent cells in the early embryo or in culture undergo natural Cell Competition according to Myc levels. In this model, Cell Competition surveils the maintenance of pluripotency at the population level by elimination of Myc-low differentiation-primed cells (losers) through confrontation with Myc-high naive pluripotent cells (winners). Molecular analysis of this model of Cell Competition identifies a p53-regulated pathway essential for the

fitness comparison between winner and loser cells. We find that the expression of BH3-family proteins NoxA and Puma, controlled by P53, regulate competitive fitness in mouse ES cells. We propose a model that integrates the p53 pathway and Myc in the definition of the loser-cell fitness "status" and suggests that a reduction in mitochondrial OXPHOS function regulated by P53-mitochondrial Puma underlies competitive fitness in pluripotent cells. In addition, to understand how Myc levels are regulated during cell competition, we identified the genomic regions involved in regulation of Myc transcription in pluripotent cells. Currently, we are investigating whether the enhancer activity of the identified regions is relevant in Cell Competition and during oncogenic Myc activation in adult tissues.

M. Torres: None. **J.A. Valverde-López:** None. **L. Li-Bao:** None.

S16 ESHG-ASHG Building Bridges: Global genetics towards a socially just practice

S16.3 Global genetics towards a socially just practice: a view from Africa

***Jantina de Vries**, Department of Medicine*

University of Cape Town, Cape Town, South Africa.

Recent years have seen a significant increase in the number of genomics studies conducted on the African continent and those activities have brought into sharp focus the importance of promoting science equity to ensure the involvement of African research participants and researchers is just. In this presentation, I will draw on concepts such as epistemic justice to sketch a picture of what science equity looks like for African genomics and associated practices such as data sharing, what the key impediments are to achieving it, and how science equity in African genomics can be fostered.

J. de Vries: None.

S17 Biases in genetic studies: Estimation and impact

S17.1 Biases in GWAS and where to find them: detecting and accounting for biases in GWAS studies beyond population structure

Nicola Pirastu

Usher Institute, University of Edinburgh, Edinburgh, United Kingdom.

In the last 15 years, genome-wide association studies (GWAS) have enabled scientists to better understand the role of genetics in determining complex traits while illuminating their underlying biology.The fact that individual genetic variants mostly have very small effects has resulted in the use of ever-larger sample sizes, with the biggest studies including millions of participants.The massive increase in power has resulted in the increase of the number of discovered associated variants from tens to thousands, and biobanks with hundreds of thousands of participants such as UK biobank or MVP have become fundamental tools in any geneticist toolbox.For a long time, we have worried mostly about the biases arising from cryptic population structure and many methods have been developed to account for it.However, with the increase in sensitivity, we have found that false associations due to other types of biases have started to appear. Generally, the effects of biases in GWAS are relatively small, however, they are similar in size to the smallest real effects we are able to detect, making the interpretation of the results more complex. This has even more important consequences and implications for methods where the effects sizes considered are even smaller than in GWAS, such as genetic correlations or Mendelian Randomization studies.

In my talk, I will examine some of the possible sources and types of biases that have been described in literature beyond population structure, such as collider bias, participation bias and reporting bias. I will examine their potential consequences, review some of the solutions which have been proposed, and what we need to implement them in future genetic studies.

N. Pirastu: None.

S17.2 Estimating direct and indirect genetic effects on birth weight

Nicole M. Warrington

University of Queensland, Brisbane, Australia.

Estimation of the direct genetic effect of an individuals' own genotype on their phenotype, independent of any contaminating indirect parental genetic effects, is becoming increasingly important. These conditional estimates are of interest in their own right, but are also useful for downstream analyses such as intergenerational Mendelian randomization or genetic correlation analyses. Until recently, such downstream methodologies have been hampered by the paucity of epidemiological cohorts with large numbers of genotyped parent-offspring pairs. We have developed a suite of methods to estimate asymptotically unbiased direct and indirect genetic effects on offspring outcomes, using individual level data, summary results data and 'virtual' parents. These methods can account for sample overlap, even when the overlap is unknown. Estimates obtained from these methods can subsequently be used in large-scale two-sample Mendelian randomization studies, such as those investigating the causal effect of maternal environmental exposures on offspring outcomes. An illustration of our methods will be presented, estimating the direct genetic effect on an individuals' own birth weight and the indirect parental genetic effects on offspring birth weight. These estimated conditional genetic effects are subsequently used in genetic correlation and Mendelian randomization analyses to investigate the relationship between birth weight and later life cardiometabolic disease. We show that without taking both the indirect and direct genetic effects into account in such Mendelian randomization analyses, the resulting causal estimate is biased in favour of a causal relationship.

N.M. Warrington: None.

S17.3 The nature of nurture

Patrick Turley

University of Southern California, Los Angeles, CA, USA.

Parental behavior can have major implications in how to interpret genetic studies. For example, if heritable child-rearing behaviors influence the children's behavior or health, SNPs associated with child-rearing will also be associated with these outcomes in children. Furthermore, when parents sort on heritable phenotypes, this can inflate genetic relationships and generate misleading results. We illustrate these sorts of confounds in several applications. First, we show that the predictive power of a polygenic score (PGS) for educational attainment (EA) is less predictive of a variety of health outcomes using a sibling design than in a population design with unrelated individuals. Second, we estimate the genetic correlation between family-based and population-based GWAS estimates and find that it is often

significantly smaller than one, especially for behavioral phenotypes. Third, we show that assortative mating inflates the genetic correlation between EA and several health phenotypes. Finally, we show that assortative mating produces spurious Mendelian Randomization estimates between EA and several outcomes. Together, these results highlight that care must be taken when interpreting population-based genetic studies, especially when they are related to behavioral phenotypes.

P. Turley: None.

S18 Overgrowth syndromes, from discovery to therapy

S18.2 Epigenetic signatures in overgrowth syndromes

Rosanna Weksberg[1,2]

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Every cell in our body has the same DNA. Yet different cell types in the body are able to develop because epigenetic marks, placed on top of the DNA, direct each cell type to read only specific sections of the DNA. There are several types of epigenetic marks including DNA methylation (DNAm), and histone modifications. These marks orchestrate normal developmental processes via the expression of several hundred epigenes (genes that function in epigenetic regulation). Rapid advances in genomic research have led to the identification of over 60 epigenes which are associated with Mendelian disorders, many of which are characterized by overgrowth and dysregulation of neurodevelopment. We and others have identified highly specific and highly sensitive genome-wide DNAm signatures for several of these epigenes including DNMT3A, NSD1, EZH2/EED, and CHD8; mutations in these genes are associated with Tatton-Brown-Rahman, Sotos, Weaver, and Autism syndromes, respectively. These DNAm signatures can be used as a "second-tier" analysis to improve molecular diagnostics by classifying sequence variants of uncertain significance (VUS) as either benign or pathogenic with efficacy far superior to most in silico programs. They can also be used for first-tier diagnostics when sequencing is uninformative. However, given the fact that DNAm signatures are still in development, DNAm-based disorder classification in this situation must be confirmed by sequencing to authenticate a molecular diagnosis. In addition to the utility of DNAm signatures for molecular diagnostics, we have also shown that gene-specific DNAm signatures reflect the functional pathophysiology of the associated genetic disorders providing opportunities to identify potential novel therapeutic targets.

R. Weksberg: None.

S18.3 Regional overgrowth

Leslie G. Biesecker

NIH, NHGRI, Bethesda, MD, USA.

Regional overgrowth associated with somatic mosaic variants raises a host of challenges for the managing clinician, including clinical diagnosis, molecular testing, clinic-molecular diagnosis, symptomatic management, and targeted therapy. Clinical diagnosis, the mainstay of the practicing clinician has not fundamentally changed. The objective is to match the pattern presented by the patient to a recognized entity through the differential diagnosis process. The difficulty is that mosaicism leads to an enormous range of expressivity that taxes the process. This can be mitigated with molecular testing, yet this process as well is challenging, due to ambiguities in patient sampling, potentially low rates of variant allele fraction (VAF), and test sensitivity and breadth. Integrating clinical and molecular findings into a final clinico-molecular diagnosis is challenging but can be made more tractable and rational by adopting a dyadic approach to diagnosis – recognizing that the molecular findings and clinical presentation are of equal weight and must be melded into a unitary diagnosis. Finally, management is challenging again due to the enormous inter-patient variability due to distinct affected genes, sometimes variable damaging or activating variants, and dissimilar distributions of VAF across tissues with wildly varying pathologic effects. The risks and benefits of treatment must be weighed against all these factors with shockingly little clinical trials data upon which to base such decisions. Yet, the clinician and patient must decide and act and these decisions require not only technical acumen, but wisdom. These larger issues surrounding mosaic overgrowth disorders will be reviewed with selected examples and illustrations to guide the practitioner challenged by these remarkable and often devastating disorders.

L.G. Biesecker: C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; Arqule Inc (Now owned by Merck, Inc.). F. Consultant/Advisory Board; Modest; Illumina Corp medical ethics board.

S20 Counselling Over Various Informatic Devices: Lessons from Covid-19

S20.1 The patient perspective

Jennifer Jones*, Amy Hunter*

Genetic Alliance UK, London, United Kingdom.

Genetic Alliance UK is a national charity working to improve the lives of patients and families affected by all types of genetic, rare and undiagnosed conditions. We are a membership organisation made up of over 200 patient groups and we aim to unify their voice around key policy issues that affect them. During the COVID-19 pandemic, Genetic Alliance UK set up the Covid-19 Information Hub (https://covid-19.geneticalliance.org.uk/). The Hub provided links to organisations providing condition specific information and updates on relevant government guidelines and legislation. They also provided practical information and links to help with the challenges of self-isolating and social distancing. The organisation hosted weekly virtual community check-ins where patient groups could raise concerns or issues, share information and work together as a community to meet the challenges of COVID-19. In June 2020, Genetic Alliance UK launched a patient experience survey to gather evidence about living with a rare condition and accessing healthcare; this included a section on the impact of the pandemic on people with rare, genetic or undiagnosed conditions.

An online survey of 102 questions was carried out with people living with or caring for someone with a rare condition. The survey instrument used a mixture of close-ended questions with pre-defined response categories and open-ended questions where respondents could supply qualitative information in text boxes. In total 1,503 people started the survey but those who did not consent to take part or indicated that they lived outside the UK were excluded. All remaining open-ended answers were included for the qualitative analysis (n=1,433). For the quantitative analysis respondents were only included if they completed more than two thirds of the survey ($n = 1{,}020$). Just under two thirds of respondents said their care had been impacted by the pandemic; around 1 in 5 said they missed hospital appointments as they were concerned about going into hospital. Many described feelings of isolation and abandonment which exacerbated their

experiences before the pandemic; others welcomed the use of telemedicine to avoid travelling for long distances to attend appointments.

There are many lessons to be learned from listening to the voices of those affected by rare conditions during the pandemic and they represent valuable opportunities to improve healthcare during the pandemic and beyond.

J. Jones: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Alexion. **A. Hunter:** None.

S20.2 Telemedicine in Genetics Services

Sofia Douzgou

Haukeland University Hospital, Bergen, Norway.

Telemedicine is defined as the delivery of healthcare services where distance is a critical factor, by all health care professionals, using technology, exchanging valid clinical or research information and in the interests of advancing the health of individuals and their communities (World Health Organization 2010). Telegenetics is the branch of telemedicine that uses an internet connection and web-based applications for clinical genetics services. The role of genomics within healthcare is rapidly expanding and it is increasingly impacting other branches of clinical practice. Better use of technology and data is a prerequisite for supporting and enabling this key development. Moreover social distancing was one of the main infection prevention and control measure during the COVID-19 crisis and this led to increased use of remote genomic clinical services. Many services transitioned to (a) virtual (remote) Clinics, (b) virtual Multidisciplinary meetings and (c) remote (home) working. This talk will focus on telegenetics initiatives working to transform IT and data systems to ensure the best services for patients; engage with patients about the role of genomics in healthcare; deliver genomics education to healthcare professionals.

S. Douzgou: None.

S21 Delivering the promise of RNA therapeutics

S21.2 Lessons learnt from the "DMD saga": from genetics to clinical trials

Annemieke Aartsma-Rus

Leiden University Medical Center, Leiden, Netherlands.

Dystrophin connects the cytoskeleton of muscle fibers to the extracellular matrix and thus provides stability to muscle fibers during contractions. Mutations (mostly deletions of one or more exons) that disrupt the reading frame of the dystrophin encoding *DMD* gene lead to non-functional dystrophin proteins and a severely progressive diseases, Duchenne muscular dystrophy (DMD). By contrast mutations that maintain the reading frame allow the production of internally deleted but partly functional dystrophins and are associated with the milder Becker muscular dystrophy. The aim of the exon skipping therapy is to enable DMD patients to produce Becker-like dystrophins. This is achieved by modulation of pre-mRNA splicing, where an antisense oligonucleotide (AON) hides an exon from the splicing machinery so it is skipped. This will enlarge the deletion, but restore the reading frame thus allowing dystrophin production. AON mediated exon skipping and dystrophin restoration has been shown in DMD patient-derived cell cultures over 20 years ago and is currently an approved therapy for DMD in the USA and Japan. However, this route was and is not without hurdles. In this lecture the presenter will outline the challenges of developing a mutation specific approach for a rare disease with very limited clinical trial expertise. She will also highlight the importance of interacting with patient advocates during drug development as well as bilateral education of the different stakeholders (patients, regulators and researchers). While DMD is used as a paradigm, these principles apply also to other rare diseases.

A. Aartsma-Rus: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Alpha Anomeric, Eisai, Italpharmaco, Silence Therapeutics, PTC Therapeutics, AstraZeneca, Santhera, Audentes. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Sarepta Therapeutics. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; PTC Therapeutics, BioMarin. F. Consultant/Advisory Board; Modest; Silence Therapeutics, Sarepta Therapeutics.

S21.3 Patisiran in clinical practice - Experience from the UK National Amyloidosis Centre

Julian D. Gillmore

University College London, London, United Kingdom.

Objectives: The purpose of this study was to determine the effect of patisiran on the cardiac amyloid load as measured by cardiac magnetic resonance and extracellular volume (ECV) mapping in cases of transthyretin cardiomyopathy (ATTR-CM).

Background: Administration of patisiran, a TTR-specific small interfering RNA (siRNA), has been shown to benefit neuropathy in patients with hereditary ATTR amyloidosis, but its effect on ATTR-CM remains uncertain.

Methods: Patisiran was administered to 16 patients with hereditary ATTR-CM who underwent assessment protocols at the UK National Amyloidosis Centre. Twelve of those patients concomitantly received diflunisal as a "TTR-stabilizing" drug. Patients underwent serial monitoring using cardiac magnetic resonance, echocardiography, cardiac biomarkers, bone scintigraphy, and 6-min walk tests (6MWTs). Findings of amyloid types and extracellular volumes were compared with those of 16 patients who were retrospectively matched based on cardiac magnetic resonance results.

Results: Patisiran was well tolerated. Median serum TTR knockdown among treated patients was 86% (interquartile range [IQR]: 82% to 90%). A total of 82% of cases showed >80% knockdown. Patisiran therapy was typically associated with a reduction in ECV (adjusted mean difference between groups: -6.2% [95% confidence interval [CI]: -9.5% to -3.0%]; $p = 0.001$) accompanied by a fall in N-terminal pro-B-type natriuretic peptide concentrations (adjusted mean difference between groups: -1,342 ng/l [95% CI: -2,364 to -322]; $p = 0.012$); an increase in 6MWT distances (adjusted mean differences between groups: 169 m [95% CI: 57 to 2,80]; $p = 0.004$) after 12 months of therapy; and a median reduction in cardiac uptake by bone scintigraphy of 19.6% (IQR: 9.8% to 27.1%).

Conclusions: Reductions in ECV by cardiac magnetic resonance provided evidence for ATTR cardiac amyloid regression in a proportion of patients receiving patisiran.

J.D. Gillmore: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Alnylam Inc. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness);

Modest; Alnylam, Eidos,. F. Consultant/Advisory Board; Modest; Alnylam, Eidos, Ionis, Intellia.

S22 Integrated approaches for ciliopathies

S22.1 Primary cilia in health and disease

Lotte B. Bang Pedersen

University of Copenhagen, Copenhagen, Denmark.

Primary cilia are dynamic microtubule-based organelles that project from the surface of most vertebrate cell types. Long thought to be evolutionary remnants of limited physiological relevance, it is now clear that primary cilia function as essential cellular signalling hubs during development and in tissue homeostasis. Well-studied examples of signalling pathways operating via primary cilia include the Sonic hedgehog (Shh) pathway and signalling mediated by polycystin 1 and 2 (PC1 and PC2), which regulate development and function of the brain, kidney and other organs and tissues. In addition, specialised primary cilia present on our photoreceptors, inner ear hair cells, and the olfactory epithelium are required for vision, hearing and smell, respectively. Not surprisingly, mutations affecting the assembly or function of cilia are associated with a growing number of diseases and syndromes, known as ciliopathies, which can affect most organs and tissues in the body. The assembly of primary cilia occurs in tight coordination with the cell cycle. Specifically, ciliogenesis is initiated in G1/G0 as the centrosome migrates towards the plasma membrane where the mother centriole docks and templates extension of the ciliary microtubule axoneme. The ciliary axoneme is surrounded by a membrane that is continuous with the plasma membrane, but contains distinct lipids and receptors involved in signalling. Establishment and maintenance of the cilium as a specialised signalling compartment depends on the intraflagellar transport (IFT) machinery as well as the transition zone (TZ), which separates the basal body from the cilium itself and controls ciliary protein entry and exit. Notably, many ciliopathies are caused by mutations in genes coding for TZ components, but disease-causing mutations have also been identified in other genes such as those coding for components of the IFT machinery or specific centrosomal proteins. For example, several studies have shown that inactivating variants of the gene coding for centrosomal protein CEP78 are associated with cone-rod dystrophy with hearing loss (CRDHL), a particular phenotype distinct from Usher syndrome. However, the molecular mechanism underlying this phenotype is unclear.

I will first provide an overview of primary cilia and associated signalling processes, with focus on the molecular mechanisms that regulate ciliogenesis. Next, I will present new results from my lab that dissects the mechanism by which CEP78 regulates cilia assembly and length, in turn providing molecular insight into how inactivating mutations in CEP78 lead to CRDHL. Our work also uncovers a new interaction between CEP78 and CEP350, thereby identifying the latter as a putative novel candidate for CRDHL.

L.B. Pedersen: None.

EDUCATIONAL SESSIONS

E01 New Technologies

E01.2 Detection of structural variation and haplotype-aware genome assembly through Strand-Seq

Ashley D. Sanders

MDC-BIMSB/BIH/Charité, Berlin, Germany.

Our genomes are constantly mutating. During normal development and ageing, genetic mutations can arise in our somatic cells, which are then propagated and clonally selected over time. How somatic mutations impact the function of our tissues and emerge in the context of disease are important questions in human health. Structural variants (SV) are a major source of somatic mutation - they are the most common cancer driver mutation type, they can arise in bursts mediating large-scale genomic rearrangements, and they can disrupt key molecular and cancer-related pathways. How somatic SV contribute to cellular heterogeneity and other complex diseases is still unclear, largely because detecting somatic SVs is challenging. Indeed, subclonal copy-neutral and complex rearrangements remain largely intractable to genetic variation studies. We overcome this by developing novel methods that harness the power of single-cell and single-strand sequencing (i.e. Strand-seq) to measure somatic mutational landscapes in human cells. By integrating complementary data layers, including read depth, template strand and haplotype phase, we can now reliably discover SV in a cell-by-cell basis and explore their functional impact in mutated cells. This advance has lead us to uncover somatic SV types that previously escaped detection and directly measure complex rearrangement processes, such as breakage-fusion-bridge cycles and subclonal chromothripsis events. Our ability to now map SV landscapes with single cell resolution positions us to study dynamic clonal expansions, genetic mosaicism and structural rearrangement processes with a new lens, all of which can provide a clearer picture of the processes behind development, ageing, and disease.

A.D. Sanders: None.

E01.3 Sequencing Genome Organization

Gabriele Girelli[1], Joaquin Custodio[1], Tomasz Kallas[1], Federico Agostini[1], Erik Wernersson[1], Bastiaan Spanjaard[2], Ana Mota[1], Solrun Kolbeinsdottir[1], Eleni Gelali[1], Nicola Crosetto[1], ***Magda Bienko***[1]

[1]Karolinska Institute, Stockholm, Sweden, [2]Max Delbrück Center, Berlin, Germany.

In the nucleus of eukaryotic cells, a significant portion of the genome consists of lamina-associated domains, which are preferentially located at the nuclear periphery. However, how the remaining parts of the genome are exactly radially organized in the nucleus remains largely unknown. Here, I will describe a method named Genomic loci Positioning by Sequencing (GPSeq), which allows genome-wide measurements of the distance to the nuclear lamina. Using GPSeq, we generated reproducible maps of the radial organization of the genome in human cells, at various resolutions, that revealed radial gradients of genomic and epigenomic features, gene expression, as well as A/B compartments and chromatin loops. We assessed the contribution of various features in predicting radiality, and found GC-content to have the strongest predictive power at all resolutions. Finally, we show that, by combining GPSeq radial information with Hi-C intra- and trans-chromosomal contacts, we are able to build whole-genome structure predictions much more accurately than possible thus far. We conclude that GPSeq is able to reveal fundamental aspects of genome architecture.

G. Girelli: None. **J. Custodio:** None. **T. Kallas:** None. **F. Agostini:** None. **E. Wernersson:** None. **B. Spanjaard:** None. **A. Mota:** None. **S. Kolbeinsdottir:** None. **E. Gelali:** None. **N. Crosetto:** None. **M. Bienko:** None.

E01.4 Single-cell multiomics data reveals extensive epigenome remodeling during cortical development

Boyan Bonev

Pioneer Campus, Helmholtz Zentrum München, Munich, Germany.

Despite huge advances in stem-cell, single-cell and epigenetic technologies, the precise molecular mechanisms that determine lineage specification remain largely unknown. Applying an integrative multiomics approach by combining **single-cell RNA-seq, single-cell ATAC-seq together with cell-type-specific DNA methylation and 3D genome measurements**, **we systematically map the regulatory landscape in the mouse neocortex in vivo**. Our analysis identifies thousands of novel enhancers and their target genes, as well as key neuronal transcription factors associated with extensive changes in chromatin accessibility, DNA methylation and 3D looping. We provide evidence that although epigenetic remodeling generally precedes transcriptional activation, true priming appears limited to a subset of lineage-determining enhancers. Finally, we discover a novel role for the transcription factor Neurog2 in mediating DNA demethylation, increasing chromatin accessibility and facilitating chromatin looping in vivo. Our work provides the first global view of the gene-regulatory logic of lineage specification in the cerebral cortex across multiple epigenome layers.

B. Bonev: None.

E02 ESHG-Y: Human organoids as genetic disease models

E02.1 Human stem cells-based organoids for personalized disease modelling in human genetics

Hans Clevers

Hubrecht Institute, Utrecht, Netherlands.

Stem cells are the foundation of all mammalian life. They come in two flavors. **Embryonic stem cells** are briefly present in the early human or mouse embryo, a few days after fertilization. These stem cells can be grown indefinitely in the lab and have the potential to build each and every tissue in our body. ES cells hold great promise in the field of regenerative medicine. **Adult stem cells**. Every organ in our body harbors its own dedicated stem cells. These adult stem cells replace tissue that is lost due to wear and tear, trauma and disease. Adult stem cells can only produce the tissue in which they reside. The adult stem cells allow us to live 80-90 years, but this comes at a cost: they easily turn into cancer. Both types of stem cells can be used to establish 'organoids', 3D structures established in a dish, that recapitulate many aspects of the original organ -including its diseases.

H. Clevers: None.

E03 Translational collaborations in hereditary cancer

E03.1 The CANGEN-CANVAR Programme: Delivering better tools for prevention and early detection in hereditary cancer syndromes

Clare Turnbull[1,2], *Jem Rashbass*[3], *Eva Morris*[4], *Antonis Antoniou*[5], *Paul Pharoah*[6], *Richard Houlston*[1], *Marc Tischkowitz*[7,8], *Gareth Evans*[9,10], *Emma Woodward*[11,12], *Diana Eccles*[13,14], *Claire Foster*[15], *Kate Tatton-Brown*[16,17], *Katie Snape*[17], *Nina Hallowell*[18], *Ingrid Slade*[19]

[1]*Division of Genetics and Epidemiology, Institute of Cancer Research, Sutton, United Kingdom,* [2]*Cancer Genetics Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom,* [3]*National Cancer Registration and Analysis Service, Public Health England, London, United Kingdom,* [4]*Big Data Institute, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom,* [5]*Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom,* [6]*Department of Public Health and Primary Care, Department of Oncology, Cambridge Cancer Centre, University of Cambridge, Cambridge, United Kingdom,* [7]*East Anglian Medical Genetics Unit, Cambridge University Hospitals NHS Trust, Cambridge, United Kingdom,* [8]*Department of Medical Genetics, National Institute for Health Research, Cambridge Biomedical Research Centre, University of Cambridge, Cambridge, United Kingdom,* [9]*Manchester Centre for Genomic Medicine, Manchester Academic Health Science Centre, Division of Evolution and Genomic Medicine, University of Manchester, Manchester, United Kingdom,* [10]*St. Mary's Hospital, Manchester Universities NHS Foundation Trust, Manchester, United Kingdom,* [11]*Manchester Centre for Genomic Medicine and NW Laboratory Genetics Hub, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom,* [12]*Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Health Innovation Manchester, Manchester, United Kingdom,* [13]*Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom,* [14]*Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton, Southampton, United Kingdom,* [15]*Macmillan Survivorship Research Group, School of Health Sciences, Faculty of Environmental & Life Sciences, University of Southampton, Southampton, United Kingdom,* [16]*St. George's University of London, London, United Kingdom,* [17]*Department of Clinical Genetics, St. George's University Hospitals NHS Foundation Trust, London, United Kingdom,* [18]*Ethox Centre, Nuffield Department of Population Health, University of Oxford, Sutton, United Kingdom,* [19]*Ethox Centre, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom.*

Background: Identifying individuals with inherited genetic cancer susceptibility prevents cancer and saves lives through effective targeting of resource for enhanced screening and/or prevention to those at highest a priori risk. Recent advances in sequencing technology have catalysed dramatic expansion in genetic cancer susceptibility testing, with imminent roll-out of population-level screening for cancer susceptibility. Vast amounts of data are being generated from individuals with different prior risks of inherited cancer susceptibility. It has become increasingly challenging how best we make value of the clinical data generated interpret and communicate the significance of variants (mutations) and associated genetic risk, and ensure the interventions offered are commensurate to risk, consistent and evidence-based. Supported by a CRUK Catalyst Award, through the 5-year CanGene-CanVar Programme (2019-2024) we seek to develop tools and resources to occupy this 'Translational Gap', spanning six distinct work-packages.

Workpackages

1) DATA COLLECTION AND LINKAGE: Aggregation of historic and prospective pan-UK data from clinical laboratory from testing for variants (mutations) in CSGs; linkage to multiple cancer datasets and hospital episode statistics (HES) datasets to produce linked life-course datasets.

2) VARIANT INTERPRETATION: Development of a national cancer variant interpretation group (CanVIG-UK) and data system (CanVar-UK), housing the national accumulated CSG variant counts, multiple functional datasets, analyses from machine-learning and consensus UK variant interpretations.

3) CLINICAL GUIDANCE: In partnership with NHS England, development of focused, dynamic, responsive national clinical management protocols for cancer susceptibility based on best currently available data. Workflows will involve horizon-scanning,

literature review, responsive data analyses and clinical consultation.

4) PATIENT DECISION AIDS: Patient-facing research using nominal group techniques leading to development of a patient-facing Clinical Decision Aids around risk and intervention options.

5) EDUCATION OF CLINICIANS: In partnership with Health Education England (HEE), development of hub-and-spoke educational resources on genetic susceptibility and communication of risk, including MOOCs (Massive Open Online Courses).

6) ETHICS: An ethical overview of this field, focused in particular on attitudes and hurdles to data-sharing.

C. Turnbull: None. **J. Rashbass:** None. **E. Morris:** None. **A. Antoniou:** None. **P. Pharoah:** None. **R. Houlston:** None. **M. Tischkowitz:** None. **G. Evans:** None. **E. Woodward:** None. **D. Eccles:** None. **C. Foster:** None. **K. Tatton-Brown:** None. **K. Snape:** None. **N. Hallowell:** None. **I. Slade:** None.

E03.2 Boosting hereditary cancer research with the European Reference Network GENTURIS

Nicoline Hoogerbrugge

Radboud university medical center, Nijmegen, Netherlands.

Approximately 27-36 million patients in Europe have one of the ~ 5.000-8.000 known rare diseases. The majority of them having a genetic origin. These patients often do not receive the care they need or they have a substantial delay from diagnosis to treatment. Currently twenty-four European Reference Networks (ERNs) are active with the aim to improve care and research for these patients. Our aim is that through the ERNs, European patients with a rare disease get access to expert care more often and more quickly, and that research will be accelerated resulting in improved diagnostics and therapies.

The ERN on Genetic Tumour Risk Syndromes (ERN GENTURIS) aims to improve research on identification, genetic diagnostics, prevention of cancer, and treatment of European patients with a genetic predisposition for cancer. The ERN GENTURIS focuses on syndromes such as hereditary breast cancer, hereditary colorectal cancer and polyposis, neurofibromatosis and more rare syndromes e.g. PTEN Hamartoma Tumour Syndrome, Li Fraumeni Syndrome and hereditary diffuse gastric cancer. Collaboration and teaming up has shown to be essential for making progress in developing new knowledge for rare diseases such as genetic tumour risk syndromes. The ERN GENTURIS research policy, successful collaborative projects as well as opportunities for European researchers will be discussed.

N. Hoogerbrugge: None.

E04 Dealing with uncertainty in genomic medicine

E04.1 Managing uncertainty in clinical genomics: toward a systematic approach

Paul K. J. Han

National Cancer Institute, Bethesda, MD, USA.

Uncertainty is a pervasive and important problem in clinical genomics and all of medicine, and managing uncertainty is one of the most challenging tasks for both the providers and recipients of health care. The objective of this presentation is to outline a systematic approach to this task that may help clinicians and patients manage uncertainty more effectively. This approach begins with understanding the nature, causes, and psychological effects of the uncertainties that arise in clinical genomics, and the various strategies people use to manage these uncertainties. It ultimately requires promoting uncertainty tolerance—which I define as the capacity to achieve an optimal balance of responses to uncertainty. I discuss key aspects of this capacity and explore potential ways of promoting uncertainty tolerance among clinicians and patients.

P.K.J. Han: None.

E04.3 Coping with uncertainty of genomic testing in a non-genetic setting

Anthony S. Wierzbicki

Guy's & St Thomas Hospitals, London, United Kingdom.

The identification of genetic disorders forms part of the work of lipid and cardiovascular disease (CVD) risk clinics. However, it forms only part of their workload which also comprises diagnosis and management of patients deemed at high CVD risk, those with non-alcoholic fatty liver disease (NAFLD) or intolerance to lipid-lowering drugs (commonly statins). In the UK the National Health Service (NHS) plan has prioritised the detection and management of monogenic familial hypercholesterolaemia (FH) with the aim being to diagnose 25% of cases by 2025. Classically FH presents with high cholesterol and an autosomal dominant family history of either hypercholesterolaemia or premature CVD. Genetic testing for FH includes screening for a panel of common genes and in the future polygenic risk scores. A number of strategies have been devised to try to identify potential index cases. Clinical scoring methods such as the Simon Broome criteria, Dutch Lipid score or the new FAMCAT calculator are useful but often confounded by other common conditions such as NAFLD, post-menopause lipid changes, familial combined hyperlipidaemia and extreme diets. Pedigree data is often difficult to acquire or vague which limits the utility of electronic health record searches. One way to clarify which patients to select for genetic testing is the use of imaging as FH is distinguished by an excess atheroma burden for age and gender. Alternatively, screening can be conducted in children where the clearest differentiation between monogenic and polygenic hyperlipidaemia occurs. Imaging is also useful to differentiate whether to treat patients with variants of unknown significance or those with elevated polygenic risk scores or other accelerants of atherosclerosis such as elevated lipoprotein (a). These approaches demonstrate that accurate phenotyping of patients is necessary to deal with the uncertainty associated with genetic testing in non-specialist settings.

A.S. Wierzbicki: None.

E05 Update on imprinting disorders

E05.1 Genetic basis of imprinting disordes

Thomas Eggermann

RWTH Aachen, Aachen, Germany.

Imprinting disorders comprise thirteen congenital disorders which are molecularly characterised by similar molecular alterations affecting the fine-tuned expression of genomically imprinted genes. Clinically, they share features from the same spectrum (growth, metabolism, pubertal timing, cognitive impairment, tumour predisposition). Their clinical overlap is mirrored by similar, in some disorders opposite molecular findings, affecting even the same imprinted loci. Four types of molecular alterations can affect the expression of imprinted genes, i.e. uniparental disomy, copy number variants and monogenic point mutations on

DNA level, and aberrant methylation (epimutations) at differentially methylated regions. Due to this heterogeneity, genetic testing of imprinting disorders is challenging, and the decision on the molecular testing strategy might be hindered. With the recent implementation of comprehensive molecular assays, the knowledge on the contribution of genetic and epigenetic alterations to the etiology of inherited growth disorders has massively expanded, making the interpretation of diagnostic data increasingly complex. These complex results lead to the discovery of unexpected and new molecular subgroups, as well as of genetic predispositions for epimutations. In the presentation, the different types of molecular alterations in patients with imprinting disorders and differential diagnosis will be reviewed, and genetic testing strategies will be illustrated. It will be emphasized that the early diagnosis of a genetically based endocrine disorder contributes to a precise management and helps the patients and their families in their self-determined planning of life. Furthermore, the identification of a causative (epi)genetic alteration allows an accurate prognosis of recurrence risks for family planning as the basis of genetic counselling. Asymptomatic carriers of pathogenic variants can be identified, and prenatal testing might be offered, where appropriate.

T. Eggermann: None.

E05.2 Diagnosis and treatment of imprinting disorders

Karen Temple

University of Southampton, Southampton, United Kingdom.

Imprinting is a natural, genomic regulatory process in humans that controls growth and development in utero. Epigenetic marks are laid down in the parental germline and maintained on the offspring's genome, affecting 35 clusters of imprinted genes throughout the genome. Consequently, these imprinted genes function differently on the maternal and paternal allele. 'Imprinting Disorders', which arise from dysregulation of imprinted genes, are a group of serious congenital conditions that affect metabolism, growth, development and behaviour, and predispose to cancer, significant short stature, obesity and the adult metabolic syndrome. 10 widely recognised imprinting disorders cluster around 5/6 key imprinted regions, but there is considerable variability in the clinical history, depending on the imprinted gene (s), mutations and parental alleles involved. •Prader Willi syndrome (PWS), Angelman syndrome (AS) - chromosome 15•Beckwith Wiedemann syndrome (BWS), Silver Russell syndrome (SRS) - chromosome 11 (also chromosome 7)•Temple syndrome (TS), Kagami Ogata syndrome (KOS) - chromosome 14•Transient neonatal diabetes mellitus (TNDM1), Maternal UPD 6 - chromosome 6•Pseudohypoparathyroidism type 1b (PHP1b), Mulchani-Bhoj-Conlin syndrome (MCBS) - chromosome 20

Early treatment and active health surveillance improves prognosis in these conditions that affect the lifecourse and yet there are a number of factors that contribute to late or missed diagnosis:-

i) many of the non-specific clinical features of imprinting disorders change with age making diagnosis difficult ii) heterogeneity and clinical overlap between conditions requires a broad diagnostic approach across many imprinted loci iii) standard diagnostic pathways that focus on genomic sequencing do not identify a significant proportion of molecular mechanisms underlying imprinting disorder siv) multi-locus imprinting disturbance (MLID) may affect the phenotype which can range from early miscarriage to less classical non-specific developmental delay and yet is not tested for routinely in most labs v) diagnostic genomic sequencing focuses on the patient and not the mother. Maternal mutations in a key network of oocyte-expressed genes can result in MLID in their offspring who do not share the mutations

This talk highlights diagnostic dilemmas and the importance of treatment in the long-term prevention of medical complications.

K. Temple: None.

E06 Pharmacogenomics in the clinic

E06.1 Pre-Emptive Pharmacogenetic Testing in Clinical Practice

Jesse J. Swen

Leiden University Medical Center, Leiden, Netherlands.

Retrospective, prospective and naturalistic studies all provide compelling evidence that genetic variation affects the way people respond to drugs. For several indications such as *DPYD* testing in oncology, PGx testing is routinely applied in clinical practice. In this educational setting, we will discuss basic principles of pharmacogenetics testing, show examples of how pharmacogenetics may be used to personalize drug treatment and outline opportunities and challenges for the field.

J. Swen: None.

E08 Variant interpretation in the clinic

E08.1 Using genomic resources to interpret the clinical significance of rare variants

Heidi L. Rehm

Massachusetts General Hospital and Broad Institute, Boston, MA, USA.

Over 800 million human genomic variants have been identified to date and we are far from discovering the full spectrum of human variation. Of those variants clinically interpreted and submitted to ClinVar, 75% have only been submitted by only one source underscoring the rarity of most human variation of potential clinical significance. As such, it is imperative that we work as a global community to aid the interpretation of genes and variants and their role in human health and disease. Before one can accurately interpret the pathogenicity of a variant, one must first curate the aggregate evidence to define a gene's role in disease. Multiple organizations, both public and private, have curated gene-disease relationships and these resources have recently been brought together for free and public access (search.thegencc.org) by the Gene Curation Coalition (GeneCC). Centralization of these curations allows comparison of claims and ultimately a more accurate resource to define valid relationships between genes and disease. Once the validity of a gene-disease relationship has been demonstrated, one can move on to curate individual variants in these genes to support or refute pathogenicity. Accurate variant classification is best facilitated through a multi-pronged strategy including 1) disseminating and applying consistent standards for variant classification, 2) submitting variant classifications and supporting evidence through a centralized database for sharing and comparing (e.g. ClinVar), 3) working to resolve discrepancies in variant classification between submitters, and 4) elevating the most challenging variants to international panels of experts with disease and gene-specific expertise. This talk will review the resources to support gene and variant curation and define progress in generating a global knowledgebase to understand the role of variation in human disease and support the application of genetic and genomic testing in the clinic.

H.L. Rehm: None.

E08.2 Towards better interpretation of variants in non-coding regions of the genome

Nicola Whiffin

Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Current clinical genetic testing approaches focus almost exclusively on the regions of the genome that code directly for protein but find a genetic diagnosis in only around half of all rare disease patients. Interpreting the effect of individual variants outside of these regions is difficult, given we have no regulatory equivalent to the amino acid code. Increasingly, however, whole-genome sequencing is being used as standard for genetically undiagnosed patients and non-coding region variants are being identified with important roles in rare disease. This talk will discuss challenges and opportunities in better interpretation of non-coding region variants, specifically focussing on (1) identification and functional characterisation of variants in 5'untranslated regions that cause a loss-of-function and lead to severe disease, (2) developing tools and resources to aid annotation of these variants in sequence data, and (3) moving towards consensus guidelines to support interpretation of non-coding region variants.

N. Whiffin: None.

E09 What's new in preimplantation genetic testing?

E09.1 Status of PGT and embryo selection in the era of NGS

Antonio Capalbo

Igenomix, Rome, Italy.

Aneuploidy is extremely common in human embryos and is one of the main causes of unsuccessful in vitro fertilization (IVF) cycles. In particular, advanced maternal age patients experience high rates of implantation failure and miscarriages. Aneuploidy in embryos mostly originates from oocyte meiotic segregation errors. Preimplantation genetic testing for aneuploidy (PGT-A) is a screening strategy employed in IVF cycles that is aimed at improving implantation and ongoing pregnancy rates per transfer through the identification and de-selection of aneuploid embryos. PGT-A implementation has rapidly grown in recent years and it is currently performed in over 20% of US IVF cycles. Two main technical developments contributed to this expansion: a) the implementation and standardization of an effective and generally safe trophectoderm (TE) biopsy approach at the blastocyst stage, b) the development of comprehensive testing platforms for the analysis of all 24 chromosomes in single-cell samples ($\simeq$ the typical TE biopsy consisting of 2-10 cells). In particular, the establishment of high-throughput, automated NGS protocols for PGT-A resulted in an unprecedented reduction of costs, allowing an increasing number of couples undergoing IVF to benefit from embryo aneuploidy testing. Although not universally applicable, comprehensive 24-chromosomes screening and single gene disorder detection can be combined in a single NGS run. Furthermore, NGS analytical capabilities were also extended to the detection of ploidy level alterations (i.e., haploid or polyploid configurations), which occur in about 1% of human blastocysts and are missed by standard NGS analysis. Remarkably, NGS has been suggested as improved technology for the diagnosis of chromosomal mosaicism in TE biopsies. When applied on controlled mixtures of fibroblast cell lines mimicking different rates of mosaicism, NGS showed increased analytical sensitivity toward intermediate chromosomal copy number values (CNV). However, the translation of algorithms developed on these stable and reproducible experimental models has not been as effective and straightforward for deriving clear diagnostic criteria when applied to embryo biopsy specimens. The use of subjective thresholds for mosaicism diagnosis has generated a critical inconsistency in detection rates reported across laboratories, with values ranging from 2 to 40%. Furthermore, the positive predictive value of a mosaicism diagnosis in PGT-A is extremely low. Among all putative mosaic embryos transferred worldwide to date (>2000), the same mosaicism finding identified at the preimplantation stage was confirmed through prenatal diagnosis (PND) only in one case. On the contrary, the high rate at which mosaicism is currently reported has shown undeniable issues in the clinical management of PGT-A cycles. These include extra costs associated with genetic counseling, anxiety, and confusion in patients and doctors due to diagnostic uncertainty, new and probably unnecessary indications for invasive PND, and more dramatically, a significant abandonment of viable embryos. Considering that all data currently available on the reproductive competence of putative mosaic embryos are retrospective and present biases at several levels (i.e., population selection), there is an urgent need for prospective non-selection studies. These trials will provide evidence-based estimates of the clinical utility of reporting intermediate chromosome CNV in NGS-based PGT-A.

A. Capalbo: A. Employment (full or part-time); Modest; Igenomix.

E11 Polygenic risks and me

E11.2 Polygenic risks in disorders of aging

Valentina Escott-Price

Cardiff University, Cardiff, United Kingdom.

Polygenic risk score (PRS) is used as a global term for a risk score including any number of genetic variants (SNPs). A major problem with disorders of aging when using PRS to categorise people at risk is the age of the study participants. In other polygenic diseases like schizophrenia, penetrance of the phenotype is mostly complete at 40 years of age, while for e.g. Alzheimer's disease (AD), even at 80 there are still individuals at risk but who have not yet developed AD. In addition, about 35% of life-time risk of dementia is modifiable by factors such as education, vascular aspects, nutrition, health care and social deprivation, etc., which has potentially led to the decrease in incidence of dementia over the last decades. The most common type of dementia, Alzheimer's disease, mainly affects the elderly population. As a consequence, comparative control samples are likely to be enriched with future AD cases who are yet to show symptoms. AD, like many other disorders of ageing, is a progressive condition which means that clinical features develop gradually over many years before diagnosis. The ability to predict disease risk before disease onset is of great importance for stratifying people for clinical trials or the selection of candidates for functional experimental studies. For AD, with the exception of *APOE*-ε4, the common genome-wide significant variants which have been discovered though GWAS have only small individual effects. Although it is clear that many genes are involved in disease development and progression, there is no agreement in the field as to whether AD is a polygenic or oligogenic disorder. We investigate various methodologies and SNP selection approaches for risk prediction. We highlight and discuss the best strategies for polygenic profiling when assessing individuals for AD risk.

V. Escott-Price: None.

E12 Bayesian methods applied in clinical settings

E12.1 How are the causes of complex disease distributed in the human genome?

David Balding

University of Melbourne, Melbourne, Australia.

I will review recent progress in using genome-wide SNPs to assess how the heritability for complex human traits is distributed across the human genome, and how it varies across traits. In particular we look at the relationship between heritability and a wide range of genome annotation features, as well as linkage disequilibrium and minor allele fraction (MAF), focusing on new results for rare variants. The relationship between MAF and heritability is informative about the effects of negative or purifying selection, for different traits and in different genome regions. I will also discuss how the heritability models that arise from our work can be used to improve genomic prediction.

D. Balding: None.

E13 Mapping the human body at the cellular level

E13.1 Deciphering liver regeneration cell-by-cell

Dominic Grün

Julius-Maximilians-University, Würzburg, Germany.

The human liver is an essential multifunctional organ, and liver diseases are rising with limited treatment options. To better characterize the cell type architecture of the human liver, we applied single-cell RNA-seq to normal human liver specimens and established a liver cell type atlas covering all known human liver cell populations. Our analysis revealed previously unknown subtypes among endothelial cells, Kupffer cells, and hepatocytes with transcriptome-wide zonation of some of these populations. We discovered heterogeneity of the $EPCAM^{+}$ population, which comprises hepatocyte-biased and cholangiocyte populations as well as a $TROP2^{int}$ progenitor population with strong potential to form bipotent liver organoids. As proof-of-principle, we utilized our atlas to unravel phenotypic changes in hepatocellular carcinoma cells and in human hepatocytes and liver endothelial cells engrafted into a mouse liver. To better understand the plasticity and differentiation pathways of liver cell populations during regeneration, and to elucidate the role of inter-cellular crosstalk in this process, we complement our human liver studies with the analysis of mouse liver damage models. Our human liver cell atlas provides a powerful resource enabling the discovery of previously unknown cell types in the normal and diseased liver.

D. Grün: None.

E13.2 Decoding the developing human immune system

Muzlifah Haniffa

Newcastle University, Newcastle Upon Tyne, United Kingdom.

Muzlifah has used functional genomics, comparative biology and single cell RNA sequencing to study human mononuclear phagocytes. In this seminar, she will demonstrate the applications of single cell genomics to decode the developing human immune system.

M. Haniffa: None.

E14 DNA methylation in Mendelian diseases

E14.1 Epivariations in the human genome

Andrew Sharp

Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Allelic changes in DNA methylation that result in gene silencing, often termed epivariations, have been recognized in a number of conditions, including imprinting disorders, CGG repeat expansions, and in a growing number of monogenic diseases. Despite this evidence, screening for epivariations is rarely performed, and very few studies have attempted to profile the contribution of epivariations to the burden of genetic disease. Our lab has recently undertaken the first large scale studies of epivariations in humans, and has investigated the methylomes of >25,000 individuals to date. Importantly, these studies show that (i) epivariations occur relatively frequently in the human genome, (ii) represent a subset of pathogenic alleles at known disease-associated genes, and (iii) would be missed by purely sequence-based approaches. I will give an overview of the current state of knowledge of the involvement of epivariations in human disease, including their potential underlying molecular causes, and insights that epivariations can provide into the regulatory architecture of the genome.

A. Sharp: None.

E15 Selection and population structure in biobank scale data

E15.2 Genetic population structure and its consequences in biobank scale data

Daniel J. Lawson

University of Bristol, Bristol, United Kingdom.

Population stratification has been well examined over the years and has some straightforward solutions at the genome-wide association-study (GWAS) level, namely principal component and linear mixed models. However, we can show that these are insufficient in at least two domains. The first is in meta-analysis, where population structure information contained in the full dataset is lost in individual meta-analysis cohorts. The second is in complex trait analysis, where the out-of-Africa bottleneck has systematically changed the frequency of variants in study populations, which is key a component of estimating selection on traits. We describe tools which make very simple changes to GWAS - providing additional meta-data - which improve their performance. We further describe models for complex traits that show that correcting for genetic drift may change our understanding of the history of complex traits.

D.J. Lawson: None.

E16 Advances in Mendelian randomisation

E16.1 Using Mendelian randomization to assess molecular mediators in causal pathways

Rebecca Richmond

University of Bristol, Bristol, United Kingdom.

Understanding mediation is useful for identifying intermediates lying between an exposure and an outcome which, when intervened upon, will block (some or all of) the causal pathway

between the exposure and outcome. Mediation approaches have been adapted to understanding the role of molecular intermediates in situations of high-dimensional omics data with varying degrees of success. One approach which is increasingly being adapted to evaluate molecular mediation is Mendelian randomization (MR). MR uses genetic variants associated with a trait of interest to estimate the causal effect of that trait on a particular outcome. Within the context of mediation, MR can be used to decompose the effect of an exposure on an outcome which acts directly, and that which acts via mediating variables. This presentation will cover existing methods for applying MR to assess molecular mediation, including the use of multivariable and two-step MR, and approaches for estimating the mediated effects, including the "difference" and "product of coefficients" methods. The use of quantitative trait loci (QTL) as causal anchors in the context of molecular traits will be elaborated on, as well as the existing resources which can be drawn upon to acquire these data and integrate them in relation to the exposure and outcome of interest. The relative advantages and limitations of using MR to conduct mediation analysis and to evaluate molecular pathways will be outlined. To date, the approach has been applied to understanding the role of the DNA methylome, metabolome and proteome in relation to disease risk. A series of examples will illustrate the application of the methods and summarise the results obtained. Finally, the importance of corroborating findings from MR with other experimental, data-based and statistical approaches, each with orthogonal sources of bias, will be highlighted in order to best triangulate evidence for the role of molecular mediation.

R. Richmond: None.

E17 Chromosomal instability across lifetime

E17.2 Genomic instability in early embryonic development

Ewart W Kuijk

Center for Molecular Medicine and Oncode Institute, University Medical Center Utrecht, Utrecht, Netherlands.

A major challenge in mammalian development is to maintain genomic integrity of the rapidly dividing cells. A failure to maintain genomic integrity can lead to widespread genetic mosaicism, the presence of multiple genetic lineages within an individual. Genetic mosaicism may impact on human health causing for example neurodevelopmental disorders or increased cancer risk. Recent advances in whole genome sequencing of individual cells and small tissue samples have provided insights into the prevalence and the underlying mutational mechanisms that drive mosaicism in embryo and fetal development. This lecture will highlight some of the recent findings on this topic.

E. Kuijk: None.

E18 Introduction to statistical analysis of genome-wide association studies (GWAS)

E18.1 What, when, and whys of genome-wide association studies (GWAS)

Marika Kaakinen[1,2]

[1]*University of Surrey, Guildford, United Kingdom,* [2]*Imperial College London, London, United Kingdom.*

Genome-wide association studies (GWAS) were introduced almost two decades ago now and their continued application to date keeps advancing our understanding of the underlying genetic architecture of many complex traits. For example, they have enabled the discovery of more than 400 genomic loci for the complex disease of type 2 diabetes, providing such clues to its pathophysiology that could not have been achieved with candidate gene studies that were dominating, along with linkage studies, in the pre-GWAS era. While GWAS were first applied predominantly to samples from individuals of European ancestry, scientists have recently understood the importance of studying genetics in diverse ancestral backgrounds. Hence, new GWAS continue to be set up in previously underrepresented populations and learning about GWAS continues to be as topical as it was at their introduction in the beginning of the 2000s. In this educational talk I will discuss things that led to the need of GWAS and the pivotal projects, such as the HapMap project, and the necessary technological advances, that finally enabled GWAS. I will then follow to outline the steps in a usual GWAS, starting from quality control and finishing in reporting the results in a meaningful way. The purpose of this talk is to give an introduction to a scientist setting up their first GWAS or someone wishing to understand GWAS better, by providing an overview of GWAS and hopefully inspiring the audience to dive further into the fascinating world of GWAS.

M. Kaakinen: None.

E19 Precision medicine in underserved populations

E19.1 Improving precision medicine for breast cancer in Africa

Samuel Ahuno[1], *Moses Kamita*[2], *Thomas Ahearn*[3], *Shahin Sayed*[4], *Francis Makokha*[2], *Beatrice Wiafe*[5], *Joel Yarney*[6], *Baffour Awuah*[7], *Paz Polak*[8], *Montserrat Garcia-Closas*[3], ***Jonine Figueroa***[9], *The Ghana and Kenya Breast Health Studies*

[1]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [2]*Mount Kenya University, Thika, Kenya,* [3]*National Cancer Institute, Bethesda, MD, USA,* [4]*Aga Khan University, Nairobi, Kenya,* [5]*Peace and Love Hospital, Kumasi, Ghana,* [6]*Korle Bu Teaching Hospital, Accra, Ghana,* [7]*Komfo Anyoke Teaching Hospital, Kumasi, Ghana,* [8]*Icahn School of Medicine at Mount Sinai, New York, NY, USA,* [9]*University of Edinburgh, Edinburgh, United Kingdom.*

Despite lower incidence rates compared to European populations, African women are more likely to die from breast cancer, which is multifaceted, and its incidence is rising. Breast cancer and its clinical complications may be preventable by mitigating factors that trigger the disease process (primary prevention), the use of therapies that reduce the risk of disease before the clinical onset (secondary prevention) or more effective treatment of the disease subtype once diagnosed (Tertiary prevention/Precision medicine). Recent technological advances in genetics, genomics, metabolomics, proteomics, and bioinformatics now offer exciting opportunities for biomarker discovery in order to accurately predict risk, prognosis and response to treatment by informing clinical-decision-making. A disproportionate number of poor-prognosis tumours, including hormone receptor negative tumours which have fewer targeted treatments, has been observed in certain populations in Africa, particularly those of West African ancestry. Coupling this with the paucity of treatment facilities and resources, primary and secondary means of prevention are needed, particularly since the early ages at onset of many breast cancers result in high-associated disability and years of life lost.

In this seminar, I will review molecular epidemiology studies of breast cancer including biomarker studies of molecular subtypes, incidence rates and exciting data on liquid biopsies. With increasing number of molecular epidemiology studies, we aim to more comprehensively understand the combination of both genetic, lifestyle and environmental determinants of breast cancer

susceptibility and prognosis to improve precision prevention and medicine for women in Africa.

S. Ahuno: None. **M. Kamita:** None. **T. Ahearn:** None. **S. Sayed:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Research Grant GSK-ERI. **F. Makokha:** None. **B. Wiafe:** None. **J. Yarney:** None. **B. Awuah:** None. **P. Polak:** None. **M. Garcia-Closas:** None. **J. Figueroa:** None.

CONCURRENT SESSIONS

C01 Developmental disorders & syndromes I

C01.1 Impaired eIF5A function causes a novel developmental syndrome partially rescued in model systems by spermidine

*Víctor Faundes[1], Martin D. Jennings[1], Siobhan Crilly[1], Sarah Legraie[1], Sarah E. Withers[1], Sara Cuvertino[1], Sally J. Davies[2], Andrew G. L. Douglas[3], Andrew E. Fry[2], Victoria Harrison[4], Jeanne Amiel[5], Daphné Lehalle[5], William G. Newman[1], Patricia Newkirk[6], Miranda Splitt[7], Judith Ranells[6], Laura A. Cross[8], Carol J. Saunders[9], Bonnie R. Sullivan[8], Jorge L. Granadillo[10], Christopher T. Gordon[11], Paul R. Kasher[1], Graham D. Pavitt[1], **Siddharth Banka**[1]*

[1]University of Manchester, Manchester, United Kingdom, [2]Institute of Medical Genetics, Cardiff, United Kingdom, [3]University of Southampton, Southampton, United Kingdom, [4]Wessex Clinical Genetics Service, Southampton, United Kingdom, [5]Hôpital Necker Enfants Malades, Paris, France, [6]University of South Florida, Tampa, FL, USA, [7]Institute of Genetic Medicine, Newcastle upon Tyne, United Kingdom, [8]University of Missouri, Kansas City, MO, USA, [9]Children's Mercy, Kansas City, MO, USA, [10]Washington University School of Medicine, St. Louis, MO, USA, [11]Institut Imagine, Paris, France.

Introduction: The structure of proline prevents it from adopting an optimal conformation for rapid protein synthesis. Polyproline-tract (PPT) associated ribosomal stalling is resolved by the highly-conserved eIF5A, the only protein to contain the amino acid, hypusine, a post-translational modification essential for eIF5A function.

Methods and Results: We describe a novel disorder caused by *de novo* heterozygous *EIF5A* variants in 7 unrelated individuals resulting in variable combinations of developmental delay, microcephaly, micrognathia and dysmorphism. To investigate the mechanism, we studied *EIF5A* mRNA expression in patient cells and expressed mutated *EIF5A* proteins in yeast cells deleted for yeast-eIF5A to perform yeast growth assays, polysome profiling, assessed total/hypusinated eIF5A levels and assessed PPT-reporter protein synthesis. These studies revealed that the variants either resulted in haploinsufficiency, or impaired hypusination or perhaps reduced protein stability. All variants ultimately resulted in reduced eIF5A-ribosome interaction and impaired the synthesis of PPT-containing proteins. We also demonstrated that genes associated with microcephaly in humans are significantly enriched in PPTs. To explore potential treatment, we generated morpholino knockdown of *eif5a* in developing zebrafish that partly recapitulated the human phenotype. Both yeast and zebrafish defects caused by eIF5A variants were partially rescued by spermidine supplementation.

Conclusions: We describe a potentially treatable novel human disorder caused by *EIF5A* mutations that cause reduced eIF5A-ribosome interactions via mutation-specific mechanisms. The phenotypes are likely explained by impaired synthesis of specific PPT-rich proteins. These findings uncover the role of eIF5A, and proteins with PPTs, in human brain and craniofacial development.

V. Faundes: None. **M.D. Jennings:** None. **S. Crilly:** None. **S. Legraie:** None. **S.E. Withers:** None. **S. Cuvertino:** None. **S.J. Davies:** None. **A.G.L. Douglas:** None. **A.E. Fry:** None. **V. Harrison:** None. **J. Amiel:** None. **D. Lehalle:** None. **W.G. Newman:** None. **P. Newkirk:** None. **M. Splitt:** None. **J. Ranells:** None. **L.A. Cross:** None. **C.J. Saunders:** None. **B.R. Sullivan:** None. **J.L. Granadillo:** None. **C.T. Gordon:** None. **P.R. Kasher:** None. **G.D. Pavitt:** None. **S. Banka:** None.

C01.2 Genomic findings in bone blood paired DNA comparison of nonsyndromic craniosynostosis

Yiran Guo[1], *Christopher L. Kalmar[1], Xiaoyan Huang[1], Bo Zhang[1], Yuankun Zhu[1], Stephanie Stefankiewicz[1], Mateusz Koptyra[1], Jennifer Mason[1], Tatiana Patton[1], Elizabeth Appert[1], Lina Lopez[1], Catherine Sullivan[1], Anna R. Carlson[1], Mychajlo S. Kosyk[1], Zachary D. Zapatero[1], Phillip B. Storm[1], Jordan W. Swanson[1], Scott P. Bartlett[1], Joseph M. Serletti[2], Adam Resnick[1], Jesse A. Taylor[1]*

[1]Children's Hospital of Philadelphia, Phiadelphia, PA, USA, [2]Perelman School of Medicine at the University of Pennsylvania, Phiadelphia, PA, USA.

Objective: The purpose of this study is to elucidate genetic variants contributing to nonsyndromic craniosynostosis (CS) by comparing samples from abnormally fused bones, unaffected bones, and parent saliva, to those from patient peripheral whole blood (PWB).

Methods: We applied whole genome sequencing, then performed best practice genomic alignment and variant calling, trio joint genotyping for germline genomic variants, consensus somatic variant calling for PWB-bone comparisons, and variant annotation. Alternative allele frequencies, variant damaging predictions, and inheritance models were used to filter variants.

Results: The study included 109 DNA samples from 26 trios, in which 17 families have affected bone tissue DNA. Patients' affected bone samples were sequenced to an average depth of 112.7X with the rest biospecimens to 35.7X. On average, 2629 somatic variants were identified in the affected bones. After filtering, we identified 40 genes with somatic pathogenic/likely pathogenic (P/LP) variants. We also detected germline P/LP variants, mostly from de novo events. We confirmed known CS genes FGFR3 and IHH (both with germline de novo variants), and FREM1 (with a somatic variant in affected bone). From a single patient, we also discovered a germline de novo CHPF variant and a somatic CHPF variant in affected bone.

Conclusions: We identified a novel candidate CS gene CHPF in the same pathway as those required for bone development and digit patterning, which shows promise for further investigation.

Y. Guo: None. **C.L. Kalmar:** None. **X. Huang:** None. **B. Zhang:** None. **Y. Zhu:** None. **S. Stefankiewicz:** None. **M. Koptyra:** None. **J. Mason:** None. **T. Patton:** None. **E. Appert:** None. **L. Lopez:** None. **C. Sullivan:** None. **A.R. Carlson:** None. **M.S. Kosyk:** None. **Z.D. Zapatero:** None. **P.B. Storm:** None. **J.W. Swanson:** None. **S.P. Bartlett:** None. **J.M. Serletti:** None. **A. Resnick:** None. **J.A. Taylor:** None.

C01.3 Phospholipase C eta-1 (PLCH1): A new gene that causes Holoprosencephaly

Ichrak Drissi[1], *Emily Fletcher[1], Ranad Shaheen[2], Fowzan Alkuraya[2], Geoffrey Woods[1]*

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Holoprosencephaly is a spectrum of developmental disorder of the embryonic forebrain in which there is failed or incomplete separation of the prosencephalon into two cerebral hemispheres. To date, dominant mutations in sonic hedgehog (SHH) pathway genes are the predominant Mendelian causes and have marked inter- and intrafamilial phenotypic variability. Here, we describe two families in which offspring had holoprosencephaly spectrum and homozygous predicted-deleterious variants in *PLCH1*. Immunocytochemistry was used to examine the expression pattern of PLCH1 in human embryos. We used SHH as a marker of developmental stage and of early embryonic anatomy. In the first family, two siblings had congenital hydrocephalus, significant developmental delay, and a mono-ventricle or fused thalami with a homozygous *PLCH1* c.2065C>T, p.(Arg689*) variant. In the second family two siblings had alobar holoprosencephaly and cyclopia with a homozygous *PLCH1* c.4235delA, p.(Cys1079ValfsTer16) variant. All parents were healthy carriers, with no holoprosencephaly spectrum features. We found that the sub-cellular localisation of PLCH1 is cytoplasmic, but the p.(Cys1079ValfsTer16) variant was predominantly nuclear. Human embryo immunohistochemistry showed PLCH1 to be expressed in the notorcord, developing spinal cord, dorsal root ganglia, cerebellum, and dermatomyosome. Furthermore, the embryonic sub-cellular localisation of PLCH1 was exclusively cytoplasmic, supporting protein mis-localisation contributing to the pathogenicity of the p.(Cys1079ValfsTer16) variant. Our data supports the contention that PLCH1 has a role in prenatal mammalian neurodevelopment, and deleterious variants cause a clinically variable holoprosencephaly spectrum phenotype. This project has been funded with support from the 2017 Cambridge NIHR Cambridge Biomedical Research Centre award.

I. Drissi: None. **E. Fletcher:** None. **R. Shaheen:** None. **F. Alkuraya:** None. **G. Woods:** None.

C01.4 Clinical delineation, sex differences and genotype-phenotype correlation in pathogenic *KDM6A* variants causing X-linked Kabuki syndrome Type 2

Víctor Faundes[1,2], *S Goh*[2], *R Akilapa*[3], *H Bezuidenhout*[4,5], *H T. Bjornsson*[6,7], *L Bradley*[8], *A F. Brady*[3], *E Brischoux-Boucher*[9], *H Brunner*[10], *S Bulk*[11], *N Canham*[12], *D Cody*[8], *M L. Dentici*[13], *M C. Digilio*[13], *F Elmslie*[14], *A. E. Fry*[15], *H Gill*[8], *J Hurst*[16], *D Johnson*[17], *S Julia*[18], *K Lachlan*[19], *R R. Lebel*[20], *M. Byler*[20], *E Gershon*[21], *E Lemire*[22], *M Gnazzo*[23], *F R. Lepri*[23], *A Marchese*[24], *M McEntagart*[14], *J McGaughran*[25], *S Mizuno*[26], *N Okamoto*[27], *C Rieubland*[28], *J Rodgers*[25], *E Sasaki*[8], *E Scalais*[29], *I Scurr*[30], *M Suri*[31], *I van der Burgt*[32], *N Matsumoto*[33], *N Miyake*[33], *V Benoit*[34], *D Lederer*[34], *S Banka*[2]

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Introduction: Kabuki syndrome is one of the most recognisable multi-system congenital disorder. Type-2 Kabuki syndrome (KS2) is a highly variable X-linked condition caused by *KDM6A* variants, and is thought to be responsible for ~5% of KS cases. Its mutation spectrum and the genotype-phenotype correlations are poorly understood. Methods: Genetic and clinical details of new and published individuals with pathogenic *KDM6A* variants were compiled and analysed.

Results: 61 distinct pathogenic *KDM6A* variants (50 truncating, 11 missense) from 80 patients (34 males, 46 females) were identified. Missense variants clustered in the TRP #2, #3, #7 and Jmj-C domains. Truncating variants were significantly more likely to be *de novo*. Thirteen individuals had maternally inherited variants and one had a paternally inherited variant. Neonatal feeding difficulties, hypoglycaemia, post-natal growth retardation, poor weight gain, motor delay, intellectual disability (ID), microcephaly, congenital heart anomalies, palate defects, renal malformations, strabismus, hearing loss, recurrent infections, hyperinsulinism, seizures, joint hypermobility and gastroesophageal reflux were frequent clinical findings. Facial features of >1/3rd patients were atypical for KS. Males were significantly more likely to be born prematurely, have shorter stature and severe developmental delay/ID. We also highlight the overlaps and differences between the phenotypes of KS2 and KS1.

Conclusion: This largest-ever KS2 series expands the *KDM6A* mutation spectrum, delineates the KS2 phenotype and demonstrates its sex and variant-dependent variability. These results will improve diagnosis for patients with KS2, especially those with inherited missense variants. In future these results will inform the development of evidence-driven management guidelines in KS.

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C01.5 Mitochondrial dysfunction in *FOXG1* syndrome

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Introduction: *FOXG1*-syndrome, previously described as the congenital form of Rett syndrome (RTT) due to overlapping clinical features, is a distinct neurodevelopmental disorder caused by pathogenic *FOXG1* variants. Forkhead box protein g1 (FOXG1) is a transcription factor important for brain development and function and is expressed in the nucleus and the mitochondria. In rodents, nuclear FOXG1 enhances mitochondrial membrane potential and induces mitochondrial fission, while mitochondrial FOXG1 promotes mitochondrial fusion. As mitochondrial dysfunction contributes to RTT pathogenesis and as FOXG1 regulates mitochondrial dynamics and bioenergetics in rodents, we hypothesized that defective *FOXG1* may affect mitochondrial function in human.

Materials-Methods: Fibroblasts were obtained from five patients with different *FOXG1* truncating or missense variants and five control individuals. Mass, membrane potential and ROS of mitochondria were quantified using high-throughput microscopy, and ATP content with luciferase-assay. Mitochondrial morphology was investigated by confocal microscopy and machine learning (skeletonization). Gene and protein expression were determined with TaqMan-assay and western blot, respectively.

Results: We observed morphological changes in mitochondrial network complexity and a significant reduction in the mitochondrial content in patient fibroblasts compared to controls. Mitochondrial depletion was further reflected as a decrease in the respiratory capacity evident by a reduction in ATP production. While biogenesis and mitophagy appeared normal, mitochondrial fusion activity was lower in the patient fibroblasts.

Conclusion: Our thorough assessment of mitochondrial function suggests involvement of mitochondrial dysfunction in the pathogenesis of *FOXG1*-syndrome in human for the first time. Further investigations are necessary to elucidate how FOXG1 deficiency impairs mitochondrial homeostasis.

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C01.6 *SPEN* haploinsufficiency causes a neurodevelopmental disorder overlapping proximal 1p36 deletion syndrome with an episignature of X chromosomes in females

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Introduction: Deletion 1p36 (del1p36) syndrome is the most common human disorder resulting from a terminal autosomal deletion. This condition is molecularly and clinically heterogeneous. Deletions involving two non-overlapping regions, known as the distal (telomeric) and proximal (centromeric) critical regions, are sufficient to cause the majority of the recurrent clinical features, although with different facial features and dysmorphisms. *SPEN* encodes a transcriptional repressor commonly deleted in proximal del1p36 syndrome and is located centromeric to the proximal 1p36 critical region.

Materials and Methods: We used clinical data from 34 individuals with truncating variants in *SPEN* to define a neurodevelopmental disorder presenting with features that overlap considerably with those of proximal del1p36 syndrome.

Results: The clinical profile of this disease includes developmental delay/intellectual disability, autism spectrum disorder, anxiety, aggressive behavior, attention deficit disorder, hypotonia, brain and spine anomalies, congenital heart defects, high/narrow palate, facial dysmorphisms, and obesity/increased BMI, especially in females. *SPEN* also emerges as a relevant gene for del1p36 syndrome by co-expression analyses. Finally, we show that haploinsufficiency of *SPEN* is associated with a distinctive DNA methylation episignature of the X chromosome in affected females, providing further evidence of a specific contribution of the protein to the epigenetic control of this chromosome, and a paradigm of an X chromosome-specific episignature that classifies syndromic traits.

Conclusions: We conclude that SPEN is required for multiple developmental processes and *SPEN* haploinsufficiency is a major contributor to a disorder associated with deletions centromeric to the previously established 1p36 critical regions.

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C02 Cardiovascular disorders

C02.1 Sudden cardiac death due to ARVC in the young: molecular autopsy by whole exome sequencing of DNA from dried blood spots (DBS) collected at birth

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Background: The genetic investigation of sudden cardiac death (SCD) is often limited by access to DNA from the proband. We evaluated the feasibility of WES on DNA from archived dried blood spots (DBS) collected at the time of newborn screening.

Methods: Through National Health Registries, we identified all Swedish cases of SCD in the young (<35 y.o.a.) with a post-mortem diagnosis of ARVC between 2000 and 2010 ($n = 22$). Medical records, family history, and autopsy findings were collected. DNA was extracted from samples in National Biobanks including DBS collected at birth ($n = 19$), post-mortem formalin-fixed paraffin-embedded (FFPE) heart ($n = 8$), and frozen blood samples ($n = 3$). Patient and parental DNA samples (when available) underwent WES.

Results: Lower yield of DNA (7.3-140 ng) was obtained from DBS compared to FFPE (515-3065 ng). However, 100% of DBS vs. 62,5% of FFPE libraries passed QC. Average mean target depth of DBS samples (160X) was similar to blood (155X) and higher than FFPE (82X); and >97% of target regions were covered. Analysis uncovered clinically relevant variants in 12 out of 19 families (63%), of which four were located in ARVC genes and six in another arrhythmogenic syndrome gene. Additionally, we identified one case with hemochromatosis and one with myotonic dystrophy.

Conclusions: DNA from archived DBS is a reliable source for WES-based molecular autopsy with a high diagnostic yield in SCD. In a proportion of cases diagnosed post-mortem with ARVC, the molecular autopsy pointed to another diagnosis, highlighting its importance for risk stratification and adequate care of surviving relatives.

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C02.2 Clustering of the cardiac transcriptome of dilated cardiomyopathy patients reveals opposite molecular signatures among patients with truncating and missense *TTN* variants

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Introduction: Truncating variants in titin *(TTNtv)* are the most prevalent genetic cause of dilated cardiomyopathy (DCM). Here, we performed unsupervised clustering on the cardiac transcriptome of DCM patients to test whether the transcriptomic profile can detect *TTNtv* and whether missense variants in *TTN* (*TTNmiss*) show a comparable transcriptomic profile. **Methods:** RNA was isolated from cardiac biopsies of 92 DCM patients (30 *TTNtv*, 12 *TTNmiss*, 7 pathogenic *LMNA*, 9 other pathogenic variants, and 34 non-genetic). The mRNA-sequencing library was generated and sequenced on the NextSeq-500. The data of 58k transcripts was subjected to dimension reduction and graph-based clustering. Transcriptomic clusters were afterwards associated with GO-biological process and phenotype-genotype data.

Results: Six distinct transcriptomic clusters were identified, among which 3 clusters (C2, C3 and C5) formed a super-cluster which was significantly enriched by *TTN* variants. Cluster 3 (C3) was dominated by *TTNtv* and 3 *TTNmiss*, reflecting a strong upregulation of mitochondrial energy metabolism pathways. Cluster 5 constituted of 5 *TTNtv* and *3 TTNmiss* samples, which showed the opposite: downregulation of metabolic and mitochondrial energy pathways. Interestingly, *TTNtv* patients in C3 had a low left ventricular mass (LVmass) in contrast to patients in C5 which had small hearts with a high LVmass and hypertension.

Conclusions: Transcriptomic clustering revealed two distinct *TTNtv* patient-clusters which show two extremities of cardiac metabolism. Hypertension could be an important mediator in determining the pathophysiology and phenotype of *TTNtv* carriers. DCM patients with *TTNmiss* show similarities to *TTNtv* in the transcriptomic signature, potentially indicating both diagnostic and therapeutic value.

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C02.3 Heterozygous and homozygous Chkb (Choline kinase beta) deficiencies are associated with cardiomyopathy: Insights from a mouse model

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Introduction: The *CHKB* gene encodes choline kinase beta, which catalyzes the first step in the pathway for the major phospholipid phosphatidylcholine (PC). Homozygous loss of function variants in *CHKB* are associated with a congenital muscular dystrophy. Variable cardiac phenotypes are reported in half of known cases and are a major reason of early death. The precise role of *CHKB* in cardiac function is not known. Methods: Heart function of 20-week-old heterozygous and homozygous ($Chkb^{+/-}$ and $Chkb^{-/-}$) mice was assessed using echocardiography. The mice were studied for potential susceptibility towards ventricular arrhythmias using ECG recordings. Lipid profile of the cardiac muscle, mitochondrial oxygen consumption rate, and gene expression of relevant genes were determined.

Results: Unlike wild type mice, 60 percent of the $Chkb^{+/-}$ and all $Chkb^{-/-}$ mice displayed arrhythmic events when challenged with isoproterenol. Lipidomic analysis revealed that Chkb deficiency altered the profile of lipids in the heart. A major change was an increase in acylcarnitines in $Chkb^{+/-}$ and $Chkb^{-/-}$ hearts (1.68-fold and 3-fold respectively), and this was accompanied by a decreased capacity to utilize fatty acids for oxygen production. There was a reduction in the expression of ANP and ANP receptor genes as well as ventricular conduction system markers (HCN4 and Cx40) in both $Chkb^{+/-}$ and $Chkb^{-/-}$ mice.

Conclusions: Here we report for the first time that Chkb deficiencies result in altered cardiac lipid metabolism, decreased oxygen consumption, defects in the ventricular conduction system development, and increased susceptibility to ventricular arrhythmias. **Sources of Support:** Canadian Institutes for Health Research

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C02.4 Whole-exome sequencing 677 aneurysm patients identifies multiple rare variants in the proprotein convertase FURIN causing impaired TGFB family signaling

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Abdominal aortic aneurysms are common and occurrence increases with age, resulting in 5-8% of men and 1-2% of women above 65 years being affected. Age-related extracellular matrix remodeling and weakening of the aortic wall is caused by genetic predispositions and risks factors like hypertension and smoking. Although familial segregation studies have identified over 40 aneurysm genes, (likely)pathogenic variants only explain ~4% of cases. In this study we have performed whole-exome sequencing of 677 abdominal aorta aneurysm patients and identified 24 (3.5%) unrelated patients with 13 different rare heterozygous variants in *FURIN*, encoding the proprotein convertase FURIN. Of these 24 patients, 14 had multiple aneurysms and 7 a rupture or dissection. Thoracic aneurysm was observed in 6. More than half of the patients showed a range of extravascular connective tissue features, like atrophic scarring, hypermobility, scoliosis or a pectus excavatum. The steady-state protein levels, protease activity and shedding of recombinant FURIN variants were affected. The consequences for maturation of pro TGFβ1 by FURIN-mediated cleavage for downstream SMAD (SMAD2) and non-SMAD (MAPK) signaling, and expression of TGFβ1-responsive *ACTA2* and *COL4A1*, were variably impaired in patient fibroblasts, indicating that TGFβ/BMP family actions are dysregulated in these aneurysms. The range of effects of the recurrent missense pR745Q FURIN in fibroblasts of different patients reflects the influence of individual genetic backgrounds in aorta aneurysms.

Conclusion: Using WES of a large cohort of unrelated patients we identified rare variants of *FURIN* with high prevalence in aortic aneurysm. This study is funded by Stichting Lijf en Leven.

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C02.5 Clinical genetic risk variants inform a functional protein interaction network for tetralogy of Fallot

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Background: Tetralogy of Fallot (TOF), the most common cyanotic heart defect in newborns, has evidence of multiple genetic contributing factors. Identifying variants that are clinically relevant is essential to understand patient-specific disease susceptibility and outcomes, and could contribute to delineating pathomechanisms.

Methods and Results: We used a clinically-driven strategy and current guidelines to re-analyze exome sequencing data from 811 probands with TOF, focused on identifying rare loss-of-function and other likely pathogenic variants in congenital heart disease

(CHD) genes. In addition to confirming a major contribution of likely pathogenic variants in *FLT4* (VEGFR3; $n = 14$) and *NOTCH1* ($n = 11$), we identified 1-3 such variants in each of 21 other CHD genes, including *ATRX*, *DLL4*, *EP300*, *GATA6*, *JAG1*, *NF1*, *PIK3CA*, *RAF1*, *RASA1*, *SMAD2*, and *TBX1*. There were also three emerging CHD/TOF candidate genes with multiple loss-of-function variants in this cohort: *KDR* ($n = 4$), *IQGAP1* ($n = 3$), and *GDF1* ($n = 8$). In total, these variants were identified in 64 probands (7.9%). Using the 26 composite genes in a STRING protein interaction enrichment analysis revealed a biologically relevant network (p-value 3.3e-16), with VEGFR2 (*KDR*) and NOTCH1 representing central nodes. Variants associated with arrhythmias/sudden death and/or heart failure indicated factors that could influence long-term outcomes.

Conclusions: The results are relevant to precision medicine for TOF. They suggest considerable clinical yield from genome-wide sequencing, and further evidence for *KDR* as a CHD/TOF gene and VEGF and Notch signaling as mechanisms in human disease. Harnessing genetic heterogeneity of single gene defects could inform etiopathogenesis and help prioritize novel candidate genes for TOF.

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C02.6 Variants in extracellular matrix and G protein coupled receptor signaling pathways associated with severe supravalvar aortic stenosis in Williams syndrome

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Introduction: Williams Syndrome (WS) is caused by a 1.5-1.8 MB deletion on 7q11.23. About 15-20% of people with WS have severe supravalvar aortic stenosis (SVAS) requiring surgical intervention. In contrast, ~40% with WS have no SVAS. Little is known about the genetic features impacting SVAS severity. Conventional statistical approaches for modifier evaluation would require thousands of cases, making it infeasible for the study of rare diseases. Here we propose an alternative approach to identify disease modifiers in smaller cohorts.

Methods: We sequenced 450 genomes from individuals with WS, including those with severe ($n = 75$) and no ($n = 186$) SVAS. Variants were jointly called using GATK procedures, identifying ~132,000 non-synonymous variants. CADD phred scores were used to filter out variants with little potential pathogenicity as well as those with minimal frequency difference between severity groups. Geneset enrichment, followed by burden testing of candidate pathway was then performed.

Results: We identified 901 genes with 1032 non-synonymous variants with a difference in allele frequency between severe SVAS and no SVAS > 5% and CADD phred scores > 10. Among the top canonical pathways enriched, we identified the extracellular matrix (FDR $q = 7.19 \times 10^{-15}$, with particular enrichment of collagens and glycoproteins), and cell-matrix interactions (FDR $q = 4.58 \times 10^{-5}$). Genes related to G protein coupled receptor signaling (FDR $q = 9.02 \times 10^{-11}$) and those implicated in the response to viral infection (FDR $q = 1.39 \times 10^{-5}$) are also notable.

Conclusions: Variants in key pathways separate people with WS with severe *vs.* no SVAS. These pathways can be targeted to understand mechanism of disease and develop future therapies.

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C03 Bioinformatics, machine learning and statistical methods

C03.1 Improved prediction of complex traits from individual-level data and summary statistics

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Most existing tools for constructing genetic prediction models (polygenic risk scores) begin with the assumption that all genetic variants contribute equally towards the phenotype. However, this represents a suboptimal model for how heritability is distributed across the genome. To address this problem, we develop eight new prediction tools, each of which allows the user to specify the heritability model. Four of our new tools use individual-level data, while four use summary statistics. We compare individual-level data prediction tools by analyzing 14 phenotypes from the UK Biobank (200k individuals per phenotype). Our new tool LDAK-Bolt-Predict performs best, outperforming the existing tools Lasso, BLUP, Bolt-LMM and BayesR for each of the 14 phenotypes. We compare summary statistic prediction tools by analyzing 225 phenotypes from the UK Biobank (average sample size 285k). Our new tool LDAK-BayesR-SS performs best, outperforming the existing tools lassosum, sBLUP, LDpred and SBayesR for 223 of the 225 phenotypes. The increase in prediction accuracy from improving the heritability model tends to be substantial. For example, when using LDAK-Bolt-Predict, changing the heritability model increases the proportion of phenotypic variance explained by on average 14% (s.d. 2%), equivalent to increasing the sample size by about a quarter. Moreover, the advantage of our new prediction tools will increase further as more realistic heritability models are developed.

D. Speed: None.

C03.2 Removing confounders from facial representations trained on the biased patient images

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Introduction: The next-generation phenotyping technology for syndromology, such as GestaltMatcher, has enabled matching patients with ultra-rare phenotypes by the facial representations learned from thousands of patient photos. However, the current available patient photos are unbalanced in ethnicity and age. For example, most of the photos are from Caucasians and taken at an early age. It results in biased models when training on an unbalanced dataset. The model might learn the ethnicity instead of facial dysmorphic features to classify the disorders. We therefore demonstrate how to remove these biases when train on an unbalanced dataset.

Methods: We compiled an unbiased dataset consisting of 1,500 patients with ten different rare disorders with equal ethnicity distribution. For each individual, a frontal photo and the molecularly confirmed diagnosis were available. We utilized deep convolutional neural networks for training a model on patients' frontal photos, and the joint learning and unlearning (JLU) technique proposed by Alvi et al. was used to remove age and ethnicity bias during the model training.

Results: We first showed that training on a dataset with unbalanced ethnicity distribution resulted in a model predicting ethnicity instead of disorder. We then proved that the JLU removed the bias. Furthermore, we benchmarked on the balanced dataset on both the network with and without JLU.

Conclusion: This study proved that the adversarial networks such as JLU unlearned the bias and generalized better on facial dysmorphic features. With this method, we could improve the disease classification on the patients of minority class in this society.

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C03.3 Enhanced SpliceMap and RNA-seq from clinically accessible tissues improves outlier prediction for non-accessible tissues

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Introduction: Aberrant splicing is a major cause of genetic disorders. However, the affected tissues are often not easily accessible, preventing direct detection of many relevant aberrant splicing events in clinical diagnostics. Deep learning models like SpliceAI and MMSplice have improved prioritizations of rare variants found by whole genome sequencing (WGS). However, their performance on predicting aberrant splicing in any given tissue has not been assessed so far.

Materials and Methods: Here, we developed the first benchmark dataset for aberrant splicing prediction by applying the aberrant splicing caller FRASER on 6931 RNA-seq samples from 48 GTEx tissues. We constructed a tissue-specific splicing map (SpliceMap) improving annotations of the GENCODE annotation by excluding unexpressed splice sites and quantifying tissue-specific reference levels of alternative splicing. Next, we built new models combining i) state-of-the-art deep learning models together with SpliceMap for WGS-only diagnostics and ii) furthermore including RNA-seq from clinically accessible tissues (CATs) for integrated WGS and RNA-seq based diagnostics.

Results: At 20% recall, out-of-the-box application of existing deep learning models, yield a precision less than 2%. However, when combined with SpliceMap, we reached 20% precision. Integrating furthermore RNA-seq of CATs (blood and skin) led to 50% precision. These results replicated in an independent mitochondriopathy cohorts of WGS with matched RNA-seq samples.

Conclusions: Altogether, our approach significantly increases performances of predicting aberrant splicing events in clinically non-accessible tissues, reaching a sufficiently high precision to guide non-invasive genetic diagnostics.

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C03.4 Accounting for temporal information and family history improves power in genome-wide association studies

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Introduction: Family history, sex and age at diagnosis are informative variables that are often overlooked in genetic analysis such as GWAS, which typically restricts the analysis to the case-control binary phenotype. We aim to develop new method that provides a refined phenotype to improve power in GWAS by including information on these variables.

Materials and Methods: Here we present LT-FH++, a flexible method for estimating a refined phenotype for GWAS that accounts for family history, age at diagnosis and sex. We apply LT-FH++ to the iPSYCH and UK Biobank cohorts by using extra information from Danish health registers for iPSYCH, and data from the British Office for National Statistics for studying mortality in the UK Biobank. Results from the LT-FH++ model are compared with the liability threshold conditional on family history (LT-FH) model and case-control status.

Results: In simulations, LT-FH++ showed a power increase of up to 17.8% over LT-FH and 60.6% over case-control status. We further applied LT-FH++ to mortality in the UK biobank and four common psychiatric disorders (ADHD, autism, schizophrenia, and depression) available in the IPSYCH cohort. Across all analyzed traits, we found 20 independent genome-wide significant associations for LT-FH++, 10 for LT-FH and 8 for case-control status across all traits.

Conclusion: Linking genetic data with information on family history, sex and age at diagnosis can significantly increase power in GWAS. As more genetic data is linked with electronic health records, we expect methods such as LT-FH++ to become more beneficial and widely used.

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C03.5 The impact of copy number variants on complex human traits

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Copy number variations (CNVs) are potent phenotypic modifiers associated with several rare genomic syndromes, but their role in complex traits remains understudied. To fill this gap, we called CNVs in 331'522 UK Biobank participants. Association studies between the copy-number (CN) of CNV-proxy probes and 57 traits revealed 134 independent signals across 47 phenotypes. Some CNVs exhibited pleiotropy, with 26 and 19 traits associating with 16p11.2-BP4-BP5 and 22q11.2, respectively. Besides recapitulating well-established associations (e.g. 16p11.2-weight), we identified new ones. For instance, deletion of *PDZK1*, which encodes a urate transporter scaffold protein, decreased urate levels ($\beta_{deletion}$ = -48.3 µmol/L, p = 5.8e-13), with a 2.6-fold stronger effect in males (p_{diff} = 2.1e-3). Furthermore, many associations mapped to rare disease regions, suggesting variable expressivity and a broad impact of these loci in the general population: *SHOX* and its regulatory region are implicated in Leri-Weill dyschondrosteosis, which is characterized by short stature, confirming the positive effect of the region's CN on height (β = 2.3 cm, p = 7.2e-16); Heterozygous deletion of the region encoding the hepatic transporters OATP1B1/3 is associated with increased total bilirubin ($\beta_{deletion}$ = 3.1 µmol/L, p = 2.2e-13), whereas homozygous carriers affected with Rotor syndrome present with hyperbilirubinemia. Finally, our approach highlights new functionalities: The CN of the interval encompassing *MARF1*, a gene essential to murine oogenesis, correlated negatively with age at menarche (β = -0.6 years, p = 8.5e-15) and menopause ($\beta_{duplication}$ = -1.8 years, p = 1.7e-6), suggesting a conserved role in female reproductive timing. Together, the numerous identified associations suggest that CNVs play an important role in shaping complex traits and that their study can reveal new gene functionalities.

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C03.6 Cancer detection by mining large cell free DNA sequencing dataset

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Introduction: Cell free DNA (cfDNA) allows non-invasive diagnosis of cancer patients. Most of the work on cfDNA focused on the detection of tumor specific mutations. The targeted nature only provides partial view and requires knowledge of known tumor mutations. In contrast, shallow whole-genome sequencing (sWGS) of cfDNA allows to interrogate copy number aberrations (CNAs) in an unbiased way. We present here a cfDNA data mining approach that enables identification of cancer-specific signals in sWGS, irrespective of the detection of CNAs.

Methods: We collected cfDNA samples from 307 healthy controls and varying stages of hematological (178) and solid (331) tumors, including breast, colorectal, gastrointestinal and ovarian samples. sWGS was carried out and genome-wide read depth profiles were assessed. Principal component analysis transformed features were utilized for clustering. A machine learning model that incorporated the profile features was trained for cancer classification.

Results: Upon clustering analysis, different cfDNA profiles grouped into cancer type specific clusters that differentiated from the profiles of healthy controls. Using the trained classifier, the overall accuracy to discriminate cancer from healthy samples was 63.8%, resulting in sensitivities ranging from 20.75% to 91.01% at 95% specificity among different cancer types and stages. This feature-based classification outperformed CNAs only based analysis.

Conclusion: We developed a novel machine learning method based on large sWGS cfDNA datasets for unbiased classification of cfDNA profiles, enabling tumor detection and typing. The method could open new opportunities for cancer management.

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C04 Unraveling the complexity of neuropsychiatric genetics

C04.1 Identification of 13 independent genetic loci associated with cognitive resilience in healthy aging in 330,097 individuals in the UK Biobank

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Cognitive resilience is the ability to withstand the negative effects of stress on cognitive functioning and is important for maintaining quality of life while aging. UK Biobank (UKB) does not have direct measurements of cognitive ability at distal timepoints. Therefore, we used number of years in education (EY) as a proxy phenotype for past cognitive performance. Current cognitive performance was determined based on processing speed. This approach captured an average time span of 40 years between past and current cognitive performance in 330,097 individuals. A confounding factor was that EY is highly polygenic and masked the genetics of resilience. To overcome this, we employed Genomics Structural Equation Modelling (GenomicSEM) to perform a GWAS-by-subtraction using two GWAS, one GWAS of EY and resilience and a second GWAS of EY but not resilience. Subtracting one from the other generated a GWAS of resilience. Replication of this approach was shown using independent discovery and replication samples and the full GWAS results were examined further using functional genomics analysis. We found 13 independent genetic loci for resilience. Functional analyses showed enrichment in several brain regions and involvement of specific cell types, including GABAergic neurons ($P = 6.59x10^{-8}$) and glutamatergic neurons ($P = 6.98x10^{-6}$) in the cortex. Gene-set analyses implicated the biological process "neuron differentiation" ($P = 9.7x10^{-7}$) and the cellular component "synaptic part" ($P = 2.14x10^{-6}$). Mendelian randomization analysis showed a causative effect of white matter volume on cognitive resilience. To our knowledge, this is the first GWAS of cognitive resilience in a large dataset. These results enhance neurobiological understanding of resilience.

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C04.2 Genetic Clustering of Repetitive Symptoms, Oppositional Defiant Disorder and Language Disorder and Delay in People with Autism: A Multivariate Genetic Variance Analysis of Genetic Relationship Matrices in the SPARK Sample

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Background: Autism spectrum disorders (autism) are complex, heritable and highly heterogeneous neurodevelopmental conditions. Common genetic variation contributes to the majority of autism liability and there is mounting evidence for considerable genetic heterogeneity. Here, we investigate whether clusters of co-occurring autism symptoms vary in their common genetic architecture and manifest as multiple, distinct, overarching genetic factors. We disentangle autism heterogeneity through multivariate genetic analysis of co-occurring autism symptoms using a case-only design.

Methods: We investigated ~5300 unrelated individuals with autism (Caucasian ancestry) from the SPARK cohort with genome-wide and phenotypic data, including 46 symptom scores related to cognitive, motor, social and language abilities and coexisting psychiatric diagnoses. Univariate and bivariate genetic variance analyses were conducted using Genome-wide Complex Trait Analysis (GCTA) software. Genetic covariance patterns across symptoms, as captured by genetic relationship matrices (GRM), were modelled with structural equation modelling (SEM) techniques (GRMSEM). The best-fitting model was identified using likelihood ratio tests.

Results: The strongest evidence for genetically predictable symptom heterogeneity was found for quantitatively assessed ritualistic behaviour (SNP-h2 = 0.38(SE = 0.12), $p = 0.00093$). Repetitive symptoms were genetically also interrelated with multiple other symptoms. Using GRMSEM, we found evidence for a shared genetic factor contributing to repetitive symptoms, behavioural problems/Oppositional Defiant Disorder (ODD) and language delay/disorder, capturing >50% of the respective SNP-h2 estimates. Replication of these findings is ongoing.

Conclusions: Our findings suggest that symptoms in autism can be understood as complex quantitative traits, where symptom heterogeneity, especially across repetitive behaviours, can be predicted by common genetic variation, assuming underlying polygenic mechanisms.

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C04.3 Polygenic risk score analysis reveals shared genetic burden between epilepsy and psychiatric comorbidities

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Background: Psychiatric comorbidities are common in people with epilepsy. Roughly one in three people with epilepsy will experience some sort of psychiatric issue, with higher rates in people with treatment-refractory epilepsy. Finding genetic links between epilepsy and psychiatric disorders has proven difficult. We used a method known as polygenic risk scoring (PRS) to test whether people with epilepsy have an increased polygenic burden for depression, psychosis, ADHD, and anxiety.

Methods: Phenotype data in the UKBiobank were assessed to identify samples with epilepsy ($n = 8{,}488$), and genotype data were taken of Caucasian samples ($n = 409{,}634$). PRS for each psychiatric trait were calculated and multinomial regression was used to compare 1) population controls, 2) people with epilepsy and no psychiatric illness, 3) people with epilepsy and the psychiatric trait of interest, and 4)people with the psychiatric trait of interest and no epilepsy. Fixed-effect meta-analysis was used to compare psychiatric PRS in refractory and responsive epilepsy samples from the UKBiobank and the EpiPGX consortium(n = 3,449).

Results: We found that people with epilepsy have elevated PRS for Depression($p < 2e^{-16}$), psychosis($p = 0.006$) and ADHD($p = 0.0002$). Refractory epilepsy cases had an increased PRS for psychosis($p = 0.002$), depression($p = 0.0004$) and ADHD($p = 0.04$), relative to responsive cases.

Conclusions: This presents the first evidence linking the common genetic basis of epilepsy to that of psychiatric conditions which are frequently comorbid in people with epilepsy. Further research will confirm these links, and assess them in non-Caucasian individuals.

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C04.4 Unravelling the cell-type-specific molecular mechanisms linked to Parkinson's Disease (PD) using bulk and single-cell gene co-expression networks (GCNs)

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Introduction: We wanted to investigate the cell-specific molecular mechanisms linked to PD, to contribute to the molecular characterization of its etiology, especially in its early stages.

Materials and Methods: We used paired bulk and single nuclei RNA-seq data from human post-mortem substantia nigra pars compacta tissue of 27 donors (30-99 years, 14 cases, 13 controls) to create a bulk GCN and a GCN for each cell type. In addition, we employed a new strategy to account for the sparsity of the single-nuclei matrix: we collapse pairs of cells from the same individual in an additive manner, create the corresponding GCN and repeat the process while the number of cells is over 100.

Results: We detected two bulk GCN modules (M1, M2) with significant overlap with Mendelian PD genes (4.282E-5 and 0.002849 Fisher-test p-values). M1 is enriched for PD (KEGG:05012, $P = 3.145e-22$), oxidation-reduction process (GO:0055114, $P = 1.169e-17$), seizure (HP:0001250, $P = 1.068e-06$), dopaminergic neurons (1.697e-07) and interneurons (4.429e-04). M2 is enriched for synaptic vesicle cycle (KEGG:04721, $P = 7.986e-09$), neuron differentiation (GO:0030182, $P = 7.423e-09$), rigidity (HP:0002063, $P = 1.836e-08$), dopaminergic neurons SN ($P = 4.853e-11$) and interneurons ($P = 2.308e-08$). On the other hand, the multi-GCN approach proved to be useful as we discovered new interesting terms such as substantia nigra development in the 1-st oligodendrocytes GCN (GO:0021762, $P = 5.916e-06$) and Lewy body core in the 4-th dopaminergic neuron GCN (GO:1990037, $P = 6.751e-04$).

Conclusions: our results provide an important framework, made up of a bulk GCN and combinated pseudo-cells GCNs of each subcluster, for detecting potential genes involved in cell-type-specific mechanisms in PD.

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C04.5 Analysis of genomic copy number variation across psychiatric disorders

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Copy number variants (CNVs) are major risk factors in neuropsychiatric disorders and are partially contributing to their shared genetic etiology. Here, we determine associations of pathogenic CNVs across five psychiatric disorders and investigate the modifying role of common genetic variants in CNV carriers. Harmonized CNV calling and quality control was performed for data from 262,190 individuals, including patients with Attention-Deficit/Hyperactivity Disorder (ADHD, $N = 5,364$), autism (ASD, $N = 15,030$), bipolar disorder (BD, $N = 25,766$), schizophrenia (SZ, $N = 32,635$), control samples ($N = 70,006$), and 113,389 individuals from clinical genetics datasets (CLIN, predominantly developmental delay). We analyzed 42 multi-genic pathogenic CNVs, including reciprocal deletions and duplications, for association with different psychiatric disorders.

Disorders clustered according to period of onset with pediatric disorders (ASD and CLIN) being highly correlated and adult-onset disorders (BP and SZ) being correlated. Groups of CNVs tend to cluster based on phenotype associations, with the most distinct CNV cluster showing larger effects in ADHD, BD and SZ. Overall, reciprocal deletions and duplications had divergent effects across diagnostic categories, suggesting that CNVs have "mirror" effects on psychiatric traits, such as CLIN and SZ. Polygenic risk scores (PRS) and CNV genotype (16p11.2 locus) showed combined effects on dimensional traits.Specific CNV alleles have distinct psychiatric risk profiles and that may be attributable to function or expression of underlying genes in the brain. PRS contributed to the variable phenotypic expressivity in CNV carriers. We will expand the range of cognitive and neuropsychiatric traits through inclusion of additional disorders and disorder-related phenotypes.

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C04.6 The impact of clinically relevant CNVs in the general population - the health consequences and personalized management of undiagnosed adult CNV carriers in the Estonian biobank

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The role of CNVs is well established in children with neurodevelopmental disorders (NDDs). However, more research is needed towards systematic understanding on how: i) CNVs affect health in the adult population and ii) to responsibly disclose findings to CNV carriers at high genetic risk for complex disorders. We screened the cohort of Estonian Biobank (EstBB; $n = 134{,}546$) for 41 recurrent CNV regions associated with susceptibility to NDDs. In the first stage, we used the 'genotype-first' approach to fetch health data from the EstBB, linked electronic health registries (EHRs) and mapped each ND-CNV with their co-occurring disease traits. In the second stage, we selected 10 ND-CNVs as a paradigm to return of genetic risk data and analysis of at-risk individuals' experience and the impact of disclosed genetic finding on their health support. Our results show that the prevalence of CNVs associated with susceptibility to NDDs in the EstBB is 2.5% ($n = 3{,}399$). Further prioritization of CNVs listed in the DECIPHER database suggested a population prevalence of 0.36% ($n = 485$) for clinically well-established CNV syndromes. According to EHRs, nearly half of them have previously documented neurological or mental and behavioural problems. Notably, only 2.7% ($n = 13$) of them are aware of their genetic diagnosis. Our results along with reports by others are showing that CNVs associated with NDDs are cumulatively common, but still understudied health problem in general population. This work was supported by the Jacobs Foundation Research Fellowship (Dr Männik), Swiss National Science Foundation grant (Dr Reymond) and the Estonian Research Council grant (Dr Tõnisson).

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C05 Reproduction is hot!

C05.1 Outcome of publicly funded nationwide first-tier non-invasive prenatal screening

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Purpose: Noninvasive prenatal screening (NIPS) using cell-free DNA has transformed prenatal care. Belgium was the first country to implement and fully reimburse NIPS as a first-tier screening test offered to all pregnant women. A consortium consisting of all Belgian genetic centers report the outcome of two years genome-wide NIPS implementation.

Methods: The performance for the common trisomies and for secondary findings was evaluated based on 153,575 genome-wide NIP tests. Furthermore, the evolution of the number of invasive tests and the incidence of Down syndrome live births was registered.

Results: Trisomies 21, 18, and 13 were detected in respectively 0.32%, 0.07%, and 0.06% of cases, with overall positive predictive values (PPVs) of 92.4%, 84.6%, and 43.9%. Rare autosomal trisomies and fetal segmental imbalances were detected in respectively 0.23% and 0.07% of cases with PPVs of 4.1% and 47%. The number of invasive obstetric procedures decreased by 52%. The number of trisomy 21 live births dropped to 0.04%.

Conclusion: Expanding the scope of NIPS beyond trisomy 21 fetal screening allows the implementation of personalized genomic medicine for the obstetric population. This genome-wide NIPS approach has been embedded successfully in prenatal genetic care in Belgium and might serve as a framework for other countries offering NIPS.

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C05.2 A single center's experience with genome-wide non-invasive prenatal screening: Results of a large unselected consecutive series of cases in Luxembourg

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Introduction: In Luxembourg, the non-invasive prenatal testing (NIPT) is nationally implemented at a single-center and reimbursed by the compulsory public health insurance for every pregnant woman. We report on our obtained insights of extended NIPT for the general population.

Method We utilize the VeriSeq NIPT Solution v2 (Illumina) for routine NIPT. This WGS-based methodology allows for the detection of common fetal aneuploidies, but also rare autosomal trisomies (RATs) and copy number variations (CNVs) ≥ 7 Mb. Positive findings trigger a comprehensive workup, including genetic counseling, pre- and postnatal follow-ups and if appropriate, placental testing.

Results: From August 2019 to December 2020 we performed NIPT in 9,796 pregnancies, corresponding to >95% of all pregnancies in the county. We detected 60 common trisomies (0.61%), 29 RATs (0.29%) and 13 CNVs (0.13%). Positive NIPT results were confirmed by prenatal invasive testing in 87% (46/53) of common trisomy, 6% (1/16) of RAT and 20% (2/10) of CNV cases.

Postnatal placental testing was performed in 35% (10/29) of RAT cases, confirming a confined placental mosaicism (CPM) in nine cases (90%). We observed adverse pregnancy outcomes in four RAT cases (2xT16, T9 and true fetal mosaic T15).

Conclusion: The expanded use of NIPT leads to the detection of medically relevant common and atypical chromosomal anomalies, but also CPM involving recurrent RATs, which are not associated with adverse outcomes. We propose the restrained reporting of established RATs without clinical consequences, for the benefit of reducing the number of invasive testing and avoiding psychological distress in expecting parents.

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C05.3 A combined omic analysis revealed autism-linked *NLGN3* as a new candidate gene associated to GnRH neuron development and disease

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During development, gonadotropin releasing hormone (GnRH) neurons are born in the nasal placode and migrate to the hypothalamus, where they position to regulate sexual reproduction. Defective GnRH neuron development may lead to GnRH deficiency (GD) which is characterized by absent or delayed puberty. Several GD causative genes have been identified so far, but half of the cases are still idiopathic. The employment of complementary research approaches may be useful to identify additional genes implicated in GD pathogenesis. Here we combined for the first time in the field the transcriptomic analysis of GnRH neurons with exome sequencing data from GD patients to identify novel candidate genes. As a proof-of-principle, we identified a pathogenic variant in the autism-linked Neuroligin 3 (*NLGN3*) gene in a patient with severe delayed puberty and autistic traits. We demonstrated that NLGN3 is enriched in GnRH neurons at late developmental stages. Further we found that NLGN3 overexpression in an immature model of immortalized GnRH neurons promoted neurite extension whereas the novel NLGN3 mutation formed a truncated protein, thus supporting its pathogenic potential. Overall, our results highlighted how the combination of gene expression and exome sequencing data is a reliable approach to identify novel candidate gene for GD such a NLGN3, an autism-linked gene that we found for the first time associated to GD.

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C05.4 A *de novo* paradigm for male infertility

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Introduction: *De novo* mutations (DNMs) are known to play a prominent role in many sporadic disorders with reduced fitness. We hypothesize that DNMs play an important role in male infertility and explain a significant fraction of the genetic causes of this understudied disorder.

Materials and Methods: In this study, we performed trio-based exome sequencing in a unique cohort of 185 males with azoospermia or oligozoospermia and their unaffected parents.

Results: In total, 145 rare protein-altering *de novo* SNVs and 2 CNVs were identified. Following a systematic analysis assessing mutational impact and protein function, 29 DNMs were classified as possibly causative of the male infertility phenotype. We observed a significant enrichment of Loss-of-Function (LoF) DNMs in LoF-intolerant genes ($p = 1.00\text{x}10^{-5}$) as well as predicted pathogenic missense DNMs in missense-intolerant genes ($p = 5.01\text{x}10^{-4}$). Additionally, a significant increase in protein-protein interactions was found amongst genes affected by predicted pathogenic DNMs, in particular involving proteins involved in RNA binding and mRNA splicing ($p = 2.35\text{x}10^{-2}$). One of the DNM genes identified is *RBM5*, an essential regulator of male germ cell pre-mRNA splicing. In a follow-up study, 5 rare pathogenic missense mutations affecting this gene were observed in a cohort of 2,279 infertile patients, whereas no such mutation was found in a cohort of 5,784 fertile men ($p = 0.009$).

Conclusions: Our results provide the first evidence for the role of DNMs in severe male infertility and point to many new candidate genes affecting fertility.

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C05.5 2006-2018: Thirteen years of prenatal diagnosis and preimplantation genetic diagnosis for single gene disorders in France

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Over the years, preimplantation genetic diagnosis (PDG) has become an alternative to prenatal diagnosis (PND) for couples at risk for monogenic disorders. In France, the bioethics law regulates the practice of both PGD and PND, the costs of which are covered by the national health insurance. PGD is carried out in authorized centres, which provide an annual report to the French Biomedicine Agency; annual reports are also drawn up by the prenatal molecular genetics laboratories. We present the data collected by the French Biomedicine Agency and compare the evolution over 13 years of PGD and PND uptake for monogenic diseases. Over the period, the number of PGD centres increased from 3 to 5. The number of molecular PND laboratories varied between 47 and 51. PGD was available for 46 diseases in 2006 and 314 diseases (345 genes) in 2018. In 2006, 134 applications were accepted (38 diseases) and 462 in 2018. Ten diseases accounted for 69.4% and 47.6% of the applications accepted in 2006 and 2018 respectively. For the more common indications, the relative proportion of PGD increased compared to PND. To our knowledge, this is the first attempt to analyse the evolution of PND and PGD practices at the level of a country in a regulated context. This study is of interest for genetic counselling, giving an order of magnitude of the adoption of PGD or PND for the most common monogenic diseases, as well as from a public health perspective. Acknowledgements: French PGD centres and PND laboratories.

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C05.6 Health outcomes at birth, 12 and 24 months of 747 children conceived after Preimplantation Genetic Testing: a single centre experience

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This is a prospective study on 747 children born between December 1999 and July 2016 after a cycle of PGT-M or PGT-SR (IVF +/− ICSI + embryo biopsy) performed at a single London reproductive centre. PGT-A is not performed in the Centre. 742/747 children were examined at birth, 444/747 at 12 months and 168/747 at 24 months. The assessment consisted of three separate questionnaires; the first one focused on the detection of congenital anomalies in newborn babies, the latter recorded growth parameters and information about the baby's health and development. We found no evidence that PGT-M and PGT-SR increased the risk of an adverse perinatal outcome when compared with children born after conventional IVF-ICSI. The overall malformation rate in our cohort of liveborn infants conceived by PGT-M and PGT-SR was 3.9%, of which 2% had major malformations. These proportions are comparable with the published data on malformation risk in children born after IVF-ICSI. We observed 10% of babies with developmental delay at 24 months which is comparable with the rate of 10% given by the WHO, but more than double the incidence (4.6%) reported in the UK by the Global Research on Developmental Disabilities in 2016. Unfortunately, the low participation rate at 24 months (23%) significantly reduced the size of our cohort, therefore significant conclusions could not be drawn from our study on the effects of PGT on early childhood development. Further studies are needed to clarify if similar results can be reproduced in larger cohorts.

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C06 COVID-19 Genomics

C06.1 Genomic sequencing and healthcare dynamics to track nosocomial SARS-CoV-2 transmission

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Background: Understanding the effectiveness of infection control methods in reducing and preventing SARS-CoV-2 transmission in healthcare settings is of high importance. Infection control is challenging in these environments due to regular contact between healthcare workers (HCWs) and patients. This is amplified by increased frequency of severe adverse responses to SARS-CoV-2 in patients with underlying health conditions.

Methods: We sequenced SARS-CoV-2 genomes for patients and HCWs across multiple geographically distinct UK hospitals. All hospitals were actively enforcing zoning approaches (SARS-CoV-2 negative and SARS-CoV-2 positive areas) as an infection control measure. We integrated patient movement and staff location data into the analysis of viral genome data in order to understand geographical and temporal dynamics of SARS-CoV-2 transmission.

Findings: We obtained 173 high-quality SARS-CoV-2 genomes from patients (n=134) and HCWs (n=39). The median number of genomic variants per sample was 11 (range=0-16), with a 61.5% average pairwise similarity in the variants (range=0-100%). Integration of patient movement identified eight patient contact clusters (PCC) with significantly increased similarity in genomic variants compared to non-clustered samples (p<0.001). Incorporation of HCW location further increased the number of individuals within PCCs. Patients within PCCs carried viruses more genetically identical to HCWs in the same ward location (p<0.001).

Interpretation: SARS-CoV-2 genome sequencing integrated with patient and HCW movement data increases identification of outbreak clusters and improved understanding of the role of patient-HCW interactions. This dynamic approach to SARS-CoV-2 outbreak monitoring in a healthcare setting is able to support infection control management strategies within the healthcare setting.

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C06.2 Genetic landscape of the ACE2 coronavirus receptor

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SARS-CoV-2 and SARS-CoV share the same cell membrane entry receptor, angiotensin-converting enzyme 2 (ACE2), which is thus key to COVID-19 virus infection, treatment, and potentially vaccine development. The genetic basis of ACE2 protein levels is not well understood, nor is its genetic link to COVID-19. We conducted a genome-wide association meta-analysis of plasma ACE2 levels in over 28,000 individuals and identified ten loci capturing 30% of the protein's heritability. We detected a positive genetic correlation (0.48; 95% confidence interval (CI), 0.12 to 0.84; P = 0.009) between soluble ACE2 and severe COVID-19 across the autosomes. A cis-pQTL-based Mendelian randomisation analysis suggests a potential causal effect of elevated ACE2 levels on severe COVID-19 (odds ratio (OR), 1.63; 95% CI, 1.10 to 2.42; P = 0.01), which was validated on COVID-19 hospitalisation (OR, 1.52; 95% CI, 1.05 to 2.21; P = 0.03) and infection (OR, 1.60; 95% CI, 1.08 to 2.37; P = 0.02). We also found that COVID-19 comorbidities such as cardiovascular phenotypes are also genetically correlated with ACE2, through established pathways functionally linked to ACE2. Our results reveal the genetic architecture of the ACE2 protein, providing a useful resource for further biological and clinical studies on this coronavirus receptor.

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C06.3 Role of HLA on COVID19 risk and outcomes in the UK Biobank

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The UK Biobank permits studying the role of HLA on COVID19 risk and outcomes courtesy of regular updates on COVID19 status for all participants, which were already HLA typed by imputation from SNP array data (Bycroft 2018).

Five case-control definitions for susceptibility and severity were assembled based on results obtained from qPCR-tests for SARS-CoV-2, COVID19 associated death and hospital admission data. Analysis of 11 classical HLA genes was performed for 336,046 unrelated individuals of European ancestry. Logistic regression was used to test the association of HLA alleles at the 2-digits or 4-digits level correcting for age and sex.

COVID19 susceptibility was tested by comparing 5,027 cases with at least one positive qPCR test vs 23,453 controls with only negative qPCR-tests. The lowest p-value was observed for HLA-C*02:02 (P-value=$2.59x10^{-4}$, OR=0.86 [95%CI 0.79-0.93]).

COVID19 severity was tested by comparing 752 cases with a COVID19 cause of death or hospitalisation vs 272,594 controls with none or only negative qPCR-tests. The lowest p-value was observed for DRB1*04:01 (P-value=$1.40x10^{-3}$, OR=0.74 [95%CI 0.62-0.89]).

Given the number of tests performed, with a minimal allotype group frequency of 0.01, these results remained above the multiplicity-adjusted significance threshold, which was set at $\alpha = 2x10\text{-}5$.

Further analyses were subsequently performed at the amino-acid residue level, revealing respectively the association of a residue at position 73 in HLA-C (P-value=9.12E-04, OR=1.39 [95% CI 1.14-1.71]) and a residue at position 13 for DRB1 (P-value=5.85E-04, OR=0.75 [95%CI 0.64-0.89]).

These results will be updated as the COVID19 phenotypic data becomes available for the UK Biobank.

O. Chazara: A. Employment (full or part-time); Significant; Astrazeneca. **A. O'Neill:** A. Employment (full or part-time); Significant; Astrazeneca. **N. Camacho:** A. Employment (full or part-time); Significant; Astrazeneca. **K. Lythgow:** A. Employment (full or part-time); Significant; Astrazeneca. **S. Petrovski:** A. Employment (full or part-time); Significant; Astrazeneca.

C06.4 More nature, less nurture: Longitudinal follow-up of mental health reveals an increasing impact of genetic variants, associated with personality traits

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In the last year several genetic risk factors have been identified for COVID-19 severity, yet little is known about the role of genetic variation in mental health and adherence to lockdown restrictions. Here, we utilized longitudinal questionnaire information from the Lifelines Corona Research project (www.coronabarometer.nl), where since March 2020, 40,000 Lifelines participants have filled in 19 extensive questionnaires on both physical and mental health and on sentiments and adherence to lockdown measures.

We studied how different polygenic scores (PGS) are correlated to >100 response items and observed highly significant relationships for PGS for educational attainment, neuroticism and risk seeking behaviour. For instance, the PGS for risk tolerance is negatively associated with "appreciation that people keep more distance" and positively associated with "number of visitors during the christmas holidays".

Surprisingly, we observed that the strength of several of these associations consistently have increased over time. For instance, the negative association between the PGS for neuroticism and "perceived quality of life" was measured at 17 different timepoints and became significantly stronger over time (Pearson correlation $R^2 = 0.69$, $P = 1.9 \times 10^{-5}$), whereas on average the quality of life measurement showed a different, seasonal characteristic.

These results indicate that PGS often explain a different proportion of trait variances, depending on when these trait measurements are made. Moreover, our results suggest that the COVID-19 pandemic, involving substantial social distancing, has amplified the role that genetics plays in determining personal emotions.

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C07 Novel insights in inherited metabolic diseases

C07.1 Biallelic variants in mitochondrial RNase P subunit PRORP cause mitochondrial tRNA processing defects resulting in pleiotropic multisystem presentations

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The human mitochondrial RNase P (mt-RNase P) complex is comprised of three protein subunits: TRMT10C, SDR5C1 and PRORP. The mt-RNase P complex is responsible for 5′-end processing of mitochondrial precursor tRNAs, a vital step in mitochondrial RNA maturation. Biallelic variants in *TRMT10C* and *SDR5C1* are associated with distinct infantile onset disorders, resulting from defects in mitochondrial tRNA processing. We report four unrelated families with multisystem disease associated with biallelic variants in *PRORP*, the metallonuclease subunit of mt-RNase P. In two families, hypomorphic variants in *PRORP* were associated with sensorineural hearing loss, accompanied by primary ovarian insufficiency in one family (Perrault syndrome). In two families, biallelic variants in *PRORP* were associated with developmental delay and brain white matter changes. In fibroblasts from affected individuals, we observed reduced steady state levels of PRORP, an accumulation of unprocessed mitochondrial transcripts and a decrease in mitochondrial protein synthesis, which was corrected by introduction of the wild type transcript. In tRNA processing assays performed using recombinant mt-RNase P proteins, the disease-associated variants resulted in reduction of tRNA processing. The severity of the molecular defect in mitochondrial protein synthesis corresponded to the severity of the phenotype in the affected families. Our data indicate that biallelic variants in all three subunits of mt-RNase P result in mitochondrial dysfunction, each with distinct pleiotropic clinical presentations.

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C07.2 Clinician led analysis of WGS data confers a diagnostic uplift in suspected primary mitochondrial disease

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Introduction: Clinical whole genome sequencing (WGS) promises unprecedented diagnostic rates. However, standard approaches in clinical laboratories cannot harness the full power of WGS data over clinical exomes. A broader approach is especially needed in Primary Mitochondrial Diseases (PMDs), where phenotypes are non-specific.

Methods and Materials: Eighty-three probands attending an NHS England Highly Specialised Service for PMDs underwent WGS (Illumina TruSeq, HiSeq 2500) via the 100,000 Genomes project following exclusion of common molecular causes of PMD. Standard virtual panel analysis was undertaken and cases were subsequently reviewed by a clinician on a clinical interpretation platform. Re-analyses included expansion of gene panels applied if appropriate, and analysis for cryptic in trans variants in strong candidate genes (assisted by splice prediction scores and copy

number variant analysis). Additionally, a somatic variant caller was used to identify heteroplasmic variants in mtDNA.

Results: The standard bioinformatic pipeline with clinical scientist-driven interpretation yielded diagnoses in 14.3% (12/83) and a partial diagnosis in 4.7% (4/83). Expanded analysis resulted in a diagnostic uplift in 16.8% (15/83) of cases; heteroplasmic mtDNA variants were identified in an additional 3/83 patients; nDNA diagnoses were confirmed in 8/83 patients; and likely pathogenic variants in novel genes in 3/83 patients.

Conclusions: To harness the full power of WGS in clinical services high throughput analysis by clinical scientists should be supplemented by in depth phenotype-focused analysis by a genomic medicine clinician. This research was made possible through access to the data and findings generated by the 100,000 Genomes Project; http://www.genomicsengland.co.uk.

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C07.3 Bi-allelic KARS1 pathogenic variants affecting functions of cytosolic and mitochondrial isoforms are associated with a progressive and multi-system disease

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KARS1 encodes a lysyl-transfer RNA synthetase (LysRS) that links lysine to its cognate tRNA. Two different KARS1 isoforms exert functional effects in cytosol and mitochondria. Bi-allelic pathogenic variants in KARS1 have been associated to sensorineural hearing and visual loss, neuropathy, seizures, and leukodystrophy. We report the clinical, biochemical and neuroradiological features of nine individuals with KARS1-related disorder carrying 12 different variants with nine of them being novel. The consequences of these variants on the cytosol and/or mitochondrial LysRS were functionally validated in yeast mutants. Most cases presented with severe neurological features including congenital and progressive microcephaly, seizures, developmental delay/intellectual disability, and cerebral atrophy. Oculomotor dysfunction and immuno-hematological problems were present in six and three cases, respectively. A yeast growth defect of variable severity was detected for most variants on both cytosolic and mitochondrial isoforms. The detrimental effects of two variants on yeast growth were partially rescued by lysine supplementation. Congenital progressive microcephaly, oculo-motor dysfunction and immuno-hematological problems are emerging phenotypes in KARS1-related disorders. The data in yeast emphasize the role of both mitochondrial and cytosolic isoforms in the pathogenesis of KARS1-related disorder and supports the therapeutic potential of lysine supplementation at least in a subset of patients.

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C07.4 A Pex7 deficient mouse series correlates biochemical and neurobehavioral markers to genotype severity - implications for the disease spectrum of rhizomelic chondrodysplasia punctata type1 (RCDP1)

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RCDP1 is a peroxisome biogenesis disorder caused by defects in PEX7 leading to impaired plasmalogen (PL) biosynthesis and phytanic acid (PA) oxidation. The degree of PL deficiency directly correlates with disease severity. Children with severe RCDP have negligible PL levels, congenital cataracts, skeletal dysplasia, profound growth and psychomotor delay, and cerebral hypomyelination and cerebellar atrophy on brain MRI. Individuals with milder RCDP forms have higher PL levels, better growth and cognitive outcomes. To better understand the human

RCDP1 spectrum, we characterized a series of Pex7 deficient mice with graded reduction in *Pex7* transcript and PEX7 protein levels, resulting in stepwise reductions in PL and docosahexaenoic acid, and increases in C26:0 lysophosphatidylcholine and PA in erythrocytes, plasma and brain. Clinically, body size and survival were reduced in accordance to genotype severity. Neurohistological examination revealed graded reduction in myelin basic protein and quantity of cerebellar Purkinje cells. Pex7 deficient mice exhibited a hyperactive behavior in the open field environment. There was significant reduction in the levels of brain neurotransmitters dopamine, norepinephrine, serotonin and GABA that were strongly correlated with the hyperactivity phenotype. In conclusion, we identified several quantitative parameters that could be used to evaluate therapeutic interventions in RCDP1. Furthermore, as even relatively low PA levels were associated with Purkinje cell loss over time, we suggest monitoring PA in PEX7 deficient patients. Lastly, our data suggest the interplay between PL, brain neurotransmitter levels and hyperactivity, which could be further investigated in RCDP patients. This project is supported by E-Rare-3 Joint Translational Call.

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C07.5 Bi-allelic missense variants in oxoglutarate dehydrogenase (OGDH) lead to a neurodevelopmental disorder characterised by hypotonia, developmental delay and metabolic abnormalities

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Introduction: The alpha-ketoglutarate dehydrogenase complex (α-KGDH) is a critical citric acid cycle enzyme which catalyses the conversion α-ketoglutarate to succinyl coenzyme A in mitochondria. The α-KGDH complex is comprised of three subunits: OGDH (E1), DLST (E2) and DLD (E3). Recently, a biallelic variant (p.N320S) in OGDH was reported in two siblings with global developmental delay, elevated lactate, hypotonia and dystonia, linking for the first time recessive variants in OGDH with α-KGDH deficiency. Here, we report an additional four unrelated individuals with similar clinical presentation and homozygous missense variants in OGDH, confirming OGDH as a novel disease gene for childhood-onset neurological disease.

Methods and Results: We present three novel missense OGDH variants in four unrelated individuals identified using whole-exome sequencing: p.P189L, p.R204Q and p.S297Y. Individuals were homozygous for rare variants (absent or MAF <0.001 in gnomAD) with high CADD Phred scores (>20). Patients presented with neurological disease characterised by hypotonia, developmental delay, movement disorder, metabolic abnormalities and abnormal MRI findings. Measurement of urine organic acids was performed for two individuals (p.P189L and p.R204Q) and revealed increased levels of α-ketoglutarate. Evidence of pathogenicity of variants was supported by *in-silico* homology modelling, overexpression of mutants in HEK293 cells, α-KGDH activity measurements in patient fibroblasts and evaluation of variants in a Drosophila model.

Conclusions: In summary, we have established OGDH as a cause of childhood-onset neurological disease. Disease mechanism remains unknown, however, OGDH protein instability and reduction in protein abundancy has been demonstrated in vitro.

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C07.6 *Ldhd*-knockout mice recapitulate human metabolomic fingerprint of Gout, providing novel model organism for the disease

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Introduction: Gout is a form of inflammatory arthritis affecting millions of people worldwide. It is caused primarily due to renal underexcretion of uric acid. We have previously demonstrated that in humans, Gout can be caused by an inactivating mutation in *LDHD* encoding D-lactate dehydrogenase (D-LDH), leading to excessive renal excretion of D-lactate in exchange to uric acid reabsorption. To study the metabolic effect of D-LDH inactivation we created *Ldhd*-knockout mice using CRISPR-Cas9. We then used metabolome analyses to compare metabolic changes characteristic of sporadic gout patients to patients with mutant *LDHD* and to *Ldhd* knockout mice.

Materials and Methods: chromatography-mass spectrometry (LC-MS), targeted metabolomics, MetaboAnalyst, CRISPR-Cas9, knockout mouse model Results: Targeted metabolomics and metabolite profiling using LC-MS were performed on blood samples obtained from sporadic Gout patients and healthy controls. After identifying all significant changes in metabolite levels, we created a metabolomic fingerprint characteristic of Gout, demonstrating typical alterations in purine, glucose, and amino acid metabolism. Metabolome analysis of mutant-*LDHD* Gout patients as well as of CRISPR-Cas9 *Ldhd*-knockout mice was then performed to comprehensively phenotype the metabolic changes that result from D-LDH inactivation and to determine if they also carry the metabolomic fingerprint of Gout.

Conclusion: *Ldhd* knockout mice share the same metabolomic fingerprint as sporadic human Gout patients, demonstrating similar alterations in key metabolites, suggesting these mice constitute a novel disease model for Gout.

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C08 Skeletal and connective tissue disorders

C08.1 Biallelic loss of function variants in *IPO8* cause a connective tissue disorder associated with cardiovascular defects, skeletal abnormalities and immune dysregulation

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Introduction: The TGF-β/BMP cytokine family exerts pleiotropic functions during embryonic development, tissue homeostasis and repair as well as within the immune system. Accordingly, dysregulation of TGF-β signaling is the cause of severe congenital disorders characterized by developmental defects with or without impaired immune regulation. This is notably the case in the Loeys-Dietz syndrome (LDS), caused by heterozygous loss-of-function (LoF) variants in *TGFBR1*, *TGFBR2*, *TGFB2* or *TGFB3*, *SMAD2* or *SMAD3*, and of the Shprintzen-Goldberg syndrome (SGS) that results from heterozygous variants in in the TGF- β repressor *SKI*. Importin-8 belongs to the karyopherin family of nuclear transport receptors and was previously shown to mediate TGF-β−dependent SMADs trafficking to the nucleus in vitro.

Materials and Methods: We used next generation sequencing (NGS) to study twelve individuals from nine unrelated families presenting with a novel syndromic association characterized by cardio-vascular anomalies, joint hyperlaxity, various degree of dysmorphic features and developmental delay as well as immune dysregulation. CRISPR/Cas9- inactivation in zebrafish was used to model the genetic defect.

Results: NGS identified bi-allelic LoF mutations in *IPO8* all the tested individuals. Consistent with IPO8 role in BMP/TGF-β signaling, *ipo8−/−* zebrafish presented mild to severe dorso-ventral patterning defects during early embryonic development. Moreover, *ipo8−/−* zebrafish displayed severe cardiovascular and skeletal defects that mirrored the human phenotype.

Conclusion: Our results provides the first evidence that IPO8 plays a critical and non-redundant role in TGF-β signaling during development and reinforces the existing link between TGF-β signaling and connective tissue defects.

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C08.2 Bi-allelic loss-of-function variants in *LTBP1*cause autosomal recessive cutis laxa syndrome

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Latent transforming growth factor β (TGFβ) binding proteins (LTBPs) are microfibril-associated proteins essential for the anchoring of TGFβ in the extracellular matrix (ECM) as well as for correct assembly of ECM components. Gene variants affecting *LTBP2*, *LTBP3*, and *LTBP4* have been identified in several autosomal recessive Mendelian disorders with skeletal abnormalities with or without impaired development of elastin-rich tissues. Thus far, the human phenotype associated with LTBP1 deficiency has remained enigmatic. In this study, we report homozygous loss-of-function (LOF) *LTBP1* variants in eight affected individuals from four unrelated consanguineous families. Affected individuals present with connective tissue features (cutis laxa and inguinal hernia), craniofacial dysmorphology, and variable heart defects and prominent skeletal features (craniosynostosis, short stature, brachydactyly and syndactyly). In vitro studies on patient dermal fibroblasts indicate distinct molecular mechanisms depending on the position of the LOF variant in the *LTBP1* gene. C-terminal variants lead to a truncated LTBP1 loosely anchored in the microfibrillar network and cause increased ECM deposition in cultured fibroblasts associated with excessive TGFβ growth factor activation and signaling. In contrast, N-terminal truncation results a loss of LTBP1 that does not alter TGFβ levels and ECM assembly. In vivo validation of two independent zebrafish lines carrying mutations in *ltbp1* induce abnormal collagen fibrillogenesis in skin and intervertebral ligaments and ectopic bone formation on the vertebrae. In addition, one of the mutant zebrafish lines show voluminous and hypo-mineralized vertebrae. Overall, our findings in humans and zebrafish show that LTBP1 is important for skin and bone ECM assembly and homeostasis.

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C08.3 Al-Gazali skeletal dysplasia constitutes the lethal end of *ADAMTSL2*-related disorders

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Lethal short-limb skeletal dysplasia, Al-Gazali type (OMIM % 601356) is an ultrarare disorder which has only been previously reported in three unrelated individuals without a known genetic cause. Through international collaborative efforts we have collected a cohort of seven patients with clinical and radiographic features consistent with Al-Gazali skeletal dysplasia. The affected individuals present with moderate intrauterine growth retardation, relative macrocephaly, hypertrichosis, large fontanelle, short neck, short and stiff limbs with small hands and feet, severe brachydactyly, and generalized bone sclerosis with mild platyspondyly. Disease-causing variants in *ADAMTSL2* were detected combining WGS and Sanger sequencing. Six individuals were compound heterozygous and one individual was homozygous for pathogenic variants in *ADATMSL2*. Three patients had variants in the pseudogene region of *ADAMTSL2*, which is poorly covered by short-read sequencing. Mutations in *ADAMTSL2* have previously been described in geleophysic dysplasia type 1 (OMIM#231050). Bioinformatic analysis of the detected variants suggests a destabilizing effect on the ADAMTSL2 protein. Protein prediction analysis indicates the existence of intrinsically disordered region in ADAMTSL2 between amino acids 341-557. Our preliminary functional data using primary dermal fibroblasts from one

individual suggests abnormal microfibrillar network organization involving FBN1, supporting the known role of ADAMTSL2 in regulating the microfibrillar network of extracellular matrix. Overall, this study sheds light on the previously unknown genetic cause of Al-Gazali skeletal dysplasia and identifies it as a lethal part of the spectrum of *ADAMTSL2*-related disorders. Furthermore, we highlight the importance of meticulous analysis of the pseudogene part of the *ADAMTSL2*, where disease-causing variants might be located.

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C08.4 Critical role of the TB5 domain of fibrillin-1 in endochondral ossification

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Introduction: Geleophysic dysplasia (GD) is characterized by short stature, thick skin and cardiac defects. We previously identified the molecular basis of GD as mutations in *Fibrillin-1* (*FBN1*) for the autosomal dominant form. All *FBN1* mutations in GD patients are localized in TB5 domain (TGFβ binding protein like domain). Moreover, the skeletal phenotype observed in GD suggests that the TB5 domain might play a role in endochondral ossification process.

Material and method: To address this hypothesis, we generated a knock-in mouse model $Fbn1^{TB5+/-}$, by introducing the mutation p.Tyr1696Cys of a GD patient in order to understand the role of i) FBN1 in GD pathophysiology and ii) TB5 domain in skeletal development.

Results: We demonstrated that the $Fbn1^{TB5+/-}$ and $Fbn1^{TB5-/-}$ mice presented a reduced stature. The point mutation of *Fbn1* affected the growth plate formation with abnormal differentiation of chondrocytes. Mainly the mutant chondrocytes failed to establish a network composed by fibrillin-1 fibrils. This novel mouse model does not present aortic disease as observed in Marfan mouse models with *Fbn1* mutations. Interestingly, the TGFβ signaling pathway was not impaired supporting that the TGFβ signaling may be not a direct pathogenic driver of GD.

Conclusions: This new model is an original mouse model with *Fbn1* mutation leading to GD-like phenotype. Our findings suggest that the underlying mechanism of GD involves the dysregulation of fibrillin microfibril deposition possibly due to the improper interactions between the TB5 domain and heparan sulfates. The proper microfibril composition in growth plate seems essential for long bone growth.

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C08.5 Biallelic variants in *ADAMTS15* cause a novel phenotype characterized by congenital contractures of the hands and feet with absent palmar creases

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The ADAMTS family comprises 19 metalloproteases involved in the modulation of the extracellular matrix. Genes for 8 members are linked to different autosomal recessive disorders. Using exome sequencing, we detected biallelic variants in *ADAMTS15* in four affected individuals from three unrelated consanguineous families with a distinctive, overlapping phenotype. The two affected members of the index family 1 carrying a homozygous early premature termination codon, c.123C>G; (p.Tyr41*), showed congenital stiffness of the finger and toe joints, absent palmar creases, especially in the interphalangeal region, spinal stiffness and scoliosis as well as foot malformations. The proband of family 2 harbors a biallelic splice site mutation, c.1903-2A>G, leading to skipping of exon 7 and thereby to a C-terminal truncation of essential domains. She developed contractures of the fingers and toes combined with absence of the palmar creases and spinal stiffness at the age of 5 years. Family 3 proband has a homozygous missense variant, c.2281G>A; p.(Gly761Ser). He had digit contractures of fingers 3-5 with missing palmar creases, stiffness of toes 2-3 and suffered two pathological fractures. Remarkably, the nonsense variant leads to the strongest phenotype, possibly indicating a null allele genotype-phenotype association. Radiographic investigations excluded a primary involvement of the interphalangeal joints. Homozygous *Adamts15* knockout mice show a reduced grip strength implicating musculotendinous alterations as a possible disease mechanism. In aggregate, our findings show that pathogenic variants in *ADAMTS15* cause a novel autosomal recessive condition of the arthrogryposis spectrum characterized by joint stiffness and missing palmar creases.

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C08.6 Vascular Ehlers-Danlos syndrome - A comprehensive natural history study in the Dutch patient cohort, preliminary results

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Introduction: Vascular Ehlers Danlos Syndrome (vEDS; OMIM 130050; ORPHA 286) is a rare connective tissue disorder, caused by heterozygous pathogenic variants in the *COL3A1* gene. The phenotype is highly variable. vEDS patients are at risk for arterial, bowel and uterine rupture.

Purpose: To perform a national multi-center cohort study in all known Dutch vEDS patients, to provide further insights into the natural history of the disease. This knowledge will allow us to optimize patient care.

Methods: After METC approval, all known Dutch patients carrying a (likely) pathogenic variant in the *COL3A1* gene were invited to participate in the study ($n = \sim 130$). The phenotype was systematically charted by retrospective and cross-sectional assessment of molecular and clinical data.

Preliminary results: Eighty-seven patients have been included thus far (44 males, mean age 48 years (4-94 years)). Thirty-one of 85 (36%) were index patients (2 missing data). Of the 87 patients, 52 (60%) had a symptomatic history. The main reasons for referral were: family member with (likely) pathogenic variant (60%), arterial dissection (13%) and aneurysm (9%). In total, 30 patients (34%) had aneurysm(s), 29 (33%) dissection(s), 28 (32%) varicose veins and 8 (9%) cardiac valve insufficiency. Six (7%) suffered from (iatrogenic) perforation of the colon. Twenty-two of 62 (34%) did not meet the 2017 criteria suggestive for vEDS.

Conclusion: This national multi-center natural history study of Dutch vEDS patients provides a basis for improving guidelines for diagnosing, follow-up and treatment of vEDS patients worldwide.

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C09 Sensory disorders: multi-omics and long-read sequencing

C09.1 NR2E3 transcription factor and photoreceptor fate: identification of gene regulatory networks causing retinal remodelling in NR2E3-associated diseases

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Inherited retinal dystrophies (IRDs) are a group of diseases associated with mutations in more than 330 genes. *NR2E3* encodes an orphan nuclear receptor with a dual function as transcriptional activator and repressor, necessary for retinal development and homeostasis. Mutations in *NR2E3* cause two different retinal diseases: enhanced S-cone syndrome and retinitis pigmentosa. However, there is no clear phenotype-genotype correlation for most *NR2E3* mutations. This gene produces a large protein isoform encoded in 8 exons. In addition, we found a previously unreported isoform of 7 exons. We dissected the *Nr2e3* function by performing CRISPR/Cas9 gene editing of the last exon and generated two different mouse models. Depending on the deleted domain, these models show two different phenotypes that correspond with the two known diseases caused by mutations in *NR2E3*. We performed single cell RNA-seq in our models to further investigate the gene regulatory networks guiding differentiation of rods and cone photoreceptors in our two phenotypes. Our results provide insight into the molecular mechanisms of the two rare diseases caused by mutations in *NR2E3* and set the basis for further epigenetic studies on the NR2E3 network imbalances that give rise to IRDs. IAM is recipient of the APIF grant (Universitat de Barcelona) and Company of Biologist travel fellowship. This research was supported by grants PID2019-108578RB-I00 (Ministerio de Ciencia e Innovación/FEDER) and 2017-SGR-738 (Generalitat de Catalunya) to GM, and the Max Planck Society, DFG Priority Programme SPP2202 Spatial Genome Architecture in Development and Disease (VA-1456/1) and the Medical Research Council (UK) to JMV.

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C09.2 Regulatory architecture of *MAB21L2:* new elements unveiled by an upstream homozygous deletion in an individual with microphthalmia

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Introduction: Monoallelic and biallelic variants in *MAB21L2* cause anophthalmia, microphthalmia and coloboma (AMC), variably associated with skeletal anomalies and intellectual delay. While the function of MAB21L2 is poorly described, its flanking regions contain tissue-specific enhancers. We report two likely pathogenic *MAB21L2* variants in unrelated AMC individuals; the missense p.(Trp113Ser), and an upstream homozygous deletion encompassing two tissue-specific enhancers, with evidence for additional regulatory elements in the region.

Methods: *MAB21L2* screening of AMC individuals was performed using Sanger and whole exome sequencing (single nucleotide variants), and SNP array genotyping (copy number variants [CNVs]). Conserved non-exonic elements were investigated using CRISPR/Cas9 in zebrafish and *Xenopus tropicalis*.

Results: We identified a monoallelic MAB21L2 p.(Trp113Ser) variant in two brothers with colobomatous microphthalmia and their mother with unilateral peripapillary coloboma. Moreover, we detected a 113,580bp homozygous deletion in a girl with bilateral microphthalmia, microcephaly, micrognathia and intrauterine growth restriction. This CNV removes two regulatory sequences controlling tissue-specific *Mab21l2* expression in mice, in addition to six other conserved elements. Recapitulation of the CNV in zebrafish resulted in transient retinal and lens developmental defects. In addition, in *Xenopus* CRISPR/Cas9 mediated disruption of a putative OTX2 transcription factor binding site contained within the deleted region induced mild coloboma in developing tadpoles.

Conclusions: We report two likely pathogenic *MAB21L2* variants in AMC individuals, including the first case of a homozygous deletion within the control region with support from animal models for its impact on gene regulation. This highlights the importance of including regulatory regions in AMC clinical genetic testing.

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C09.3 CTG18.1-mediated Fuchs Endothelial Corneal Dystrophy: defining signatures of transcriptomic dysregulation in a common repeat-mediated disease

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Purpose: Fuchs endothelial corneal dystrophy (FECD) is predominantly caused by an expanded (≥ 50 copies) CTG repeat (termed CTG18.1) in an intron of the transcription factor *TCF4* (E-FECD). E-FECD is relatively common when compared to other repeat expansion diseases with a pathophysiology limited to corneal endothelial cells (CECs). Here we generated transcriptome data from primary CEC cultures to identify potential key biomarkers, enhancing our understanding of the pathophysiology of E-FECD.

Methods: RNA was extracted from primary CEC cultures of 10 unrelated individuals: 4 unaffected controls, 3 CTG18.1-expansion negative FECD (NE-FECD) and 3 E-FECD patients. RNA-seq libraries were prepared using oligo(dT) beads to enrich for poly-A mRNA and sequenced with standard Illumina paired-end protocols. Differential gene expression was assessed via DeSeq2 (with independent hypothesis weighting), while pre-mRNA splicing was analyzed with IsoformSwitchAnalyzeR.

Results: In the E-FECD cohort, 1,248 out of 4,497 significantly (adj. p-value≤0.05) differentially expressed genes were identified when compared with NE-FECD. Similarly, isoform analysis revealed 268 genes with significant differences in isoform usage between E-FECD and controls, 179 of which are predicted to produce a functional difference. Included within this dataset are genes with known MBNL1 splicing regulation, suggesting that dysregulation could be due to sequestration of splicing factors by the CTG18.1-expanded transcript.

Conclusions: A comparison of transcriptomic profiles of control, E-FECD and NE-FECD provides a dataset of differentially expressed transcripts and aberrantly regulated pre-mRNA splicing events that were only detected in E-FECD. These data provide insight into disease mechanisms and highlight targets for CTG18.1 expansion-specific translational interventions.

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C09.4 Cas9-targeted nanopore sequencing for the repetitive coding region of *RPGR*, a major cause of X-linked retinal dystrophy

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Introduction: Despite the advent of massively parallel sequencing, many repetitive/low-complexity regions remain intractable to short, paired-end sequencing technologies. One clinically significant example is ORF15, a 1734bp AG-rich repetitive exon of *RPGR*, in which variants cause approximately 50% of X-linked retinopathy. We established a Cas9-targeted long-read sequencing (LRS) assay to effectively read through ORF15 and enable variant detection without the need for complicated sequencing strategies.

Methods: Seven consecutive males with molecularly confirmed (Sanger sequencing) pathogenic variants in *RPGR*-ORF15 were recruited for LRS analysis. Oxford Nanopore Technologies (ONT)

library preparations were targeted to a 45kb region containing the entire *RPGR* coding region by dephosphorylation and CRISPR-guided Cas9 cleavage using multiplex guides flanking *RPGR*. Libraries were sequenced using a MinION flowcell for approximately 12h. Read data was processed using the ONT Guppy, Minimap2 and Medaka packages.

Results: Sequencing runs generated a median of 74 reads (min = 37, max=182 [one sample was allowed to run for 24h]) on-target reads, with a median read length of 7442bp, (min = 242, max=59,677). Across the 45Kb region, a median 99.7% (min = 89.7%, max=99.9%), and for ORF15, a median 100% (min = 99.9%, max=100%) of bases were covered by ≥5 reads. After filtering, 1-3 coding variants (including indels and SNVs) were detected per individual with 100% sensitivity for pathogenic variants and a single apparently false positive call.

Conclusions: This study demonstrates that Cas9-targeted ONT-LRS is effective for variant detection within the repetitive region of *RPGR*. This represents a proof of principle for LRS approaches to study clinically relevant and otherwise intractable genomic regions.

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C09.5 Long-read sequencing to unravel complex structural variants of *CEP78* leading to Cone-Rod Dystrophyand Hearing Loss

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Inactivating variants as well as a missense variant in *CEP78* have been identified in autosomal recessive Cone-Rod Dystrophy with Hearing Loss (CRDHL), a syndromic inherited retinal disease distinct from Usher syndrome. In addition, a complex structural variant (SV) implicating *CEP78* has been reported in CRDHL. Here we aimed to expand the genetic architecture of typical CRDHL by the identification of *CEP78* SVs. Approaches used are shallow whole genome sequencing (sWGS) combined with quantitative PCR and long-range PCR, or ExomeDepth analysis on whole exome sequencing data. Targeted or whole genome long-read sequencing (LRS) was used to delineate breakpoint junctions at the nucleotide level. Two *CEP78* SVs were identified in three unrelated individuals with CRDHL: a heterozygous total gene deletion (235 kb) and a partial gene deletion (15 kb) in both heterozygous and homozygous state. Assessment of the molecular consequences of the SVs on patient's materials displayed a loss-of-function effect. Delineation and characterization of the 15 kb deletion using targeted LRS revealed the previously described complex *CEP78* SV, for which a founder haplotype was demonstrated. The novel 235 kb deletion was delineated using whole genome LRS. Breakpoint analysis showed microhomology suggesting a replication-based mechanism. Moreover, data mining of bulk and single-cell transcriptional datasets and immunostaining, linked the CEP78 expression domain with its phenotypic manifestations. Overall, this study supports that the *CEP78* locus is prone to SVs and suggests SV analysis in a genetic work-up of CRDHL. Finally, it demonstrates the power of sWGS and LRS in identifying and characterizing complex SVs.

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C09.6 Long-read technologies identify a hidden inverted duplication in a family with choroideremia

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The lack of molecular diagnoses in rare genetic diseases can be explained by limitations of current standard genomic technologies. Upcoming long-read techniques have complementary strengths to overcome these limitations, with a particular strength in identifying structural variants (SVs). By using optical genome mapping (Bionano Genomics) and long-read HiFi genome sequencing (Pacific Biosciences), we aimed to identify the pathogenic variant in a large family with X-linked choroideremia, a progressive and rare form of chorioretinal degeneration. In the respective family, aberrant splicing of exon 12 of the choroideremia gene *CHM* was detected in 2003, but the underlying genomic defect remained elusive using targeted short-read sequencing. Optical genome mapping followed by long-read sequencing now revealed an intragenic 1,752 bp inverted duplication including exon 12 and surrounding intronic regions, located downstream of the wild-type copy of exon 12 (c.1510+693_1510+694ins1414-1244_1510+402inv). Both breakpoint junctions were confirmed with Sanger sequencing. The inverted duplication segregates with the X-linked inheritance in the family, and is predicted to result in a hairpin-

formation of the pre-mRNA with the wild-type exon 12, leading to exon skipping in the mature mRNA. This exemplifies the potential for SVs to unravel novel mutational mechanisms. We believe that the identified SV represents the hidden pathogenic cause of disease in this family, that was missed to be identified for nearly 20 years. Our study shows that optical genome mapping and long-read sequencing have significant potential for the identification of hidden structural variants in rare genetic diseases that escaped molecular diagnoses so far, even for well-known disease-associated genes.

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C10 Genome-wide Association Studies

C10.1 Parent-of-origin inference for biobank scale datasets

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Genome-wide association studies (GWAS) have shown that identical genetic variations can have different effects on a phenotype, depending on their parent-of-origin (PO). To determine the PO of an individual's haplotypes, researchers usually rely on the genome of the parents or the close relatives when the genealogy is known. However, these informations are rarely available in biobanks, preventing the study of PO.

Here, we present a novel probabilistic approach that (i) identifies surrogate parents regardless of genealogy using identity-by-descent sharing between individuals, (ii) uses a Hidden Markov Model to represent a specific haplotype as a mosaic of haplotypes of its close relatives, (iii) assigns paternal or maternal origin to these shared haplotype segments, and (iv) extends the PO inference to entire chromosomes.

Using the UK Biobank dataset, we inferred the PO for ~25k samples, increasing by 5 times the sample size compared to classical approaches on this dataset. We assessed the accuracy of our method using a total of 1397 duos and trios, reaching a call rate >80% (% of sites with PO assignment) and an error rate <1% (% heterozygous sites with incorrect PO assignment). Using the resulting callset, we performed association scans for PO effects across hundreds of phenotypes and discovered known and novel imprinted variants, many of which reached genome-wide significance thanks to the large sample size offered by our approach.

By leveraging relatedness in biobanks, our approach allows studying PO effects on large sample sizes, increasing our ability to further characterize the genetic architecture of complex traits. Grant:SNSF-PP00P3_176977

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C10.2 Genetic investigation of fibromuscular dysplasia identifies risk loci and shared genetics with common cardiovascular diseases

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Introduction: Fibromuscular dysplasia (FMD) is a non-atherosclerotic arteriopathy of unknown etiology, affecting mostly middle-aged women. It is characterized by stenotic lesions of the vascular wall in middle-size arteries, sometimes associated with dissection, aneurysm or tortuosity.

Methods: We report results from the first genome-wide association meta-analysis of six studies including 1962 FMD cases and 7100 controls. We integrated genetic association with arterial gene expression using transcriptome-wide association (TWAS). To identify FMD associated variants located in regulatory elements, we determined open chromatin regions in artery-derived primary cells using ATAC-Seq and estimated heritability and genetic correlation of FMD with other vascular traits and diseases using LD Score regression.

Results: We found an estimate of SNP-based heritability compatible with a polygenic feature for FMD and report four robustly associated loci (*PHACTR1*, *LRP1*, *ATP2B1*, and *LIMA1*). TWAS identified one additional locus (*SLC24A3*). We found that FMD associated variants were located in arterial-specific regulatory elements. Target genes were broadly involved in mechanisms related to actin cytoskeleton and intracellular calcium homeostasis, central to vascular contraction. We found significant genetic overlap between FMD and hypertension, one of the most frequent consequences of FMD lesions. We also report an important genetic overlap with migraine, intracranial aneurysm, coronary artery disease and LDL cholesterol.

Conclusion: We identified several loci related to vascular contraction, suggesting that altered vascular tonicity may play a role in the pathogenesis of FMD. We find that FMD is genetically close to several vascular diseases where vascular integrity is impaired. This work was funded by ERC grant ERC-Stg-ROSALIND-716628.

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C10.3 Genome-wide association study of more than 40,000 patients with bipolar disorder provides novel biological insights

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Bipolar disorder (BD) is a complex mood disorder characterized by recurrent episodes of mania and depression. Genetic factors contribute substantially to BD (estimated heritability of around 60-85%). Previous genome-wide association studies (GWAS) identified a number of susceptibility loci for BD. However, the disease-relevant genes and molecular mechanisms are still largely unknown.

In the present study, the BD Working Group of the Psychiatric Genomics Consortium (PGC) conducted the largest GWAS of BD to date (Mullins et al., medRxiv, 2020). Our meta-analysis included 57 cohorts comprising 41,917 BD patients and 371,549 controls of European ancestry. For PGC cohorts, quality control, imputation and statistical analyses were performed using RICOPILI. Five external cohorts were processed using comparable procedures. Meta-analysis was performed using an inverse variance-weighted fixed effects model in METAL.

We identified 64 independent genome-wide associated loci. 33 of these were novel including the extended MHC region on chromosome 6. Pathway analysis using MAGMA revealed significant enrichment for four gene-sets including synaptic signaling. In addition, we found significant enrichment in genes encoding targets of psycholeptics, antiepileptics, anesthetics and calcium channel blockers. Integrative bioinformatic analyses using eQTL data provided converging evidence for 15 genes whose BD association could be mediated by changes in gene expression (e.g., *FURIN*). Subtype analyses identified one genome-wide significant locus for BD II that did not reach significance in our main BD GWAS.

Our findings advance the understanding of the biological basis of BD, identify novel therapeutic approaches and prioritize genes for subsequent functional studies. Grants: NIMH (U01 MH109528; U01 MH094421).

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C10.4 Genome-wide association study and replication of liver enzyme loci

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Introduction: Serum concentration of liver enzymes are linked to liver dysfunction, metabolic and cardiovascular diseases. To identify and replicate genetic variations associated with serum concentration of liver enzymes, we performed genome-wide association study on serum levels of alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT).

Methods: The analysis was performed using linear mixed models (LMM) association testing on 437,438 participants of European descent with the UK Biobank. We additionally sought replication of the findings in 315,572 individuals from European descent from several studies including the Million Veteran Program, Rotterdam Study and Lifelines study.

Results: We replicated 517 liver enzyme variants. We performed several secondary analyses on the identified variants including genetic risk score analysis which showed association with higher fat percentage of body, trunk, and liver and body mass index. Additional genetic risk score analysis in two independent European descent studies (The Airwave Health Monitoring study and the Northern Finland Birth Cohort 1966) confirmed strong association with liver enzymes. We additionally performed gene-set enrichment analysis which highlighted the role of the identified variants in liver development and function, lipid metabolism, insulin resistance, and vascular formation. We performed Mendelian randomization analysis which showed association of liver enzyme variants with coronary heart disease and ischemic stroke.

Conclusions: Our study highlighted the importance of molecular pathways regulated by the liver in metabolic disorders and cardiovascular disease.

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C10.5 Discovery and prioritization of variants and genes for kidney function in >1.2 million individuals

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Introduction: Large genome-wide association studies (GWAS) for kidney function were performed in the past providing promising targets for functional studies, but systematically prioritizing thousands of variants and genes remains challenging. We aimed to develop a comprehensive tool to perform that prioritization.

Methods: We performed large GWAS meta-analyses for estimated glomerular filtration rate based on serum creatinine (eGFRcrea) and cystatin (eGFRcys) and blood urea nitrogen (BUN) using data from the CKDGen consortium and the UK Biobank (total $n = 1{,}201{,}909$, $n = 460{,}826$ and $n = 852{,}678$, respectively). Second signal analyses, fine mapping and comprehensive bioinformatic follow-up analyses (e.g. CADD score, functional annotation, kidney-tissue eQTLs, comparison with monogenic kidney diseases) were performed and combined into a Gene PrioritiSation (GPS) database that can evaluate each gene underneath an associated locus based on individual weighing of the different evidence.

Results: We identified 424 genetic loci (201 novel) including 634 independent signals significantly associated ($P<5x10^{-8}$) with eGFRcrea, of which 343 received support for kidney function relevance by eGFRcys or BUN analyses. Fine mapping narrowed down association signals to five or less 99% credible variants for 138 signals. The GPS highlighted 18 genes mapping to small credible sets containing a protein-altering or kidney-tissue regulatory variant.

Conclusion: We identified novel genetic loci with strong evidence of relevance to kidney function that may generate new biological hypotheses. The searchable and customizable GPS database supports the translation of GWAS results into actionable candidate genes and variants for functional studies.

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C10.6 The genetic relationship between the Vitamin D binding protein and Vitamin D

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The vitamin D binding protein (VDBP) is the major blood transport protein for vitamin D and its metabolites. VDBP concentration influences total 25 hydroxyvitamin D (25OHD) concentrations, which has been linked to a range of adverse health outcomes, including mental disorders. Here we perform a genetic analysis of neonatal VDBP blood level measurements ($n = 77{,}509$) that were quantified from dried blood spots. We performed a genome-wide association study (GWAS) of VDBP levels and identified 9 independent genome-wide significant associations. The most significant association was in the GC gene on chromosome 4, responsible for the VDBP protein itself, where 3 frequent alleles explain about 50% of the phenotypic variance. By conditioning on these haplotypes, we further identify 8 additional loci. A pheWAS analysis of these loci revealed associations with lipid metabolism (mainly cholesterol) and blood protein and cell levels. We then performed a heritability analysis where we estimated the heritability of VDBP to be 67%, and it's genetic correlation to vitamin D levels to be 0.4. We also performed Mendelian randomisation using GSMR (Zhu et al., Nat Comm 2018) to study the causal relationship between VDBP and vitamin D levels, where we found strong evidence for a causal effect of VDBP on 25OHD concentration, explaining 11% of the variation. Because the SNP-heritability of vitamin D levels is also 11%, this result suggests that much of the variance in 25OHD concentration may be explained by VDBP protein concentration.

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C11 New technologies and better diagnostics

C11.1 Comprehensive detection of variants in unsolved rare disease cases with PacBio HiFi reads

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Introduction: PacBio HiFi reads (99.9% accuracy, 15-25 kb) enable comprehensive variant detection in human genomes, extending to repetitive regions of the genome not accessible with short-read WGS (srWGS). HiFi reads match or surpass srWGS for single nucleotide variant and small indel detection while also improving detection of structural variants (SVs), with recall far exceeding that of srWGS. To support large-scale, reproducible application of HiFi WGS, we developed an automated workflow for variant analysis from HiFi reads and applied it to 80 rare disease cases unsolved by srWGS.

Materials and Methods: We used the workflow management system Snakemake to link tools for 1) sequencing quality control, 2) sample quality control, 3) alignment and variant calling, 4) genome assembly, and 5) variant filtering and prioritization. Small variants were called with DeepVariant/GLnexus and phased with WhatsHap, and SVs were detected by PBSV. Variants were annotated with population frequency from gnomAD and other public datasets and with predicted functional consequence based on Ensembl gene models.

Results: Across 80 rare disease cases, the workflow identified a median of 4,064,900 SNVs, 931,879 indels, and 21,737 SVs. Variants of interest were identified in 30 cases, with confirmed diagnostic pathogenic variants including a start codon loss, a novel repeat expansion, and compound heterozygous loss-of-function variants.

Conclusions: Our analysis workflows support broad application of HiFi reads, which we expect will continue to identify diagnostic variants in many rare disease cases unsolved by srWGS. The Snakemake workflow and a WDL port for cloud execution are available on GitHub.

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Significant; Pacific Biosciences. **R. Lleras:** A. Employment (full or part-time); Significant; Microsoft. **M.H. McLoughlin:** A. Employment (full or part-time); Significant; Microsoft. **B.M. Moskowitz:** A. Employment (full or part-time); Significant; Microsoft. **E. Farrow:** A. Employment (full or part-time); Significant; Children's Mercy Hospital. **N. Miller:** A. Employment (full or part-time); Significant; Children's Mercy Hospital. **I. Thiffault:** A. Employment (full or part-time); Significant; Children's Mercy Hospital. **S. Chakraborty:** A. Employment (full or part-time); Significant; Pacific Biosciences. **C. Lambert:** A. Employment (full or part-time); Significant; Pacific Biosciences. **P. Baybayan:** A. Employment (full or part-time); Significant; Pacific Biosciences. **T. Pastinen:** A. Employment (full or part-time); Significant; Children's Mercy Hospital.

C11.2 Splicing noise is detectable across human tissues and modelling its characteristics is likely to improve the detection of pathogenic splicing within patient-derived samples

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Alternative splicing is a characteristic of most multi-exonic human genes. However, RNA-sequencing data from human tissues, suggests that this process can be inaccurate leading to the presence of reads which cannot be completely mapped to known transcripts. Here, we characterised splicing noise using RNA-sequencing data from 42 human tissues, provided by the Genotype-Tissue Expression Consortium and processed by recount2. We focused on reads mapping with a gapped alignment to the genome that could be assigned to a gene through sharing of a known acceptor or donor splice site, termed partially unannotated junctions (PUJs). Using MaxEntScan to score novel splice sites, we verified that PUJs contained flanking sequences likely to be recognised by the spliceosome. We assessed the proportion of PUJs resulting from misrecognition of acceptor versus donor sites and found that this was consistently higher across all samples studied (p-value<2.2e-16) with significant tissue differences (p-value<2.2e-16). Furthermore, we showed that the novel donor and acceptor sites implied by PUJs were located in close proximity to annotated sites. However, whereas mis-splicing appeared to be symmetrical around known donor sites (mode=3bp), there was an asymmetric distribution at known acceptor sites (mode=20bp/3bp), compatible with the well-recognised AG-exclusion-zone at acceptor sites. These findings suggested that mis-splicing is less tolerated at donor sites, which is consistent with the significantly higher proportion of donor as compared to acceptor splice site mutations in ClinVar (p-value=1.8e−2). Taken together, our findings imply that the detection of pathogenic splicing events in patient-derived RNA-sequencing data could be improved by modelling splicing noise.

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C11.3 Whole Blood RNA Sequencing in a Cohort of Patients with Undiagnosed Genetic Conditions

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Objectives: Several studies have shown that analysis of the transcriptome by RNA sequencing (RNA-seq) can improve our ability to interpret the functional and clinical importance of the genetic variants identified by WES and WGS. Our aim was to develop an RNA-seq method that can be used in a clinical diagnostic laboratory and identify clinically relevant RNA isoforms and gene expression.

Methods: The NEBNext® Ultra™ II DNA Library Prep Kit for Illumina was used. Sequencing was performed using the NovaSeq Illumina instrument. The cohort consisted of 134 pediatric clinical blood samples collected through the Genome Clinic (Centre for Genomic Medicine) at the Hospital for Sick Children. The samples were collected as part of a cohort of patients with a wide spectrum of undiagnosed genetic conditions and had previously been tested with microarray and WGS.

Results: Our data shows that >70% of the genes associated with these clinical indications were expressed in blood. Notably, 79.5% of genes involved in neurological disorders ($N = 995$) were expressed. We developed an approach to identify aberrant gene expression and clinically relevant splicing variants by comparing patient data to large sets of data from controls. After integrating with WGS results, we show that RNA-seq analysis has identified several clinically relevant candidate variants even without availability of the most appropriate tissue type.

Conclusions: We have developed an RNA-centric strategy to identify clinically relevant splicing and expression variants in a cohort of undiagnosed patients for whom WGS and microarray testing had previously been performed.

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C11.4 Practical considerations for utilising ClinVar in variant prioritisation: evidence from the 100,000 Genomes Project

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Background: Public archives, such as Clinvar, can be used to enhance pathogenic variant prioritisation in rare disease, but requires consideration of potential annotation inconsistencies, hypomorphic variants and incidental findings.

Materials and Methods: Genomic data from 29,880 families with rare diseases from the 100,000 Genomes Project were scanned for ClinVar variants in phenotypically relevant gene panels. ClinVar variants with star rating 1-4 and at least one

"pathogenic" or "likely pathogenic" annotation were reviewed in the context of clinical data. The impact on the number of prioritised variants was modelled using 58 families for whom a large Paediatric Disorders gene panel was applied.

Results: We identified ClinVar pathogenic variants not previously prioritised in the 100,000 Genomes Project in 2,147 families. 55 families carried homozygous recessive variants, of which 17 (30.91%) likely represent a new diagnosis, while 9 (16.4%) were already reported as explaining the phenotype. 43 further families carried potentially diagnostic autosomal dominant variants and one an X-linked variant. Variants not considered to be clinically relevant were primarily monoallelic variants for recessive conditions, variants with low evidence of pathogenicity or hypomorphic variants. Modelling the prioritisation of ClinVar variants at various levels of stringency results in an increase in prioritised variants between 0.41 (±0.17) and 2.52 (±0.42) per proband when applying a large gene panel.

Conclusions: ClinVar is a useful resource for improving variant prioritisation algorithms. Refining prioritisation rules for variants with lower evidence of disease association increases specificity. Inclusive criteria result in only a modest increase in the number of prioritised variants.

L. Abrahams: A. Employment (full or part-time); Significant; Genomics England. **M. McEntagart:** A. Employment (full or part-time); Significant; Genomics England. **D. Kasperaviciute:** A. Employment (full or part-time); Significant; Genomics England. **S. Walker:** A. Employment (full or part-time); Significant; Genomics England. **M. Bleda:** A. Employment (full or part-time); Significant; Genomics England. **A. Rueda Martin:** A. Employment (full or part-time); Significant; Genomics England. **J. Lopez:** A. Employment (full or part-time); Significant; Genomics England. **J. Almeida:** A. Employment (full or part-time); Significant; Genomics England. **M. Imprialou:** A. Employment (full or part-time); Significant; Genomics England. **D. Rhodes:** A. Employment (full or part-time); Significant; Genomics England. **A. Stuckey:** A. Employment (full or part-time); Significant; Genomics England. **E. Thomas:** A. Employment (full or part-time); Significant; Genomics England. **A. Taylor Tavares:** A. Employment (full or part-time); Significant; Genomics England. **H. Brittain:** A. Employment (full or part-time); Significant; Genomics England. **A. Tucci:** A. Employment (full or part-time); Significant; Genomics England. **R.H. Scott:** A. Employment (full or part-time); Significant; Genomics England. **Z. Deans:** A. Employment (full or part-time); Significant; NHS England and NHS Improvement. **R. Mein:** A. Employment (full or part-time); Significant; NHS England and NHS Improvement. **S.L. Hill:** A. Employment (full or part-time); Significant; NHS England and NHS Improvement. **M.J. Caulfield:** A. Employment (full or part-time); Significant; Genomics England. **A. Rendon:** A. Employment (full or part-time); Significant; Genomics England.

C11.5 Chimeric transcript formation as a new pathogenetic mechanism of rare and undiagnosed diseases: Analysis using whole genome sequencing and long-read transcriptome sequencing

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Background: Part of two distinctive genes can be transcribed into a new chimeric transcript due to chromosomal aberrations. The importance of chimeric transcript formation remains unknown in constitutional genetic disorder contrast to cancer research.

Methods: We screened for chimeric transcripts by re-analyzing RNA-seq data derived from peripheral blood of 153 patients whose diagnosis remained undetermined after standard exome analysis. When chimeric transcripts were identified using dedicated software ChimPipe, whole genome sequencing for characterizing structural abnormalities and the entire length of the chimeric transcripts sequenced by nanopore long read sequencing were performed.

Results: Two patients were found to harbor chimeric transcripts. 1) The RNA-seq of the first patient revealed reads spanning exon 5 of *ZEB2* and exon 7 of *GTDC1*. Whole genome sequencing revealed a 436-kb deletion spanning intron 4 of *ZEB2* and intron 7 of *GTDC1* and the diagnosis of Mowat-Wilson syndrome was made. 2) The RNA-seq of the second patient revealed reads spanning exon 21 of *TRAPPC9* and exon 1 of *KCNK9*. Whole genome sequencing revealed a 186-kb deletion spanning intron 20 of *TRAPPC9* and intron 1 of *KCNK9* and the diagnosis of Birk-Barel syndrome was made.

Conclusions: The approach of detecting chimeric transcripts from RNA-seq data, is unique in that the approach does not rely on any prior information on the presence of genomic deletion. Chimeric gene formation plays an important role in the pathogenesis of congenital malformation syndromes. RNA-seq, when combined with chimera analysis, represents promising modality to decipher genetic etiology of undiagnosed patients. [JP17ek0109151, JP19K17342, JP20ek0109485]

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C11.6 Evaluating the performance of a clinical genome sequencing programme for diagnosis of rare genetic disease, seen through the lens of craniosynostosis

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Introduction: Genome sequencing (GS) for diagnosis of rare genetic disease is being introduced into the clinic, but the complexity of the data poses challenges for developing routine pipelines with high diagnostic sensitivity. The UK's 100,000 Genomes Project, delivered by NHS England through national Genomic Medicine Centres (GMCs) and Genomics England (GE), represents the first implementation of GS into a national

diagnostic programme at scale. We evaluated the GE/GMC diagnostic rare disease triaged bioinformatics pipelines, using craniosynostosis as a test case.

Methods: GS data from 114 probands with craniosynostosis or their relatives (314 samples), negative on routine genetic testing, were intensively scrutinised by a specialized research team and compared to diagnoses identified through the GE/GMC pipeline.

Results: Fourteen variants classed as likely pathogenic/pathogenic were identified by the GE/GMC pipeline. Eighteen additional likely pathogenic/pathogenic variants were identified by the research team, indicating that for craniosynostosis, the GE/GMC pipeline diagnostic sensitivity was only 44%. Measures that could have increased diagnostic success include comprehensive analysis of *de novo* small variants (+31% sensitivity) and copy number/structural variants (+12%), improved scrutiny of existing panel genes (+15%), and review of updated panels (+12% sensitivity). Current consensus NHS practice, which partially incorporates these measures, would have achieved a 78% sensitivity.

Conclusion: GS identified likely pathogenic/pathogenic variants in 28% of the craniosynostosis cohort, with over half of diagnoses missed by the GE/GMC pipelines. Improved phenotype capture, variant calling, and scrutiny of variants outside restricted gene panels will be essential to leverage the full potential of GS in clinical diagnostics.

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C12 Counselling, communication and service delivery

C12.1 Developing a genomic-competent physician workforce: immersive and structured experiences as part of a multi-faceted approach

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A multifaceted approach is required to ensure non-genetic physicians have sufficient genomic expertise to adopt genomic medicine. Our workforce development strategy for physicians, informed by adult learning and implementation science theories, includes immersive and structured experiences. The program aims to produce a cohort of physicians across a spectrum of genomic expertise, from basic familiarity to speciality-experts who can guide change within their discipline. We evaluated this strategy using mixed methods. Workplace-learning participants ($n = 10$ *clinical fellows*; $n = 12$ *immersion fellows*) completed post-program interviews (RR 100%). *Workshop participants* ($n = 311$) completed surveys (pre-workshop, 179/265, 68%; post-workshop, 189/251, 71%). *Blended course participants* (n = 71) completed surveys (baseline, 64/71, 90%; post-online, 40/71, 56%; post-course, 63/71, 88%). Quantitative data were analysed using descriptive statistics. Open comments and interview data were analysed using inductive content analysis. Workplace-learning participants reported improved genomic capability and recognition as credible genomic experts within their discipline. Five clinical fellows subsequently contributed to curriculum design for structured learning. Workshops included foundational concepts and discipline-specific cases. The blended course was cross-disciplinary, with online foundational modules plus generic case-based workshops. Both structured learning experiences improved participants' self-rated and objective genomic knowledge, skills and confidence. Blended learning participants showed gains from the self-directed online learning, plus further gains after workshop attendance. Contextualisation of foundational learning is supported by generic and discipline-specific cases. Immersion experiences develop speciality genomic experts who can support peer-learning and champion genomics. A workforce development strategy that incorporates both immersion and structured approaches develops physicians with a spectrum of genomic expertise.

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C12.2 Genetic counselling by video consultation during COVID-19 pandemic: the perceived quality

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Introduction: The number of video consultations (VCs) in genetic counselling have increased significantly since the start of the COVID-19 pandemic. We investigated the perceived quality and suitability of genetic counselling by VC.

Methods: We sent a questionnaire (containing 31 items) to all patients who were counselled via VC between May-June of 2020 ($n = 1188$) and a separate questionnaire (containing 45 items) to the genetic counsellors involved ($n = 40$).

Results: Patient response rate was 33% ($n = 387$). 93% of respondents were positive about the quality of the VC, concerning both technical aspects as well as content, and would recommend a VC to others. Patients aged below 56 years were more positive compared to older patients ($p = 0{,}033$). Travel distance did not impact satisfaction consistently with both patients with a short (<30 minutes) and long (90-120 minutes) travel distance being significantly less positive about VCs compared to patient with other travel distances ($p = 0{,}008$). Educational level did not impact satisfaction ($p = 0{,}086$). Genetic counsellor response rate was 90% ($n = 36$), of which the majority was positive (75%) and indicated that the quality of VCs was equally good with respect to content and understanding of the information by patients compared to physical consultations. The disadvantages reported most often were less satisfying doctor-patient relationships and limitations in physical examination (31% experienced this limitation in ≥25% of consultations).

Conclusions: VCs are well received and are a suitable alternative for physical consultations for most patients referred for genetic counselling.

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C12.3 Women's preferences for receiving uncertain results from prenatal genomic testing: An international discrete choice experiment

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We conducted a survey-based discrete-choice experiment (DCE) to examine the impact of diagnostic uncertainty on women's preferences for advanced tests e.g. fetal exome sequencing. The DCE comprised five attributes identified through literature review and interviews as being important for decision-making. Responders were given 13 scenarios and chose between Test A, Test B (both invasive tests) or No Test. Women from eight countries who delivered a baby in the previous 24 months were recruited through a market research company to complete the survey. Choices were modelled using conditional logit regression analysis. Surveys from 1239 women (Australia: $n = 178$; China: $n = 179$; Denmark: $n = 88$; Netherlands: $n = 177$; Singapore: $n = 90$; Sweden: $n = 178$; UK: $n = 174$; USA: $n = 175$) were analysed. Participants selected an invasive test (Test A or B) in 93% of all choices. The key attribute affecting women's preferences was a test with the highest diagnostic yield ($p<0.0001$). Women preferred tests with short turnaround times and tests where maximum results were reported (including variants of uncertain significance (VUS) and secondary findings (SF)). Women from all countries except Denmark preferred VUS to be reported ($p<0.004$), and women from all countries except the USA preferred receiving SF ($p<0.02$). Women from China preferred results returned by a genetics specialist, whereas in Denmark they preferred a maternity specialist. Whilst most women want to receive maximum information, our findings also highlight country based differences. Therefore, a global consensus on returning results from fetal exome/genome sequencing is not necessarily realistic or desirable. Funding: Wellcome Trust Small Grant in Humanities and Social Science [211288/Z/18/Z].

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C12.4 Personality traits predict psychological impact derived from germline cancer genetic testing

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Introduction: Integration of personality traits (Neuroticism, Extraversion, Openness, Agreeableness, Conscientiousness) in the genetic counselling of germline cancer testing is key to understand personalized medicine as an adaptation to individuals' psychosocial needs. Previous studies suggested more uncertainty in carriers of moderate versus high-penetrance breast cancer (BC) genes. We aimed to investigate the association between personality traits and the psychological impact from hereditary germline cancer genetic testing(GCGT); and compare the psychological impact between moderate and high-penetrance pathogenic variant carriers

Materials and Methods: Prospective multicentre study including individuals who underwent GCGT. The NEO-FFI (personality) and the Cancer Worry (CWS) Scales were analysed at baseline; and the MICRA (psychological impact) and CWS scales immediately, 3 and 12 months after results disclosure. Multivariate analysis was used to evaluate predictors of psychological impact.

Results: Overall, 714 patients were analysed: 532 (74%) female, 394 (55%) diagnosed with cancer, 404 (56%) underwent panel testing, 44% single gene testing, and 187 (26%) resulted positive. Female sex, cancer diagnosis, and high levels of neuroticism correlated with higher psychological impact (Table 1). Among mutation carriers, no differences in uncertainty between high and moderate BC risk carriers (p-values>0.05).

Conclusions: Levels of neuroticism is an independent factor to predict the psychological impact of GCGT. There are no differences in uncertainty levels between high and moderate BC risk carriers. **Grants** Carlos III National Health Institut and Ministerio de Educación y Ciencia (PI16/01363)

	Liner mixed model to estimate MICRA score (n = 714)					
	Univariate			*Multivariate*		
	Estimate	*CI (95%)*	*P value*	*Estimate*	*CI (95%)*	*P value*
Age (10-years increment)	0,1	[−0.43, 0.63]	*0,72*	–	–	–
Gender (male vs. female)	−4,01	[−5.6, −2.42]	***<0.001***	−2,78	[−4.41, -1.15]	**<0.001**
High education (yes vs. no)	−0,88	[−2.37, 0.61]	*0,25*	–	–	–
Result (positive vs. negative)	0,31	[−1.29, 1.92]	*0,71*	–	–	–
Cancer diagnosis (yes vs. no)	3,51	[2.12, 4.9]	**<0.001**	2,62	[1.21, 4.04]	**<0.001**
Neuroticism (vs low)						
- Medium	1,78	[−0.30, 3.86]	0,09	1,97	[−0.07, 4.01]	0,06
- High	5,45	[3.67, 7.27]	**<0.001**	5,14	[3.31, 6.97]	**<0.001**
Extraversion (vs low)						
- Medium	−0,19	[−1.80, 1.42]	0,81	–	–	–
- High	-0,8	[−2.64, 1.04]	0,4	–	–	–
Conscientious (vs low)						
- Medium	−1,39	[−3.13, 0.35]	0,12	–	–	–
- High	−1,74	[−3.52, 0.04]	0,05	–	–	–
Openness (vs low)						
- Medium	1,14	[−0.51, 2.79]	0,18	–	–	–
- High	−0,63	[−2.37, 1.11]	0,48	–	–	–
Agreeableness (vs low)						
- Medium	−2,32	[−3.93, -0.71]	**0,005**	−1,66	[−3.25, -0.07]	**0,04**
- High	−1,79	[−3.55, -0.02]	0,05	−0,57	[−2.31, 1.17]	0,52

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C12.5 Breast cancer polygenic risk scores: A 12-month prospective study of patient reported outcomes and risk management behavior

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Introduction: Polygenic risk scores (PRS) for breast cancer risk have emerged as a potential tool for informing disease risk management. However, little is known about women's responses to receiving this information. This study aimed to prospectively assess patient reported outcomes and risk management behavior of women choosing to receive (receivers) or decline (decliners) their breast cancer PRS. Methods: Women either unaffected or affected by breast cancer and from families with no identified pathogenic variant in a breast cancer risk gene were invited to receive their PRS. Genotyping for 62 common variants was performed, from which a PRS and relative risk were calculated. All participants completed a questionnaire at study enrollment. Receivers completed questionnaires at two-weeks and 12-months after receiving their PRS, and decliners a second questionnaire at 12-months post study enrollment.

Results: Of the 208 participants, 165 (79%) received their PRS. Among receivers there were no changes in anxiety or distress following testing. Receiving a low PRS was associated with reduced perceived breast cancer risk at 12-months ($p = 0.030$). At 12-months, breast screening and uptake of risk-reducing strategies were consistent with current Australian guidelines of breast cancer risk management. Compared to receivers, decliners reported significantly higher decisional regret regarding receipt of their PRS ($p<0.001$). Reasons for declining PRS included being unable to attend the appointment in person and concerns over potential emotional response.

Conclusion: Our findings provide additional support for the suitability of PRS in clinical practice, while highlighting the issues that need to be addressed when providing this information.

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C12.6 A tailored approach towards informing relatives at risk of inherited cardiac diseases: results of a randomised controlled trial

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Introduction: If undetected, inherited cardiac diseases can lead to sudden cardiac death, while preventive and treatment options are available. Currently, probands are asked to inform relatives, with only half of relatives attending genetic counselling. Direct contact by the genetic counsellor may enhance uptake, although there are legal, ethical and psychological concerns.

Methods: Current practice was compared to a tailored approach, in which probands were asked to decide to (initially) inform relatives themselves or by the counsellor, and family letters were sent directly by the counsellor 1 month after disclosure of the patients' test result. Outcomes were uptake of counselling in the first year, impact on psychological and family functioning, and evaluation of the approach (measured with surveys at 3 (T1) and 9 (T2) months after disclosure).

Results: 96 probands were included, yielding 549 relatives eligible for counselling and genetic testing. We observed no significant difference in uptake of genetic counselling between the current (38%) and the tailored (37%) approach. Significantly more probands in the tailored group felt satisfied and supported with the used approach, while they also felt more pressure to inform relatives and perceived room for improvement. No differences in psychological and family functioning were observed.

Conclusions: Surprisingly, the tailored approach showed a similar uptake as the current approach. There were no differences observed in impact on psychological/family functioning, but probands in the tailored group more often felt satisfied and supported. Further research is needed to identify the most optimal approach to reach at-risk relatives. Funding: CVON 2015-12 eDETECT

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C13 Cancer susceptibility: From mechanisms to clinic

C13.1 Dissecting mutational mechanisms underpinning signatures caused by replication errors and endogenous DNA damage

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Mutational signatures are imprints of pathophysiological processes arising through tumorigenesis. Here, we generate isogenic CRISPR-Cas9 knockouts (Δ) of 43 genes in human induced pluripotent stem cells, culture them in the absence of added DNA damage, and perform whole-genome sequencing of

173 daughter subclones. Δ*OGG1*, Δ*UNG*, Δ*EXO1*, Δ*RNF168*, Δ*MLH1*, Δ*MSH2*, Δ*MSH6*, Δ*PMS1*, and Δ*PMS2* produce marked mutational signatures indicative of being critical mitigators of endogenous DNA changes. Detailed analyses reveal that 8-oxo-dG removal by different repair proteins is sequence-context-specific while uracil clearance is sequence-context-independent. Signatures of mismatch repair (MMR) deficiency show components of C>A transversions due to oxidative damage, T>C and C>T transitions due to differential misincorporation by replicative polymerases, and T>A transversions for which we propose a 'reverse template slippage' model. Δ*MLH1*, Δ*MSH6*, and Δ*MSH2* signatures are similar to each other but distinct from Δ*PMS2*. We validate these gene-specificities in cells from patients with Constitutive Mismatch Repair Deficiency Syndrome. Based on these experimental insights, we develop a classifier, MMRDetect, for improved clinical detection of MMR-deficient tumors.

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C13.2 *CCNF* (Cyclin F) as a candidate gene for familial Hodgkin lymphoma

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Hodgkin lymphoma (HL) is a hematological malignancy that affects 2.7 individuals per 100,000 per year. It is characterized by the presence of rare, morphologically distinctive tumor cells surrounded by reactive immune cells. We assessed for rare variants with the potential to confer predisposition to familial HL, in whole exome sequencing (WES) data from a series of 22 affected members from 11 families. Genes with rare, predicted-pathogenic, disease-cosegregating variants in two or more families were considered candidates. Intriguingly, a significant fraction of the 35 genes identified act in mitosis, mitotic checkpoints and DNA repair, with a second fraction acting in metabolic processes. *CCNF* (Cyclin F), a widely-expressed cell cycle-regulated E3 ubiquitin ligase component, belongs to the first category and was prioritized for in vitro functional testing. The ubiquitination targets of the Skp1-Cul1-F box$^{\text{Cyclin F}}$ ($SCF^{CyclinF}$) complex function at precise points during mitosis and are then degraded; inappropriate persistence of these proteins can cause genomic instability, focal DNA damage, micronuclei, and formation of multinuclear cells, a hallmark of certain HL tumors. We find that both of the variants identified in familial HL disrupt binding of CCNF to three different ubiquitination targets tested: RRM2 (Ribonucleotide Reductase Regulatory Subunit M2), involved in deoxyribonucleotide production and homeostasis), CDC6 (Cell Division Cycle 6, involved in regulation of DNA replication) and EXO1 (Exonuclease 1, involved in DNA repair, telomere maintenance). CRISPR/Cas9-mediated disruption of CCNF in lymphoblasts is being used to confirm the induction of HL-like cellular phenotypes, supporting a tumor-suppressor role for CCNF.

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C13.3 Interpreting *TP53* variants identified in HBOC panels: a challenge for geneticists and a major issue for patients

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Since 2017, a large gene panel including the *TP53* gene has been validated by the French Genetic and Cancer Group to explore patients suggestive of hereditary breast and ovarian cancer predisposition (HBOC). As breast cancer belongs to the tumour spectrum of the Li-Fraumeni or heritable *TP53*-related cancer syndrome, we embarked upon a large scale collection and classification of *TP53* variants found in a HBOC context. Sequencing of 22,500 HBOC panels in France led to the detection, after exclusion of circulating tumour DNA and clonal haematopoiesis of 232 heterozygous *TP53* variants (1%). Most of them remained unclassified by ClinGen Expert Panel and therefore were classified according to *TP53* specifications adapted from ACMG. Thirty three percent were deleterious (classes 4 and 5) and the rest were VUS requiring further investigations. Interestingly, 45% of deleterious variants have never been described in LFS, suggesting a different mutational spectrum in HBOC patients. Unexpectedly, similar rates of deleterious variants were found between the group of patients fulfilling the Chompret diagnosis criteria for LFS and the group who did not fulfil these criteria (33% and 29% of detected *TP53* variants, respectively). We also identified in late breast or ovarian cancer (median ages 46 and 79 years, respectively) several loss-of-function or dominant negative missense *TP53* variants, already described in childhood cancers. Identification of pathogenic, childhood cancer-prone *TP53* variants in HBOC adult patients suggests the existence of modifying factors and obviously casts a shadow of complexity over genetic counselling and clinical follow up of these patients and their families.

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C13.4 Surveillance recommendations for *DICER1* pathogenic variant carriers: a report from the SIOPE Host Genome Working Group and CanGene-CanVar Clinical Guideline Working Group

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DICER1 syndrome is a rare genetic disorder that predisposes to a wide spectrum of tumors. Developing surveillance protocols for this syndrome is challenging because uncertainty exists about the clinical efficacy of surveillance, and appraisal of potential benefits and harms vary. In addition, there is increasing evidence that germline *DICER1* pathogenic variants are associated with lower penetrance for cancer than previously assumed. To address these issues and to harmonize DICER1 syndrome surveillance programs within Europe, the Host Genome Working Group of the European branch of the International Society of Pediatric Oncology and Clinical Guideline Working Group of the CanGene-CanVar project reviewed current surveillance strategies and evaluated literature. Consensus was achieved for a new surveillance protocol and information leaflet that informs patients about potential symptoms of *DICER1*-associated tumors. The surveillance protocol comprises a minimum program and an extended version for consideration. The key recommendations of the minimum program are: annual clinical examination from birth to age 20 years, six-monthly chest X-ray and renal ultrasound from birth to age 6 years, and thyroid ultrasound every 3 years from age 8 to age 40 years. The surveillance program for consideration comprises additional surveillance procedures, and recommendations for *DICER1* pathogenic variant carriers outside the ages of the surveillance interval. Patients have to be supported in choosing the surveillance program that best meets their needs. Prospective evaluation of the efficacy and patient perspectives of proposed surveillance recommendations is required to expand the evidence base for surveillance protocols. This work was supported by Cancer Research UK C61296/A27223.

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C13.5 *APC* mosaicism testing in milder polyposis phenotypes reveals *pks+ E.coli* bacteria as a possible additional explanation for the development of colorectal adenomas

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Introduction: Mosaic mutations in the *APC* gene have been identified as a common cause (25%) for unexplained polyposis in patients with >20 adenomas. The frequency of *APC* mosaicism remains unknown in milder phenotypes.

Materials and Methods: To test for *APC* mosaicism, we analyzed the *APC* gene in multiple lesions of patients with unexplained colonic polyposis using Next Generation Sequencing. Additionally, patients with milder phenotypes, e.g. >20 adenomas at age >70, were included.

Results: The mosaicism detection rate was 12% (27/232) in the entire cohort, 5.7% in patients with <10 adenomas (2/35) and 7.7% in those with 10-20 adenomas (8/104). Stratified for age, 2.8% (1/36) of patients aged >70 showed with a mosaicism. Besides these "true" mosaicism cases, 21% (50/232) of patients showed a so-called hybrid mosaicism, where multiple, but not all lesions share an identical variant. Interestingly, 46% (23/50) of hybrids have a specific *APC* splice variant c.835-8A>G in multiple lesions. Together with 7 other recurring *APC* variants, this variant was compatible with the recently described mutational signature caused by colibactin, a compound produced by *pks+ E.coli*. The possible influence of colibactin needs further exploration. Therefore, we are now performing additional analyses like Whole Genome Sequencing.

Conclusions: Our results indicate that *APC* mosaicism also plays a role in milder polyposis phenotypes. Furthermore, a substantial

proportion of our cohort had a hybrid mosaicism of which the clinical consequences are not yet clear. In some patients, the presence of *pks+ E.coli* might be the explanation for the development of polyps. **Grant:** DCS

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C13.6 Germline chromothripsis of the APC locus in a patient with adenomatous polyposis

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Many patients with clinical attenuated adenomatous polyposis coli remain undiagnosed after standard genetic testing. Here, we describe a patient with normal results for Sanger sequencing and NGS finally carrying complex structural rearrangements of the *APC* locus apparently arisen from rare localized germline chromothripsis.

By short-read sequencing (Agilent SureSelectXT, Illumina), benign heterozygous variants were identified in *APC* exons 11, 13 and 15, which were used to determine the allelic expression of *APC* mRNA. RT-PCR and targeted long-read RNA-Seq (Oxford Nanopore Technologies, ONT) showed an allelic imbalance with ratios of 20:80 and 30:70, suggesting an allelic reduction of mRNA expression. A deletion of ~105 kb upstream of *APC* was found by chromosomal microarray but deemed too distant (~990 kb) to disrupt *APC* mRNA expression.

WGS with ONT identified several genomic rearrangements indicative of an insertion of *APC* from chr.5q22 into chr.10q21.3, which was confirmed by FISH. Ultimately, optical mapping and *de novo* assembly (Bionano Genomics) was combined with long-read WGS data to reconstruct both *APC* loci. Whereas one allele was retained in a normal configuration presumably transcribing physiological levels of *APC* mRNA, the second allele was deleted from chromosome chr.5q22.1-q22.3 and inserted into chr.10q21 (*CTNNA3*). The inserted fragment harbored multiple complex deletions, duplications, and inversions separating the *APC* promoter/exon 1B from the remaining exons and attenuating transcription.

Combining Bionano optical mapping, ONT WGS, and RNA-seq provides a powerful tool set for the diagnosis of hitherto genetically unresolved patients with rare mutation mechanisms.

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C14 Advances in neurogenetics: From diagnosis to treatment

C14.1 DNA methylation episignature testing improves molecular diagnosis of mendelian chromatinopathies

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Chromatinopathies define genetic neurodevelopmental conditions caused by genetic alterations of components of the epigenetic machinery. Majority of these disorders exhibit highly specific genome wide DNA methylation profiles detectable in peripheral blood, referred to as episignatures or EpiSigns. Here we assessed the DNA methylation episignatures in a cohort of patients with clinical features of a chromatinopathy disorder. The 129 patients were grouped in: i) the discovery cohort included patients with a certain clinical diagnosis associated with a pathogenic variant, ii) the validation cohort consisted of patients carrying pathogenic or likely pathogenic variant with no definitive clinical diagnosis, iii) the uncertain cohort included patients with a clinical diagnosis carrying VUS, and iv) negative cohort consisted of patients with no definitive clinical diagnosis and no genetic findings. EpiSign analysis was concordant with sequencing findings in 98/101 samples. For 3 cases without previous genetic findings, EpiSign analysis identified a unique DNA methylation profile, as a sole molecular diagnostic finding explaining their clinical presentation. In the uncertain cohort, twenty-five samples had no known episignature suggesting that the identified VUS has likely no clinical relevance and that those patients need to be clinically and molecularly reconsidered. Two *KDM6A* VUSs were reclassified as likely pathogenic after EpiSign analysis, while in one patient with a Kabuki VUS, an alternate episignature associated with Wolf-Hirschhorn syndrome was detected and subsequently confirmed. We demonstrated that DNA methylation episignature provides an effective diagnostic modality in Chromatinopathies, helping establish, improve, and correct molecular diagnosis achieved by standard DNA sequencing.

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C14.2 Integrative approach to interpret *DYRK1A* variants, leading to a frequent neurodevelopmental disorder

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Introduction: Intellectual disability (ID) is a highly heterogeneous group of neurodevelopmental disorders with substantial genetic contributions which overlap strongly both at the clinical and genetic levels. *DYRK1A*-related ID is among the most frequent monogenic form of ID. We refined the molecular and clinical description of this disorder and developed tools to better interpret missense variants, which remains a major challenge in human genetics.

Methods: We reported clinical and molecular data of forty individuals with ID harboring *DYRK1A* variants and developed i) a specific *DYRK1A* clinical score, ii) amino acid conservation data generated from one hundred of DYRK1A sequences across different taxa, iii) in vitro overexpression assays to study level, cellular localization, and kinase activity of DYRK1A mutant proteins, and iv) a specific blood DNA methylation signature.

Results: This integrative approach was successful to reclassify several variants as pathogenic. However, we questioned the involvement of some others, such as p.Thr588Asn, yet reported as pathogenic, and showed it does not cause protein impairment or obvious phenotype in mice.

Conclusion: Our study demonstrated the need for caution when interpreting variants in *DYRK1A*, even those occurring *de novo*. The tools developed will be useful to interpret accurately the variants identified in the future in this gene.

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C14.3 SUFU heterozygous loss of function variants cause a dominantly inherited neurodevelopmental disorder at the mildest end of Joubert Syndrome

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Joubert syndrome (JS) is a recessively inherited ciliopathy characterized by a unique cerebellar and brainstem malformation, the "molar tooth sign" (MTS), associated to ataxia, oculo-motor apraxia (OMA), developmental delay/intellectual disability (DD/ID) and variable multiorgan involvement. Over 40 JS genes are known, accounting for ~60% cases.

In 2018, we reported homozygous hypomorphic missense variants of the SUFU gene in 2 families with mild JS and polydactyly. A recent study identified heterozygous loss of function (LOF) SUFU variants in families with dominantly inherited OMA occasionally associated with mild cerebellar hypoplasia and DD/ID.

To test the hypothesis that SUFU haploinsufficiency may be responsible for a neurodevelopmental disorder at the mildest end of the JS spectrum, we reanalyzed NGS data in a large cohort of 442 JS patients, and identified SUFU heterozygous LOF variants in 6 patients (1.4%). This frequency is significantly higher than that reported in GnomAD (0.002%, $p<0.00001$). The phenotype was characterized by mild, purely neurological JS, with OMA and mild DD/ID. While most variants were

de novo, two variants were inherited inherited from mothers, which reported mild DD, clumsiness and learning difficulties in childhood.

We propose SUFU heterozygous LOF variants as responsible of a spectrum of neurodevelopmental disorders ranging from isolated OMA to a mild, purely neurological form of JS.

Given the overall "benignity" of the phenotype, this condition can be transmitted from a carrier parent unaware of being affected. Awareness of this genetic condition will increase the diagnostic yield of mild JS, allowing appropriate genetic counselling and risk estimates.

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C14.4 Mitochondrial dysfunction and oxidative stress may explain cognitive and muscle impairment in FOXP1 syndrome

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Impaired mitochondrial function can affect cognitive and motor performance in people with neurodevelopmental disorders. FOXP1 syndrome is a neurocognitive disorder that includes motor malfunction, as well as intellectual disability, autism and language impairment. In this study, we used a *Foxp1*$^{+/-}$ mouse model to investigate whether cognitive and motor deficits in FOXP1 syndrome are associated with mitochondrial dysfunction and oxidative stress. Here we show that several genes that regulate mitochondrial biogenesis and dynamics (e.g. *Foxo1*, *Pgc-1a*, *Tfam*, *Opa1*, and *Drp1)* were dysregulated in the striatum of *Foxp1*$^{+/-}$ mice at different postnatal stages. Furthermore, in the striatum of *Foxp1*$^{+/-}$ animals, mitochondrial membrane potential was disrupted, and reactive oxygen species, lipid peroxidation and cytochrome c release were significantly elevated. These features can

explain the reduced neurite branching, learning and memory, endurance, and motor coordination that we observed in these animals. Taken together, we provide strong evidence of mitochondrial dysfunction in *Foxp1*$^{+/-}$ mice, suggesting that insufficient energy supply and excessive oxidative stress may underlie the cognitive and muscle impairment in patients with FOXP1 deficiency.

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C14.5 Antisense oligonucleotides targeting *SNCA* reduce alpha-synuclein and associated cellular pathology in Parkinson's patient iPSC-derived midbrain dopaminergic neurons

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder and the fastest growing neurological disease, with a prevalence in the general population aged ≥ 65 years of 2-3 % already. With no approved curative or disease-modifying therapies currently available, PD presents a major unmet clinical need. Overwhelming evidence suggests that the abnormal aggregation of the protein α-synuclein, encoded by the *SNCA* gene, is the causative agent of PD, driving the neuronal death and cellular dysfunction characteristic of the disorder. Given that multiplications of the *SNCA* gene are known to cause familial PD, and that PD risk loci identified through genome-wide association studies have been shown to regulate *SNCA* expression, there has been growing interest in novel therapies directly targeting α-synuclein. Here, we use novel antisense oligonucleotides (ASOs) targeted to human *SNCA* to reduce α-synuclein expression in midbrain dopaminergic (mDA) neurons generated from PD patient-derived induced pluripotent stem cells. We demonstrate that an ASO-induced reduction in α-synuclein expression reverses established α-synuclein pathology and rescues cell death in these neurons. We further demonstrate that this improvement in neuronal health may be mediated through an amelioration of mitochondrial function. In summary, our data supports the continued exploration of ASO technology targeted to *SNCA* as a novel disease-modifying therapy for PD and other related synucleinopathies.

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C14.6 Yield of clinically reportable genetic variants in cerebral palsy by whole genome sequencing

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Despite increasing evidence for a major contribution of genetics to cerebral palsy etiology, genetic testing is not performed systematically. We assessed the diagnostic rate of genome sequencing (GS) in a heterogeneous cohort of singleton cerebral palsy patients without prior genetic testing. 150 participants were retrospectively recruited after formal diagnosis of cerebral palsy at >4 years of age, with parents also recruited where possible. Cases were clinically unselected, except for exclusion of post-neonatal causes of CP, and diagnosis confirmed by a paediatric rehabilitation specialist. PCR-free GS was performed on the proband, and single nucleotide, indel and copy number variants called. Variants were filtered, and classified according to American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG-AMP) guidelines. Variants classified as ACMG pathogenic or likely pathogenic (P/LP) were assessed for their contribution to the reported clinical presentation. Clinically relevant genetic variants were identified in a heterogeneous group of genes, including genes associated with hereditary spastic paraplegia, clotting and thrombophilic disorders, small vessel disease, and other neurodevelopmental disorders. In total, 24.7% of individuals carried a variant considered to cause or increase risk of cerebral palsy, with 4.7% of the cohort resolved by copy number variant analysis and 20% carrying single nucleotide or indel variants. A further 34% of probands carried one or more rare, high impact variants of uncertain significance (VUS) in variation intolerant genes. Future reanalysis of GS data will increase diagnostic yield, largely due to increasing associations of emerging genes with CP etiology enabling reclassification of high impact VUS.

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C15 Pleiotropic diseases: diagnosis and mechanisms

C15.2 Evaluating positive and negative predictive values of expanded carrier screening: lessons learned from the ciliopathies

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Next generation sequencing allows determination of carrier status for rare recessive disorders, informing couples about their reproductive risk. To justify its broad application, any screening test must demonstrate sufficient positive and negative predictive values (PPV/NPV). Predicting the clinical significance of identified variants represents a challenge for expanded carrier screening (ECS), since variant pathogenicity interpretation relying on ACMG criteria is limited in healthy individuals because criteria like "phenotypic match" or "familial segregation" are unapplicable. To evaluate PPV and NPV of ECS, we focused on the ciliopathies, a well-studied group of recessive disorders with ~130 associated genes causing severe pediatric phenotypes. Performing WES on 397 healthy individuals, we found 37% to be carriers of ≥1 "reportable" ciliopathy variant. In 5/177 couples, both partners carried variants in the same gene. However, classifications frequently differed between databases, questioning the pathogenicity of identified variants and limiting the PPV. Novel missense variants are typically classified as VUS and not reported. They represent however putative false negatives, increasing the residual risk of being a carrier after a "negative" test. How much this decreases the NPV is unclear. Including rare novel missense

variants predicted as deleterious by multiple algorithms, the carrier rate rose to 71% with 11/177 couples at potential risk. Ciliopathies being rare disorders, the majority of variants cannot be disease alleles; To identify disease-causing variants, we included gene-specific and structural information to prioritize variants for functional evaluation. In conclusion, current limitations in variant pathogenicity prediction substantially decrease PPV and NPV of ECS. **Funding**: KFSP-UZH *Praeclare*

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C15.3 The Bardet-Biedl protein Bbs1 control protein and lipid composition of zebrafish photoreceptor outer segments

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Bardet-Biedl syndrome (BBS) is a ciliopathy characterized by retinal dystrophy, intellectual disability, polydactyly, obesity and renal anomalies. In photoreceptors (PR), light sensation occurs in outer segments (OSs), which are specialized primary cilia. *BBS1*, the major BBS gene, is part of a protein complex called "BBSome", which is involved in intracellular protein transport. However, the precise function of BBS1/BBSome in controlling trafficking of ciliary proteins in PRs remains unclear.

In zebrafish *bbs1* mutants, we observed impaired visual function starting at 5 days post fertilization (dpf), before appearance of any morphological changes. With time, progressive PR-cell layer disorganization and dysmorphic OSs appeared. PRs differentiated normally, indicating that Bbs1 is predominantly required for their function and/or maintenance. Eye-specific transcriptomic analyses showed no differentially expressed genes at 5dpf, indicating that developmental signaling is not affected by Bbs1 loss. At 10dpf, mostly non-specific degenerative signatures were present in the transcriptome. Proteomics on isolated adult OSs revealed the loss of the entire BBSome from mutant OSs and an overall accumulation of non-ciliary proteins. Membrane-associated proteins were overrepresented in our dataset, supporting the role of BBS1/the BBSome in controlling ciliary transport of membrane-associated proteins. Interestingly, lipid binding/transport proteins were significantly enriched in mutant OSs. LC-MS lipidomics revealed an increase of unesterified cholesterol in mutant OSs. Given the exquisitely controlled lipid composition of OSs, this disruption is expected to interfere with visual function. In conclusion, we have identified a novel putative function for Bbs1/BBsome in lipid homeostasis, which suggests a new mechanism underlying decreased visual function. Grant: SNF:_PP00P3_170681

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C15.4 Beyond syndromic optic atrophy: expanding the ocular phenotype caused by biallelicvariants in *FDXR* and reporting retinal dystrophy as a novel feature

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Introduction: Biallelic *FDXR* (ferredoxin reductase) variants were recently identified to lead to a novel mitochondrial disorder characterised by sensorineural hearing loss, visual impairment and systemic manifestations. Since the first reports in 2017, other researchers identified further individuals diagnosed with variable severity mitochondrial disorder consequent upon pathogenic biallelic *FDXR* variants totalling 34 cases worldwide. This study reports novel pathogenic variants in *FDXR* and details the association of *FDXR* variants with retinal dystrophy in an expanded spectrum of mitochondrial iron-sulfur synthesis disease.

Methods: Patients carrying biallelic *FDXR* variants were identified by genome sequencing (GS) as part of the National Institute for Health Research BioResource rare-disease and the UK's 100,000 Genomes Project with an additional case identified by exome sequencing. Retrospective clinical data was collected from the medical records. Haplotype reconstruction was performed in families harboring the same missense variant.

Results: 10 individuals (8 unrelated families) with biallelic variants in *FDXR* were identified. In addition to bilateral optic atrophy and variable extra-ocular findings, 7/10 (70%) individuals had retinal dystrophy, not well characterised in previous reports. The previously unreported missense variant (c.1115C>A) was found in 5/8 (62.5%) study families. Haplotype reconstruction using GS data demonstrated a likely ancestral haplotype.

Conclusions: *FDXR*-associated disease is a phenotypically heterogeneous disorder with retinal dystrophy as a frequent clinical feature observed in this cohort. In addition, we hypothesise that multiple factors may drive the pathogenesis of optic atrophy, retinal degeneration and perhaps the systemic manifestations.

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C15.5 A Mouse Model of Brittle Cornea Syndrome caused by mutation in *Zfp469*

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The collagen-rich corneal stroma accounts for 90% of corneal thickness in humans, and is a major determinant of visual acuity. Brittle Cornea Syndrome (BCS) is a rare recessive condition characterised by extreme thinning of the cornea and sclera. BCS results from loss-of-function (LOF) mutations in the poorly understood genes *ZNF469* or *PRDM5*. We created a mouse model of BCS in order to determine the function of Zfp469 in the cornea, and to elucidate pathogenic mechanisms in BCS. CRISPR-Cas9 genome editing was used to recapitulate a human *ZNF469* BCS mutation in the orthologous gene in mouse, *Zfp469*. The resulting mouse line has been subject to ophthalmic phenotyping, including anterior segment optical coherence tomography, histology, immunohistochemistry and transmission electron microscopy (TEM), alongside transcriptomic and proteomic profiling of stromal keratocytes. Homozygous Zfp469 LOF causes significant corneal thinning, arising from reduced stromal thickness, established during corneal development. The expression of genes with important roles in keratocytes was altered in homozygous mutant lines, changing the composition of ECM in vivo and in vitro. Primary keratocytes show a proliferation defect, suggesting possible mechanisms that contribute to disease pathology in BCS. A mouse model of Zfp469 dysfunction offers a unique entry point for investigating regulatory processes shaping the stroma in health and disease, and highlight pathways contributing to the development and maintenance of a healthy corneal stroma. Work remains to determine whether these pathways may be modulated for diagnostic or therapeutic benefit in BCS or conditions such as keratoconus where the cornea thins progressively over time.

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C15.6 Whole exome sequencing reveals a monogenic cause in 57% of individuals with laterality disorders and associated congenital heart defects

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Introduction: The molecular basis of heterotaxy and congenital heart malformations associated with disruption of left-right (L-R) asymmetry is broad and heterogenous, with over 25 genes implicated in its pathogenesis thus far. *Objective:* We sought to elucidate the molecular basis of laterality disorders and associated congenital heart defects in a cohort of 28 unrelated probands of Arab-Muslim descent, using next generation sequencing techniques. *Methods:* Detailed clinical phenotyping followed by Whole Exome Sequencing (WES) was pursued for each of the probands and their parents (when available). Sanger sequencing was used for segregation analysis of disease-causing mutations in the families.

Results: Using WES, a molecular diagnosis was reached for 16 of the 28 probands (57%). Genes known to be associated with heterotaxy and/or primary ciliary dyskinesia in which homozygous pathogenic or likely-pathogenic variants were detected, included: *CFAP53 (CCDC11), CFAP298* (*C21orf59*), *CFAP300, LRRC6, GDF1, DNAAF1, DNAH5, CCDC39, CCDC40* and *TTC25*. Additionally, we detected a homozygous disease causing mutation in *DAND5*, as a novel recessive monogenic cause for heterotaxy in humans. Three additional probands were found to harbor variants of uncertain significance. These included variants in *DNAH6, HYDIN, CELSR1* and *CFAP46*.

Conclusions: Our findings contribute to the current knowledge regarding monogenic causes of heterotaxy and its associated congenital heart defects and underscore the role of next generation sequencing techniques in the diagnostic workup of such patients, and especially among consanguineous families.

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C16 Monogenic neurodevelopmental disorders

C16.1 Pathogenic variants in *SMARCA5*, a chromatin remodeler, cause a syndromic neurodevelopmental disorder

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Chromatin remodelers regulate chromatin states and DNA accessibility, to achieve precise gene transcription. We describe pathogenic variants in *SMARCA5*, encoding the ATPase motor of the ISWI chromatin remodeler, as a cause of a novel neurodevelopmental disorder. By exome sequencing and international matchmaking, we identified twelve individuals from ten unrelated families with nine *de novo* or dominantly inherited variants in *SMARCA5*. All of the variants were absent in population genomics resources. Accompanying phenotypes include varying degrees of developmental delay, frequent postnatal short stature and microcephaly, and recurrent dysmorphic features. Three-

dimensional modelling suggested that all of the identified amino-acid substitutions may disrupt interactions of SMARCA5 with the nucleosome and in turn affect ATPase binding. Loss-of-function mutation of its *Drosophila* orthologue *Iswi* leads to decreased larval body size and sensory dendrite complexity, together with a tiling defect. Moreover, neural-specific knockdown of *Iswi* in adult flies exhibit smaller brain, mushroom body patterning defects and impaired locomotion. These phenotypes mimic the salient features observed in this patient cohort. Remarkably, these phenotypes seen in flies can be rescued by wild type human SMARCA5 but not the variants identified in humans, highlighting the pathogenic nature of the identified variants in patients. Our findings in a fly in vivo model highlight the important and previously underappreciated role of the ISWI family proteins in dendrite morphogenesis, neural circuit formation, and diverse behaviors. In conclusion, this study expands the spectrum of ISWI-related disorders and extends the tally of causative genes in neurodevelopmental disorders.

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C16.2 Biallelic *TTI1* pathogenic variants cause a microcephalic neurodevelopmental disorder

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Telomere maintenance 2 (TELO2 or Tel2), Tel2 interacting protein 2 (TTI2) andTel2 interacting protein 1 (TTI1) are the three components of the conserved Triple T (TTT) complex that regulates activities of phosphatidylinositol 3-kinase-related protein kinases (PIKKs), including mTOR, ATM and ATR, by regulating the assembly of mTOR complex 1 (mTORC1) and is essential for the expression, maturation and stability of ATM and ATR in response to DNA damage. *TELO2* and *TTI2*-related autosomal recessive encephalopathies have been recently described in 8 and 10 patients respectively, presenting with moderate to severe intellectual disability and postnatal microcephaly, associated to a movement disorder for *TELO2* patients and short stature for *TTI1* patients. We present clinical and functional data in a series of seven patients belonging to five unrelated families. All harbor *TTI1* biallelic variants and present with intellectual deficiency, microcephaly, a short stature, and a movement disorder. Functional studies were conducted. We evaluated the activity of TTI1 clinical mutants, Leu767Ser, Ser838Leu and Asp921Asn, and of 3 additional TTI1 variants, Val123Glu, Ser402Pro and Asp887Glu in HEK293T cell lines. We also showed functional impairment of TTT complex and mTor pathway activity in cells derived from 4 patients. With these results, we describe *TTI1*-related encephalopathy for the first time and introduce the concept of TTT-pathy.This work was supported by UM1 HG006542 (JRL) from the National Human Genome Research Institute (NHGRI)/National Heart Lung and Blood Institute (NHLBI) to the Baylor Hopkins Center for Mendelian Genomics (BHCMG). SEA was partially supported by the ChildCare Foundation and an ERC grant.

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C16.3 Monoallelic variants in *TFAP2E* cause central nervous system and craniofacial anomalies

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TFAP2E encodes the transcription factor AP-2. This transcription factor's role in the formation of the central nervous system (CNS) and craniofacial structures in humans has not been investigated. Here, we report 4 individuals with novel heterozygous missense variants in *TFAP2E*, all presenting with CNS malformations and craniofacial anomalies. For one family, parental samples were available to confirm *de novo* occurrence of the missense variant. All variants were identified by exome sequencing. We initiated functional characterisation in zebrafish larvae including splice and translational blocking morpholinos (MO) in different transgenic zebrafish lines. Fluorescent signals of neuronal, glial and neural crest cells at 2 dpf were analysed in vivo using a two-photon point scanning microscope and subsequent 3D modelling with IMARIS analysis software. Alcian blue cartilage staining was done at 3 dpf. Shared phenotypic features among patients comprised malformations of the CNS (4/4) and craniofacial anomalies (4/4). MO knockdown of *tfap2e* in zebrafish larvae recapitulated the human CNS and craniofacial phenotype. Major effects on vasculogenesis were excluded after MO injections into vascular reporter lines, suggesting the tissue specificity of the observed effects. Immunohistochemistry showed the expression of Tfap2e in the developing CNS of zebrafish and mice. Our findings indicate that *TFAP2E* plays an important role in CNS and neural crest development and suggest *TFAP2E* as a novel candidate gene for human CNS malformations and craniofacial anomalies. Grant: J.C.K: BonnNI Q614.0754, BONFOR O-167.0023. HR, HT: DFG RE 1723/5-1, TH 1327/2-1. J.R.L: NHGRI and NHLBI grant to the Baylor-Hopkins Center for Mendelian Genomics [UM1 HG006542]

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C16.4 Biallelic *TRAPPC10* variants are associated with a microcephalic TRAPPopathy disorder in humans and mice

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The highly evolutionarily conserved transport protein particle (TRAPP) complexes (TRAPP II and III) perform fundamental roles in secretory and endocytic subcellular trafficking pathways. TRAPPC9 and TRAPPC10 are subunits specific to the mammalian TRAPP II complex. Our genomic studies confirmed biallelic *TRAPPC10* variants as the cause of disease in ten individuals from two families affected by an autosomal recessive neurodevelopmental disorder. The cardinal clinical features include microcephaly, short stature, severe intellectual disability, prominent speech delay, hypotonia, seizures, pervasive behavioural abnormalities and reduced white matter volume including thinning of the corpus callosum. The clinical features of *TRAPPC10*-related disorder display notable overlap with those associated with biallelic *TRAPPC9* variants, and other 'TRAPPopathies'. Studies of *Trappc10*$^{-/-}$ mice revealed significant neuroanatomical brain defects and microcephaly, paralleling those seen in the human condition and in a *Trappc9*$^{-/-}$ mouse model. While both *Trappc10*$^{-/-}$ and *Trappc9*$^{-/-}$ mice display increased BMI (particularly notable in females), only ~60% of individuals with biallelic *TRAPPC9* variants are overweight, and no *TRAPPC10* patients identified to date display a raised BMI. Yeast two hybrid and membrane trafficking assays in HEK293 TRAPPC10$^{-/-}$ and patient lymphoblastoid cell lines revealed an absence of TRAPPC10 alongside an unexpected concomitant absence of TRAPPC9, and a weakened interaction between mutant TRAPPC10 and its putative adaptor protein TRAPPC2L, in addition to membrane trafficking defects. Taken together our studies confirm biallelic *TRAPPC10* variants as causative of a human 'TRAPPopathy' disorder, and define TRAPP-mediated pathomolecular outcomes of importance to TRAPPC9 and TRAPPC10 mediated neurodevelopmental disorders in humans and mice. (Wellcome Trust:209083/Z/17/Z; MRC:G1002279,G1001931)

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C16.5 A clustering of missense variants in the crucial chromatin modifier WDR5 defines a new neurodevelopmental disorder

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WDR5 is a broadly studied, highly conserved protein involved in a wide array of biological functions. Among these functions, WDR5 is a part of several protein complexes that affect gene regulation via post-translational modification of histones. We collected data from ten unrelated individuals with six different rare *de novo* missense variants in *WDR5*; one identical variant was identified in four individuals, and another variant in two individuals. All ten individuals had neurodevelopmental disorders including speech/language delays ($N = 10$), intellectual disability ($N = 8$), epilepsy ($N = 6$) and autism spectrum disorder ($N = 4$). Additional phenotypic features included abnormal growth parameters ($N = 6$), heart anomalies ($N = 2$) and hearing loss ($N = 2$). All six missense variants occurred in regions of the *WDR5* locus known to be extremely intolerant to variation. Three-dimensional structure analyses indicate that all the residues affected by these variants are located at the surface of one side of the WDR5 protein. Five out of the six amino acid substitutions are predicted to disrupt interactions of WDR5 with RbBP5 and/or KMT2A/C, as part of the COMPASS family complexes. Thus, we define a new neurodevelopmental disorder associated with missense variants in *WDR5* characterized by a broad range of associated features including intellectual disability, speech/language impairments, epilepsy and autism spectrum disorders. This finding highlights the important role of COMPASS family proteins in neurodevelopmental disorders.

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C16.6 Biallelic *PI4KA* variants cause a novel neurodevelopmental syndrome with hypomyelinating leukodystrophy

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Introduction: Phosphoinositides (PIPs) are lipids that play a critical role in processes such as cellular signalling, ion channel activity and membrane traffic. When mutated, several genes that encode proteins that participate in the metabolism of these lipids give rise to neurological or developmental phenotypes. PI4KA is a PIP kinase that is highly expressed in the brain and is essential for life.

Materials and Methods: We used whole exome or genome sequencing to identify biallelic PI4KA variants in 10 independent patients. Western Blot, Immunofluorescence and targeted lipidomics to test for PI4KA activity were performed on PBMC ($n = 4$) and fibroblast samples ($n = 5$).

Results: The mutations discovered in PI4KA caused a spectrum of conditions ranging from severe global neurodevelopmental delay with hypomyelination to pure spastic paraplegia. Some patients presented immunological deficits or genito-urinary abnormalities. Functional analyses showed decreased PI4KA and phosphoinositol-4-phosphate levels in the patients' fibroblasts and a diminished PI4KA activity was found in fibroblasts and peripheral blood mononuclear cells.

Conclusions: We report a novel severe neurometabolic disorder caused by *PI4KA* malfunction, highlighting the importance of phosphoinositide (PIP) signalling in human brain development and the myelin sheath. **Acknowledgements:** This study was supported by URD-Cat SLT002/16/00174, the Center for Biomedical Research on Rare Diseases (CIBERER ACCI19-759), the Instituto de Salud Carlos III FIS PI20/00758, the Hesperia Foundation, and the Asociación Española contra las Leucodistrofias (ALE-ELA España).

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C17 Population genetics and genetic epidemiology

C17.1 Estimation of penetrance for known gene-phenotype relationships using large-scale exome sequencing data in 394K UK Biobank participants

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Penetrance estimates for causal variants implicated in monogenic human diseases have traditionally relied on small, family-based case series and are acknowledged to be susceptible to ascertainment bias. As such, the population-based nature of the UK Biobank (UKB) cohort study reduces this ascertainment bias and could enable better estimates of the population-level penetrance for some of the well-known gene-phenotype relationships. We estimated penetrance for certain well-established gene-phenotype relationships using the exome sequence data from ~394K unrelated UKB participants of European ancestry. UKB participants were recruited at age 40-80 years and detailed medical history was obtained from self-report data, general practitioner, hospital and death records. Gene-level penetrance was generated by calculating the proportion of carriers of a specific class of variant

(e.g., rare [MAF<0.1%] protein-truncating variants [PTVs]) in a gene that have a particular disease. For instance, the penetrance of rare PTVs in *MYBPC3*, a predominant cause of hypertrophic cardiomyopathy, is estimated to be 10.5% (11/105 carriers) [95% CI: 5.4%-18%] in the UKB dataset. In comparison, the penetrance for rare PTVs in *PKD1* and polycystic kidney disease is 50.4% (61/121 carriers) [95% CI: 41.2%-59.6%], and that for rare PTVs in *HBB* and thalassemia is 71.1% (59/83 carriers) [95% CI: 60.1%-80.5%]. The UKB can thus provide a complementary resource to assess the population-level penetrance for established gene-phenotype relationships, which may be further optimised through careful variant curation and phenotypic refinement beyond ICD-10 codes. This could also help broaden our understanding of the underlying genetic architecture and improve risk prediction for "monogenic" diseases.

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C17.2 Public health impact of genetic variants

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In genome wide association studies attention is typically focused on measures of relative risk that do not inform us of the total health burden associated with carrying risk alleles. We developed a new framework for estimating the attributable disease burden of genetic variants based on disability-adjusted life-years (DALYs) from the Global Burden of Disease 2019 Study, which depict the amount of healthy life years lost due to premature death and living with disability.

We use information from the FinnGen study (n = 260 391) and UK Biobank (n = 426 464). We meta-analyze hazard ratio estimates from the cohorts, apply a Bayesian spike-and-slab type prior, and estimate the attributable DALYs for each variant or polygenic score. We consider 2580 fine-mapped disease or risk factor associated variants. Additionally, we consider 80 HLA region variants and 57 selected polygenic scores in FinnGen. We estimate the DALYs attributable for 72 major diseases from the Global Burden of Disease 2019 Study.

Rs3798220 (*LPA*) had the highest effect on loss of healthy life years quantified as DALYs at 1.249 (95% CI: 1.090 - 1.407) through increasing risk of ischemic heart disease (1.151 DALYs), valvular heart disease (0.047 DALYs), aortic aneurysm (0.035 DALYs), and peripheral artery disease (0.016 DALYs). The top 3 polygenic scores with highest number of attributable DALYs when comparing top 10% vs bottom 90% individuals were 1) coronary artery disease (3.90), type 2 diabetes (3.29), and educational attainment (-2.76).

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C17.3 Differentially expressed genes reflect disease-induced rather than disease-causing changes in the transcriptome

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Comparing transcript levels between cases and controls allows the identification of differentially expressed genes, which may be causes, consequences or mere correlates of the disease under scrutiny. Whereas we have previously integrated summary-level data from GWAS and eQTLs in a Transcriptome-Wide Mendelian Randomization (TWMR) approach to elucidate gene expression to trait causal effects, here we propose a reverse TWMR (revTMWR) to shed light on causal imprints of complex traits on transcript levels. Combining TWMR and revTWMR results revealed that whole-blood gene expression-trait correlation is mainly driven by causal effect from the phenotype on the expression rather than the reverse. For example, BMI- and triglycerides-gene expression correlation coefficients robustly correlate with trait-to-expression causal effects (r=0.09, P=1.54x10^{-39} and r=0.09, P=1.19x10^{-34}, respectively), but not detectably with expression-to-trait effects. Genes implicated by revTWMR confirmed known associations, such as rheumatoid arthritis and Crohn's disease induced changes in expression of *TRBV* and *GBP2*, respectively. They also revealed intricate feedback loops controlling the levels of clinical biomarkers. For instance, we observed that high levels of HDL cholesterol lower the expression of genes involved in cholesterol biosynthesis (*SQLE, FDFT1*) and increase the expression of genes responsible for cholesterol efflux (*ABCA1, ABCG1*), two key molecular pathways in determining HDL levels. In conclusion, our method disentangles the gene expression-phenotype relationship and reveals that complex traits have more pronounced impact on gene expression than the reverse, demonstrating that studies comparing the transcriptome of cases and controls are more prone to reveal disease-induced gene expression changes rather than disease causing ones.

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C17.4 The penetrance of age-dependent monogenic disease variants depends on ascertainment context

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Aim: Accurate penetrance of monogenic disorders is often unknown due to a phenotype-first approach to genetic testing. We use a genotype-first approach in large-scale exome-sequenced cohorts to accurately estimate penetrance of monogenic diabetes variants. We contrast the two commonest causes of monogenic diabetes, *HNF1A*-MODY which causes an age-related progressive diabetes and *GCK*-MODY, which causes life-long mild hyperglycaemia.

Method: We identified *HNF1A* and *GCK* pathogenic variants in clinically-selected probands ($N = 1742$) and three clinically-unselected cohorts with different background rates of diabetes: family members of the proband ($N = 2192$;51% diabetes), a hospital-based Geisinger cohort, ($N = 132{,}194$;24% diabetes) and a population-based UK Biobank cohort ($N = 198{,}748$;6% diabetes). We used age of diabetes-onset as disease outcome for *HNF1A*-MODY and raised glucose (HbA1c≥6%/fasting glucose≥6 mmol/L) for *GCK*-MODY.

Results: For *HNF1A-MODY*, the penetrance of diabetes was 98% in the probands at age 40. This reduced to 86% in family members, 49% in the Geisinger study, and 32% in the UK Biobank (all$P<3x10^{-9}$vs. proband). The consistency of hazard ratios across studies suggested that the variation in background rate of diabetes could explain the variation in penetrance (family members: 11 [95%CI:8-14]; Geisinger: 7.6 [4-16]; UK Biobank 15.6 [8-24,],$P=0.4$). In contrast, for *GCK*-MODY, penetrance of mild hyperglycaemia was similar in all four cohorts (94%, 93%, 86%, 89% respectively,$P>0.05$) and showed remarkably similar HbA1c (6.4% [95%CI:6.3-6.4], 6.6 [6.5-6.7], 6.6 [6.2-6.9], 6.5 [6.4-6.6] respectively) despite the substantial variation in background rate of diabetes and HbA1c across cohorts.

Conclusion: Ascertainment context is crucial when interpreting the consequences of monogenic disorders variants for age-related variably penetrant disorders.

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C17.5 Genetic and environmental determinants of drug adherence and drug purchasing behaviour

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One of the major factors behind the efficacy of pharmacological treatments is patients adherence to the prescribed therapy regimen. Many demographic and socioeconomic factors may play a role in determining adherence, however, there have been few investigations on the potential effects of genetic variation on drug adherence. By leveraging genetic data from the FinnGen study ($N = 260{,}405$), data from Finnish nation-wide health registries and the drug purchase registry (59,605,493 total purchases), we provide a systematic investigation of adherence determinants across multiple medications.

For each individual drug-trajectory, we defined adherence as the ratio between the total purchased quantity and the total days of purchasing. For each medication, we run a GWAS of adherence and estimated its genetic correlation (rg) with 27 publicly available traits. Overall, these results suggest that adherence is related to behavioural aspects. For example, higher adherence to statins resulted in association with a higher educational attainment level (rg=0.23, $P = 1.6x10\text{-}3$) and a lower risk tolerance (rg=-0.41, $P = 1x10\text{-}4$), while self-rated health showed a positive correlation with adherence to blood pressure medications (rg=0.24, $P = 3.7x10\text{-}3$).

This study provides better insights into the factors affecting adherence and allows for a better identification of patients at high risk of non-adherence in drug taking.

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C17.6 Revealing the recent demographic history of Europe via haplotype sharing in the UK Biobank

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Haplotype-sharing has recently been successfully leveraged to interrogate fine-scale genetic structure - revealing the impact of specific historical events on the human genetic landscape, particularly in Europe. However, the study of population structure in Europe has been largely limited to western-Europe - restricting our understanding of the genetic landscape and demographic history of the wider European continent. Utilising haplotypes from ~5,000 individuals within the UK Biobank with selected European birthplace and ancestry phenotypes, we sought to apply haplotype and Identity-by-Descent (IBD) analyses to investigate the landscape of recent demographic history across Europe, from Portugal in the west, to Russia in the east.

We report analysis of the broad genetic structure of Europe using haplotype-based methods. We highlight novel results from eastern Europe, the Balkans, and Malta - finding eastern Europe a crossroads of different European ancestry clines, and footprints of genetic isolation in Malta. Obtaining genetically homogenous groups with clustering analysis, we detected IBD-segment sharing within and between genetic regions of Europe. We demonstrate region-specific demographic trajectories captured by IBD-sharing, showing a demographically heterogenous landscape across Europe. Finally, we further investigate the ancestry of previously under-reported populations using haplotype-based modelling methodology, further quantifying ancestral components with IBD-sharing patterns to investigate demographic histories.

Our work aids the understanding of the general haplotypic landscape of Europe, identifying regions with sharing patterns of interest to gene-mapping efforts, describing the varied demographic history across the continent, and finally highlighting the potential use of the UK Biobank as a reference of European variation beyond Britain.

E.H. Gilbert: None. **G.L. Cavalleri:** None.

C18 Functional genomics and transcriptomics

C18.1 An epigenome-wide view of osteoarthritis in primary tissues

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Osteoarthritis is a complex degenerative joint disease. To promote the development of novel, efficient therapeutic approaches, it is first necessary to unravel the genomic architecture of osteoarthritis. Here, we investigate genome-wide DNA methylation from macroscopically intact (low-grade) and degraded (high-grade) osteoarthritis cartilage, as well as synovium tissue from a total of 98 knee osteoarthritis patients undergoing joint replacement surgery. We conducted an epigenome-wide association study (EWAS) comparing matched low-grade and high-grade osteoarthritis cartilage within the same individual to identify methylation markers of cartilage degeneration. We further constructed random forest-based classifiers of cartilage degeneration. We generated genome-wide cis-methylation QTL (mQTL) maps in synovium, low-grade, and high-grade osteoarthritis cartilage. We used Mendelian randomization (MR) and colocalisation to identify epigenetic mechanisms mediating the effects of genotype on disease risk. The EWAS discovered 15,328 differentially methylated sites and 2,477 regions linked to cartilage degeneration. These regions were mapped to genes enriched in 76 Gene Ontology terms including a potential novel process linked to epithelium development. Machine learning models distinguished low-grade from high-grade osteoarthritis cartilage with high accuracy (90%). The mQTL analysis revealed widespread associations between genetic variants and methylation in all three examined tissues. Furthermore, mQTLs colocalised with 20 osteoarthritis-associated genetic loci, potentially elucidating their regulatory targets. Applying MR identified 19 methylation loci with a putative causal influence on osteoarthritis. This study presents the largest EWAS for knee cartilage degeneration, the first genome-wide mQTL map in knee OA affected tissues, and evidence for a role of epigenetic mechanisms in osteoarthritis.

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C18.2 Dynamics of gene regulatory organization in longitudinal twin RNA-seq data

Anna Ramisch[1,2], *Julia S. El-Sayed Moustafa*[3], *Diogo Ribeiro*[4], *Gwenaël Leday*[5], *Yunlong Jiao*[6,7,8], *George Nicholson*[8], *Michael Stevens*[1,2], *Moustafa Abdalla*[6,7,8], *Cristina Menni*[3], *Ana Viñuela*[9], *Chris Holmes*[8], *Timothy D. Spector*[3], *Mark I. McCarthy*[6,7,10], *Sylvia Richardson*[11], *Olivier Delaneau*[4], *Emmanouil D. Dermitzakis*[1,2]

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The human genome can be partitioned into domains of regulatory elements necessary to control gene expression. Previously, we identified cis-regulatory domains based on coordinated chromatin activity, offering a great insight into the regulatory organization of the human genome. However, chromatin data are not available for most human genetics' datasets. Therefore, to characterize cis-regulatory organization based on gene expression and genotypes, we used whole-blood RNA-seq from 335 twins (MultiMuTHER study, TwinsUK). The longitudinal study design also permits exploration of the temporal dynamics of cis-coordinated expression patterns. We identified 3,979 dynamic co-expressed cis-gene pairs (COPs) in whole-blood with a significant longitudinal change in correlation. The majority of pairs (51%) shows decreasing positive correlation and only 6% decreasing negative correlation over time, while 32% show longitudinal increase in negative and 11% in positive correlation. Dynamic COPs involved 3,335 unique genes, of which one third were differentially expressed (DE) over time. However, 45% of dynamic COPs do not contain any DE gene, suggesting that their correlation dynamics is due to change of variance rather than of mean gene expression. We observed that decreasing/increasing correlation often coincides with decreasing/increasing variance of expression over time, respectively. Moreover, we assessed the temporal changes in genetic regulation by identifying longitudinal-eQTLs, i.e., SNPs associated with gene expression change. The longitudinal eQTL-genes were significantly enriched in dynamic COPs, identifying genetic regulatory elements modulating dynamics of coordinated cis-regulation.

We plan to assess the genetic correlation of our dynamic COPs using the twin relatedness to disentangle genetic from environmental regulatory factors.

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C18.3 Repeated genomic elements characterization at the single-cell level along normal brain development

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Introduction: Repeated genomic elements (RGE) make up more than half of the human genome, yet the understanding of their biological function and behavior is still elusive. Some of them however have been shown to play major roles in coding genes regulation, especially during development and cell type differentiation.

Material and methods: We used single-cell or single-nuclei sequencing, followed by our in-house protocol for RGE quantification, to characterize their expression in a developmental atlas that includes multiple time points from E10 to P6 in the mouse forebrain and hindbrain, as well as several human fetal samples.

Results: Our approach allowed us to identify repeats families with defined expression dynamics in some cell types and developmental windows. For example, Long Interspersed Nuclear Elements 1 (LINE1) retrotransposons show a consistent increase of

expression along differentiation of neuronal lineages. This dynamic is specific, since Short Interspersed Nuclear Elements, which are comparable from the technical point of view, display a rather constant profile of expression.

Conclusions: Our pipeline for RGE quantification allows us to reliably detect repeat families in single-cell data across development. Our results are consistent with the known role of some RGE in cell type differentiation. We will discuss potential biological process that may be driving the dynamics we observe and explore the technical feasibility of pinpointing specific roles for given elements. Our strategy builds up promising avenues towards a better understanding of the functional consequences of this large, unexplored fraction of the genome at the single-cell level.

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C18.4 A global map of the impact of deletion of post-translational modification sites in genetic diseases

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There are >200 protein post-translational modifications (PTM) types described in eukaryotes, having diverse species conservation levels, proteome coverage, number of high-throughput experiments and functional roles. From a clinical perspective, a number of diseases have been associated to deregulated PTM sites and missense rare variants are globally enriched in PTMs. We hypothesize that some genetic diseases may be caused by deregulation of particular functions produced by the removal of specific PTM types by genomic variants. We collected >320,000 human PTMs of 59 types and cross them with >4M missense DNA variants annotated with pathogenic predictions and disease associations. We report >1.74M PTM-variant concurrences in >16,500 proteins that an enrichment analysis distributed in 217 pairwise significant associations between 18 PTM types and 150 genetic diseases. Around 23% of these associations are already described in the literature, 34% have partial evidences based on single variants, related diseases or regulatory evidences, and 43% are novel. Removal of acetylation presents the highest effect, still low studied PTM types like S-glutathionylation or S-nytrosylation show relevance. A network of PTM types and phenotypes associations is also discussed. Using pathogenicity predictions we identified potential PTM sites to produce particular diseases if genomic variants remove them. Our results show an important impact of PTM removal producing genetic diseases and phenotypes that is PTM type specific. We describe for the first time a general scenario of PTM types and genetic diseases direct associations, >40% novel, that provides new capacities to understand and diagnose these disorders.

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C18.5 Transcriptome profiling using long-read sequence to dissect the interplay between genetic variant and transcript variations

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Over the last decade, RNA-seq technologies have vastly increased our knowledge of gene expression and transcript isoform signatures. However, current short-read RNA-seq methods show limitations in identifying complex transcript isoforms, as full-length transcripts and modifications are not retained. In this study, we address these limitations exploiting the advantages of long reads native poly(A) RNA-seq using the Oxford Nanopore Technologies (ONT) platform and created a dataset of direct RNA-seq from 60 lymphoblastoid cell lines from European ancestry.A pilot analysis on 42 samples generated ~3.5 million aligned sequencing reads per sample with a read N50 length of 1,350 bases. We identified 10,882 protein coding genes expressed in at least 90% of the samples, 96% overlap with those identified for the same samples with Illumina platform. Gene expression was highly concordant between ONT and Illumina (median R^2=0.68) showing good reproducibility. Preliminary isoforms analysis was performed using FLAIR. We identified 18,355 isoforms representing 10,882 genes with 6,987 non-annotated transcripts, the majority of them being linked to genes with more than one annotated transcript.Ongoing allele-specific expression analysis will yield novel insights on the effect of common and rare genetic variants into the specific transcript alterations with the advantage of high resolution given by long-read sequence technology. Moreover, we plan to identify RNA modifications from our Nanopore direct RNA-sequencing, and combine these with ChIP-seq data from our previous work to dissect the contribution of RNA and histone modifications to gene regulation.

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C18.6 Substantial somatic genomic variation and selection for *BCOR* mutations in human induced pluripotent stem cells

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Human Induced Pluripotent Stem Cells (hiPSC) are an established patient-specific model system where opportunities are emerging for cell-based therapies. We compared and contrasted hiPSCs derived from skin and blood from the same individual. We show extensive single-nucleotide mutagenesis in all hiPSC lines, although fibroblast-derived hiPSCs (F-hiPSCs) are particularly heavily mutagenized by ultraviolet (UV)-related damage. We utilized genome sequencing data on 454 F-hiPSCs and 44 blood-derived hiPSCs (B-hiPSCs) to gain further insights. Across 324 whole genome sequenced F-hiPSCs derived by the Human Induced Pluripotent Stem Cell Initiative (HipSci), UV-related damage is present in ~72% of cell lines, sometimes causing substantial mutagenesis (0.25-15 per Mb). Furthermore, we find remarkable genomic heterogeneity between independent F-hiPSC clones derived from the same reprogramming process, due to oligoclonal populations within fibroblasts. In all, we identify 272 predicted pathogenic mutations in cancer-related genes, of which 21 genes were hit recurrently three or more times, involving 77 (17%) lines. Notably, 151 of 272 mutations were present in starting fibroblast populations suggesting that more than half of putative

driver events in F-hiPSCs were acquired in vivo. In contrast, B-hiPSCs reprogrammed from erythroblasts show lower levels of genome-wide mutations (0.28-1.4 per Mb), no UV damage, but a strikingly high prevalence of acquired *BCOR* mutations in ~57% of lines, indicative of strong selection pressure. All hiPSCs had otherwise stable, diploid genomes, highlighting how copy-number-based approaches do not have the required resolution to detect widespread nucleotide mutagenesis.Funding: This work was funded by Cancer Research UK (CRUK) Advanced Clinician Scientist Award (C60100/A23916),

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C19 ELSI in genomics

C19.1 Participant experiences of genome sequencing for rare diseases in the 100,000 Genomes Project: A mixed methods study

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Background: The 100,000 Genomes Project (100kGP) delivered whole genome sequencing (WGS) for rare disease and cancer in a hybrid research-clinical setting. In this mixed-methods longitudinal study, we assessed the attitudes, understanding and experiences of participants consenting for WGS for rare disease diagnosis.

Methods: Participants (patients, parents, relatives) taking part in the 100kGP at six hospitals were recruited. A survey at time 1 measured participants' ($n = 504$) knowledge, attitude and decisional conflict about WGS. At time 2 (at least 12 months later), a survey measured knowledge, attitude, decisional regret and psychological outcomes. Participants who received a main finding were invited to a qualitative interview.

Results: Of 296 survey participants, attitudes remained positive and knowledge remained stable over time. Few reported decisional regret and 26% had received main findings with minimal negative psychological impacts. Interviews from 38 participants further substantiated this, though distress was greater for parents than patients. Results could bring relief and gratitude as well as disappointment and worry, and though a diagnosis did not impact clinical care for many, social, practical, and emotional benefits were observed.

Conclusions: Whilst a genetic diagnosis can be transformative, it is not the ultimate endpoint for patients and families: Appropriate measures should be developed to support the varied emotional and psychological needs of patients irrespective of the outcome of WGS results.

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C19.2 Changing a single word in prenatal microarray agreement form significantly decreases the rate of reported variants of questionable significance

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Objective: To examine the effect of patient selected opt-in vs. opt-out choice on the rate of reported variants of uncertain clinical significance (VOUS) and high-frequency low-penetrant (HFLP) findings in prenatal microarray. Methods: A standard microarray agreement form in Israel includes requirement to note patients' choice to be or not to be informed of VOUS and HFLP variants. The original form was built as an opting-out method, i.e. the women had to actively mark if they did not want to be informed about such findings. Since October 2019, the form was changed into opting-in option. In this study we have compared the rates of reported VOUS and HFLP variants between these two periods.

Results: Of the 1014 prenatal CMA tests, 590 (58.2%) were performed in the opt-out period. A decrease of 49.2% in the rate of women requesting to be informed of VOUS findings was noted (66.8% in opt-out period vs. 34.0% in opt-in period), yielding a relative risk (RR) of 0.46 (95% confidence interval (CI) 0.39-0.53), $p<0.0001$. Rate of women preferring to be informed of HFLP variants decreased by 36.1% (from 75.3% to 48.1%, RR 0.52 (95% CI 0.45-0.60), $p = 0.0002$. More significant decrease was noted in counselling conducted by doctors compared to genetic counsellors.

Discussion: In this study we present a simple and effective method to decrease the rate of reported findings of questionable significance. Varying rates of decrease between doctors vs. genetic counsellors imply differing levels of communication and understanding, yielding potential points that need to be improved.

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C19.3 Healthcare professionals' views about responsibility in the sharing of genetic information to patients' relatives

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Although the sharing of information about genetic risks with patients' relatives is key in genetic health care, research exploring healthcare professionals' views on this topic is still limited. We conducted 10 focus groups with 34 healthcare professionals working in Portuguese genetic services. We aimed at exploring their views and reasoning about the sharing of genetic information with patients' relatives, their perceived responsibilities to patients and their families, confidentiality of genetic information, and how those considerations relate to their practice. Data were analysed thematically. Discussions centred mostly on conditions with a clinical intervention. Most participants perceived to be their professional responsibility to provide objective information on genetic risks to their patients, their patients having a moral

responsibility to share relevant information with relatives at risk. They did not consider having a direct responsibility to patients' relatives; but, when identifying barriers to communication, they actually used several strategies to encourage disclosure. Direct contact from genetic services with at-risk relatives was recognised as potentially "more productive", but difficult to implement due to insufficient multidisciplinary professionals in genetic services and lack of infrastructural resources. Participants, however, had a strict individual approach to confidentiality. They observed that a familial approach to confidentiality - treating personal genetic information and familial information differently - would be problematic. Some demanded for broader discussion and clear national guidelines. These are relevant results, as a growing number of individuals are being identified at-risk for hereditary diseases, and the need for healthcare professionals to communicate this information to patients grows accordingly.

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C19.4 The impact of the GDPR on genomic medicine and research

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Introduction: The COVID-19 pandemic has powerfully illustrated that collecting and sharing genomic data is now essential to improve population and individual health. However, reconciling this with the EU's comprehensive data protection law—the General Data Protection Regulation (GDPR)—is complex. Funded by the UK's data protection authority, the Information Commissioner's Office, our research identifies and evaluates aspects of the GDPR that are challenging for uses of genomic data in healthcare or health research, as well as measures that could be adopted to mitigate or reduce negative impacts.

Methods: We conducted comprehensive legal analysis, interviews with key stakeholders and convened a multidisciplinary workshop of clinical/scientific professionals, policy makers, regulators and academic experts.

Results: We identified a wide range of challenges for genomic healthcare and research determining: when and where the GDPR applies; when genetic data are 'personal data'; appropriate legal bases for processing genomic data; how data subject rights should be fulfilled, and; legal mechanisms for transfer of data outside the EU/EEA. We also identified some promising mitigations, including technical measures to safeguard data while facilitating analysis (such as homomorphic encryption) and sector-led initiatives such as the development of legally recognised codes of conduct (art 40) or certification mechanisms (art 42).

Conclusions: The genomics community, regulators and policymakers must work together to establish data protection standards that are appropriate for genomic medicine and research. Both informal methods and more formal codes of conduct or certification schemes should be pursued to reach consensus and crystallise best practice as quickly as possible.

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C19.5 Loosening the purse strings: Influencing public funders about the value of genomic testing for intellectual disability

***Deborah Schofield**[1], Owen Tan[1], Rupendra Shrestha[1], Sarah West[1], Jackie Boyle[2], Louise Christie[2], Natalie Hart[1], Katherine Lim[1], Melanie Leffler[2], Lucinda Murray[2], Radhika Rajkumar[3], Morgan Rice[4,5], Robert Tanton[6], Jinjing Li[6], Tony Roscioli[7,8,9], Mike Field[2]*

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Introduction: Given financial pressures on governments worldwide, robust evidence of the cost of genetic disorders and cost-effectiveness of genomic testing is important when seeking public funding. We collected data on the cost of familial Intellectual Disability (ID) to be subsequently used in assessment of the cost effectiveness of Whole Genome Sequencing (WGS) for diagnosis of patients of the Genetics of Learning Disability state-wide service.

Materials and methods: As part of the Economic and Psychosocial Impacts of Caring for Families Affected by Intellectual Disability (EPIC-ID) study, economic modelling using microsimulation methods was developed to assess the lifetime cost of familial ID. Carer surveys were used to obtain data on health and social costs (e.g. education, employment, aids and appliances, and welfare payments) as well as linked medical, pharmaceutical and hospital data.

Results: Familial ID was found to have a lifetime cost of about AU$11.6 million per household. A total of about $AU3.7 million was borne by private households with about AU$2.5 million per household being due to lost income for patients and carers. Government bore the remainder of the costs, with the largest cost being for supported accommodation at about AU$2.7 million per household. These findings have influenced several public funding decisions to support access to genomics for patients with ID.

Conclusions: The cost of familial ID is very substantial for households and for government. Robust economic data such as that collected in the EPIC-ID study can play an important role in public funding allocation decisions.

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C19.6 Genomics in society: Engaging children in dialogue about human germline editing

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Introduction: Dialogic communication with children is a novel field of study and seems a promising method to engage and acknowledge children as stakeholders in complex social issues, such as the question what to do with human germline editing technology (HGGE). As part of the Dutch DNA dialogues, we organized two dialogues to enable formation and assembly of children's opinions on HGGE.

Methods: Twenty-two healthy, and 12 children affected by (hereditary) disease took part in two dialogues (age 8-12 years). Psychological safety was established by involving play and including attachment figures, such as peers. Furthermore, connection with the children was established by an introduction game. The adult moderator pretended not to know anything about the particular subject and adopted an interested and curious attitude, to stimulate children's critical thinking. Dialogue was fueled by age-appropriate scenarios, fitting the concept of 'let's pretend' play.

Results: Children highly appreciated the acknowledgement that their opinions mattered and stated perceiving themselves as essential stakeholders of this topic. Whereas parents often stressed feeling responsible for giving their child the best possible health, children put more emphasis on their right to self-determination. Most children expressed that HGGE was acceptable only for severe genetic disease. Most children also expressed resistance against the idea of parents having altered their DNA.

Discussion: Children are essential yet often overlooked stakeholders in many facets of the HGE debate. Despite the relatively small groups, we found that child perspectives differ from adult perspectives, thus contributing important insights for societal alignment of HGGE.

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C20 From mechanisms to therapeutic insights in cancer

C20.1 Decrypting the breast cancer cellular complexity by single-cell transcriptomics of tumor-derived organoids

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Introduction: Organoids are a valuable model for cancer research and drug screening since they incorporate environmental conditions (hypoxia and lack of nutrients in the tumor core respect to areas close to blood vessels) and comprise a heterogeneous cell population differently responding to the tumor micro-niche. To study this complex model, the Single Cell Analysis (SCA) approach for transcriptomic profiling has become mandatory.

Materials and Methods: Organoids were obtained from 4 breast tumor cases by partial mechanic/enzymatic tissue dissociation, resulting in small portions recapitulating the parental organ structure. Organoids were separated from original tissue by filtration, dissociated into single cells and processed for SCA using 10X Genomics technology. SC data were analysed with 10xGenomics CellRanger, Seurat, Monocle and our developed tool scMUFFIN, which includes CNV estimation.

Results: SCA approach sorted out the cellular complexity of the breast cancer organoids revealing the existence of different populations related to specific cell sub-types of the normal mammary gland (basal, secretory luminal and hormone-responsive luminal cells) and tumor peculiar populations, showing distinct CNV profile. We performed differential expression analysis comparing the same cell types in tumor and normal breast organoids and identified cell-type-specific genes and pathways involved in tumorigenesis. Pseudotemporal algorithms revealed trajectories and lineage hierarchy among populations, thus allowing to identify precursor cells and their specific expression profile.

Conclusions: Single-cell transcriptomics of breast cancer organoids revealed pathways active in specific cancer cells that play pivotal roles in tumor progression, providing candidate targets for more accurate therapeutic approaches.

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C20.2 Identifying and characterizing EZH2 as a druggable dependency factor for desmoid tumors in a genetic *Xenopus tropicalis* model for Gardner's Syndrome

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Introduction: Desmoid tumors are soft-tissue neoplasms strictly driven by Wnt signaling network hyperactivation and can occur sporadic (harboring activating missense mutations in the *CTNNB1* gene), or can be associated with Gardner's syndrome due to germline loss-of-function mutations in the tumor suppressor gene *APC*. Despite the clearly defined genetic etiology and the strict and unique implication of the Wnt/β-catenin pathway, no systemic specific molecular therapy for the tumors exists.

Materials and Methods: We developed a fast and semi-high throughput genetic *Xenopus tropicalis* model for identifying and/or characterizing drug targets for desmoid tumors. The platform also permits pre-clinical assessment of candidate therapeutic compounds. The methodology uses multiplexed CRISPR/Cas9 based genome editing by simultaneously targeting a tumor suppressor gene (*apc*) and a candidate dependency gene. It thereby exploits the fact that for a genetic dependency biallelic frameshift mutations in this gene will never be recovered from sampled tumors.

Results: Using an in vivo negative-selection screen we identified the epigenetic regulator *EZH2* and the transcription factor *CREB3L1* as genetic dependencies for desmoid tumors. Interestingly, inhibition of the enzymatic activity of EZH2 by the compound Tazemetostat (Tazverik) reduced the size of established desmoid tumors in the *Xenopus* model as monitored by MRI. Using in vitro models of patient derived desmoid cells, the mode of action of Tazemetostat was further investigated, indicating a direct effect on Wnt pathway activity. Since Tazemetostat is well-tolerated and FDA-approved for treatment of epithelioid carcinomas, it could offer prospects for treating patients with desmoid tumors. Research support: DTRF and SOS Desmoïde.

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C20.3 Neuroblastoma somatic mutations enriched in cis-regulatory elements collectively affect genes involved in embryonic development and immune system response

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The ever-growing interest towards noncoding cis-regulatory variants as cancer drivers is currently hampered by numerous challenges and limitations of variant prioritization and interpretation methods and tools. To overcome these limitations, we focused on active cis regulatory elements (aCREs) to design a customized panel for deep sequencing of 56 neuroblastoma tumor and normal DNA sample pairs. CREs were defined by a reanalysis of H3K27ac ChiP-seq peaks of 25 neuroblastoma cell lines. This provided a small subset of genomic regions with evident regulatory functions in which to search for driver mutations. We tested these regions for an excess of somatic mutations and assessed the statistical significance with a global approach accounting for chromatin accessibility and replication timing. Additional validation was provided by analyzing whole genome sequences of 151 neuroblastomas. For the mutated regions, we determined their candidate target genes through HiC data analysis. We identified a significant excess of somatic mutations in aCREs of diverse genes including *IPO7*, *HAND2*, and *ARID3A*. A gene expression signature built on basis of these three, and nearby, interacting genes strongly correlated with negative prognostic markers and low survival rates of patients affected by neuroblastoma. Moreover, we observed a convergence of biological functions of the target genes of mutated aCREs and transcription factors with binding motifs altered by mutations towards processes related to embryonic development and immune system response. Our strategy led us to identify somatic mutations in regulatory elements that collectively can drive neuroblastoma onset.

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C20.4 Investigation of tumor suppressor gene loss on chromosome 8p in hepatocellular carcinoma using chromosome editing

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Chromosome 8p (chr8p) is frequently deleted in a variety of cancer entities and correlates with poor patient survival in hepatocellular carcinoma (HCC). The extent and complexity of chr8p loss of heterozygosity (8pLOH) suggests that multiple genes with tumor suppressive properties can be affected by this large-scale deletion. Co-suppression of multiple genes might synergistically promote tumor growth. Exploiting the CRISPR-Cas9 technology, we introduced genomic deletions of chr8p into HCC cells resulting in distinct 8pLOH clones. Successful chromosome editing was confirmed by PCR, Sanger sequencing and fluorescence in situ hybridization. The gene expression in wildtype and 8pLOH cells was determined by RT-qPCR and RNA-seq. A genome-wide CRISPR-Cas9 knockout screen substantiated gene dependencies specific for 8pLOH harboring tumors. Gene set enrichment analysis of differentially expressed genes in RNA-seq as well as TCGA data identified major deregulations in migration and extracellular matrix signaling upon chr8p loss. Subsequent functional assays revealed increased metastasizing potential in 8pLOH cells which may account for poor prognosis. Investigation of gene dependencies specific for 8pLOH tumors unveiled the tight junction aPKC-PAR3-PAR6 complex as novel synthetic lethality making 8p deleted tumors strongly susceptible for inhibition of these proteins. We established a model system to study large-scale genomic deletions and loss of heterozygosity in liver cancer. By integrating RNA-seq and CRISPR-Cas9 screening data, we showed that 8pLOH may increase patient mortality and inhibition of the aPKC-PAR3-PAR6 complex may be a new therapeutic option in patients with 8pLOH. *Funded by German Research Foundation (DFG) - Project-ID 314905040 - TRR209*

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C20.5 tRNA^{Ser} overexpression induces adaptive mutations in NSCLC tumors

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Introduction: tRNA deregulation causes Protein Synthesis Errors (PSEs) and is associated to worse patient prognosis. In yeast, PSE levels, driven by tRNA deregulation increase genomic alterations. PSE are naturally increased in tumors and can foster tumor growth. Therefore, we hypothesized that tRNA deregulation may contribute to the increased genome instability observed in tumors.

Materials and Methods: H460 cells with tRNA^{Ser} overexpression (tRNA^{Ser}OE) and Mock (control) were inoculated nude mice as described in the following table to study the effect of tRNA^{Ser}OE alone and in the presence of a trigger:

Time-point 1	Time-point 2	Time-point 3							
Naive	Vehicle	Cisplatin	Vehicle	Cisplatin					
Mock	tRNA^{Ser}OE	Mock	tRNA^{Ser} OE	tRNA^{Ser} OE	Mock	Mock	tRNA^{Ser} OE	Mock	tRNA^{Ser} OE
5	5	5	5	5	5	5	5	5	5

DNA and protein were extracted for WES and proteomics.

Results: The tumor mutation burden remained unchanged along time, however, tumors with tRNA^{Ser}OE treated twice with cisplatin, showed a higher mutation burden. Although in Time-point1 tumors have a similar number of variants, in Timepoints 2&3, tRNA^{Ser}OE tumors display two times more variants than Mock tumors regardless of treatment. These variants are mostly low impact variants but they are predicted to have impact in distinct

pathways. For instance, tRNA^{Ser}OE exclusive variants will favor proliferation and therapy resistance, which is in line with the phenotypes observed and the proteomics data.

Conclusion: tRNA^{Ser}OE increases the tumor mutation burden and the variants detected favor tumor growth, proving tRNA deregulation is enough to induce adaptive mutations in the genome. Acknowledgments: PTDC/MED-ONC/28834/2017

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C20.6 Characterization of fusion-circRNAs in childhood acute lymphoblastic leukemia: *f-circMLL-AF4*

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Childhood acute lymphoblastic leukemia (cALL), the most frequent cause of death by disease in children, is a heterogeneous disease comprising multiple molecular subtypes defined by specific genetic alterations. Gene fusions arising from chromosomal rearrangements represent more than 50% of common driver events in cALL. As recently discovered, these fusion genes can generate linear RNA or fusion-circularRNAs (f-circRNAs) via back-splicing of exons. These f-circRNAs are implicated in malignant transformation, cell survival, and therapeutic resistance. Apart from their relevance for cancer biology, f-circRNAs are also promising biomarkers for liquid biopsies due to their increased stability relative to linear transcripts. Therefore, we propose to characterize f-circRNAs in cALL, which might allow a more precise diagnosis and prognosis.

Here, we identified a novel f-circRNA generated from the MLL-AF4 fusion gene (*f-circMLL-AF4*) by screening leukemia cell lines carrying common rearrangements using primers diverging from the breakpoint of translocations. Functional studies through gain-of-function strategy showed that *f-circMLL-AF4* promotes cell migration in leukemia cells, but has little effect on cell proliferation. We also found that it confers resistance to chemotherapeutic drugs commonly used to treat leukemia. Using a bead-based multiplexed immunoassay system, we determined that *f-circMLL-AF4* overexpression decreased phosphorylation of p38 protein, involved in apoptosis Interestingly, we also demonstrate the presence of *f-circMLL-AF4* in extracellular vesicles, pointing to a biomarker potential.

In conclusion, our study not only expand the current knowledge of molecular mechanisms underlying leukemia progression, but also provides potential diagnostic and therapeutic new targets.

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C21 Clinical immunology and novel therapies of genetic diseases

C21.1 Hypermorphic heterozygous variants in the tyrosine kinase *ZAP-70* underlie autoimmune disease

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Introduction: T lymphocytes require intact T cell antigen receptor (TCR) signalling to successfully activate and eliminate pathogens. The tyrosine kinase ZAP70 plays a crucial role in relaying proximal TCR signalling. Biallelic loss-of-function mutations in *ZAP-70* therefore cause a severe combined immunodeficiency (SCID) with early-onset recurrent infections caused by defective $CD4^+$ T cell functioning and $CD8^+$ T cell lymphopenia. In addition, there has been a single report of a patient with a severe autoimmune syndrome that carried a heterozygous hypermorphic variant (Arg360Pro).

Materials and Methods: An exome-wide research-based reanalysis was performed in three unrelated index patients with various, partly overlapping autoimmune disorders. Segregation of rare, non-synonymous candidate genes in affected family members was investigated using Sanger sequencing. Tailored functional tests were conducted to test pathogenicity and encompassed the isolation of patient peripheral blood mononuclear cells (PBMCs), including ex vivo stimulation and phospho-flow cytometry analysis.

Results: In the index patient of family 1, a private, heterozygous missense variant in *ZAP-70* (NM_001079.3:c.340G>T;p.(Val114Phe), CADD score 23.1) was discovered with complete segregation in the affected family members. In both index patients of family 2 and 3, an identical heterozygous missense variant (NM_001079.3: c.973C>T;p.(Leu325Phe); allele frequency 0.00004243, CADD score 25.7) was identified, also showing complete segregation. In PBMCs isolated from the patients, hyperphosphorylation of ZAP70 was observed, indicative for constitutive activation and consequently suggestive for a loss of autoinhibition of ZAP-70.

Conclusion: In three families of patients with autoimmune disease novel heterozygous variants in ZAP-70 were identified that led to augmented T-cell signalling, thereby acting as hypermorphs.

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C21.2 Gene therapy in a novel large animal model of Stargardt disease

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Stargardt disease type 1 (STGD1), due to bi-allelic mutations in *ABCA4*, is the most common form of inherited macular degeneration, for which no therapeutic options are currently available. We have recently developed a gene therapy approach for STGD1 which relies on the use of two adeno-associated viral vectors (AAV) each encoding for one of the two halves of the ABCA4

protein flanked by split-inteins, which catalyze trans-splicing of the two ABCA4 half proteins following AAV administration. We have shown that subretinal administration of the AAV intein vectors results in precise reconstitution of the full-length ABCA4 protein and in therapeutic efficacy in *Abca4−/−* mice. However, the mouse retina largely differs from that of humans both in terms of size and retinal architecture. Given the similarity between the swine and human retina, we have recently generated pigs through somatic cell nuclear transfer from primary fibroblasts in which ABCA4 has been knock-out (KO) using CRISPR/Cas9. We have found that ABCA4 KO pigs have increased lipofuscin accumulation in the retinal pigmented epithelium region, as it also occurs in STGD1 patients macula, up to 36 months, the longest time-point evaluated. In addition, subretinal delivery of AAV-*ABCA4* intein vectors in ABCA4 KO pigs results in efficient reconstitution of the ABCA4 protein at 2 months post-injection. Further analysis of the impact of subretinal delivery of AAV intein on lipofuscin accumulation will provide important insights towards the clinical translation of this platform for gene therapy of STGD1. This project was supported by European Union's Horizon 2020 (grant No.813490)

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C21.3 Tasimelteon Safely and Effectively Improves Sleep in Smith Magenis Syndrome: a Double-Blind Randomized Trial Followed by an Open-Label Extension

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Smith-Magenis Syndrome (SMS; OMIM #182290) is a rare genetic disorder that results from an interstitial deletion of 17p11.2 and, in rare cases, from a retinoic acid induced 1 (*RAI1*) gene variant (Slager et al 2003). Individuals with SMS present with a distinct pattern of mild to moderate intellectual disability, delayed speech and language skills and, almost uniformly, significant sleep disturbances. Patients exhibit low overall melatonin concentrations and abnormal timing of peak plasma melatonin concentrations. To assess the efficacy of tasimelteon, a melatonin receptor agonist, to improve sleep in SMS, a 9-week, double-blind, randomized, two-period crossover study was conducted at four clinical centers. Genetically-confirmed SMS patients, aged 3 to 39, with sleep complaints participated in the study. Over three years, fifty-two patients were screened and twenty-five patients completed the randomized portion of the study. Improvement of sleep quality (DDSQ50) significantly improved over placebo (0.4, $p = 0.0139$) and total sleep time (DDTST50) also improved (18.5 min, $p = 0.0556$). Average sleep quality (0.3, $p = 0.0155$) and actigraphy-based total sleep time (21.1 min, $p = 0.0134$) improved significantly. Patients treated for ≥ 90 days in the open label study showed persistent efficacy. Tasimelteon safely and effectively improved sleep in SMS. The 17p11.2 deletion encompasses *RAI1*, leading to haploinsufficiency, which is considered the primary cause for most features of SMS, including dysregulation of the molecular clock via its effect on *CLOCK* expression. The results of this study suggest that treatment with a circadian regulator can, in part, ameliorate the circadian deficiencies caused by RAI1 haploinsufficiency.

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C21.4 Attenuation of dysfunctional DNA damage response and PARP1 signaling by minocycline reduces ectopic calcification in pseudoxanthoma elasticum

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Aim: Pseudoxanthoma elasticum (PXE) is a hereditary ectopic calcification disorder characterized by deposition of calcium crystals in skin, eyes and blood vessels. Recently, the DNA damage response (DDR), in particular poly(ADP-ribose) polymerase 1 (PARP1) activation, was shown to be involved in aberrant mineralization raising the hypothesis that dysfunctional DDR/PARP1 signaling contributes to PXE pathogenesis. Methods: Expression analysis of DDR targets, PARP1 effectors and calcification markers was performed on PXE patient-derived and healthy control fibroblasts using qPCR, western blot and enzyme activity assays; before and after treatment with the PARP1 inhibitor minocycline. Effect of minocycline treatment on calcium deposition was evaluated in vitro and in *abcc6a−/−* zebrafish.

Results: DDR targets PARP1, p21, p53 and ATM were significantly upregulated in PXE while the DDR inhibitor SIRT1 was suppressed. PARP1 effectors STAT1/3, IL-6, TET1 and RUNX2 were also upregulated with decreased expression of the RUNX2 antagonist microRNA-204. Minocycline significantly attenuated p21, p53, ATM and the PARP1-STAT1/3-TET1-RUNX2 axis leading to reduced levels of ALPL, MSX2, CTGF, MMP2 and BMP2 and preventing aberrant mineralization in vitro. Minocycline-treated *abcc6a−/−* zebrafish showed a 60% reduction in ectopic calcification compared to untreated controls.

Conclusion: We demonstrated for the first time the involvement of dysfunctional DDR and PARP1 signaling in PXE showing that excessive activation of DDR/PARP1 evokes a STAT-driven cascade with upregulation of the epigenetic modifier TET1 and pro-calcifying transcription factor RUNX2. Minocycline attenuated this deleterious molecular mechanism and reduced ectopic calcification in vitro and in vivo, fueling the exciting prospect of a novel therapeutic compound for PXE.

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C21.5 New uses for old drugs: Added value of celiprolol and pravastatin in vascular EDS

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Introduction: Patients affected by the rare connective tissue disorder vascular Ehlers-Danlos syndrome (vEDS) are at increased risk for fatal aortic ruptures. Recently, we have established an objective read-out system for measuring the biomechanical integrity of the murine thoracic aorta, allowing us to identify new uses for market-approved drugs (cheap with high level of knowledge) in the medical treatment of vEDS.

Materials and Methods: Mice modelling vEDS were treated with the beta-blockers celiprolol or bisoprolol, the angiotensin-II-type-1-receptor-blocker losartan, or the HMG-CoA-reductase-inhibitor pravastatin for 4 weeks. 1.5-mm-long sections of the

ascending and descending murine thoracic aorta were mounted on a tissue puller and uniaxially stretched until rupture. For the tested drugs, we considered pharmacogenetic information from the PharmGKB website and DPWG/CPIC guidelines.

Results: The rupture force (mN) was significantly lower in untreated heterozygous compared to wild-type mice, while celiprolol and pravastatin but neither bisoprolol nor losartan increased the thoracic aortic rupture force in heterozygous mice. While losartan and bisoprolol undergo cytochrome-P450-mediated metabolism and the plasma concentration of pravastatin may be affected by *SLCO1B1*, celiprolol is secreted unmetabolized.

Conclusions: Unlike losartan and bisoprolol, celiprolol and pravastatin have added value regarding the strengthening of the weakened murine vEDS aorta. Despite this potential added value of pravastatin, celiprolol prevails due to its favourable pharmacokinetic profile and, hence, should currently be the medical therapy of choice for vEDS, until further evidence emerges. Our results exemplify that drug repositioning/repurposing can be a powerful source to identify old drugs for potential new therapeutic applications in rare diseases.

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C21.6 Increased CHIP prevalence amongst people living with HIV

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People living with human immunodeficiency virus (PLWH) have accelerated biologic aging and significantly increased risk for cardiovascular disease. Clonal hematopoiesis of indeterminate potential (CHIP), the age-related acquisition and expansion of hematopoietic stem cells due to leukemogenic driver mutations, increases risk for both hematologic malignancy and coronary artery disease (CAD). Since increased inflammation is hypothesized to be both a cause and consequence of CHIP, we hypothesized that PLWH have a greater prevalence of CHIP. Here, we searched for CHIP in multi-ethnic cases from the Swiss HIV Cohort Study (SHCS, $n = 600$) and controls from the Atherosclerosis Risk in the Communities study (ARIC, $n =$ 8,111) from blood DNA-derived exome sequences. First, we observed that HIV is associated with increased CHIP prevalence, both in the whole study population and in a subset of 230 cases and 1002 controls with similar sequence coverage and matched by age, sex and ethnicity (SHCS 7%, ARIC 3%, $p = 0.005$). Second, unlike in controls, ASXL1 was the most commonly mutated CHIP gene among PLWH. Third, we observed a positive association of duration of antiretroviral therapy (ART) with CHIP prevalence. Among people with HIV, the rate of somatic mutagenesis may be increased, the fitness landscape of hematopoietic stem cells changed or the effective population size of blood cells decreased. Whether other mechanisms are responsible for increased CHIP prevalence among PLWH requires further study. Altogether, we propose that CHIP may be one mechanism through which PLWH are at increased biological aging and risk for CAD.

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C22 Developmental disorders & syndromes II

C22.1 Expanding the genotypic spectrum of *TXNL4A* variants in Burn-McKeown syndrome

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The ultra-rare developmental disorder Burn-McKeown Syndrome (BMKS) is characterised by choanal atresia and characteristic craniofacial features. BMKS is caused by biallelic variants in the pre-mRNA splicing factor *TXNL4A*. Most patients have a loss-of-function variant on one allele in *trans* with a 34bp deletion (type 1 Δ34) in the promoter region. Some patients are homozygous for a different 34bp promoter deletion (type 2 Δ34). Here, we identified two new BMKS patients ascertained through the Genomics England 100,000 Genomes Project (100K). Neither patient was initially diagnosed through the tiering system as the non-coding *TXNL4A* promoter region was not included in standard 100K variant filtering/prioritization pipelines. One patient has a *TXNL4A* c.93_94delCC, p.His32Argfs*21 variant combined with a type 1 Δ34. The other has an intronic *TXNL4A* splice site variant

(c.258-3C>G) with a type 1 Δ34. We show the c.258-3C>G variant, and a previously reported c.258-2A>G variant, cause skipping of the final exon of *TXNL4A* in a minigene assay. Furthermore, we identify transcription factor binding sites within the 56bp of the *TXNL4A* promoter affected by the type 1 and type 2 Δ34, and luciferase assays identified a 22bp repeated motif essential for *TXNL4A* expression within this region. We propose that additional variants affecting critical transcription factor binding nucleotides within the 22bp repeated motif could be relevant to BMKS etiology. Overall, we have expanded the genotypic spectrum of BMKS and highlighted the importance of considering non-coding variation in analysis of whole-genome sequencing data to improve diagnostic yield.

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C22.2 Mesenchymal enhancer adoption at the ARHGAP36 locus causes connective tissue to bone transformation

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Fibrodysplasia ossificans progressiva is characterized by early-onset heterotopic ossification (HO), spontaneous or precipitated by trauma, and is caused by mutations in *ACVR1*. Here we report on a girl presenting with a novel progressive HO phenotype. At 5 months, whole body imaging showed progressive calcifications affecting exclusively the muscle tissues surrounding the joints. At 5 years, imaging analysis demonstrated calcifications progression, with many of her skeletal muscles transformed into bone. Array CGH, FISH and WGS analyses revealed a *de novo* ~820kb duplication on chromosome 2 that was inserted into the X chromosome, within a topologically associating domain (TAD) containing two genes, *ARHGAP36* and *IGSF1*. We performed chromosome confirmation capture analysis (Hi-C) in fibroblasts of the proband and observed a new chromatin domain (neo-TAD) on the der(X) containing *ARHGAP36* and part of the 820kb duplication. This neo-TAD contained several predicted mesenchymal enhancers that could potentially drive ectopic *ARHGAP36* expression. Indeed, RNA-sequencing and Western blot analysis in proband fibroblasts revealed ectopic activation of *ARHGAP36*. RNA-seq also showed dysregulation of signalling pathways related to bone formation in the HO proband such as TGF-beta, Hedgehog and WNT signalling. Osteogenic differentiation of fibroblasts revealed a faster and stronger calcification in proband cells in comparison to cells from healthy controls. Finally, *ARHGAP36* overexpression in C2C12 and NIH/3T3 cells was sufficient to reduce TGF-beta activity upon TGFβ1 induction. In summary, here we report on a rare HO case caused by an insertional duplication causing enhancer adoption at the *ARHGAP36* locus and subsequent connective tissue to bone transformation.

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C22.3 Pathogenic *MYCN* gain-of-function variants are responsible for a mirror phenotype of the Feingold syndrome, resulting in a novel megalencephaly-polydactyly-hydrocephalus syndrome

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Introduction: Loss-of-function variants in *MYCN* have been associated to the Feingold syndrome characterized by, amongst other signs: microcephaly and absent/hypoplastic middle phalanx. MYCN function relies on two sequential phosphorylations, in position 62 (Serine) and then 58 (Threonine). Dephosphorylation of Ser62 and subsequent phosphorylation of Thr58 leads to proteasomal degradation. We report two patients with *de novo* missense variants in *MYCN* and ventriculomegaly, macrocephaly and postaxial polydactyly.

Materials and methods: We performed trio exome-sequencing and data sharing. Western Blot and phosphorylation assay were performed on HEK293 cells transfected with plasmids, after site-directed mutagenesis of the patients variants. A knock-in mouse was generated to assess and compare the phenotype.

Results: Trio exome-sequencing combined with data sharing allowed the identification of 2 patients harboring rare *de novo* missense variant in *MYCN*. The core phenotype included: ventriculomegaly, macrocephaly and postaxial polydactyly. In cellular analysis, the level of phosphorylation of Thr58 was drastically reduced compared to wild type and the level of MYCN was increased in both mutant MYCN proteins. The knock-in mouse also displayed the same biological anomalies. In addition, the knock-in mouse presented with postaxial polydactyly as with the case of two patients.

Conclusions: We report 2 patients with ventriculomegaly, macrocephaly, postaxial polydactyly and a concordant knock-in mouse model. The molecular mechanism is underlined by a Thr58 phosphorylation defect leading to an accumulation of MYCN. This novel phenotype, caused by GoF variants in *MYCN*, leads to a mirror syndrome (at the phenotypic and molecular level) of the Feingold syndrome.

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C22.4 A homozygous hypomorphic *BNIP1* variant results in disproportionate short stature and skeletal dysplasia and causes an increase in autophagosomes and reduced autophagic flux

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We investigated two apparently unrelated probands, a 6-year-old girl and a 10-month-old boy, both with normal neuromotor development and disproportionate short stature. Radiographs showed ovoid vertebrae, small and squared ilia, narrow sciatic notches, very short femoral necks, mild metaphyseal dysplasia and delayed epiphyseal ossification. Exome sequencing identified the homozygous intronic variant c.84+3A>T in *BNIP1* in both probands. BNIP1 is a member of the proapoptotic BH3-only protein family located in the endoplasmic reticulum (ER). Fibroblasts of the girl were available for further investigations. *BNIP1* transcript analysis using fibroblast-derived RNA revealed (i) wild-type mRNAs, (ii) mRNAs with an *in-frame* deletion of the last 18 bp of exon 1, and (iii) mRNAs with insertion of the first 55 bp of intron 1 causing a frameshift and introduction of a premature stop codon. Immunoblotting demonstrated a 52% to 59% reduction of BNIP1 in patient compared to control cells. BNIP1 showed the typical ER staining in patient fibroblasts and ER morphology was apparently normal. We also identified lysosomal alterations as the levels of lysosomal enzymes were increased and lysosomal localizations were changed with ~77% of patient cells showing scattered lysosomes throughout the cytoplasm and ~23% showing clustered lysosomes in the perinuclear region. Immunocytochemistry and LC3B turnover assay revealed an increase in autophagosomes and reduced autophagic flux. These data suggest a block at the terminal stage of autolysosome formation and/or clearance in patient cells and demonstrate the importance of BNIP1 in regulating lysosomal degradation and autophagy.

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C22.5 WNT11 is associated to idiopathic osteoporosis by inhibiting the Wnt canonical and non-canonical pathways

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Introduction: Primary or idiopathic Osteoporosis (IO) is a rare bone disease with an increased risk of fractures. Several genes were identified as involved in IO, but many cases are still unexplained. Therefore, our aim is to identify new genes related to IO and to analyze the function of the variants.

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

Results: Exome sequencing revealed variants in *WNT11* in two IO patients with fractures. Heterozygous variants were found in a 4-year-old boy with a loss-of-function *de novo* mutation (NM_004626.2:c.677_678dup p.Leu227Glyfs*22) and in a 48-year-old woman with a missense mutation (NM_004626.2:c.217G>A p.Ala73Thr). Heterozygous *WNT11* mutant (32bp deletion) osteosarcoma cells, expressing both the wild type and the mutant protein, showed a ~30% decrease in proliferation and a ~80% decrease in osteoblast differentiation. The mutant cells displayed reduced expression of genes within the Wnt non-canonical and canonical pathways. When mutated cells were treated with the WNT11 recombinant protein, we obtained a recovery in the proliferation and in several target gene's expressions. Results from RNAseq using the patient's primary fibroblast allowed us to identify new WNT11 targets involved in bone remodeling. Among those, we identified genes involved in the extracellular matrix synthesis (MMPs), osteoblast function (SNX10, RSPOs, SOST, MEOX1, SMOC1) and activation (FGFR2 and FGF10).

Conclusions: this is the first time that variants in WNT11 are described as related to any disease, most specially osteoporosis, by inhibiting the canonical and non-canonical pathways. European Union's Horizon 2020 No 766347

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C22.6 Impact of glycosaminoglycan biosynthesis defects on the endochondral ossification in a *Slc10a7*$^{-/-}$ mouse model with skeletal dysplasia

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Introduction: Chondrodysplasia with multiple dislocations are a group of rare disorders characterized by severe growth retardation, joint dislocations and scoliosis. Most of these disorders have been linked to pathogenic variants in genes encoding proteins required for glycosaminoglycan (GAG) biosynthesis. We identified homozygous mutations in *SLC10A7* in six individuals with a skeletal dysplasia with dislocations and amelogenesis imperfecta. The aim of the project is to clarify the impact of the GAG biosynthesis impairment on the endochondral ossification process using a *Slc10a7* deficient mouse model that mimics the human bone phenotype. Methods: Exome sequencing, PCR, immunohistology.

Results: Analyzing mouse femurs, we showed a significant reduction of the growth plate length associated with a reduced Safranin O staining in embryonic and juvenile null mice compared to controls. In particular, null mice showed a significant and progressive reduction of the hypertrophic zone during development and a significantly reduced type X collagen expression, specific for mature chondrocytes. Type II collagen expression was unchanged throughout the growth plate of null mice at all ages, but was reduced, as type I collagen expression, in the trabecular

bone. We did not find any significant difference in chondrocyte proliferation but the apoptosis was significantly increased in null hypertrophic chondrocytes compared to controls.

Conclusion: Slc10a7 is involved in GAG biosynthesis and suggests an impairment of chondrocyte differentiation and maturation processes responsible for an advanced ossification in our model. Further in vitro experiments are required to confirm this defect and to better characterize the affected signaling pathways involved in bone development.

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C23 Internal organs - Kidney, bowel, fat

C23.1 Biallelic *TMEM72* variants in patients with nephronophthisis-like phenotype

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Introduction: Causes for monogenic kidney diseases are heterogenous and distinguishing the different causes based on phenotype can be challenging. Nephronophthisis, an autosomal recessive kidney disease resulting from primary cilium defects, is hard to diagnose due to unspecific and variable symptoms. Currently, <50% of cases can be diagnosed by assessing known genes.

Material and Methods: We performed genetic testing in patients with a phenotype suggestive for nephronophthisis. We carried out immunohistochemistry, in silico, in vitro and in vivo experiments to further interpret our findings.

Results: We present eight patients from five families carrying biallelic TMEM72 variants. Four families presented with kidney failure at a young adult age. One family had a more severe phenotype with prenatal onset of kidney failure and neurological symptoms. We show that the truncating variant results in a short TMEM72 isoform. With immunohistochemistry staining in kidney biopsy material we show that TMEM72 is localized and expressed differently in cases compared to controls, in cells known to be affected in nephronophthisis. Furthermore, using mass-spectrometry we found significant and altered binding of TMEM72 with proteins known to be involved in renal ciliopathies.

Conclusion: We present the first genomic evidence and functional work that link TMEM72 variants to kidney disease. Based on our patients' phenotype, immunohistochemistry findings and mass-spectrometry results we can link TMEM72 to ciliary function.

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C23.2 Rare variant burden influences the rate of disease progression in Polycystic Kidney Disease

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is caused primarily by variants in *PKD1*, and *PKD2*. Disease severity ranges from *in-utero* onset to preserved kidney function into old age. It is well established that the type of diagnostic ADPKD variant can influence disease severity and variants have been identified which modify disease severity or can cause autosomal recessive disease. Here we demonstrate, using robust statistical analyses, that rare variant burden in the gene *PKD1* contributes to ADPKD phenotypic variability.

Methods: Patients ($n = 449$) with an established genetic diagnosis of ADPKD due to a pathogenic variant in *PKD1* or *PKD2* were recruited from five international centres (Dublin Ireland, Genomics England/ Royal Free Hospital London UK, Leipzig Germany, Bologna Spain, Sydney Australia). The association between the presence of rare, additional *PKD1* variants and age at end-stage renal disease (ESRD) was tested using a Cox mixed effect regression model.

Results: The presence of rare, additional bioinformatically-inferred damaging *PKD1* variants was associated with a lower age at ESRD, in patients with an established genetic diagnosis (hazard ratio: 1.61 (1.14-2.27), $p=5.9\text{x}10^{-3}$).

Conclusions: Rare, additional bioinformatically-inferred damaging *PKD1* variants impact disease severity in ADPKD. Further work is required to evaluate the impact of rare variation in other PKD-related genes. These findings have important implications for patient counselling and the assessment of variant pathogenicity in clinical genetics services.

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C23.3 Diagnostic yield and benefits of whole-exome sequencing in patients with congenital anomalies of the kidney and urinary tract (CAKUT) diagnosed in the first thousand days of life

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Introduction: Congenital anomalies of the kidney and urinary tract (CAKUT) are the predominant cause of chronic kidney disease (CKD) in children and adolescents. CAKUT can occur in an isolated form or with extrarenal comorbidities. Although over 50 genes are known to cause CAKUT if mutated, the diagnostic yield of whole-exome sequencing (WES) studies is typically lower than 15%. Here, we asked for the diagnostic yield in young CAKUT patients and whether an early genetic diagnosis may impact patient management.

Materials and Methods: In 100 patients diagnosed with CAKUT in the first 1,000 days of life, WES was performed and variants in 58 established CAKUT-associated genes were extracted and classified according to the ACMG guidelines. The translational value of the genetic findings was assessed.

Results: In 26% of patients diagnosed with CAKUT early in life, we identified a likely pathogenic (LP) or pathogenic (P) rare variant in one or two of 17 CAKUT-associated genes, including *HNF1B*, *LIFR*, *SALL1* and *UMOD*. Of the 24 different variants detected, 12 were loss-of-function and five *de novo* variants. Thirteen of the affected genes were potentially associated with extrarenal morbidities, e.g. diabetes mellitus or hyperuricemia. Patients with LP/P variants were significantly more likely to have end-stage CKD under three years of age and extrarenal manifestations than those without.

Conclusions: Our data demonstrate a relatively high diagnostic yield of WES in children diagnosed with CAKUT early in life, particularly with end-stage CKD and extrarenal manifestations, and suggest a benefit for managing comorbidities (Else Kröner-Fresenius-Stiftung, grant no. 2018_Kolleg.12).

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C23.4 Single cell RNA sequencing of the enteric nervous system shows selective preservation of schwann cell progenitor-like cells in*ret*mutant zebrafish

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Introduction: Hirschsprung disease (HSCR) is a congenital defect characterized by the absence of the enteric nervous system (ENS) in the distal colon. The ENS was long believed to be solely derived from the vagal neural crest, however trunk crest derived Schwann cell progenitors (SCPs) were found to have enteric neurogenic potential as well. The role of SCPs in HSCR remains unexplored, therefore we aimed to determine the effect of mutations in *ret*, the major gene involved in HSCR, on the ENS.

Methods: We optimized and performed single cell (sc)RNA sequencing on intestines isolated from 5-day-old wildtype and *ret* mutant zebrafish to analyze their ENS. We validated our findings by live-imaging in zebrafish and by immunohistochemistry using human intestinal material.

Results: Based on the scRNA sequencing data, we were able to identify clusters with "differentiated neurons", clusters of cells that undergo neuronal commitment or differentiation and clusters of "progenitor cells", including SCPs. As expected, we found that *ret* mutant larvae lack the majority of these clusters. However, the SCP cluster was selectively persevered. We are currently investigating whether SCPs remain present in the aganglionic bowel of HSCR patients.

Conclusion: Our data show that SCPs in the intestine, develop independently of Ret expression. Therefore, the presence of these cells brings new insights that could be used to develop novel intervention options for enteric neuropathies, such as HSCR.

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C23.5 The *OSMR* gene is involved in Hirschsprung Associated Enterocolitis susceptibility through an altered downstream signaling

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Hirschsprung (HSCR) Associated Enterocolitis (HAEC) is a common life-threatening complication in HSCR, a congenital intestinal aganglionosis. The loss of gut homeostasis caused by an impaired immune system hypothesised for HAEC and the susceptibility to HAEC in HSCR patients suggest a genetic background. Yet, no gene has been claimed to contribute to HAEC. We investigated the genetics of HAEC by omics approaches: Whole Exome Sequencing (WES) and proteomics analysis. WES on 12 HSCR patients with enterocolitis (HAEC) and 12 without (HSCR-only) and replication in a panel of 65 HAEC and 105 HSCR-only patients, identified a susceptibility variant in the Oncostatin-M receptor (OSMR) gene, finding allelic frequencies of 14.6% in HAEC and 5.1% in HSCR-only patients, $p = 0.0024$. The Transmission Disequilibrium Test on 38 HAEC and 26 HSCR-only trios supported an overtransmission of the variant allele to the affected patients, $p = 0.01255$. Proteomic analysis on lymphoblastoid cells from one HAEC patient homozygote and one HAEC patient wt for this variant pointed out two well distinct clusters of proteins significantly up or downregulated upon OSM stimulation. Pathways analysis showed an enrichment in immune response related

proteins in the HAEC OSMR wt cells that was completely lost in the OSMR variant cells, where proteins related to pathogen infection and inflammation pathways were instead over-expressed. In conclusion, OSMR is implicated in the susceptibility to HAEC likely perturbating the downstream signalling cascade necessary for the gut immune response and homeostasis maintenance. (Italian Ministry of Health grant GR-2011-02347381).

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C23.6 Lipodystrophy due to genetic deficiency of picornavirus host factor and obesity regulator PLAAT3

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Background: PLAAT3 (also known as PLA2G16) is a phospholipid modifying enzyme mainly expressed in white adipose tissue (WAT). PLAAT3 represents an important potential drug target as its deficiency in mice protects against picornavirus infection and diet-induced obesity. The consequences of PLAAT3 deficiency in humans are not known.

Methods: Unbiased genome-wide approaches including homozygosity mapping, whole exome and genome sequencing were applied to identify the causal mutation in 4 patients from two consanguineous families with unexplained partial lipodystrophy. Lipidomics and histopathological analysis of patient WAT were performed.

Results: In the first family we identified a homozygous 5092 bp deletion encompassing exon 2 of the *PLAAT3* gene (c.16-4823_118 +167del, p.Pro6ValfsTer15) within a shared ~43 Mb homozygous region at 11q12.3. In the second family a homozygous duplication of a single base leading to a frameshift in exon 3 of *PLAAT3* (c.286dupG, p.Ala96GlyfsTer16) was observed. The patients presented clinically with partial lipodystrophy, severe insulin resistance and hyperlipidemia. Demyelinating neuropathy and chronic pain were additional disabling features. PLAAT3-deficient WAT showed inflammation, irregular adipocyte morphology and an abnormal lipidomic profile indicative of a failure to liberate arachidonic acid (AA) from membrane phosphatidylcholines (PC) resulting in increased levels of AA containing lyso-PCs (LPC).

Conclusions: Genetic PLAAT3 deficiency in humans causes a novel type of autosomal recessive familial partial lipodystrophy. This finding introduces an important caveat when considering PLAAT3 as a therapeutic target.

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C24 Genome variation and architecture

C24.1 What is the best solution to manage failures of chromosomal structural variations detection by short-read strategy?

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Short-read Whole Genome sequencing (WGS) affords the efficient and accurate characterization of balanced chromosomal rearrangement (BCR) breakpoints. However, in 9 to 11% of cases, the rearrangement cannot be detected by this strategy. Among the 117 BCR we studied in patients with abnormal phenotype, fourteen (11.9%) could not be detected by our current strategy that includes short-read WGS, alignment against GRCh38 genome

and SV detection using Breakdancer v1.4.5. These BCRs were all reciprocal translocations, 10 of which implicated constitutive heterochromatin of chromosomes 1, 9 or 16, acrocentric short arms or pericentromeric regions. The data of these BCRs were re-analyzed using 3 others bio-informatic tools (GRIDDS, LUMPY and SvABA). In parallel, eleven BCRs were further characterized using different techniques, either isolated or combined, including FISH, linked-read sequencing (10X Genomics), long-read sequencing (PacBio, Nanopore sequencing) or optical mapping (Bionano). We were able to characterize the breakpoints at the base-pair level for 11 translocations. For 10 translocations, at least one breakpoint involved highly repetitive elements such as alpha-satellites, segmental duplications, satellite repeats or other poorly mapped regions. For 5/11 patients, one of the breakpoints could explain the phenotype either by gene disruption (*NLGN4*, *DYRK1A*, *CAMTA1*) or position effect (*BMP2*, *SIX3*). In conclusion, failure of the short read strategy is due to the implication of highly repetitive genomic regions, either absent from the reference genome or not attributed to a unique position. The resolution of these cases are of essential for patients' care since we concluded to a pathogenic variant in 45% of the patients.

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C24.2 How silence shapes the brain: synonymous variants alter codon usage and translation of Sonic Hedgehog in holoprosencephaly

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Despite their clinical relevance, synonymous Single Nucleotide Variants (sSNVs) are still largely ignored in clinical studies, unless directly associated with splicing defects. In particular, their impact on codon usage and protein translation remains unelucidated. In this work, we explore the clinical significance of sSNVs in the Sonic Hedgehog gene (*SHH*) identified in patients affected by holoprosencephaly - a developmental defect resulting from incomplete forebrain cleavage. Genetic screening was performed in 931 patients and revealed 8 different *SHH* sSNVs, selectively enriched in our cohort as compared to healthy individuals, suggesting their role in disease etiology. We systematically assessed their deleterious impact at both transcriptional and translational levels using a series of in silico and in vitro approaches. Although no evidence of impact of these sSNVs on splicing, mRNA structure or miRNA regulation was found, five sSNVs were predicted to impact protein translation by introducing significant changes in codon usage. Cell assays demonstrated that these five sSNVs result in a significantly reduced expression of the SHH protein (up to 23%, p<1e^-9). Inhibition of the proteasome rescued the protein levels for four out of five sSNVs, confirming their impact on protein stability and folding. Remarkably, we found a significant correlation between experimental values of protein reduction and computational measures of codon usage, indicating the relevance of our *in silico* predictions. Our findings underline the clinical relevance of sSNVs and the necessity to investigate their impact on translation. This study was supported by Fondation Maladie Rares (PMO1201204) and the Agence de la Biomedecine (AMP2016).

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C24.3 *De novo* alpha satellite insertions and the evolutionary landscape of centromeres

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Human centromeres are composed of alpha satellite DNA hierarchically organized as higher-order repeats and epigenetically specified by CENP-A binding. Current evolutionary models assert that new centromeres are first epigenetically established and subsequently acquire an alphoid array. Differently, we identified during routine aneuploidy FISH testing a *de novo* insertion of alpha satellite DNA array (~100-200 kbp) from the centromere of chromosome 18 (D18Z1) into chromosome 15q26 euchromatin. Although bound by CENP-B, this locus did not acquire centromeric functionality as demonstrated by lack of constriction and absence of CENP-A binding. We characterized the rearrangement by FISH and sequencing using Illumina, PacBio, and Nanopore adaptive sampling which revealed that the insertion was associated with a 2.8-kbp deletion and occurred in the paternal germline. Notably, the site was located ~10 Mbp distal from the location where an ancestral centromere was seeded ~25 million years ago in the common ancestor of Old World monkeys and apes. Long reads spanning either junction showed that the D18Z1 higher-order repeat structure was more homogeneous at the proximal end and more divergent at the distal one. The rearrangement did not disrupt any gene or regulatory element and did not alter the epigenetic status of the surrounding region, consistent with the absence of phenotypic consequences in the carrier. This case demonstrates a likely rare but new class of structural variation that we name alpha satellite insertion. It also expands our knowledge about the evolutionary life cycle of centromeres, suggesting that alphoid arrays can relocate near vestigial centromeric sites.

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C24.4 Integration of Hi-C and long-read sequencing reveals the structure of highly rearranged chromosomes in patients with germline-chromothripsis

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Chromothripsis commonly observed in cancer may also occur as a rare event in the germline. An important step towards a more thorough understanding, which rearrangements have a pathogenic effect, is the precise mapping of breakpoints and the reconstruction of the shattered chromosomes.

Here, we investigated 12 cases with complex genomic rearrangements in the germline, including chromothripsis, by combining short-read and long-read PacBio WGS with Hi-C. Large-scale genomic rearrangements were readily identified in Hi-C interactions maps, allowing for an independent assessment of breakpoint calls from the WGS methods. This resulted in the detection of >300 large-scale genomic junctions in total (4 to 62 per sample). Additionally, Hi-C was effective in guiding the reconstruction of rearranged chromosomes telomere to telomere. The Hi-C derived 3D chromatin structure of the mutant chromosomes showed in more than 2/3 of the instances a reshuffling of topologically associated domains (TADs). To investigate the effect of these rearrangements on gene regulation, we used phased RNA-seq data generated from patients' lymphoblastoid cells. For most of the cases, less than 1/4 of the detectable genes +-500kb around breakpoints show significant expression differences between the alleles, which are partly associated with gene disruptions. The fraction of genes with expression differences decreases quickly with increasing distance to the breakpoint. This indicates that the genome can be relatively resistant against the effects of SVs and that even large-scale genomic rearrangements appear to be tolerated to a surprising extend.

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C24.6 22q11.2 inversion in a mosaic 22q11.2 deletion patient provides insights in LCR22-mediated rearrangements

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Introduction: The 22q11.2 Deletion Syndrome (22q11.2DS), the most common genomic disorder, is thought to be caused by non-allelic homologous recombination between low copy repeats on chromosome 22 (LCR22s). Due to their complexity, LCR22s have remained elusive for sequencing and as a consequence, the mechanistic origin has never been charted. The identification of a mosaic 22q11.2DS individual provides a unique opportunity to infer the deletion origin since both non-rearranged and rearranged alleles are present.

Materials and Methods: cfDNA analysis, arrayCGH, SNParray, and 22q11.2del FISH uncovered an aberrant maternal profile in the 22q11.2 locus. An LCR22-specific fiber-FISH test was performed to assemble the haplotypes and results were validated by Bionano optical mapping and targeted interphase-FISH. Family members were tested for the presence of rearrangements. Nanopore sequencing is used to map the (rearranged) LCR22s.

Results: Genetic testing detected a LCR22-A/B deletion (1.5Mb) in 50% of the white blood cells. Haplotype assembly and interphase-FISH uncovered an inversion between LCR22-A/B in the cells without the deletion. The inversion occurred *de novo* and was not transmitted to the fetus. Long read sequencing is applied to map the LCR22s, currently still gaps in the human reference genome, and pinpoint the rearrangement breakpoints.

Conclusions: Inversions have never been described in 22q11.2DS and somatic mosaicism is rare. This inversion probably renders the locus unstable, driving the somatic deletion. Presence of the inversion and deletion allele in the same individual provides a unique opportunity to map the LCR22s and identify the *cis* acting elements driving 22q11 deletions. Funding: FWO GOE1117N

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C25 Using genomics to personalise medicine

C25.1 Pharmacogenetics to Avoid Loss of Hearing (PALOH): A Prospective Observational Trial to Assess the Implementation of Rapid Genotyping to Avoid Aminoglycoside Induced Ototoxicity in Newborns

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Introduction: Aminoglycosides are first-choice antibiotics for empirical treatment of neonatal sepsis. The m.1555A>G variant predisposes to profound ototoxicity after aminoglycoside administration. Current genotyping takes days, an unacceptable delay in acute settings. We aimed to develop and implement a point of care test (POCT) to genotype the m.1555A>G variant in a clinically relevant timeframe, facilitating tailored prescribing.

Methods: A loop-mediated isothermal amplification reaction was designed to amplify the target region before genotyping via melt-curve analysis. This was packaged into the Genedrive POCT system and integrated into clinical pathways at two major UK neonatal centres over 11-months. All admissions were eligible for recruitment. The primary outcome was the number of neonates successfully tested for the variant out of all babies prescribed antibiotics. Secondary outcomes include measures of clinical timings to assess whether implementation negatively impacted normal care.

Results: The POCT identifies the m.1555A>G genotype in 26 minutes with performance comparable to gold standard genotyping. 81% of admissions receiving antibiotics were successfully tested for the variant and other clinical timings, including time-to-antibiotic, were equivalent to previous practice. Three babies with m.1555A>G were identified, all of whom received an alternative antibiotic regimen. A failure rate of 15% persisted throughout the study, however this rate could be almost eliminated after adjustments to assay chemistry.

Conclusions: We can identify the m.1555A>G variant in a practice-changing timeframe and demonstrate the importance of implementation trials in genomics. To our knowledge, this represents the first example of a pharmacogenetic assay employed in the acute neonatal setting to alter management.

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C25.2 Family history and polygenic risk scores are not interchangeable measures of genetic susceptibility

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Family history (FH) is a risk factor for most common diseases, capturing both genetic and other familial risk. Polygenic risk scores (PRS) identify individuals at substantially increased risk of disease, but whether these two measures of genetic susceptibility are interchangeable remains unclear. We studied the interplay of FH and PRS for 20 common diseases, including common cancers, cardiometabolic diseases, and mental disorders. From FinnGen ($N = 260{,}405$), we identified 30,517 individuals with a first-degree relative in the dataset, and evaluated the effect of FH by linkage to nationwide healthcare registries. For each disease, we systematically constructed genome-wide PRSs. For the majority of diseases, adjusting the FH effect size with PRS resulted in moderate decreases (mean decrease -10.9%;s.d. 6.7%). Similarly, adjusting the PRS effect with FH had negligible impact on the PRS (-2.9%;1.3%). Examples for three diseases are in the table. None of the 20 diseases showed evidence of interaction between FH and PRS (p-value range 0.07-0.87). For 5/20 diseases, PRS showed larger improvements in risk prediction than FH. In none did FH and PRS jointly perform better than PRS alone. Results were similar for parental causes of death ($N = 163{,}973$, out of whom 61.3% had a dead parent). For a wide range of diseases, this systematic comparison demonstrates that family history and polygenic risk provide complementary information in risk assessment, supporting the clinical utility of polygenic risk scores.

Effect sizes for family history and polygenic risk scores

	Family history	Family history adjusted with PRS	PRS	PRS adjusted with family history
	OR (95% CI)	OR (95% CI)	OR per SD (95% CI)	OR per SD (95% CI)
Breast cancer	2.46 (2.01-3.00)	2.06 (1.68-2.54)	1.76 (1.63-1.91)	1.71 (1.58-1.85)
Venous thromboemolism	1.80 (1.46-2.21)	1.70 (1.38-2.09)	1.40 (1.32-1.48)	1.39 (1.31-1.47)
Glaucoma	2.50 (2.05-3.06)	2.25 (1.84-2.76)	1.64 (1.53-1.77)	1.61 (1.50-1.74)

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C25.3 Breast and prostate cancer risk: the interplay of polygenic risk, high-impact monogenic variants, and family history

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Genetic predisposition to breast cancer (BC) has been associated with both high-impact monogenic variants across susceptibility genes, and the polygenic risk due to the additive effects of many common variants. While the two types of genetic risks are well-established, few studies have explored the extent to which polygenic background can modify the risk of high-impact monogenic variants in BC. Here, we show that among 96,344

women with 3,654 BC cases (data from UK Biobank), polygenic risk score (PRS) modifies the penetrance of monogenic variants across 12 BC susceptibility genes (*BRCA1, BRCA2, ATM, PALB2, CHEK2, RAD51, TP53, RAD51D, RAD51C, BRIP1, RAD54L, BARD1*). A polygenic risk score for BC was calculated and used to categorise individuals into low, intermediate, and high risk groups. We then compared how the PRS stratification among carriers of high-impact variants influence the BC risk. The prevalence of BC is significantly higher in carriers of high-impact variants with a high PRS (>90% percentile) [(Odds ratio (95% CI), 4.41 (3.19-5.97), $P = 3.96\times10-21$) and (Hazard ratio (95% CI), 3.76 (2.84-4.98), $P = 2\times10-16$)], compared to carriers with low PRS (<10% percentile) [(OR (95% CI), 1.98 (1.05-3.41), $P = 2\times10-2$) and (HR (95% CI), 2.23 (1.34-4.00), $P = 3\times10-3$)].

These findings highlight the potential benefit of including PRS in the risk assessment of familial BC.

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C25.4 FAIR Genomes: a metadata model and guidelines for reuse of NGS data

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The genomes of thousands of Dutch individuals are now profiled annually in healthcare and research. However, this valuable genomic and associated clinical data is captured in different ways and fragmented across many organizations. Discovering additional rare patient cases is difficult and reusing data for personalized medicine is nearly impossible. Moreover, establishing research cohorts based on specific parameters is extremely time-consuming. FAIR Genomes aims to unlock genomes data full potential for everyone's health benefit. Together with clinicians, laboratory specialists, patient organizations and bioinformaticians we defined which meta data are essential to find, share and reuse NGS data. We evolved a semantic schema of essential data elements reusing existing ontologies whenever possible and focussed on being harmonized with international initiatives such as EJP, RD3, PhenoPackets, X-omics, BBMRI, ELIXIR, Solve-RD and EJP-RD. Currently FAIR genomes schema has 107 elements in 9 modules (Study, Personal, Leaflet and consent form, Individual consent, Clinical, Material, Sample preparation, Sequencing, Analysis), all linked to common ontologies such as NCIT, DUO, and EDAM. The schema is represented by a YAML file and is transformed into templates for EDCs so you can easily implement FAIR genomes into your EPD, MOLGENIS, Castor, OpenClinica and REDCap systems. Currently, we are writing a guideline to help institutes implement FAIR Genomes in practice. By providing guidelines and practical resources, FAIR Genomes expects to increase reuse of genomic data for healthcare and research while addressing ELSI issues, providing a great basis for participation in e.g. Europe's Beyond 1+Million Genomes. Join us at: https://github.com/fairgenomes.

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C25.5 Analysis of medically actionable variants in the 59 ACMG genes using 6045 whole genomes from the Qatar Genome Program

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Introduction: The American College of Medical Genetics (ACMG) identified 59 genes in which incidental findings of medically actionable pathogenic variants is recommended. The prevalence of these variants from Arab and other Middle Eastern populations is still lacking. The Qatar Genome Program (QGP), which is a large, population-based whole genome sequencing project with comprehensive phenotypic information, allowed us to identify and analyze the medically actionable variants in the Qatari population.

Materials and Methods: We used data from 6,045 whole genomes from the QGP and integrated it with phenotypic data collected by the Qatar Biobank. We identified known pathogenic and likely pathogenic variants based on ClinVar and the Human Gene Mutation Database professional entries. Additionally, we identified novel variants, assessed their phenotypic associations, and functionally characterized two novel variants in zebrafish model.

Results: We identified a total of 64 pathogenic and likely pathogenic variants in 27 ACMG genes in 170 individuals. Overall, 2.8% of the QGP-sequenced participants carried a pathogenic or likely pathogenic variant in one of the 59 ACMG genes. We found 75 novel, potentially pathogenic variants. We prioritized two novel cardiovascular variants, *DSP* c.1841A>G (p.Asp614Gly) and *LMNA* c.326T>G (p.Val109Gly) for functional characterization. Our results

showed that both variants resulted in abnormal zebrafish heart development, heart rate and rhythm.

Conclusion: The prevalence of known medically actionable variants in the Qatari population is slightly higher than in other populations. There is a comparable number of predicted pathogenic novel variants. We confirmed the pathogenicity of two novel variants in zebrafish.

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C25.6 Genetic testing and its impact on therapeutic decision making in childhood-onset epilepsies - a study in a tertiary referral centre

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Introduction: To assess the distribution of genetic epilepsies and accordingly adjusted medical treatment at the only tertiary epilepsy referral centre in Denmark.

Material and Methods: The records of children born between 2006-2011 and followed at the centre in 2015 were systematically analysed. Genetic approaches used were karyotyping, array and next-generation sequencing (NGS) approaches such as gene panels and whole-exome sequencing.

Results: 357 children were identified, however 7 % did not have epilepsy, and 11 % had epilepsy due to an acquired brain lesion. These patients were excluded from further analysis. The remaining 290 children were most frequently diagnosed with developmental and epileptic encephalopathies (35 %), focal or multifocal epilepsy (41 %), and generalized epilepsy (8 %). 211/290 patients consented for genetic testing aiming to find a genetic explanation for their illness. A genetic cause was found in half of the consented patients across 36 different genes encompassing 14 copy-number variants and 86 single-nucleotide variants. Commonly affected genes affected were *SCN1A* and *TSC1/2*. Tailored treatment approaches were possible in half of patients with a molecular diagnosis. Exome sequencing led to molecular diagnosis in several genetically unexplained children and identified three new candidate genes.

Conclusions: Almost 50% reached a genetic diagnose. NGS approaches have much higher yield than an array (detection rate of 40% vs 7%). We shows that 1 out of 4 patients referred to a tertiary epilepsy centre can benefit from early genetic testing in order to receive tailored treatment and prevent unnecessary and potentially harmful diagnostic procedures and managements.

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C26 Late Breaking

C26.1 Sediment DNA reveals Neandertal population history

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The study of hominin history has progressed through both archaeological and genetic insights. However, many archaeological sites lack associated hominin fossils, frustrating genetic analyses. Even when fossils are found, they often do not cover the full time-span of a site, or sampling them for DNA may not be possible. Here we present targeted enrichment and sequencing of hominin nuclear DNA from sediments, and insights into human history derived from this DNA. We developed methods to capture hominin DNA even in the presence of homologous faunal DNA, and evaluate the extent of microbial and faunal DNA in our data. We applied these methods to sediment samples from Galería de las Estatuas, a site in northern Spain, and Denisova and Chagyrskaya caves, in the Altai Mountains in southern Siberia, and identified and sequenced Neandertal nuclear DNA in stratigraphic layers spanning 55k - 200 thousand years ago. We then placed each sample on the Neandertal phylogenetic tree, inferring the most likely divergence date from a lineage. In Estatuas we demonstrate a population transition, and associate this change with specific layers. In Chagyrskaya, all layers are associated with a single Neandertal lineage, suggesting a more homogenous occupation. This work demonstrates that detailed genetic analyses may be possible from many more archaeological sites than was previously thought, and is particularly encouraging for time-series studies of single sites, or for sites with a sparse fossil record.

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C26.2 Comprehensive PGT for patients with *de novo* pathogenic variants following single-molecule long read amplicon sequencing based haplotyping

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Preimplantation genetic testing for monogenic disorders (PGT-M) aims to select embryos devoid of pathogenic inheritable variants from parents to the offspring. Different generic PGT methods, such as Karyomapping, haplarithmisis and OnePGT, cannot be applied for couples where one of the partners is carrying a de *novo* pathogenic. The absence of affected close relatives entangles the process of constructing accurate haplotype using linkage analysis by Single Nucleotide Polymorphisms (SNPs) located upstream and downstream of the pathogenic variant.

Current practices for the handling of couples with *de novo* variants are mostly targeted, require the combination of direct and indirect approaches and often require multiple biopsies. To overcome this limitation, we developed a new method: long-range PCR and subsequent long read sequencing of the pathogenic variant region enabled haplotype reconstruction and imputation in the genome-wide haplotypes. From January 2017 until December 2019, twenty-two couples with one of the partners carrying a pathogenic *de novo* variant causing a Mendelian disorder were enrolled for PGT-M in the Centre for Human Genetics, UZ Leuven. Genomic DNA from the partner carrying the variant and his/her parents was used for targeted long-read sequencing on a PacBio RSII/Oxford Nanopore to impute the disease-carrying allele. Subsequently, genotyping of the prospective parents and both grandparents, and DNA amplified from blastomere or trophectoderm biopsies via Illumina HumanCytoSNP-12v2.1 beadchips enabled genome-wide haplotyping using haplarithmisis. Unaffected embryos were transferred. Hence, our novel approach consequently allows comprehensive PGT for *de novo* variants in a single workflow.

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C26.3 Genetic analysis of blood molecular phenotypes reveals regulatory networks affecting complex traits: a DIRECT study

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To investigate the interplay between molecular intermediaries that define the pathways from genetic variation to disease, we evaluated the shared genetic regulation of mRNA molecules, proteins and metabolites derived from whole blood from 3,029 human donors. We observed extensive allelic heterogeneity, with 94.4% genes, 97.3% proteins and 49.2% metabolites associated with two or more variants, and pleiotropy, where variants were associated with multiple local (cis) and distal (trans) molecular phenotypes. Estimates of the proportion of shared genetic regulation ranged from 66.6% for expression and proteins, 33.3% for expression and metabolites, to 27% for proteins and metabolites. Compared to gene expression in a diverse set of 44 tissues, the highest proportion of sharing of genetic regulation was observed for gene expression (from 81% in testis to 98% in brain); followed by plasma proteins (from 20.5% spleen to 100% in liver or skeletal muscle) and plasma metabolites (from 0% brain to 16.88% testis).

To define pathways from genetic variation to disease, we constructed networks of genetic variants and molecular phenotypes connected by significant QTL associations. The complete network included 79,733 nodes: 15,254 genes, 373 proteins, 172 metabolites and 63,795 SNPs, of which 2,828 were known GWAS variants. One sub-network included the trans relationship between rs149007767 and *RETN*, and identified *GRB10* and *IKZF1* as candidate mediating genes. Our study provides a roadmap to understand GWAS networks and the underlying mechanism of action of GWAS variants as DNA information is passed across molecular processes.

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C26.4 Mutational bias in human spermatogonia impacts the anatomy of enhancers active in neural development

Vera Kaiser, Lana Talmane, Yatendra Kumar, Fiona Semple, Marie MacLennan, Deciphering Developmental Disorders Study, David FitzPatrick, Martin Taylor, ***Colin Semple***

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Mutation in the germline is the ultimate source of genetic variation, but little is known about the influence of germline chromatin structure on mutational processes. Using ATAC-seq, we profile the open chromatin landscape of human spermatogonia, the most proliferative cell-type of the germline, identifying transcription factor binding sites (TFBSs) and PRDM9-binding sites, a subset of which will initiate meiotic recombination. As expected we observe an increase in rare structural variant (SV) breakpoints at PRDM9-bound sites, implicating meiotic recombination in the generation of structural variation. However, many germline TFBSs, such as NRF1, are also associated with increased rates of SV breakpoints, apparently independent of recombination. In addition, singleton short insertions (>=5 bp) are highly enriched at TFBSs, particularly at sites bound by testis active TFs, and their rates correlate with those of structural variant breakpoints. Short insertions often duplicate the TFBS motif, leading to clustering of motif sites near regulatory regions in this male-driven evolutionary process. Increased mutation loads at germline TFBSs disproportionately affect neural enhancers with activity in spermatogonia, potentially altering neurodevelopmental regulatory architecture. Local chromatin structure in spermatogonia is thus pervasive in shaping both evolution and disease.

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C26.5 C2orf69 mutations disrupt mitochondrial function and cause a multisystem human disorder with recurring autoinflammation

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Background: Deciphering the function of the many genes previously classified as uncharacterized "open reading frame" (orf) completes our understanding of cell function and its pathophysiology.

Methods: Whole-exome sequencing, yeast 2-hybrid and transcriptome analyses together with molecular characterization are used here to uncover the function of the *C2orf69* gene.

Results: We identified loss-of-function mutations in the uncharacterized *C2orf69* gene in eight individuals with brain abnormalities involving hypomyelination and microcephaly, liver dysfunction and recurrent autoinflammation. C2orf69 contains an N-terminal signal peptide that is required and sufficient for mitochondrial localization. Consistent with mitochondrial dysfunction, patients showed signs of respiratory chain defect and a CRISPR-Cas9 knockout cell model of *C2orf69* had similar respiratory chain defects. Patient-derived cells revealed alterations in immunological signaling pathways. Deposits of PAS-positive material in tissues from affected individuals together with decreased glycogen branching enzyme 1 (GBE1) activity indicated an additional impact of C2orf69 on glycogen metabolism.

Conclusion: Our study identifies C2orf69 as an important regulator of human mitochondrial function and suggests an additional influence on other metabolic pathways.

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C26.6 High-throughput screening identifies suppressors of mitochondrial fragmentation in *OPA1* fibroblasts

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Mutations in OPA1 cause autosomal dominant optic atrophy (DOA) as well as DOA+, a phenotype characterized by more severe neurological deficits. OPA1 deficiency causes mitochondrial fragmentation and also disrupts cristae, respiration, mitochondrial DNA (mtDNA) maintenance, and cell viability. It has not yet been established whether phenotypic severity can be modulated by genetic modifiers of OPA1. We screened the entire known mitochondrial proteome (1531 genes) to identify genes that control mitochondrial morphology using a first-in-kind imaging pipeline. We identified 145 known and novel candidate genes whose depletion promoted elongation or fragmentation of the mitochondrial network in control fibroblasts and 91 In DOA+ patient fibroblasts that prevented mitochondrial fragmentation, including Phosphatidyl Glycerophosphate Synthase (*PGS1*). PGS1 depletion reduces CL content in mitochondria and rebalances mitochondrial dynamics in OPA1-deficient fibroblasts by inhibiting mitochondrial fission, which improves defective respiration, but does not rescue mtDNA depletion, cristae dysmorphology or apoptotic sensitivity. Our data reveal that the multifaceted roles of OPA1 in mitochondria can be functionally uncoupled by modulating mitochondrial lipid metabolism, providing novel insights into the cellular relevance of mitochondrial fragmentation.

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