ABSTRACTS COLLECTION

# Abstracts from the 56th European Society of Human Genetics (ESHG) Conference: Oral Presentations

*European Journal of Human Genetics* (2024) 32:3–90; https://doi.org/10.1038/s41431-023-01480-z



Volume 32 | Supplement 1

Glasgow, Scotland, United Kingdom

June 10-13, 2023

**Sponsorship:** Publication of this supplement was sponsored by the European Society of Human Genetics. All content was reviewed and approved by the ESHG Scientific Programme Committee, which held full responsibility for the abstract selections.

**Disclosure Information:** In order to help readers, form their own judgments of potential bias in published abstracts, authors are asked to declare any competing financial interests.

Contributions of up to EUR 10 000.- (Ten thousand Euros, or equivalent value in kind) per year per company are considered "Modest". Contributions above EUR 10 000.- per year are considered "Significant".

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

## PLENARY SESSIONS

### PL1 ESHG AWARD LECTURE

#### PL1.1 ESHG Award Lecture

***Magdalena Zernicka-Goetz***

I will talk about our lab's work to establish methods for culturing human and mouse embryos beyond implantation in vitro and how results coming from these studies enabled us to put together multiple stem cell types, programmed to form embryonic and extra-embryonic tissues, to self-organise into complete embryo-like structures. I will detail how we are using these complete mouse and human embryo models to determine the mechanisms behind embryo self-organisation, bringing insight into the cellular and molecular mechanisms that control previously unexplored aspects of early mammalian development until early organogenesis.

### PL2 OPENING PLENARY

#### PL2.1 Terrestrial and extraterrestrial genomics and multiomics

***Christopher E. Mason***

In this talk, cellular and molecular details on the human and microbial responses to short and long-duration spaceflight will be detailed, which span several NASA, SpaceX, and Axiom missions and provide key lessons for upcoming lunar and Mars missions. Then, recent advances in biotechnology, multi-omics, data modeling, and cross-species genome engineering will be highlighted, which have shown novel means of extremophile adaptation in space and chimeric human cells with increased radioresistance. Together, these tools, methods, and data support an ethical and ambitious, 500-year plan of reengineering biology to enable life on other worlds, and also reveal the best candidate planets for life in new solar systems.

https://mitpress.mit.edu/books/next-500-years

### PL3 WHAT'S NEW? HIGHLIGHT SESSION

#### PL3.1 Analysis of RNAseq from 4400 individuals in the 100,000 Genomes Project identifies new potential diagnoses

***Jenny Lord***[1], *Carolina Jaramillo Oquendo*[1], *Niall McGinness*[2], *Alexander Ho*[2], *Terena James*[3], *Mark Ross*[3], *Lily Hoa*[2], *Greg Elgar*[2], *Diana Baralle*[1]

[1]*University of Southampton, Southampton, United Kingdom;* [2]*Genomics England, London, United Kingdom;* [3]*Illumina Cambridge Ltd., Cambridge, United Kingdom*

**Background**: Diagnosis of rare disorders has been revolutionised by whole exome and genome sequencing (WES/WGS), yet despite these advances, around half of sequenced patients do not receive a molecular diagnosis. Interpretation of variants which affect

splicing and gene regulation has lagged behind coding variation, and improvement in this area will boost diagnostic yields. Here we present early analyses of whole blood based transcriptome sequencing data from over 4,400 individuals with various rare disorders recruited to the 100,000 Genomes Project lacking a molecular diagnosis from WGS.

**Methods:** Gene expression outliers were identified by OUTRIDER via DROP and splicing outliers were identified using LeafCutterMD. Candidate outlier events were assessed in combination with WGS data to identify underlying genetic causation, and gene panels were applied to flag candidate diagnostic events. Application of additional tools for identifying splicing outliers (rMATS-turbo and FRASER) is in progress.

**Results:** An average of 5.4 expression and 5.3 splicing outliers were identified per proband genome wide, with 0.2 expression, 0.2 splicing outliers per proband when restricting to relevant gene panels. Filtering and assessment of candidates is ongoing, but early estimates suggests 21% of the cohort have a credible diagnostic candidate for review. Updated analyses will be presented and interesting examples highlighted, including expression outliers tagging structural variants and deep intronic variants causing complex splicing abnormalities.

**Discussion:** Although analyses are ongoing, early work has found new diagnostic candidates in ~20% of probands, which is expected to lead to a significant uplift in diagnostic yield.

**Grant References:** NIHR RP-2016-07-011

**Conflict of Interest:** Jenny Lord: None declared, Carolina Jaramillo Oquendo: None declared, Niall McGinness Genomics England, Alexander Ho Genomics England, Terena James Illumina Cambridge Ltd., Mark Ross Illumina Cambridge Ltd., Lily Hoa

### PL3.2 Rapid, definitive treatment of phenylketonuria in variant-humanized mice with corrective editing

***Dominique Brooks***[1], *Kiran Musunuru*[1], *Xiao Wang*[1]

[1]*Perelman School of Medicine at the University of Pennsylvania, Cardiovascular Institute, Department of Medicine, and Department of Genetics, Philadelphia, United States*

Phenylketonuria (PKU), an autosomal recessive disorder caused by pathogenic variants in the phenylalanine hydroxylase (*PAH*) gene, results in the accumulation of blood phenylalanine (Phe) to neurotoxic levels. Current dietary and medical treatments are chronic and reduce, rather than normalize, blood Phe levels. Among the most frequently occurring *PAH* variants in PKU patients is P281L (c.842C > T). Using a CRISPR prime-edited hepatocyte cell line and a humanized PKU mouse model, we demonstrate efficient in vitro and in vivo correction of the P281L variant with CRISPR adenine base editing. With delivery of ABE8.8 mRNA and either of two guide RNAs in vivo using lipid nanoparticles (LNPs) in humanized PKU mice, we observe complete and long-term durable normalization (>90%) of blood Phe levels within 48 h of treatment, resulting from corrective *PAH* editing in the liver. We have comprehensively assessed off-target editing by ABE8.8 with one of the guide RNAs and have made modifications to the guide RNA to eliminate off-target editing while preserving efficacy. These studies nominate a drug candidate that we are further developing for testing in early-phase clinical trials with PKU patients. We are extending this work to additional humanized mouse models with *PAH* variants that are not amenable to standard base editing, including R408W (c.1222C>T) and c.1066-11G > A, the most frequent variants reported in PKU patients worldwide. With delivery of a prime editor using adeno-associated viral (AAV) vectors, we observe complete and durable normalization of blood Phe levels.

**Conflict of Interest:** Dominique Brooks: None declared, Kiran Musunuru Verve Therapeutics, Variant Bio, Verve Therapeutics, Variant Bio, Xiao Wang: None declared

### PL3.3 SMAD4-associated Myhre-syndrome mutations are under positive selection in the male germline

***Katherine Wood***[1;2;3], *Ronald Spencer Tong*[1;2;3], *Viviana Cordeddu*[4], *Alice Traversa*[5], *Marialetizia Motta*[6], *Viviana Caputo*[5], *Valérie Cormier-Daire*[7], *Andrew Wilkie*[1;2;3], *Stephen Twigg*[1;2;3], *Marco Tartaglia*[6], *Anne Goriely*[1;2;3]

[1]*University of Oxford, MRC-Weatherall Institute of Molecular Medicine, Oxford, United Kingdom;* [2]*University of Oxford, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, Oxford, United Kingdom;* [3]*University of Oxford, NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom;* [4]*Istituto Superiore di Sanità, Department of Oncology and Molecular Medicine, Rome, Italy;* [5]*Sapienza University, Department of Experimental Medicine, Rome, Italy;* [6]*Bambino Gesu` Children´s Hospital, Molecular Genetics and Functional Genomics, Rome, Italy;* [7]*Institut Imagine, Hôpital Necker-Enfants Malades, Service Genetique Clinique, Paris, France*

While it is widely thought that de novo mutations (DNMs) occur randomly, we have previously shown that some DNMs are preferably transmitted because they are positively selected in ageing testes. These "selfish" mutations cause disorders with shared features including: (1) high apparent germline mutation rate, (2) significant increase of the father's age and (3) exclusive paternal origin; pointing to their origin in the ageing male germline. To date, all known selfish genes cluster within the RTK-RAS-MAPK pathway, a critical modulator of testicular homeostasis.

In previous screens for selfish genes, we identified variants in *SMAD4*, a key effector of Activin/BMP/TGFB signalling, associated with Myhre syndrome, suggesting that these DNMs may be clonally expanding in ageing testes.

To assess the selfish nature of Myhre syndrome-causing *SMAD4* mutations, we asked whether they fulfil the 3 criteria defined above. We developed an ultra-sensitive enrichment-based assay to analyse 65 sperm and blood samples from individuals of different ages. We show that the c.1498A>G (p.I500V) mutation was commonly detected in sperm with 75% samples carrying mutation levels $>10^{-6}$; reaching ~1:9650 in the semen of a 60-year old man; and correlated positively with donor age. Using a dual-luciferase reporter assay, we demonstrate that positively-selected variants in the germline exhibit gain-of-function properties. Finally, analysis of 14 informative trios demonstrates that, in all cases, the causative mutation is present on the paternally-derived allele.

Taken together, these data suggest that *SMAD4* is the first gene outside the RTK-RAS-MAPK pathway associated with selfish selection.

**Funding:** Wellcome 219476/Z/19/Z; Italian Health Ministry 2019_5x1000

**Conflict of Interest:** None declared

### PL3.4 Early evolutionary branching across spatial domains predisposes to clonal replacement under chemotherapy in neuroblastoma

*Jenny Karlsson*[1], *Hiroaki Yasui*[1;2], *Adriana Manas Nunez*[3], ***Natalie Andersson***[1], *Karin Hansson*[3], *Kristina Aaltonen*[3], *Caroline Jansson*[1], *Geoffroy Durand*[1], *Minjun Yang*[1], *Subhayan Chattopadhyay*[1], *Kajsa Paulsson*[1], *Diana Spierings*[4], *Floris Foijer*[4], *Anders Valind*[1;5], *Daniel Bexell*[3], *David Gisselsson Nord*[1;6]

[1]*Lund University, Instution of Laboratory Medicine, Department of Clinical Genetics., Lund, Sweden;* [2]*Nagoya University Graduate*

*School of Medicine, Department of Obstetrics and Gynecology, Nagoya, Japan; [3]Lund University, Division of Translational Cancer Research, Department of Laboratory Medicine, Lund, Sweden; [4]University of Groningen, University Medical Center Groningen, European Research Institute for the Biology of Ageing (ERIBA), Groningen, Netherlands; [5]Skåne University Hospital, Department of Pediatrics, Lund, Sweden; [6]Laboratory Medicine Skåne, Department of Pathology, Lund, Sweden*

**Background:** Neuroblastoma (NB) is the most common pediatric solid malignancy outside the central nervous system. Little is known about how NB cells surviving chemotherapy in the primary tumor are related to the lineages dominating prior to treatment.

**Methods:** We performed whole genome genotyping across 89 tumor regions from 12 NBs. Targeted resequencing and single cell whole genome sequencing (scWGS) was performed on a subset of the samples. This was followed by phylogenetic analyses and spatial mapping of subclone geographies before and after chemotherapy to assess the impact of treatment on the clonal landscape.

**Results:** Under effective treatment and tumor shrinkage, densely packed territories of closely related subclones present at diagnosis were replaced by distantly related survivor subclones, a pattern denoted collateral clonal replacement. Tumors that progressed under treatment instead displayed linear evolution where the ancestors of subclones dominating later in disease were present already at diagnosis. These patterns were confirmed both in xenograft models and in cell culture. Phylogenies based on scWGS illustrated that a rich repertoire of subclones emerges early and lays the foundation for clonal replacement under treatment.

**Conclusion:** We reveal two contrasting response scenarios following chemotherapy, depending on whether the tumor initially responds significantly or not. We also show that evolutionary branching early in NB pathogenesis sets the stage for clonal replacement under effective therapy, which has direct implications for how to sample these tumors for genomic profiling.

**Grant references:** Swedish Research Council, Cancer Society, Childhood Cancer Fund, Royal Physiographic Society and LMK Foundation.

**Conflict of Interest:** None declared

### PL3.5 Limb girdle muscular disease caused by HMGCR mutation and statin myopathy treatable with mevalonolactone

***Yuval Yogev***[1], *Zamir Shorer*[2], *Ohad Wormser*[1], *Max Drabkin*[1], *Daniel Halperin*[1], *Arie Koifman*[3], *Geula Davidov*[4;5], *Hila Nudelman*[4;5], *Raz Zarivach*[4;5], *Ilan Shelef*[6], *Ohad Shmuel Birk*[1;3]

*[1]Ben-Gurion University of the Negev, The Morris Kahn Laboratory of Human Genetics at the National Institute of Biotechnology in the Negev, Be'er Sheva, Israel; [2]Soroka University Medical Center, Department of Pediatric Neurology, Be'er Sheva, Israel; [3]Soroka University Medical Center, Genetics Institute, Be'er Sheva, Israel; [4]Ben Gurion University of the Negev, Department of Life Sciences, Be'er Sheva, Israel; [5]Ben Gurion University of the Negev, National Institute for Biotechnology in the Negev and Ilse Katz Institute for Nanoscale Science and Technology, Be'er Sheva, Israel; [6]Soroka University Medical Center, Department of Radiology, Be'er Sheva, Israel*

**Background:** Myopathy is the main adverse effect of the widely prescribed statin drug class. Statins exert their beneficial effect through inhibiting HMG CoA-reductase, the rate-controlling enzyme of the mevalonate pathway. The mechanism of statin myopathy is yet to be resolved, and its treatment is insufficient.

**Methods:** We performed phenotypic delineation and genetic studies of a novel form of human limb girdle muscular dystrophy (LGMD), through homozygosity mapping and whole exome sequencing, followed by functional analysis of the disease-causing variant using confocal microscopy, biochemical and biophysical methods. We then biochemically synthesized and purified mevalonolactone, and tested the safety of its oral administration in mice. Finally, we evaluated the efficacy of oral administration of the synthesized and purified mevalonolactone in human LGMD and in a statin myopathy model in mice

**Results:** We demonstrate using genetic, statistical, and functional methods that a pathogenic homozygous loss-of-function missense mutation in *HMGCR*, encoding HMG CoA-reductase, causes adult-onset severe progressive LGMD. We synthesized and purified mevalonolactone, never administered to humans before, and establish the safety of its oral administration in mice. We then show that its oral administration is effective in treating a human patient with no significant adverse effects. Furthermore, we demonstrate that oral mevalonolactone resolved statin-induced myopathy in mice.

**Conclusions:** *HMGCR* mutation causes a novel late-onset severe progressive muscular dystrophy, which shows similar features to statin-induced myopathy. Our findings indicate that Mevalonolactone is effective both in the treatment of hereditary *HMGCR* myopathy and in a murine model of statin myopathy.

**Conflict of Interest:** Yuval Yogev Folks foundation grant, A patent has been filed for an invention in this study for YY and OSB, Zamir Shorer: None declared, Ohad Wormser: None declared, Max Drabkin: None declared, Daniel Halperin: None declared, Arie Koifman: None declared, Geula Davidov: None declared, Hila Nudelman: None declared, Raz Zarivach: None declared, Ilan Shelef: None declared, Ohad Shmuel Birk The research was funded by the Israel Science Foundation (grant no. 2034/18) awarded to OSB, as well as by the National Knowledge Center for Rare/Orphan Diseases of the Israel Ministry of Science, Technology and Space, Ben-Gurion University of the Negev, Beer-Sheva, Israel, A patent has been filed for an invention in this study for YY and OSB

### PL3.6 Eye2Gene: a novel AI algorithm enables phenotype-driven gene prioritisation directly from retinal scans in inherited retinal diseases

***Nikolas Pontikos***[1;2], *William Woof*[1], *Miriam Bauwens*[3], *Saoud Al-Khuzaei*[4], *Behnam Javanmardi*[5], *Michalis Georgiou*[1;2], *Malena Daich Varela*[1;2], *Thales Antonio Cabral De Guimaraes*[1;2], *Muhammad Moghul*[1;2], *Alice Davidson*[1], *Panos Sergouniotis*[6], *Jamie Ellingford*[6], *Konstantinos Balaskas*[1;2], *Alison J. Hardcastle*[1], *Susan Downes*[4], *Gavin Arno*[1;2], *Peter Krawitz*[5], *Damian Smedley*[7], *Elfride De Baere*[3], *Andrew Webster*[1;2], *Michel Michaelides*[1;2], *Omar Mahroo*[1;2]

*[1]UCL Institute of Ophthalmology, London, United Kingdom; [2]Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom; [3]Ghent University, Ghent, Belgium; [4]Oxford Eye Hospital, John Radcliffe Hospital, Oxford, United Kingdom; [5]University Hospital Bonn, Institute for Genomic Statistics and Bioinformatics, Bonn, Germany; [6]University of Manchester, Division of Evolution, Infection and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester, United Kingdom; [7]Queen Mary University of London, Clinical Pharmacology and Precision Medicine, London, United Kingdom*

**Background/Objectives:** The ACMG PP4 criterion is invoked in the assessment of the pathogenicity of a variant when the phenotype is deemed specific to the gene and is relevant to inherited retinal diseases (IRDs). Approaches such as Exomiser use Human Phenotype Ontology (HPO) terms to prioritise genes based

on gene-phenotype relationships. However, identifying specific HPO terms is often challenging and time-consuming, and requires specialist knowledge. We have developed an AI algorithm, Eye2Gene, capable of automatically identifying likely causative genes directly from retinal scans of patients with IRDs without the need for HPO terms.

**Methods:** We benchmarked Eye2Gene on IRD cases with a known gene diagnosis for which whole exome/genome, retinal scans and detailed HPO descriptions were available. We included 130 IRD cases with a known gene diagnosis, each having at least 3 HPO terms available which were specified by experienced clinicians. HPO gene scores, as provided by Exomiser, were compared to the Eye2Gene gene scores.

**Results:** We found that Eye2Gene provided a rank for the correct gene higher or equal to the HPO-only score in over 75% of the cases, showing that image-based gene predictions can outperform HPO-only predictions for IRDs.

**Conclusion:** We have developed an AI approach, Eye2Gene, able to infer directly from retinal scans the probability of a causative gene. Using Eye2Gene to score genes, it is now possible to incorporate PP4 into variant interpretation in an objective way, without relying on phenotypic labels such as HPO for IRDs. This approach could potentially increase the diagnostic yield in IRDs.

**Conflict of Interest:** Nikolas Pontikos full-time University College London and part-time Moorfields Eye Hospital, Principal investigator on NIHR AI Award (AI_AWARD02488), Phenopolis Ltd unsalaried co-founder and director, William Woof full-time UCL, co-investigator NIHR AI Award (AI_AWARD02488)., Miriam Bauwens: None declared, Saoud Al-Khuzaei: None declared, Behnam Javanmardi full-time Bonn, by IIR-DE-002818 from Shire/Takeda and by the European Reference Network for Rare Malformation Syndromes, Intellectual and Other Neurodevelopmental Disorders (ERN-ITHACA), Michalis Georgiou full-time UCL and Moorfields Eye Hospital, Malena Daich Varela full-time UCL and Moorfields Eye Hospital, Thales Antonio Cabral De Guimaraes full-time UCL and Moorfields Eye Hospital, Muhammad Moghul UCL and Moorfields Eye Hospital, Phenopolis Ltd unsalaried co-founder and director, Alice Davidson full-time UCL, UKRI Future Leader Fellowship, Panos Sergouniotis full-time, Jamie Ellingford full-time, Konstantinos Balaskas full-time Moorfields Eye Hospital, co-investigator NIHR AI Award (AI_AWARD02488), Alison J. Hardcastle full-time UCL, Susan Downes full-time Oxford University, Gavin Arno full-time Moorfields Eye Hospital and UCL, Peter Krawitz full-time Bonn University, GeneTalk GmbH co-founder, FDNA, Damian Smedley full-time QMUL and Genomics England, Elfride De Baere full-time University of Ghent, Andrew Webster full-time UCL and Moorfields Eye Hospital, Michel Michaelides UCL and Moorfields Eye Hospital, NIHR AI Award (AI_AWARD02488), MeiraGTx co-founder, MeiraGTx advisor, Omar Mahroo UCL and Moorfields Eye Hospital, Wellcome Trust (206619/Z/17/Z)

## PL6 ELPAG AWARD LECTURE

### PL6.1 ELPAG Award Lecture

***Tara Clancy***

My presentation will focus on what has made the most difference to and has had the greatest influence on my clinical practice and academic career. I will discuss the importance of developing and sustaining good working relationships with colleagues—including trainees and students - from a range of backgrounds and from other specialties in the UK, the rest of Europe and globally. I will speak about what I have learned from the patients and families I have worked with, and the impact this has had on me. I will also talk about some of the things that I wish I had known more about earlier in my working life.

## CONCURRENT SESSIONS

## C01 RNA AND EPIGENETICS IN INTELLECTUAL DISABILITY

### C01.1 Biallelic variants in INTS11 are associated with a novel complex neurological disorder

***Marcello Niceta***[1], *Tepe Burak*[2], *Erica Macke*[3], *Monika Hubshman*[2], *Oguz Kanca*[2], *Laura Schultz-Rogers*[4], *Yuri Zarate*[5], *Bradley Schaefer*[6], *Jorge Luis Granadillo De Luque*[7], *Daniel Wegner*[8], *Benjamin Cogne*[9], *Brigitte Gilbert-Dussardier*[10], *Xavier Le Guillou*[10], *Eric Wagner*[11], *Lynn Pais*[12], *Jennifer Neil*[12], *Ganeshwaran Mochida*[12], *Chris Walsh*[12], *Nurit Magal*[13], *Valerie Drasinover*[13], *mordechai shohat*[14], *Tanya L. Schwab*[4], *Christopher Schmitz*[4], *Karl J. Clark*[4], *Anthony Fine*[15], *Brendan C. Lanpher*[3], *Ralitza Gavrilova*[3], *Pierre Blanc*[16], *lydie BURGLEN*[16], *Alexandra Afenjar*[16], *Dora Steel*[17], *Manju Kurian*[17], *Prab Prabhaker*[18], *Sophie Gößwein*[19], *Nataliya Di Donato*[19], *Enrico Bertini*[20], *Michael Wangler*[2], *SHINYA YAMAMOTO*[2], *Marco Tartaglia*[1], *Eric W. Klee*[3], *Hugo J Bellen*[2]

[1]*Bambino Gesù Children's Hospital, IRCCS, Molecular Genetics and Functional Genomics Research Unit, Rome, Italy;* [2]*Baylor College of Medicine, Houston, United States;* [3]*Mayo Clinic, Rochester, United States;* [4]*Jan and Dan Duncan Neurological Research Institute, Houston, United States;* [5]*University of Kentucky, Lexington, United States;* [6]*UAMS Medical Center, Little Rock, United States;* [7]*Washington University School of Medicine, St. Louis, United States;* [8]*Washington University in St. Louis, St. Louis, United States;* [9]*Centre hospitalier universitaire de Nantes, Nantes, France;* [10]*Poitiers University Hospital, Poitiers, France;* [11]*University of Rochester School of Medicine and Dentistry, Rochester, United States;* [12]*Harvard Medical School, Boston, United States;* [13]*Raphael Recanati Genetics Institute, Petah Tikva, Israel;* [14]*Sheba Medical Center, Ramat Gan, Israel;* [3]*Mayo Clinic, Rochester, United States;* [16]*Hospital Armand Trousseau Ap-Hp, Paris, France;* [17]*UCL Great Ormond Street Institute of Child Health, London, United Kingdom;* [18]*Great Ormond Street Hospital for Children, London, United Kingdom;* [19]*UniversitätsCentrum für Orthopädie und Unfallchirurgie, Dresden, Germany;* [20]*Bambino Gesù Pediatric Hospital, Roma, Italy*

**Background**: The Integrator complex is a multi-subunit protein complex that regulates the processing of nascent RNAs transcribed by RNA polymerase II, including small nuclear RNAs, enhancer RNAs, telomeric RNAs, viral RNAs, and protein coding mRNAs. Integrator subunit 11 (*INTS11*) is the catalytic subunit that cleaves nascent RNAs. To date, three integrator complex subunits have been associated with human disease (*INTS1*, *INTS8* and *INTS13*). Here, we describe 15 individuals from 10 unrelated families with biallelic variants in *INTS11*, who present with global developmental delay, intellectual disability and progressive pontocerebellar atrophy, and detail functional studies assessing these variants.

**Methods**: We generated null alleles in flies and show that *IntS11* is an essential gene expressed in a subset of larval and adult CNS neurons and glia. Finally, we tested seven of the human *INTS11* variants using orthologous modeling in Drosophila.

**Results**: Loss of *dIntS11* is lethal but can be fully rescued by a wild-type fly cDNA. Two of the changes fail to rescue lethality of null mutants indicating that they are strong loss-of-function variants. Five other substitutions rescue lethality but cause a short lifespan. When these variants are expressed at reduced levels, the

flies display bang sensitivity and impaired locomotor activity indicating that they are partial loss-of-function variants (LoF).

**Conclusions**: These data suggest that individuals with biallelic LoF alleles die at an early age whereas most individuals compound heterozygous for hypomorphic alleles have milder symptoms. Here we provide data on the functional consequence of biallelic *INTS11* variants underlying a novel complex neurological disorder.

**Conflict of Interest:** None declare

### C01.2 Biallelic variants in NSUN6 cause an autosomal recessive neurodevelopmental disorder

***Francesca Mattioli***[1], *Lina Worpenberg*[1], *Cai-Tao Li*[2], *Nazia Ibrahim*[1;3], *Shagufta Naz*[3], *Saima Sharif*[3], *Saghar Ghasemi Firouzabadi*[4], *Shohreh Vosoogh*[4], *Radoslava Saraeva-Lamri*[5], *Laure Raymond*[5], *Carlos Trujillo*[6;7], *Nicolas Guex*[8], *Stylianos Antonarakis*[9], *Muhammad Ansar*[10], *Hossein Darvish*[4], *Ru-Juan Liu*[2], *Jean-Yves Roignant*[1;11], *Alexandre Reymond*[1]

[1]*Center for Integrative Genomics—UNIL, Lausanne, Switzerland;* [2]*Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China;* [3]*Lahore College for Women University, Department of zoology, Lahore, Pakistan;* [4]*Golestan University of Medical Sciences, Gorgan, Iran;* [5]*Eurofins Biomnis, Lyon, France;* [6]*Universidad CES, Departmento de Genetica, Medellin, Colombia;* [7]*Genome Unit, KFMRC, Jeddah, Saudi Arabia;* [8]*Bioinformatics Competence Center-UNIL, Lausanne, Switzerland;* [9]*University of Geneva, Department of Genetic Medicine and Development, Geneva, Switzerland;* [10]*University of Lausanne, Department of Ophthalmology, Lausanne, Switzerland;* [11]*Johannes Gutenberg-University Mainz, Institute of Pharmaceutical and Biomedical Sciences, Mainz, Germany*

**Background/Objectives**:While the leading causes of neurodevelopmental diseases (NDD) in Western countries are autosomal dominant de novo mutations, autosomal recessive (AR) inheritance is common in countries with frequent parental consanguinity. Due to the extreme heterogeneity of NDD about half of the cases remain without diagnosis.

RNA modifications have emerged as an important post-transcriptional regulation mechanism. Among these, the 5-methylcytosine ($m^5C$) modification, driven by NSUN methyltransferases is a widespread RNA modification found in mRNA, rRNA, and tRNA and implicated in RNA stability, processing and translation. *NSUN2* and *NSUN3* were previously associated with Mendelian NDD.

**Methods**: We combined exome sequencing of consanguineous families with functional characterization to identify a new NDD gene.

**Results:** We identified three unrelated consanguineous families with deleterious homozygous variants in *NSUN6*. Two of these variants are truncating. One maps to the first exon and is predicted to lead to the absence of NSUN6 via non-sense mediated decay, while the second one is in the last exon and leads to a protein unable to fold correctly. Likewise, we demonstrated that the missense variant identified in the third family has lost its enzymatic activity and is unable to bind the S-adenosyl-L-methionine methyl donor. The affected individuals present with developmental delay, intellectual disability, motor delay, and behavioral anomalies. We engineered an animal model by homozygously ablating the *Drosophila NSUN6* ortholog. Mutants displayed locomotion defects and learning impairment.

**Conclusion:** Overall, our data provide evidence that biallelic pathogenic variants in *NSUN6* cause one form of AR intellectual disability and establish another link between RNA modification and cognition.

**Conflict of Interest:** Francesca Mattioli: None declared, Lina Worpenberg: None declared, Cai-Tao Li: None declared, Nazia Ibrahim: None declared, Shagufta Naz: None declared, Saima Sharif: None declared, Saghar Ghasemi Firouzabadi: None declared, Shohreh Vosoogh: None declared, Radoslava Saraeva-Lamri Eurofins Biomnis, Laure Raymond Eurofins Biomnis, Carlos Trujillo: None declared, Nicolas Guex: None declared, Stylianos Antonarakis Co-founder and CEO of Medigenome, Swiss Institute of Genomic Medicine, Muhammad Ansar: None declared, Hossein Darvish: None declared, Ru-Juan Liu: None declared, Jean-Yves Roignant: None declared, Alexandre Reymond: None declare

### C01.3 De novo missense variants in phosphatidylinositol kinase PIP5KIγ underlie a novel neurodevelopmental syndrome associated with altered phosphoinositide signaling

***Manuela Morleo***[1;2], *Rossella Venditti*[1;3], *Evangelos Theodorou*[4], *Lauren C. Briere*[4], *Marion Rosello*[5], *Alfonsina Tirozzi*[2;6], *Roberta Tammaro*[1], *Nour Al-Badri*[5], *Frances A. High*[7], *Jiahai Shi*[8], *Viviana Cetrangolo*[1], *Annalaura Torella*[1;2], *Melissa A. Walker*[9], *Romano Tenconi*[10], *Maria Iascone*[11], *Davide Mei*[12], *Renzo Guerrini*[12], *jasper van der Smagt*[13], *Hester Kroes*[13], *Koen L. I. van Gassen*[13], *Muhammad Bilal*[14], *Muhammad Umair*[15], *Veronica Pingault*[16], *Tania Attie-Bitach*[16], *Jeanne Amiel*[16], *Resham Ejaz*[17], *Lance Rodan*[18], *Marcella Zollino*[19], *Pankaj B. Agrawal*[20], *Filippo Del Bene*[5], *David A. Sweetser*[4], *Brunella Franco*[1;21;22], *Vincenzo Nigro*[1;2]

[1]*Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy;* [2]*University of Campania "Luigi Vanvitelli", Department of Precision Medicine, Naples, Italy;* [3]*University of Napoli "Federico II", Department of Molecular Medicine and Medical Biotechnology, Naples, Italy;* [4]*Massachusetts General Hospital, Harvard Medical School, Center of Genomic Medicine, Divisions of Pediatric Hematology/Oncology and Medical Genetics, Department of Pediatrics, Boston, United States;* [5]*Sorbonne Université, INSERM U968, CNRS UMR 7210, Institut de la Vision, Paris, France;* [6]*IRCCS NEUROMED, Department of Epidemiology and Prevention, Pozzilli, Italy;* [7]*Massachusetts General Hospital for Children, Division of Medical Genetics & Metabolism, Boston, United States;* [8]*Yong Loo Lin School of Medicine, National University of Singapore, Department of Biochemistry, Singapore, Singapore;* [9]*Massachusetts General Hospital, Department of Neurology, Division of Neurogenetics, Boston, United States;* [10]*University of Padova, Clinical Genetics Unit, Department of Women and Children's Health, Padova, Italy;* [11]*ASST Papa Giovanni XXIII, Medical Genetics, Bergamo, Italy;* [12]*Meyer Children's Hospital IRCCS, Neuroscience Department, Florence, Italy;* [13]*University Medical Center Utrecht, Department of Genetics, Utrecht, Italy;* [14]*Quaid-i-Azam University, Department of Biochemistry, Faculty of Biological Sciences, Islamabad, Pakistan;* [15]*King Abdullah International Medical Research Center & King Saud Bin Abdulaziz University for Health Sciences, Medical Genomics Research Department, Riyadh, Saudi Arabia;* [16]*Hôpital Necker-Enfants Malades, Service de Médecine Génomique des Maladies Rares, et Institut Imagine, Paris, France;* [17]*McMaster Children's Hospital, Division of Genetics, Department of Pediatrics, Hamilton, Canada;* [18]*Boston Children's Hospital, Department of Neurology & Division of Genetics and Genomics, Department of Pediatrics, Boston, United States;* [19]*A. Gemelli School of Medicine, Catholic University of the Sacred Heart, Institute of Medical Genetics, Rome, Italy;* [20]*Boston Children's Hospital, Harvard Medical School, Divisions of Newborn Medicine & Genetics and Genomics, The Manton Center for Orphan Disease Research, Boston, United States;* [21]*Scuola Superiore Meridionale (SSM, School of Advanced Studies), Genomics and Experimental Medicine program, Naples, Italy;* [22]*University of Naples 'Federico II', Medical Genetics, Department of Translational Medicine, Naples, Italy*

**Background**: Phosphoinositides (PIs) are membrane phospholipids produced through the local activity of PI kinases and

phosphatases that add or remove phosphate groups from the inositol headgroup. PIs play key roles in many cellular processes. Mutations in PI metabolizing enzymes cause a broad spectrum of neurodevelopmental disorders, such as Lowe and Joubert syndrome, both of which are due to mutations in phosphatidylinositol 4,5 bisphosphate [PI(4,5)P2] phosphatases, and thus associated with increased levels of PI(4,5)P2.

**Methods:** Whole exome or genome sequencing was performed. Research and clinical teams were connected with GeneMatcher. Immunofluorescence experiments and quantification were performed on patient's fibroblasts. A zebrafish (*Danio rerio*) model was generated.

**Results:** We identified three recurrent de novo heterozygous missense variants in the *PIP5K1C* gene, which encodes an isoform of the phosphatidylinositol 4-phosphate 5-kinase (PIP5K1γ), in nine unrelated children exhibiting intellectual disability, developmental delay, microcephaly, seizures, visual abnormalities and dysmorphic features. We provide evidence that the *PIP5K1C* variants result in an increase of the endosomal PI(4,5)P2 pool, giving rise to ectopic recruitment of filamentous actin at early endosomes (EEs) that in turn causes dysfunction in EE trafficking. In addition, we generated an in vivo zebrafish model that recapitulates the disorder.

**Conclusions:** Our data provide compelling evidence that these three *PIP5K1C* variants result in a novel neurodevelopmental disorder associated with aberrant PI signaling.

**Grant References**: Telethon Undiagnosed Diseases Program (GSP15001); the NIH (U01HG007690,1UL1TR001102); Programme Investissements d'Avenir IHU FOReSIGHT (ANR-18-IAHU-01); Tuscany Region Call for Health 2018 (DECODE-EE); France Genomic Medicine Plan 2025 (SeqOIA); AIRC (MFAG2020_25174); RD-Connect (FP7-N.305444).

**Conflict of Interest:** None declared

### C01.4 CRISPR-engineered disruption of POGZ in neuronal lines alters synaptic pathways through indirect epigenetic effects

***Mariana Moysés-Oliveira***[1;2;3], *Yating Liu*[1;2], *Serkan Erdin*[1;2], *Dadi Gao*[1;2], *Riya Bhavsar*[1;3], *Kathryn O'Keefe*[1], *Kiana Mohajeri*[1;2;3], *Phillip Boone*[1;2;4], *Gabriela Xavier*[1;2;3], *Calwing Liao*[1;3], *Aiqun Li*[5;6], *Rachita Yadav*[1;2], *Monica Salani*[1], *Benjamin Currall*[1], *Celine De Esch*[1], *Derek Tai*[1;2;3], *James Gusella*[1;7;8], *Douglas Ruderfer*[9;10], *Kristen Brennand*[5;6;11], *Michael Talkowski*[1;2;3]

[1]*Massachusetts General Hospital, Center for Genomic Medicine, Boston, United States;* [2]*Massachusetts General Hospital and Harvard Medical School, Department of Neurology, Boston, United States;* [3]*Broad Institute of MIT and Harvard, Stanley Center for Psychiatric Research, Cambridge, United States;* [4]*Boston Children's Hospital, Division of Genetics and Genomics, Boston, United States;* [5]*Icahn School of Medicine at Mount Sinai, Department of Genetics and Genomic Sciences, New York, United States;* [6]*Icahn School of Medicine at Mount Sinai, Nash Family Department of Neuroscience, New York, United States;* [7]*Blavatnik Institute, Harvard Medical School, Department of Genetics, Boston, United States;* [8]*Harvard Stem Cell Institute, Cambridge, United States;* [9]*Vanderbilt Genetics Institute, Vanderbilt University Medical Center, Division of Genetic Medicine, Department of Medicine, Nashville, United States;* [10]*Vanderbilt University Medical Center, Department of Biomedical Informatics and Department of Psychiatry and Behavioral Sciences, Nashville, United States;* [11]*Yale University New Haven, Department of Psychiatry, New Haven, United States*

*POGZ* (Pogo transposable element-derived protein with ZNF domain) is strongly associated with autism spectrum disorder (ASD) and related neurodevelopmental disorders (NDDs), yet little is known about its direct and indirect regulatory mechanisms. On two independent human induced pluripotent stem cell (hiPSC) backgrounds, we created an extensive allelic series of 36 CRISPR-engineered clones harboring *POGZ* loss of function (LoF) variants and 30 unaltered clones exposed to identical editing conditions. All 66 hiPSC models were differentiated into neural stem cells (NSCs) and *Ngn2*-induced glutamatergic neurons (iNs), followed by RNA-sequencing and ATAC-sequencing. *POGZ* disruption resulted in both reduced and increased expression of its regulatory targets, with the direction of effect dependent on cell-type and predicted POGZ interactors. Differential gene expression analyses indicated that heterozygous and homozygous *POGZ* LoF disturbed genes associated with synaptic function in iNs, and extracellular matrix organization in NSCs and iNs. In heterozygous models, ATAC-seq revealed unchanged chromatin accessibility for POGZ regulatory targets, but footprinting disruption of other transcription factors that occurred more often at promoters of differentially expressed genes associated with synaptic function. Neurite arborization and synaptic activity were increased in iNs with *POGZ* homozygous LoF mutations but unaltered in heterozygous models. In iNs derived from the same hiPSC background, we observed significant convergence on disruption of cytokine-related pathways by *POGZ*, *MEF2C*, and *SCN2A* heterozygous LoF mutants. Overall, the signatures observed in these isogenic allelic series provided insights into cell-type-specific POGZ regulatory mechanisms and shared molecular consequences between different ASD/NDD-associated genes, suggesting key points of convergence on neurodevelopmental pathologies.

**Conflict of Interest:** None declared

### C01.5 De novo variants in KDM2A cause a syndromic neurodevelopmental disorder - virtual

*Konrad Platzer*[1], *Eric Anderson*[2], *Sukhleen Kour*[2], *Deepa Rajan*[2], *Senta Schönnagel*[1], *Laura-Marie Pilgram*[1], *Karen Stals*[3], *Deirdre Donnelly*[4], *Siobhan O'Sullivan*[5], *John Mantovani*[6], *Pia Zacher*[7], *Nicolas Chatron*[8], *Pauline MONIN*[8], *severine drunat*[9], *Julie Cohen*[10], *Kirsty McWalter*[11], *Pedro Sanchez-Lara*[12], *Katheryn Grand*[12], *Hyung-Goo Kim*[13], *Rebecca Belles*[14], *Klaske D. Lichtenbelt*[15], *Anita Rauch*[16], *Ivan Ivanovski*[16], *Isabelle Thiffault*[17], *Frédéric Tran Mau-Them*[18], *Aurore Garde*[19], *Rachel Rabin*[20], *John Pappas*[20], *Maximilian Radtke*[1], *Stephan Drukewitz*[1], *Heather Mefford*[21], ***Julia Hentschel***[1], *Udai Pandey*[2]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany;* [2]*Department of Pediatrics, Childrens Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, United States;* [3]*Exeter Genomics Laboratory, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom;* [4]*Northern Ireland Regional Genetics Centre, Belfast Health and Social Care Trust, Belfast City Hospital, Belfast, United Kingdom;* [5]*Department of Paediatric Metabolic Medicine, Belfast Health and Social Care Trust, Royal Belfast Hospital for Sick Children, Belfast, United Kingdom;* [6]*Division of Child Neurology, Washington University School of Medicine, St. Louis, United States;* [7]*Epilepsy Center Kleinwachau, Radeberg, Germany;* [8]*Department of Medical Genetics, Hospices Civils de Lyon, Lyon, France;* [9]*Département de Génétique, Hôpital Universitaire Robert Debré, Paris, France;* [10]*Department of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, United States;* [11]*GeneDx, Gaithersburg, United States;* [12]*Division of Medical Genetics, Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, United States;* [13]*Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Doha, Qatar;* [14]*Geisinger Health System, Medical Genetics, Danville, United States;* [15]*Department of Genetics, University Medical Center Utrecht, Untrecht, Netherlands;* [16]*Institute of Medical Genetics, University of Zurich, Zurich, Switzerland;* [17]*Genomic Medicine Center, Children's Mercy*

*Hospital, Kansas City, United States; [18]INSERM UMR1231, Equipe Génétique des Anomalies du Développement, Université de Bourgogne-Franche-Comté, Dijon, France; [19]Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, Centre Hospitalier Universitaire Dijon, Dijon, France; [20]Department of Pediatrics, NYU Grossman School of Medicine, New York, United States; [21]Center for Pediatric Neurological Disease Research, St. Jude Children's Research Hospital, Memphis, United States*

**Background:** Lysine-demethylase 2A (*KDM2A*) is responsible for demethylation of lysine residues on histone tails that constitutes a key dynamic chromatin modification impacting gene regulation. Variants in genes of the epigenetic machinery, including multiple KDMs, have previously been described to cause neurodevelopmental disorders. Therefore, *KDM2A* was an appealing candidate gene for further investigation after the initial identification of a de novo missense variant in an individual with a neurodevelopmental disorder.

**Methods:** Through international collaboration and matchmaking, we identified heterozygous de novo variants in *KDM2A* in twelve unrelated individuals. We use cell and fly models as well as genome-wide methylation analysis to establish gene disease causality.

**Results:** Affected individuals showed mild to severe intellectual disability and a spectrum of epilepsy, microcephaly, autism and facial dysmorphism. De novo variants comprise seven missense variants and five truncating variants. We provide several lines of evidence for causation through (1) in silico prediction and structural modelling of variants in a gene constraint for both missense and truncating variants; (2) an aberrant subcellular distribution pattern of mutated KDM2A with patient specific variants in a mammalian cell model; (3) expression of patient specific mutant KDM2A in Drosophila melanogaster caused a more severe external eye degenerative phenotype as compared to WT KDM2A and (4) aberrant genome-wide methylation profiles in blood derived patient DNA. Further testing of developmental abnormalities and motor defects in Drosophila melanogaster are ongoing.

**Conclusion:** Taken together, we firmly establish heterozygous de novo variants in *KDM2A* as a novel cause of a syndromic neurodevelopmental disorder.

**Conflict of Interest:** None declared

### C01.6 Biallelic loss of function variants in WBP4, encoding a spliceosome protein, identified in individuals with syndromic neurodevelopmental delay

*Eden Engal[1], Kaisa Teele Oja[2;3], Ophir Geminder[1], Thuy Linh Le[4], Naama Elefant[5], Naama Zvi[5], Maha Zaki[6], Joseph Gleeson[7;8], Tamar Harel[1;5], Orly Elpeleg[1;5], Kai Muru[2;3], Sander Pajusalu[2;3], Monica H Wojcik[9], Reza Maroofian[10], Chris Gordon[4], Katrin Ounap[2;3], Maayan Salton[5],* ***Hagar Mor-Shaked*** *[1;5]*

*[1]Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel; [2]Tartu University Hospital, Genetics and Personalized Medicine Clinic, Tartu, Estonia; [3]Institute of Clinical Medicine, University of Tartu, Tartu, Estonia; [4]Institute Imagine, Paris, France; [5]Hadassah Medical Organization, Department of Genetics, Jerusalem, Israel; [6]Human Genetics and Genome Research Institute, Department of Clinical Genetics, Cairo, Egypt; [7]University of California San Diego Core Bio, Department of Neurosciences, San Diego, United States; [8]Rady Children's Institute for Genomic Medicine, San Diego, United States; [9]Broad Institute of MIT and Harvard, Cambridge, United States; [10]UCL Queen Square Institute of Neurology, Department of Neuromuscular Disease, London, United Kingdom*

**Background:** The spliceosome is a complex of RNA and proteins responsible for promoting accurate splicing. Over two dozen spliceosome proteins are involved in human diseases, also referred to as spliceosomopathies. WBP4 (WW Domain Binding Protein 4) is part of the early spliceosomal complex, and was not described before in the context of human pathologies.

**Methods:** Following informed consent, individuals underwent exome or genome sequencing. Protein analysis included immunoblotting with anti-WBP4. RNA Sequencing and analysis was performed on primary fibroblasts.

**Results:** Eleven patients, from nine families ascertained through GeneMatcher, were diagnosed with a severe neurodevelopmental syndrome with variable manifestations. Clinical manifestations included hypotonia, global developmental delay, severe intellectual disability, brain abnormalities, musculoskeletal and gastrointestinal abnormalities. Genetic analysis revealed overall four different homozygous loss-of-function variants in WBP4. Immunoblotting on patient fibroblasts from two patients with different genetic variants demonstrated complete loss of protein. RNA sequencing analysis uncovered shared abnormal splicing patterns, including enrichment for abnormalities of the nervous system and musculoskeletal system genes, suggesting that the overlapping differentially spliced genes are related to the common phenotypes of the probands.

**Conclusions:** Biallelic variants in WBP4 cause a spliceosomopathy. Further functional studies are called for better understanding of the mechanism of pathogenicity.

**Funding:** PRG471.

**Conflict of Interest:** None declared

## C02 NEW TREATMENTS AND CLINICAL APPROACHES

### C02.1 Discovery and targeted rescue of functional signatures associated with X-Linked dystonia-parkinsonism in postmortem brains and neuronal models

***Rachita Yadav*** *[1;2;3], Christine Vaine[2;3], Aloysius Domingo[1;2;3], Dadi Gao[1;2;3], Shivangi Shah[2;3], Ellen Penney[2;3], Siddharth Reed[1], Serkan Erdin[1], John Lemanski[1], Riya Bhavsar[1], Kathryn O'Keefe[1], Celine De Esch[1], Moira McMahon[4], Michaela Jackson[4], Margo Courtney[4], Joseph Ochaba[4], Holly Kordasiewicz[4], C. Frank Bennett[4], D. Cristopher Bragg[2;3], Michael Talkowski[1;2;3]*

*[1]Massachusetts General Hospital, Center for Genomic Medicine, Boston, United States; [2]Massachusetts General Hospital, Department of Neurology, Boston, United States; [3]Massachusetts General Hospital, The Collaborative Center for X-Linked Dystonia-Parkinsonism, Boston, United States; [4]Ionis Pharmaceuticals Inc., Carlsbad, United States*

X-linked Dystonia-Parkinsonism (XDP) is an adult-onset neurodegenerative disorder indigenous to the Philippines that exhibits features of both dystonia and parkinsonism. From de novo assembled XDP genome and transcriptome datasets, we discovered aberrant splicing (AS_i32) and intron retention (IR) that was specific to XDP cases and localized proximal to novel SVA insertion within intron 32 of the *TAF1* gene on a founder haplotype, as well as a modest but significant reduction (~18%) of *TAF1* expression. We generated patient-specific induced pluripotent stem cell-derived neuronal models and demonstrated rescue of these disrupted splicing and expression signatures through CRISPR excision of the causal SVA. We have now replicated these XDP-specific hallmarks in 21 postmortem brain tissues across the 15 brain regions from Filipino cases and saw the strongest signatures in brain regions associated with dystonia, such as cerebellum and striatum. Motivated by the convergence of in vitro and post-mortem tissue results, we designed a neural stem-cell based platform for testing molecular therapy for this

disease in partnership with Ionis Therapeutics using 37 antisense oligonucleotides (ASO) that tiled the intron 32 region of *TAF1* proximal to the causal SVA mutation. Overall, targeted and capture RNA sequencing of 1556 samples revealed that ASO therapy could ameliorate the aberrant AS/IR of *TAF1* and 43% of the transcriptome-wide XDP expression signatures, including rescue of established neurodegenerative pathways such as GPCR signaling, cation channel and neuropeptide hormone activity. This study highlights the potential of exploiting simultaneous genomics discovery and precision therapeutic research in Mendelian disorders and rare disease research.

**Conflict of Interest:** Rachita Yadav: None declared, Christine Vaine: None declared, Aloysius Domingo: None declared, Dadi Gao: None declared, Shivangi Shah: None declared, Ellen Penney: None declared, Siddharth Reed: None declared, Serkan Erdin: None declared, John Lemanski: None declared, Riya Bhavsar: None declared, Kathryn O'Keefe: None declared, Celine De Esch: None declared, Moira McMahon Ionis Pharmaceuticals, Carlsbad, Ca, USA, Michaela Jackson Ionis Pharmaceuticals, Carlsbad, Ca, USA, Margo Courtney Ionis Pharmaceuticals, Carlsbad, Ca, USA, Joseph Ochaba Ionis Pharmaceuticals, Carlsbad, Ca, USA, Holly Kordasiewicz Ionis Pharmaceuticals, Carlsbad, Ca, USA, C. Frank Bennett Ionis Pharmaceuticals, Carlsbad, Ca, USA, D. Cristopher Bragg: None declared, Michael Talkowski Levo Therapeutics, Microsoft Inc, and Illumina Inc, Levo Therapeutics, Microsoft Inc, and Illumina Inc

### C02.2 Replantation of teeth in patients with papillon lefevre syndrome using a regenerative approach

*ahmad abd Elazeem*[1], *Mohamed Abdel Kader*[1], *yasmin khalil*[1], ***Nermeen Ahmed***[1]

[1]*Human Genetics and Gnome Research Institute, National Research Centre, oro-dental genetics, Giza, Egypt*

**Background/Objectives:** Papillion LeFevre syndrome (PLS) is an autosomal recessive disorder that is characterized by early-onset severe periodontitis and consequently premature loss of teeth. Early teeth loss is followed by progressive bone resorption, atrophied ridges, and reduced vertical dimension of occlusion hindering the construction of a suitable prosthesis. Bone monocular cells (BMMNCs) have been used for bone regeneration as they contain a fraction of stem cells. Replantation is a technique used to preserve avulsed teeth, it showed success however, usually followed by ankylosis and root resorption. Here, a new technique is introduced in which a modified replantation technique is used in combination with BMMNCs.

**Methods:** This study included 2 patients diagnosed with PLS, their canines were extracted, endodonticaly treated, disinfected using a 980 nm diode laser, and replanted. A combination of; autologous BMNCs, platelet-rich fibrin (PRF), and nano-hydroxyapatite was added to the bony socket before the replantation. Clinical and radiographic assessment was performed at 6 and 12 months postoperatively.

**Results:** All the replanted teeth were successfully fixed with no mobility after 6 months. No signs of ankylosis or root resorption were detected either clinically or radiologically throughout the follow-up period.

**Conclusion:** Introducing this modified replantation technique using stem cells provides hope for patients with PLS to preserve their own teeth and subsequently the alveolar bone expanding their restoration options which will help improve the lifestyle and health of those patients.

**Grant References:** Science and Technology Development Funding Authority (STDF), young Researcher Grant (STDF - YRG–Call 10, Grant number 33438, 2019).

**Conflict of Interest:** ahmad abd Elazeem full, member in a project, Mohamed Abdel Kader Full, Member in a project, yasmin khalil full, member in a project, Nermeen Ahmed full, Principle investigator

### C02.3 Challenges, insights and outcomes of a clinically integrated multi-omic rare disease program, RDNow, for individuals who remain undiagnosed after clinical genomic sequencing

***Tiong Yang Tan***[1;2;3], *Michelle De Silva*[1;2;3], *Katrina Bell*[2;3], *Lyndon Gallacher*[1;2;3], *Rocio Rius*[2;3;4], *Kirsten Allan*[1;2], *Natasha J Brown*[1;2;3], *Natalie Tan*[1;2;3], *Smitha Kumble*[1;2;3], *Russell Gear*[1;2;3], *Cas Simons*[2;3;4], *David Stroud*[1;2;5], *David Thorburn*[2;3], *John Christodoulou*[1;2;3], *Sue White*[1;2;3]

[1]*Victorian Clinical Genetics Services, Parkville, Australia;* [2]*Murdoch Children's Research Institute, Parkville, Australia;* [3]*University of Melbourne, Department of Paediatrics, Parkville, Australia;* [4]*Garvan Institute of Medical Research, Centre for Population Genomics, Darlinghurst, Australia;* [5]*University of Melbourne, Department of Biochemistry & Pharmacology, Parkville, Australia*

**Background:** Genomic technologies are transforming diagnostic efficiency and can guide precision child health by enabling targeted treatments. However, we need to improve diagnostic yield and overcome the barriers of knowledge, systems and lack of natural history data to access clinical trials. The Rare Diseases Now (RDNow) program is a multifaceted, clinically integrated rare disease pathway from diagnosis to clinical trials and therapies.

**Methods:** Individuals who remain undiagnosed after clinical exome/genome sequencing are recruited on referral from genetics or other medical subspecialties. A bespoke testing strategy is designed from a suite of multi-omic technologies and informed consent obtained. Genomic reanalysis may be undertaken prior to additional tests, while family-based genomic sequencing, transcriptomics, proteomics, deep sequencing, long-read sequencing or other functional analyses are deployed depending on clinical phenotype and prior testing.

**Results:** Testing strategies: ES/GS reanalysis, $n = 67$; Singleton ES/GS, $n = 22$; Family-based ES, $n = 149$; Family-based GS+RNA-seq, $n = 96$; Deep sequencing, $n = 9$; Long-read sequencing, $n = 5$; Transcriptomics, $n = 8$; Proteomics $n = 14$. Of the 256 families recruited, sequencing and analysis is complete for 65 families and for 37 of them we have identified the genetic cause for their condition (57% diagnostic yield).

**Conclusion:** We have demonstrated that a personalised testing strategy informed by deep phenotyping increases diagnostic yield for patients with undiagnosed rare monogenic diseases. Scalability remains an ongoing priority to deliver integrated multi-omics for rare disease diagnostics.

**Grant references:** The RDNow program acknowledges financial support from The Royal Children's Hospital Foundation and the Murdoch Children's Research Institute.

**Conflict of Interest:** None declare

### C02.4 Development of a Pharmacogenetic Service for the NHS: The PROGRESS Programme

***John McDermott***[1;2], *Jessica Keen*[1], *Videha Sharma*[3], *Duncan Stoddard*[4], *Rachel James*[1], *Stuart Wright*[5], *Algy Taylor*[1], *Garima Dalal*[5], *Paul Wilson*[6], *Katherine Payne*[5], *Munir Pirmohamed*[7], *William Newman*[1;2]

[1]Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester, United Kingdom; [2]Division of Evolution, Infection and Genomics, School of Biological Sciences, Manchester, United Kingdom; [3]Centre for Health Informatics, Division of Informatics, Imaging and Data Science, Manchester, United Kingdom; [4]DS Analytics and Machine Learning Ltd, Hereford, United Kingdom; [5]Manchester Centre for Health Economics, Division of Population Health, Health Services Research, and Primary Care, Manchester, United Kingdom; [6]Centre for Primary Care and Health Services Research, Division of Population Health, Health Services Research and Primary Care, Manchester, United Kingdom; [7]The University of Liverpool, Department of Pharmacology and Therapeutics, Liverpool, United Kingdom

**Introduction:** The effectiveness and safety of many medicines show considerable inter-personal variation. Strategies are required to reduce this variability. One approach is to leverage genetic data to support medicines optimisation, a concept known as pharmacogenetics. Evidence for pharmacogenetic-guided prescribing exist for many medicines. Despite this, implementation is limited. The PROGRESS programme aims to develop an end-to-end solution for the return of pharmacogenetic data into clinical practice to inform prescribing.

**Methodology:** Pharmacogenetic programmes were assessed via literature review and semi-structured interviews. Genotyping technologies were assessed for analytical performance, turnaround-time, and cost. Bespoke software was developed to handle genotype data and, via dedicated open application programming interfaces, convert this into actionable prescribing guidance. The system's performance was appraised at stakeholder workshops before implementation in primary care settings across the North West of England.

**Results:** Forty pharmacogenetic programmes were characterized, revealing a heterogenous implementation landscape. Several challenges unique to the NHS were identified, including the importance of developing an interoperable system able to function across institutional boundaries. All genotyping platforms had excellent analytical performance, but there was a notable variation in turnaround times and cost. The software solution (Genomic Prescribing Advisory System) can agnostically convert genotype data into meaningful prescribing guidance, acceptable to primary care stakeholders. The system is being deployed into four general practices as part of the PROGRESS trial, scaling to additional recruiting sites across England in 2024.

**Conclusion:** Implemented correctly, pharmacogenetics can potentially better use NHS resources and improve health outcomes. The PROGRESS programme provides an approach to achieve this.

**Conflict of Interest:** None declared

### C02.5 Polygenic risk scores from multi-ancestry GWAS of >450 phenotypes in the Pan-UK Biobank Project offer insights into impacts of genetic architecture, ancestry, and statistical methodology on prediction

***Kristin Tsuo***[1;2;3], *Ying Wang*[1;2], *Konrad Karczewski*[1;2], *Rahul Gupta*[1;2;3], *Masahiro Kanai*[1;2], *Nikolas Baya*[1;2], *Raymond Walters*[1;2], *Patrick Turley*[1;2], *Shawneequa Callier*[4], *Duncan Palmer*[1;2], *Jacqueline Goldstein*[1;2], *Gopal Sarma*[1;2], *Matthew Solomonson*[1;2], *Nathan Cheng*[1;2], *Wenhan Lu*[1;2], *Sam Bryant*[1;2], *Claire Churchhouse*[1;2], *Caroline Cusick*[1;2], *Daniel King*[1;2], *Timothy Poterba*[1;2], *John Compitello*[1;2], *Wei Zhou*[1;2], *Cotton Seed*[1;2], *Mark Daly*[1;2;5], *Hilary Finucane*[1;2], *Benjamin Neale*[1;2], *Elizabeth Atkinson*[1;2], *Alicia Martin*[1;2]

[1]Broad Institute, Cambridge, United States; [2]Massachusetts General Hospital, Boston, United States; [3]Harvard Medical School, Boston, United States; [4]George Washington University, Washington, D.C., United States; [5]Institute for Molecular Medicine Finland, Helsinki, Finland

Most polygenic risk scores (PRS) developed using large-scale biobanks, like UK Biobank (UKB), are trained in primarily individuals of European (EUR) ancestry and consequently show decreased predictive power in other ancestries. To facilitate more diverse genetic studies, the Pan-UKB project conducted thousands of genome-wide association studies (GWAS) across 441,331 individuals from 6 genetic ancestry groups. With this resource of multi-ancestry GWAS, we performed a comprehensive and systematic evaluation of PRS across these ancestry groups in UKB. We first conducted extensive heritability-based QC to prioritize 452 phenotypes, spanning biomarker, categorical, continuous, electronic health record, and prescription categories, and estimated their polygenicity using SBayesS. To elucidate governing principles for PRS accuracy as a function of multiple ancestries, genetic architectures, and PRS methods, we applied heuristic and Bayesian methods to construct PRS and evaluated accuracies in holdout ancestries not included in the discovery GWAS. Comparing PRS constructed from heuristic methods, we identified a few phenotypes, mostly of lower polygenicity, for which multi-ancestry GWAS improved accuracy over EUR-only analyses, including LDL (1.4-fold increase in the African ancestry group) and pulse wave reflection index (2-fold in East Asians). We found that Bayesian methods outperformed heuristic ones to substantially varying degrees across target ancestries. For some traits, like vitamin D, heuristic methods using EUR-only and multi-ancestry GWAS performed better than Bayesian methods by almost 2-fold in some non-EUR ancestries. Altogether, our study provides extensive accuracy benchmarks and guidelines for PRS for hundreds of phenotypes in UKB to advance the development of more accurate and generalizable PRS.

**Conflict of Interest:** Kristin Tsuo: None declared, Ying Wang: None declared, Konrad Karczewski Vor Biopharma, Rahul Gupta: None declared, Masahiro Kanai: None declared, Nikolas Baya: None declared, Raymond Walters: None declared, Patrick Turley: None declared, Shawneequa Callier: None declared, Duncan Palmer: None declared, Jacqueline Goldstein: None declared, Gopal Sarma: None declared, Matthew Solomonson: None declared, Nathan Cheng: None declared, Wenhan Lu: None declared, Sam Bryant: None declared, Claire Churchhouse: None declared, Caroline Cusick: None declared, Daniel King: None declared, Timothy Poterba: None declared, John Compitello: None declared, Wei Zhou: None declared, Cotton Seed: None declared, Mark Daly Maze Therapeutics, Hilary Finucane: None declared, Benjamin Neale Deep Genomics, Camp4 Therapeutics, Takeda Pharmaceutical, Biogen, Elizabeth Atkinson: None declared, Alicia Martin: None declare

### C02.6 Early prevention for 9 common diseases via combined genomic and metabolomic prediction

***Jeffrey Barrett***[1], *Heli Julkunen*[1], *Sini Kerminen*[1], *Sara Lundgren*[1], *Kirsten Schut*[1], *Peter Wurtz*[1]

[1]Nightingale Health, Helsinki, Finland

**Background/Objectives:** Early identification of individuals at high risk of chronic disease is essential to personalised disease prevention. Cost effective interventions can be aimed at the right people before they are sick. There has been a lot of recent attention to using polygenic scores for this purpose but less attention on combining genetics with other easily measured biomarkers.

**Methods:** We derive and validate prediction models for nine leading causes of disability-adjusted life years using both genomic and metabolic biomarker data (measured by NMR spectroscopy on blood samples) on 275,000 individuals from the UK Biobank. We replicate our findings in independent cohorts with equivalent data.

**Results:** For each of nine diseases with the highest disability adjusted life years lost in the WHO European region we can identify individuals with substantially elevated risk. For example, the individuals in the highest risk 10% of individuals have a 10-fold risk of liver cirrhosis, 8-fold risk of diabetes, 4-fold risk of COPD and 1.8-fold risk of depression. The metabolomic and genetic data complement each other, and except colon cancer, metabolomics provides more information than genetics, even 8–10 years after the blood sample is taken, demonstrating that multi-omic prediction is valuable even for events far in the future. The same approach can also be used to find clinically useful sub-groups, such as recently diagnosed diabetics at elevated risk of subsequent kidney disease.

**Conclusion:** Population-level risk screening across multiple diseases is effective with affordable technologies, and is substantially boosted by combining genetic and metabolomic data.

**Grant References:** NA

**Conflict of Interest:** Jeffrey Barrett Nightingale Health, Nightingale Health, Heli Julkunen Nightingale Health, Nightingale Health, Sini Kerminen Nightingale Health, Nightingale Health, Sara Lundgren Nightingale Health, Nightingale Health, Kirsten Schut Nightingale Health, Nightingale Health, Peter Wurtz Nightingale Health, Nightingale Health

## C03 MULTIPLE CONGENITAL ANOMALIES

### C03.1 The recurrent p.Ser470Phe gain-of-function variant in DCAF15 leads to Cornelia de Lange syndrome by targeting SMC3 for aberrant ubiquitination

***Sahar Da'as***[1;2], *Frédéric Ebstein*[3;4], *Constance Wells*[5], *Aymeric MASSON*[6], *Julien Paccaud*[6], *Waseem Hassan*[1], *Sixto Garcia-Minaur*[3], *Emi Rikeros Orozco*[7], *Elena Mansilla*[7], *Mamen Sanchez-Gomez*[7], *Miranda Splitt*[8], *Ruth Richardson*[8], *Florent Fuchs*[9;10;11], *Yannis Duffourd*[6], *Raissa Relator*[12;13], *Elke Krüger*[3], *Christophe Philippe*[6;14], *Laurence Faivre*[6;15], *Christel Thauvin-Robinet*[6;14;16], *Bekim Sadikovic*[12;13], *María Palomares-Bralo*[7;17], *Khalid Fakhro*[1;2;18], *Antonio Vitobello*[6;14]

[1]*Sidra Medicine, Human Genetics Department, Doha, Qatar;* [2]*Hamad Bin Khalifa University, College of Health and Life Sciences, Doha, Qatar;* [3]*University Medicine Greifswald, Institute of Medical Biochemistry and Molecular Biology, Greifswald, Germany;* [4]*Nantes Université, Institut du Thorax, Nantes, France;* [5]*CHU Montpellier, Univ Montpellier, Department of Medical Genetics, Rare diseases and Personalized medicine, Montpellier, France;* [6]*Université de Bourgogne Franche-Comté, INSERM UMR1231 GAD, Dijon, France;* [7]*Hospital Universitario La Paz, Institute of Medical and Molecular Genetics (INGEMM), Madrid, Spain;* [8]*Newcastle Upon Tyne Hospitals NHS Trust, Northern Genetics Service, Newcastle, United Kingdom;* [9]*University Hospital Center, Department of Obstetrics and Gynecology, Montpellier, France;* [10]*Reproduction and child development, Inserm, CESP Center for research in Epidemiology and Population Health, Villejuif, France;* [11]*Univ Montpellier, INSERM, (CHU Montpellier), Desbrest Institute of Epidemiology and Public Health (IDESP), Montpellier, France;* [12]*Western University, Department of Pathology and Laboratory Medicine, London, Canada;* [13]*London Health Sciences Centre, Verspeeten Clinical Genome Centre, London, Canada;* [14]*FHU-TRANSLAD, CHU Dijon Bourgogne, Unité Fonctionnelle Innovation en Diagnostic Génomique des Maladies Rares, Dijon, France;* [15]*FHU-TRANSLAD, CHU Dijon Bourgogne, Centre de Référence Maladies Rares "Anomalies du Développement et Syndromes Malformatifs", Centre de Génétique, Dijon, France;* [16]*FHU-TRANSLAD, CHU Dijon Bourgogne, Centre de référence maladies rares « déficiences intellectuelles de causes rares », Centre de Génétique, Dijon, France;* [17]*ITHACA-European Reference Network, ITHACA-European Reference Network, Madrid, Spain;* [18]*Weill Cornell Medical College, Doha, Qatar*

Cornelia de Lange syndrome (CdLS) is a clinically and genetically heterogeneous developmental disorder with multiple defects. Through whole exome sequencing, we identified three unrelated individuals with the same de novo rare variant in *DCAF15* (NM_138353.3:c.1409C>T; p.Ser470Phe). Clinical features included severe developmental delay, microcephaly, congenital heart defect, and craniofacial features consistent with CdLS. In one case, termination of pregnancy was performed following a diagnosis of fetal malformation syndrome. DCAF15 (DDB1 and CUL4 Associated Factor 15) is the substrate-recognition component of the DCX complex, a cullin-4-RING E3 ubiquitin-protein ligase that mediates ubiquitination of target proteins, including proteins of the cohesion family. The p.Ser470Phe variant is located within a highly conserved protein fold that is predicted to recognize these target proteins. Protein analysis in primary cells from two affected individuals demonstrated DCAF15/Cullin-4 (E3 Ubiquitin Ligase) influenced ubiquitination and accumulation of SMC3, suggesting a gain-of-function mechanism and thus, providing a functional link with CdL syndrome. EpiSign analysis demonstrated DNA methylation signature consistent with CdLS. Functional validation in the zebrafish model showed accumulated smc3 and phenotypically mimicked the patients' presentations of stunted growth, altered head width, visual and auditory impairments, and structural heart defects. As well, the crispants displayed neurological presentations including reduced cerebellar neurons and shorter spinal motor neuron axons. Remarkably, induslam treatment rescued the upregulated SMC3 in both the cellular and zebrafish models. In summary, these findings broaden the CdLS genetic heterogeneity, describe a novel *DCAF15* variant, and provide insights into the disease mechanism that can serve as a potential treatment.

**Conflict of Interest:** Sahar Da'as These results reported here were generated using funding received from the Solve-RD project within the European Rare Disease Models & Mechanisms Network (RDMM-Europe). The Solve-RD project (https://solve-rd.eu/) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 779257., Frédéric Ebstein: None declared, Constance Wells: None declared, Aymeric MASSON: None declared, Julien Paccaud: None declared, Waseem Hassan: None declared, Sixto Garcia-Minaur: None declared, Emi Rikeros Orozco: None declared, Elena Mansilla: None declared, Mamen Sanchez-Gomez: None declared, Miranda Splitt: None declared, Ruth Richardson: None declared, Florent Fuchs: None declared, Yannis Duffourd: None declared, Raissa Relator: None declared, Elke Krüger: None declared, Christophe Philippe: None declared, Laurence Faivre: None declared, Christel Thauvin-Robinet: None declared, Bekim Sadikovic: None declared, María Palomares-Bralo: None declared, Khalid Fakhro: None declared, Antonio Vitobello These results reported here were generated using funding received from the Solve-RD project within the European Rare Disease Models & Mechanisms Network (RDMM-Europe). The Solve-RD project (https://solve-rd.eu/) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 779257.

### C03.2 A novel gene for autosomal dominant primary lymphoedema (PL)

***Ege Sackey***[1], *Daniela Pirri*[2], *Kazim Ogmen*[1], *Silvia Martin Almedina*[1], *Ruby Moy*[1], *Sara Dobbins*[1], *Dionysios Grigoriadis*[1], *John Dean*[3], *Claire Turner*[4], *Sian Ellard*[4], *Frances Elmslie*[5], *Vaughan Keeley*[6], *Sahar Mansour*[5], *Kristiana Gordon*[5], *Graeme Birdsey*[2], *Pia Østergaard*[1]

[1]*St. George's University of London, Molecular and Clinical Sciences, London, United Kingdom;* [2]*Imperial College London, National Heart and Lung Institute, London, United Kingdom;* [3]*Aberdeen Royal Infirmary, Medical Genetics, Aberdeen, United Kingdom;* [4]*Royal Devon & Exeter Hospital, Peninsula Clinical Genetics Service, Exeter, United Kingdom;* [5]*St George's Healthcare NHS Trust, South West Thames Regional Genetics Service, London, United Kingdom;* [6]*University Hospital of Derby and Burton NHS Foundation Trust, Derby, United Kingdom*

Primary lymphoedema (PL) is caused by abnormal development of lymphatic vessels or failure of lymphatic function due to genetic abnormality. To date, the genetic causes of only 30% of PL cases are known. Through whole genome analysis of PL cases included in the 100,000 Genomes Project, six proband with novel variants in the *ERG* gene were identified.

Verification and co-segregation analysis of the four frameshift and two nonsynonymous variants were completed by Sanger sequencing. Plasmids containing wildtype-ERG or mutant-ERG were overexpressed in human dermal lymphatic endothelial cells (HDLECs), and qRT-PCR, western blotting and immunofluorescence carried out. Wildtype-ERG was correctly localised in the nucleus with endogenous ERG. However, overexpression of mutant-ERG resulted in a diffuse distribution in the cytosol of HDLECs.

ERG is a transcription factor, and its role is well known in the blood vascular endothelium as preserving adhering cell junctions, angiogenesis and blood vessel stability. However, the role of ERG in the lymphatic system is not clearly established. Our studies showed that ERG expression colocalise with PROX1, a LEC marker, in vivo by immunostaining of ear skin from mice.

In conclusion, this study identifies novel heterozygous pathogenic variants in *ERG* causing dominantly inherited primary lymphoedema. There is no treatment for PL and patients need lifelong management. Understanding the underlying causes plays a huge role in the accurate diagnosis and the potential for the development of future therapies.

This work was funded by the Swiss Federal National Fund for Scientific Research (CRSII5_177191/1), the Medical Research Council (MR/P011543/1) and BHF (RG/17/7/33217).

**Conflict of Interest:** None declared

### C03.3 Large-scale data-driven analysis to understand the contribution of rare variants to congenital heart disease

***Enrique Audain Martinez***[1;2], *Anna Wilsdon*[3], *Gregor Dombrowsky*[1;2], *Alejandro Sifrim*[4], *Jeroen Breckpot*[4], *Yasset Perez-Riverol*[5], *Allan Daly*[6], *Pavlos Antoniou*[6], *Philipp Hofmann*[1], *Anne-Karin Kahlert*[1], *Ulrike Bauer*[7], *Thomas Pickardt*[7], *Sabine Klaassen*[8;9], *Felix Berger*[9], *Ingo Dähnert*[10], *Sven Dittrich*[11], *Brigitte Stiller*[12], *Hashim Abdul-Khaliq*[13], *Frances Bu'Lock*[14], *Anselm Uebing*[1], *Hans-Heiner Kramer*[1], *Vivek Iyer*[6], *Lars Allan Larsen*[15], *J David Brook*[3], *Matthew Hurles*[6], *Marc-Phillip Hitz*[1;2]

[1]*University Hospital of Schleswig-Holstein, Department of Congenital Heart Disease and Pediatric Cardiology, Kiel, Germany;* [2]*Klinikum Oldenburg, Institute for Medical Genetics, Oldenburg, Germany;* [3]*University of Nottingham, School of Life Sciences, Nottingham, United Kingdom;* [4]*University of Leuven, Department of Human Genetics, Leuven, Belgium;* [5]*European Bioinformatics Institute (EBI), Cambridge, United Kingdom;* [6]*Wellcome Sanger Institute, Cambridge, United Kingdom;* [7]*Competence Network for Congenital Heart Defects, Berlin, Germany;* [8]*Charité - Universitätsmedizin Berlin and Max Delbrück Center, Experimental and Clinical Research Center (ECRC), Berlin, Germany;* [9]*Deutsches Herzzentrum der Charité, Department of Congenital Heart Disease-Pediatric Cardiology, Berlin, Germany;* [10]*University of Leipzig, Department of Pediatric Cardiology and Congenital Heart Disease, Heart Center, Leipzig, Germany;* [11]*University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Pediatric Cardiology, Erlangen, Germany;* [12]*University Heart Center Freiburg—Bad Krozingen, Department of Congenital Heart Disease and Pediatric Cardiology, Freiburg, Germany;* [13]*Saarland University Hospital, Department of pediatric Cardiology, Homburg, Germany;* [14]*East Midlands Congenital Heart Centre and University of Leicester, Glenfield Hospital, Congenital and Paediatric Cardiology, Glenfield, United Kingdom;* [15]*University of Copenhagen, Department of Cellular and Molecular Medicine, Copenhagen, Denmark*

**Background/Objectives:** Congenital Heart Disease (CHD) is a global health problem, affecting ~1–2% of live births worldwide. Despite recent advances in our understanding of underlying disease aetiology, a significant proportion of CHD cases remain unexplained. Here we have assembled and analyzed a case-control cohort to further investigate the contribution of rare genetic variants to CHD at single gene and digenic level.

**Methods:** We have compiled an exome cohort of 57,628 samples (4747 CHD cases and 52,881 controls). Case and control data was processed and harmonized using the same alignment (BWA), calling (GATK), annotation (VEP) and quality control (Hail) pipelines. A gene-based collapsing approach was used to discover significant genome-wide associations. In addition, the contribution of digenic interactions was evaluated using the RareComb framework. The analysis was complemented with an enrichment analysis on cardiac-specific cell populations.

**Results:** Our analysis revealed 14 genes significantly enriched at *FDR* 5% for rare LOF or constrained missense variants associated with CHD. Furthermore, digenic interactions contributed more to non-syndromic forms of CHD, than syndromic ($P = 6.7 \times 10^{-3}$, proportion *Z*-test). We observed distinct enrichment patterns of different cardiac-specific cell populations for syndromic (e.g., cNCCs) and non-syndromic (e.g., capillary endothelial cells) CHD.

**Conclusions:** In summary, we analysed ~57,000 exomes and complemented this with transcriptomic data at single-cell resolution. The findings have strengthened previously described genes with CHD, identified novel candidate genes, and provide a deeper understanding of the pathophysiological mechanisms underlying CHD at gene and digenic level.

**Grand references:** German Center for Cardiovascular Research (DZHK).

**Conflict of Interest:** Enrique Audain Martinez: None declared, Anna Wilsdon: None declared, Gregor Dombrowsky: None declared, Alejandro Sifrim: None declared, Jeroen Breckpot: None declared, Yasset Perez-Riverol: None declared, Allan Daly: None declared, Pavlos Antoniou: None declared, Philipp Hofmann: None declared, Anne-Karin Kahlert: None declared, Ulrike Bauer: None declared, Thomas Pickardt: None declared, Sabine Klaassen: None declared, Felix Berger: None declared, Ingo Dähnert: None declared, Sven Dittrich: None declared, Brigitte Stiller: None declared, Hashim Abdul-Khaliq: None declared, Frances Bu'Lock: None declared, Anselm Uebing: None declared, Hans-Heiner Kramer: None declared, Vivek Iyer: None declared, Lars Allan Larsen: None declared, J David Brook: None declared, Matthew Hurles M.E.H. is a co-founder of, consultant to and holds shares in Congenica, a genetics diagnostic company., Marc-Phillip Hitz: None declared

### C03.4 Expanding genotype-phenotype associations in biglycan-related Meester-Loeys syndrome

*Josephina (Jeannette) Meester[1], **Anne Hebert**[1], Maaike Bastiaansen[1], Laura Rabaut[1], Jarl Bastianen[1], Nele Boeckx[1], Antoine Benichou[2], Jan D. Blankensteijn[3], Paul Brennan[4], Solène Conrad[5], Stephanie L. Curtis[6], Carolyn L. Dent[7], Himanshu Goel[8], Shuxiang Goh[8], arjan houweling[9], Bertrand Isidor[5], Nicola Jackson[10], Pieter Koopman[11], Anita Korpioja[12;13;14], Liina Kuuluvainen[15], Karen Low[16], Stephen P. Oakley[17;18], Nicole M. Organ[17], Eline Overwater[9;19], Nicole Revencu[20], Minna Kraatari-Tiri[21], Alison Trainer[22], Claire Turner[23], Rebecca Whittington[7], Andreas Zankl[24;25;26], Lut Van Laer[1], Aline Verstraeten[1], Bart Loeys[1;27]*

*[1]Center of Medical Genetics, University of Antwerp and Antwerp University Hospital, Edegem, Belgium; [2]Department of Internal and Vascular Medicine, CHU Nantes, Nantes Université, Nantes, France; [3]Department of Vascular Surgery, Amsterdam University Medical Center, Amsterdam, Netherlands; [4]Northern Genetics Service, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom; [5]Service de Génétique Médicale, CHU Nantes, Nantes, France; [6]Bristol Heart Institute, University Hospitals Bristol & Weston, NHS Foundation Trust, Bristol, United Kingdom; [7]South West Genomic Laboratory Hub, Bristol Genetics Laboratory, Bristol, United Kingdom; [8]Hunter Genetics, Waratah, Australia; [9]Department of Human Genetics, Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Amsterdam, Netherlands; [10]Clinical Genetics Service, University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom; [11]Department of Cardiology, Heart Centre Hasselt, Jessa Hospital, Hasselt, Belgium; [12]Department of Clinical Genetics, Oulu University Hospital, Oulu, Finland; [13]Research Unit of Clinical Medicine, University of Oulu, Oulu, Finland; [14]Medical Research Center Oulu, Oulu, Finland; [15]Department of Medical and Clinical Genetics, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [16]Clinical Genetics Department, University Hospitals Bristol and Weston NHS Foundation Trust St Michael's Hospital, Bristol, United Kingdom; [17]John Hunter Hospital, New Lambton Heights, Australia; [18]College of Health, Medicine and Wellbeing, School of Medicine, University of Newcastle, Newcastle, Australia; [19]Department of Genetics, University Medical Center Groningen, Groningen, Netherlands; [20]Center for Human Genetics, Cliniques universitaires Saint-Luc and Université catholique de Louvain, Brussels, Belgium; [21]Department of Clinical Genetics, Research Unit of Clinical Medicine, Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland; [22]Department of Genomic Medicine, The Royal Melbourne Hospital, Parkville, Australia; [23]Department of Clinical Genetics, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom; [24]The Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia; [25]Department of Clinical Genetics, The Children's Hospital at Westmead, Sydney, Australia; [26]Garvan Institute of Medical Research, Sydney, Australia; [27]Department of Clinical Genetics, Radboud University Medical Center, Nijmegen, Netherlands*

Loss-of-function variants in *BGN*, an X-linked gene coding for biglycan, are associated with Meester-Loeys syndrome (MRLS), a syndromic form of aortic aneurysm/dissection. Since the initial publication of five families in 2017, we identified eleven additional MRLS families. All sixteen probands, except two, are male and had an average age at presentation of 38 years. Thirteen males and one female presented with aortic ($n = 10$) and/or arterial ($n = 6$) aneurysms/dissections, one male (11y) presented with syndromic features without cardiovascular symptoms (yet), and one female proband (42y) was identified as part of comprehensive prenatal testing. An additional 33 *BGN* variant-harbouring family members (M/F:6/27) were identified with cascade screening. Their phenotype ranged from no cardiovascular or connective tissue phenotype to death due to aortic dissection. Identified *BGN* mutations causing a stop codon insertion, frameshift, or splicing defect and partial *BGN* deletions were shown to result in loss-of-function by cDNA and Western Blot analysis of skin fibroblasts of seven probands, or were strongly predicted to lead to loss-of-function based on the nature of the variant. Interestingly, a male proband with a deletion encompassing exon 2–8 of *BGN* presented with a more severe skeletal phenotype. RNA sequencing revealed expressional activation of a downstream ATPase (*ATP2B3*; normally repressed in skin fibroblasts) driven by the remnant *BGN* promotor as a possible explanation. These observations indicate that extensive analysis at RNA, cDNA and protein level is required before concluding on the pathogenicity of *BGN* variants; and distinct mutational mechanisms may underlie the wide phenotypic spectrum of MRLS patients.

**Conflict of Interest:** None declared

### C03.5 Rare heterozygous variants in PTCH1 are associated with bladder exstrophy-epispadias complex

***Glenda Beaman**[1;2], Rebecca Yarwood[1], Filipa Lopes[3], Raimondo Cervellione[4], David Keene[4], Imran Mushraq[5], Paul Romitti[6], Martin Lowe[1], Heiko Reutter[7], Adrian S. Woolf[3;8], William Newman[1;2]*

*[1]University of Manchester, School of Biological Sciences, Manchester, United Kingdom; [2]Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine, Manchester, United Kingdom; [3]University of Manchester, Division of Cell Matrix Biology and Regenerative Medicine, Manchester, United Kingdom; [4]Central Manchester University Hospitals NHS Foundation Trust, Paediatric Urology, Manchester, United Kingdom; [5]Great Ormond Street Hospital for Children NHS Foundation Trust, Department of Paediatric Urology, London, United Kingdom; [6]The University of Iowa, Department of Epidemiology, Iowa, United States; [7]Friedrich-Alexander-University Erlangen-Nürnberg, Division of Neonatology and Pediatric Intensive Care Medicine, Erlangen, Germany; [8]Manchester University NHS Foundation Trust, Royal Manchester Children's Hospital, Manchester, United Kingdom*

Bladder exstrophy-epispadias complex (BEEC) is a clinically and genetically heterogeneous devastating developmental disorder which presents at birth in its classic form with a bladder exposed through the abdominal wall. Most affected children have no family history and it usually occurs as an isolated developmental anomaly. A small number of affected children have duplications at chromosome 22q11.

Through exome and Sanger sequencing, we identified 9 of 277 individuals with BEEC with very rare (MAF $<2 \times 10^{-5}$) heterozygous variants in *PTCH1*. This contrasts with 7 in 1199 healthy controls ($p = 0.0008$, odds ratio of 5.6 (95% Confidence interval 2.1–14.9)). The variants clustered at the 3′ end of the gene and have not been associated with Gorlin syndrome or other phenotypes.

Zebrafish embryo studies of a recurrent de novo BEEC associated variant c.4246T>C (p.Phe1416Leu) in *PTCH1* demonstrated that injection of mutant and wild type human transcripts resulted in a disrupted cloaca, whereas injection of wild type or mutant sequences alone resulted in no phenotype.

We present genetic and preliminary functional evidence that putative dominant negative variants in *PTCH1* result in BEEC in a subset of individuals. We predict that these variants result in disrupted hedgehog signalling, a process integral to urogenital and midline developmental processes.

**Conflict of Interest:** None declared

### C03.6 TUBB4B variants specifically impact ciliary function, causing a ciliopathic spectrum

***Isabelle Perrault***[1], *Méchaussier Sabrina*[1], *Dodd Daniel*[2], *Yeyati Patricia*[3], *McPhie Fraser*[3], *Shoemark Amelia*[4], *Zariwala Maimoona*[5], *Marie Legendre*[6], *Lucas Fares Taie*[1], *Josseline Kaplan*[1], *Descartes Maria*[7], *Jabs Ethylin*[8], *Jean-François Papon*[9], *Laurent Pasquier*[10], *Marlène Murris*[11], *Heymut Omran*[12], *Amjad Horani*[13], *Jean-Michel Rozet*[1], *Pleasantine Mill*[14]

[1]*Imagine, Laboratory of Genetics in ophthalmology, Paris, France;* [2]*University of Edinburgh, The University of Edinburgh MRC Human Genetics Unit, Edinburg, United Kingdom;* [3]*University of Edinburgh, Edinburg, United Kingdom;* [4]*University of Dundee, Royal Brompton Hospital, London, United Kingdom;* [5]*University of North Carolina, North Carolina, United States;* [6]*Sorbonne Université, Hôpital Armand Trousseau, Paris, France;* [7]*University of Alabama at Birmingham, Genetics, Birmingham, United States;* [8]*Icahn School of Medicine at Mount Sinai, New York, United States;* [9]*Bicêtre Hospital, Paris, France;* [10]*CHU Pontchaillou, Medical Genetics Department, Rennes, France;* [11]*Larrey Hospital, Department of Pulmonology, Transplantation, and Cystic Fibrosis Centre, Toulouse, France;* [12]*University Children's Hospital Münster, Department of General Pediatrics, Munster, Germany;* [13]*Washington University School of Medicine, Department of Pediatrics, Saint Louis, United States;* [14]*University of Edinburgh, MRC Human Genetics Unit, Edinburg, United Kingdom*

Cilia are small microtubule-based structures found on the surface of most mammalian cells, which have key sensory and sometimes motile functions. Primary ciliary dyskinesia (PCD) is a type of ciliopathy caused by defects in motile cilia. The genetic basis of PCD is only partially understood. Studying a cohort of 11 human patients with PCD, we find that de novo mutations in *TUBB4B*, a beta tubulin isotype, cause three distinct classes of ciliopathic disease: a sensorineural disease, a PCD or a syndromic PCD. In vivo studies in mice show that *Tubb4b* plays a specific role in cilia, building centrioles and axonemes in multiciliated cells. Examining the effects of specific TUBB4B variants in cells and in mice, we further demonstrate that distinct *TUBB4B* mutations differentially affect microtubule dynamics and cilia formation in a dominant negative manner. Finally, structure-function studies reveal that different TUBB4B mutations disrupt distinct tubulin interfaces. Importantly, these molecular differences correlate with disease features. We show that tubulin heterodimer-impairing TUBB4B variants underlie nonsyndromic PCD, whilst additional renal and sensorineural ciliopathic features in a syndromic PCD subtype arise from microtubule lumenal interface-impaired TUBB4B variants. These findings suggest that specific tubulin isotypes have distinct and non-redundant subcellular functions, and demonstrate that human tubulinopathies can be drivers of ciliopathic syndromes.

**Conflict of Interest:** None declared

## C04 CLINICAL GENETIC COUNSELING

### C04.1 Participant experiences of receiving looked for additional findings in the 100,000 Genomes Project: a mixed methods study

***Melissa Hill***[1;2], *Bethany Stafford-Smith*[1], *Jana Gurasashvili*[1;2], *Morgan Daniel*[1], *Blanche Griffin*[1], *Rashida Baptiste*[1], *Michelle Peter*[1;2], *Dame Lyn Chitty*[1;2]

[1]*Great Ormond Street Hospital NHS Foundation, NHS North Thames Genomic Laboratory Hub, London, United Kingdom;* [2]*UCL Great Ormond Street Institute of Child Health, Genetics and Genomic Medicine, London, United Kingdom*

**Background/Objectives:** Participants in the 100,000 Genomes Project were able to consent to receive looked for additional findings (AFs) relating to genes associated with susceptibility to cancer and heart disease. We aimed to evaluate the experiences of participants who chose to receive AFs to inform clinical practice.

**Methods:** Our mixed-methods study, conducted at 18 sites across England, comprised a cross-sectional survey and in-depth interviews with participants who received a positive AF (PAF) or a no additional findings (NAF) result.

**Results:** To date 131 surveys have been returned and 34 interviews with participants with a PAF and 28 interviews with participants who received an NAF are complete. Survey findings indicate that most participants felt their PAF result was useful and agreed their result would influence their health management in the future (82%). The majority (90%) reported they had shared the result with family members. Interviews highlight that some patients with a PAF were initially anxious about their results and worried about informing their children and wider family, however, with time they could see benefits from receiving the AF result. For interview participants with an NAF result, most were positive about the experience, but we also found many had misinterpreted the result.

**Conclusion:** Analysis is ongoing and the overall results of this evaluation will be important in determining *whether* and *how* additional findings should be offered in the English NHS Genomic Medicine Service and the clinical implementation of genome sequencing more widely.

**Grant reference:** NHS England and the GOSH NIHR Biomedical Research Centre

**Conflict of Interest:** None declared

### C04.2 Exploring uncertainties regarding unsolicited findings in genetic testing

***Vyne van der Schoot***[1], *Eline van der Meer*[2], *Marij Hillen*[3], *Helger Yntema*[4], *Han Brunner*[4], *Anke Oerlemans*[2]

[1]*Erasmus MC, Clinical genetics, Rotterdam, Netherlands;* [2]*Radboud University Medical Center, IQ Healthcare, Radboud Institute for Health Sciences, Nijmegen, Netherlands;* [3]*Academic Medical Center, University of Amsterdam, Department of Medical Psychology, Amsterdam, Netherlands;* [4]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands*

**Background/Objectives:** Non-normative uncertainty (uncertainty about empirical facts) and normative uncertainty (uncertainty about moral values or beliefs) regarding 'unsolicited findings' (UFs) might play an important role in clinical genetics. Identifying normative uncertainty is of special interest since it might guide towards novel directions for counseling practice. This study aims to gain insight into the role of non-normative and normative uncertainty regarding UFs, as expressed by counselees and counselors.

**Methods:** We performed a secondary qualitative analysis of 40 interviews with counselees and counselors who had been confronted with UFs. Following a deductive approach, we used an existing theoretical framework of uncertainty, in which we additionally incorporated normative uncertainty.

**Results**: Major issues of non-normative uncertainty were practical and personal for counselees, whilst counselors' uncertainty pertained mainly to scientific issues. Normative uncertainty was a major theme throughout the interviews. We encountered the moral conflicts of autonomy vs. beneficence and non-maleficence and of autonomy vs. truthfulness.

**Conclusion:** Non-normative uncertainty regarding UFs highlights the need to gain more insight in their penetrance and clinical utility. Importantly, even if uncertainty about empirical facts regarding UFs were to be resolved, the identified moral conflicts should be addressed when counseling UFs. Exploring counselees' non-normative uncertainties and normative conflicts seems a prerequisite to optimize genetic counseling. This study suggests an important role for moral conflicts as a source of feelings of uncertainty in clinical genetics beyond the scope of UFs.

**Conflict of Interest:** None declared

### C04.3 Multidisciplinary healthcare professionals' views on a psychiatric genetic counselling service within the northwest of England

***Hanna Quigley***[1;2], *ramona moldovan*[1;2]

[1]*Manchester university NHS foundation trust, Genomic medicine, Manchester, United Kingdom;* [2]*University of Manchester, Faculty of Biology, Medicine and Health, Manchester, United Kingdom*

**Background:** Psychiatric Genetic Counselling (PGC) is not a service currently offered in England despite psychiatric conditions being very prevalent and highly heritable. Psychiatric conditions are complex conditions, due to a combination of genetic and environmental factors. Despite genetic testing not currently being available to confirm psychiatric diagnoses, genetic counselling has been consistently shown to be valuable for both patients and family members. This study explores healthcare professionals' views on setting up a PGC service within the northwest of England.

**Methods:** Purposive sampling was used to select participants with relevant background and/or experience with patients diagnosed with psychiatric or neuropsychiatric conditions. The 8 participants included a clinical psychologist, two geneticists, and five genetic counsellors. All participants were employed by NHS trusts in the northwest of England. Interviews were transcribed verbatim and analysed using thematic analysis.

**Results:** Data analysis indicated four themes: benefits of PGC; challenges for PGC; the clinical and cultural context of PGC; and opportunities for a PGC service. Thirteen subthemes were identified, and included increased knowledge, therapeutic gains, therapeutic barriers, expectation management, resources, appropriate collaboration, society and mental health, clinician training, structural challenges, clinical models, service formats, scope of practice and research advancements.

**Conclusion:** Participants agreed that PGC could be of great benefit to patients and families. Participants detailed the main opportunities in setting up a PGC service, as well as the main barriers to consider, including cultural context.

**Conflict of Interest:** None declared

### C04.4 The decision-making of at-risk marriages after pre-marital screening for beta-thalassemia and sickle cell disease in alqatif, saudi arabia - virtual

***Sara Alkaff***[1], *mohammed aldarwish*[2], *saud takroni*[3], *Shirin Al Sharfa*[4], *Moeen Al-Sayed*[3]

[1]*King Saud Medical City, Genetic and metabolic, Riyadh, Saudi Arabia;* [2]*Alqatif Central Hospital, Alqatif, Saudi Arabia;* [3]*King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia;* [4]*Alqatif Central Hospital,, Alqatif, Saudi Arabia*

**Background/Objectives:** The Kingdom of Saudi Arabia has the highest prevalence of sickle cell disease and beta-thalassemia major in the Middle East. Because of this, the Saudi government established the Saudi Premarital Screening and Genetic Counseling (PMSGC). In Saudi Arabia there's decreased in the number of at-risk marriages, but in some areas, the rate remains as high as 80%. Here, we aimed to identify the factors that affect couples at risk of proceeding with their marriage knowing the test results.

**Methods:** Descriptive, cross-sectional study Al-Qatif Central Hospital, Eastern Province, Saudi Arabia. A total of 124 Saudi couples underwent a premarital screening from 2004 (when the pre-marital screening became mandatory) to 2019 and were deemed incompatible. Seventy-three completed a self-administered questionnaire in the outpatient paediatric hemoglobinopathies clinic at the Al-Qatif Central Hospital, and 51 couples were telephonically interviewed.

**Results:** Of the 124 at-risk Saudi couples, 113 (91%) received an "incompatible" certificate, and 31 (25%) were aware of their medical status before the screening. Factors influencing at-risk couples to proceed with marriage included a lack of awareness of the severity of the disease (33%), familial commitment or pressure (20%), a pre-existing love story (12%), the wedding had already been arranged and was non-cancellable (7%), religious considerations (7%), and learning of methods of having a healthy child (7%).

**Conclusion:** These findings suggest the need to conduct an effective educational program and optional screening at high schools, educating the community using social media. Qualified genetic counsellors should be assigned to all premarital screening centres.

**Conflict of Interest:** None declared

### C04.5 Preferences of people with inherited genetic conditions and family members in Portugal toward informing at-risk relatives of genetic risk

*Mara Pinto*[1], *João Parente Freixo*[2], *Filipa Júlio*[3], *Tamara Hussong Milagre*[4;5], *Luís Sousa*[6], *Liliana Sousa*[1], ***Álvaro Mendes***[2;7]

[1]*CINTESIS@RISE, Universidade de Aveiro, Department of Education and Psychology, Aveiro, Portugal;* [2]*IBMC – Institute for Molecular and Cell Biology, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, CGPP – Centre for Predictive and Preventive Genetics,, Porto, Portugal;* [3]*Associação Portuguesa dos Doentes de Huntington, Lisboa, Portugal;* [4]*Evita – Associação de Apoio a Portadores de Alterações nos Genes Relacionados com Cancro Hereditário, Lisboa, Portugal;* [5]*ePAG ERN GENTURIS - European Patient Advocacy Group, European Reference Network for all Patients with one of the Rare Genetic Tumour Risk Syndromes, Nijmegen, Netherlands;* [6]*APAHE – Associação Portuguesa de Ataxias Hereditárias, Vila Franca de Xira, Portugal;* [7]*IBMC – Institute for Molecular and Cell Biology, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, UniGENe, Porto, Portugal*

Patients commonly shoulder the dissemination of information on genetic risk among relatives, yet genetic counselling and testing of at-risk relatives is under-utilised. This study aimed at exploring the preferences of persons with inherited genetic conditions and family members about informing relatives of genetic risk.

Seventeen patients' organisations in Portugal advertised online surveys between November 2022 and January 2023. Data were analysed through descriptive and comparative statistics.

After exclusions, 293 participants were considered: mean age 45.5 (range: 18–77, SD 11.54), 74.7% female, 72% with children, 59% with higher education. Participants (45.4% symptomatic, 33.2% family members, 21.4% pre-symptomatic carriers) were mainly from families with Transthyretin amyloidosis (15%) Huntington disease (14%), cystic fibrosis (13%), and hereditary cancer predisposition syndromes (11%). Most participants (39%) thought that all relatives should be informed, 32% believed that

direct relatives should be contacted first and 26% that only direct relatives should be informed. Most participants (45%) also thought that probands should make the first contact and the health professional the following, while 28% thought the proband should choose whether health professionals would be involved in informing relatives. A large majority (84%) believed that health professionals should actively offer support to inform relatives, and that health professionals should inform relatives directly when patients are unable or unwilling to do it (for un/treatable conditions) (83.6%).

Results highlight preferences toward involvement of patients and health professionals in informing relatives, including direct contact of health professionals. This study contributes for discussions on how to appropriately cascade relevant information to at-risk relatives.

**Conflict of Interest:** Mara Pinto University of Aveiro, João Parente Freixo CGPP-IBMC, i3S, Univ. Porto, Filipa Júlio APDH and EHA, Tamara Hussong Milagre Evita, Luís Sousa: None declared, Liliana Sousa University of Aveiro, Álvaro Mendes i3S, Univ. Porto, Research grants from the FCT - Fundação para a Ciência e Tecnologia: CEECIND/02615/2017 and (2022.04025.PTDC.

### C04.6 Hope lost and found, a qualitative exploration of the role of hope in parents offered genome sequencing

*Jana Gurasashvili[1;2], Sergio Silverio[3;4], Melissa Hill[1;2], Michelle Peter[1;2], Bethany Stafford-Smith[1;2], Celine Lewis[1;5]*

*[1]NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom; [2]UCL Great Ormond Street Institute of Child Health, Genetics and Genomic Medicine, London, United Kingdom; [3]King's College London, Department of Women & Children's Health, School of Life Course Sciences, Faculty of Life Sciences & Medicine, London, United Kingdom; [4]University College London, Elizabeth Garrett Anderson Institute for Women's Health, Faculty of Population Health Sciences, School of Life and Medical Sciences, London, United Kingdom; [5]UCL Great Ormond Street Institute of Child Health, Population, Policy and Practice Department, London, United Kingdom*

**Background/Objectives:** Genome sequencing (GS) has the potential to reduce the 'diagnostic odyssey' that parents of children with rare undiagnosed conditions experience. Research has considered the impact of receiving a diagnostic result, but rarely focused on the impact of receiving a no primary finding (NPF) result. This study aimed to investigate the experience of parents of children with rare undiagnosed conditions following an NPF result from GS.

**Methods:** Parents ($n = 9$) whose child had an NPF result were recruited to semi-structured telephone interviews through a patient organisation. Interviews explored motivations for testing and experience of receiving results. They were transcribed verbatim and analysed using Grounded Theory.

**Results:** Analysis suggested a central theory: 'the disequilibrium of hope' reflecting the peaks and troughs parents experience in their GS journey. Two sequential themes were identified: (1) 'Hoping to Solve the Unsolved Puzzle' comprising the subthemes 'Hoping for Diagnosis' and 'Hopeful, yet Fearful'; and (2) 'Hope, Lost and Found' including the subthemes 'Hopes Dashed', 'Isolation Revisited' and 'Hope out of Darkness'. Hope was identified as in important coping mechanism for parents.

**Conclusion:** Our research highlights the dynamic, complex, and important role hope plays in the parent journey. Health professionals must tread a delicate path to both nurture hope to maintain healthy coping and manage expectations. For parents receiving an NPF, ways of promoting hope e.g., discussing healthcare options, practical support, and psychological wellbeing, is valuable. Further research should consider how to support parents after an NPF result.

**Grant Reference:** Celine Lewis: NIHR Advanced Fellowship Grant (NIHR300099)

**Conflict of Interest:** Jana Gurasashvili Full time, Sergio Silverio Full time, Melissa Hill Full time, Michelle Peter Full time, Bethany Stafford-Smith Full time, Celine Lewis Full time

## C05 INTERNAL MEDICINE GENETICS

### C05.1 DAAM2 mutations cause androgen insensitivity syndrome by interfering with transcriptional droplet formation at androgen receptors

*Julian Knerr[1], Ralf Werner[2;3], Carsten Schwan[1], Hong Wang[4], Peter Gebhardt[4], Helga Grötsch[2;5], Almuth Caliebe[6], Malte Spielmann[6], Paul-Martin Holterhus[7], Robert Grosse[1;4], **Nadine Hornig**[6]*

*[1]Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Freiburg, Germany; [2]Department of Pediatrics and Adolescent Medicine, University of Lübeck, Lübeck, Germany; [3]Institute of Molecular Medicine, University of Lübeck, Lübeck, Germany; [4]Centre for Integrative Biological Signalling Studies, University of Freiburg, Freiburg, Germany; [5]Veraxa Biotech GmbH, Heidelberg, Germany; [6]Institute of Human Genetics, Universitätsklinikum Schleswig-Holstein, Kiel, Germany; [7]Department of Paediatrics, University Hospital of Schleswig-Holstein, Kiel, Germany*

**Background/Objectives:** Androgen insensitivity syndrome (AIS) is a 46,XY difference of sex development classically caused by mutations in the X-chromosomal androgen receptor (*AR*) gene. Nevertheless, in over 50% of individuals with clinical AIS no *AR* coding gene mutation can be detected. We previously established an assay that measures androgen dependent AR activity in genital skin fibroblasts (GSFs). Using this assay, we identified a group of GSFs with reduced AR function in the absence of an *AR* coding gene mutation (AIS type II). Exome sequencing on patients affected by AIS type II revealed variants in the formin and actin nucleator DAAM2 in two unrelated individuals. We hypothesized that DAAM2 is a co-factor of AR activity.

**Methods:** DAAM2 variant cells lines were analyzed through AR dependent reporter assays, colocalizations between the AR, DAAM2 and actin were visualized by super resolution microscopy of live and fixed cells.

**Results:** DAAM2 is required for AR transcriptional activity in GSF and prostate cancer cells. It localizes to the nucleus and colocalizes with the AR to form actin-dependent transcriptional droplets in response to dihydrotestosterone. DAAM2 polymerizes actin directly at the AR to promote DAAM2-AR droplet fusion in a highly dynamic manner and the droplets associate with active RNA polymerase II.

**Conclusion:** Our data uncover signal-regulated nuclear actin assembly at the AR necessary for transcription. This is also the first AR-cofactor description involved in AIS type II.

**Grant References:** German Research Council to NCH (HO 6028/2-1) and to RG (GR 2111/13-1) and Germany's Excellence Strategy (EXC-2189, project-ID:390939984) to RG.

**Conflict of Interest:** None declared

### C05.2 Influence of primary kidney disease in Chronic Kidney Disease pregnancies: pregnancy outcomes in women with COL4A3-5 related disease (Alport Syndrome)

***Margriet Gosselink***[1;2], *Rozemarijn Snoek*[1;2], *Agne Cerkauskaite-Kerpauskiene*[3], *Sophie Van Bakel*[1;2], *Renee Vollenberg*[1;2], *Henk Groen*[4], *Rimante Cerkauskiene*[3], *Marius Miglinas*[3], *Rossella Attini*[5], *Kálmán Tory*[6], *Kathleen Claes*[7], *Kristel Van Calsteren*[8], *Aude Servais*[9], *Margriet de Jong*[10], *Valentine Gillion*[11], *Liffert Vogt*[12], *Antonio Mastrangelo*[13], *Monica Furlano*[14], *Roser Torra*[14], *Kate Bramham*[15], *Kate Wiles*[15], *Elizabeth Ralston*[15], *Matthew Hall*[16], *Rhea O'Neill*[17], *Michelle Hladunewich*[17], *Titia Lely*[1], *Albertien M. van Eerde*[2]

[1]*University Medical Centre Utrecht, Obstetrics, Utrecht, Netherlands;* [2]*University Medical Centre Utrecht, Clinical Genetics, Utrecht, Netherlands;* [3]*Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania;* [4]*University Medical Centre Groningen, Department of Epidemiology, Groningen, Netherlands;* [5]*University of Turin = Campus Luigi Einaudi, Department of Nephrology, Turin, Italy;* [6]*Semmelweis University, Department of Nephrology, Budapest, Hungary;* [7]*University Hospital Leuven, Department of Nephrology, Leuven, Belgium;* [8]*University Hospital Leuven, Department of Obstetrics, Leuven, Belgium;* [9]*Necker Hospital, Department of Nephrology, Paris, France;* [10]*University Medical Centre Groningen, Department of Internal Medicine, divison of Nephrology, Groningen, Netherlands;* [11]*Cliniques Universitaires Saint-Luc (UC Louvain), Department of Nephrology, Bruxelles, Belgium;* [12]*Amsterdam University Medical Centre, Department of Internal Medicine, division of Nephrology, Amsterdam, Netherlands;* [13]*Fondazione IRCCS ca'Granda Ospedale Maggiore Policlinico Milano, Department of Nephrology, Milan, Italy;* [14]*Fundació Puigvert, IIB-Sant Pau, Medicine Department, Universitat Autonoma de Barcelona, Department of Nephrology, Inherited Kidney Diseases, Barcelona, Spain;* [15]*King's College Hospital, Department of Nephrology, London, United Kingdom;* [16]*Nottingham University Hospitals, Department of Nephrology, Nottingham, United Kingdom;* [17]*Sunnybrook Health Sciences Centre, Department of Nephrology, Toronto, Canada*

**Background:** Individualized pre-pregnancy counseling and antenatal care for women with chronic kidney disease require disease-specific data. This study aimed to investigate pregnancy outcomes and long-term kidney function in women with *COL4A3-5* related disease (Alport Syndrome, AS) in a large multicenter cohort.

**Methods:** The ALPART-network (mAternaL and fetal PregnAncy outcomes of women with AlpoRT syndrome), an international collaboration of 15 centers, retrospectively investigated *COL4A3-5* related disease pregnancies >20 weeks. Outcomes were stratified per inheritance pattern (X-Linked AS (XLAS), Autosomal Dominant AS (ADAS), Autosomal Recessive AS (ARAS)). Influence of pregnancy on estimated glomerular filtration rate (eGFR)-slope was assessed.

**Results:** 192 pregnancies in 116 women were included (XLAS $n = 119$, ADAS $n = 47$, ARAS $n = 14$). Mean eGFR pre-pregnancy was >90ml/min/1.73m$^2$ (114 IQR 31.5). Neonatal outcomes were favorable: 100% live births, median gestational age 39.0 weeks (IQR 2.6), mean birth weight 3135 gram (SD 724). New-onset hypertension occurred during 23% of pregnancies (reference: 'general' CKD-stage 1&2 pregnancies incidence 4–20%), preeclampsia in 19%. Though eGFR declined after pregnancy, mean eGFR remained >90ml/min/1.73m$^2$. Pregnancy did not affect eGFR-slope (pre-pregnancy $\beta = -0.943$, post-pregnancy $\beta = -1.309$, $p = 0.302$). ARAS-pregnancies show less favorable outcomes (early-preterm birth incidence 3/13 (23%)). ARAS is an independent predictor for lower birth weight (β-617, SEM 295, $p = 0.036$), adjusted for pre-pregnancy kidney function, proteinuria and chronic hypertension.

**Conclusion:** This is the largest study to date on *COL4A3-5* related disease pregnancy outcomes. Though overall results are reassuring, inheritance patterns should be considered in counseling. These findings support personalized reproductive care and highlight the importance of investigating kidney disease specific pregnancy outcomes.

**Conflict of Interest:** Margriet Gosselink Fulltime employment UMC Utrecht, Alexion Symposium invited speaker, EUR 500, Rozemarijn Snoek: None declared, Agne Cerkauskaite-Kerpauskiene: None declared, Sophie Van Bakel: None declared, Renee Vollenberg: None declared, Henk Groen: None declared, Rimante Cerkauskiene: None declared, Marius Miglinas: None declared, Rossella Attini: None declared, Kálmán Tory: None declared, Kathleen Claes Astra Zeneca, Astellas, GSK, Fresenius Kabi, Sanofi, Vifor, Kristel Van Calsteren: None declared, Aude Servais: None declared, Margriet de Jong: None declared, Valentine Gillion: None declared, Liffert Vogt: None declared, Antonio Mastrangelo: None declared, Monica Furlano: None declared, Roser Torra Sanofi, Reata, Enyo Pharma, Sanofi, Kate Bramham: None declared, Kate Wiles: None declared, Elizabeth Ralston Kings College London, Matthew Hall Research equipment provided by Sienco., Speaker fees : Astellas, Vifor Pharma, Consultancy fees : Astellas, Vifor Pharma, Rhea O'Neill: None declared, Michelle Hladunewich Yes, Yes

Public Health Agency of Canada – Study of COVID in CKD patients

Calliditas and Ionis – IgA studies

Pfizer – FSGS study

Roche – Preeclampsia Study,

Uptodate Contributor – pregnancy and CKD sections, Medical Lead for Glomerulonephritis – Ontario Renal Network, Titia Lely Parttime 90% UMC Utrecht, PI several grant Dutch Kidney Foundation, Achmea Zilver Kruis, Interreg, (all not related to this project), ZonMW Open, Freyburg (private gift related to project co-PI), Alexion, Albertien M. van Eerde Yes

### C05.3 Re-analysis of whole-exome sequencing data and optical genome mapping in genetically undiagnosed patients with congenital anomalies of the kidney and urinary tract (CAKUT)

***Helge Martens***[1], *Leonie Greipel*[1;2], *Jonathan Lühmann*[1], *Lina Werfel*[1;2], *Anne Christians*[1], *Winfried Hofmann*[1], *Robert Geffers*[3], *Alejandro Hofmann*[4], *Doris Steinemann*[1], *Anna Bjerre*[5], *Dieter Haffner*[2], *Ruthild G. Weber*[1]

[1]*Hannover Medical School, Department of Human Genetics, Hannover, Germany;* [2]*Hannover Medical School, Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover, Germany;* [3]*Helmholtz Centre for Infection Research, Genome Analytics Research Group, Braunschweig, Germany;* [4]*Hannover Medical School, Department of Pediatric Surgery, Hannover, Germany;* [5]*Oslo University Hospital, Division of Paediatric and Adolescent Medicine, Oslo, Norway*

**Background/objectives:** Congenital anomalies of the kidney and urinary tract (CAKUT) are the predominant cause of chronic kidney disease in children. Today, over 60 genes are known to cause CAKUT if mutated. Nevertheless, causative aberrations are commonly identified in only ~16% of patients. To increase the diagnostic yield and identify new CAKUT-associated genes, this study revisited a cohort of 101 CAKUT patients who initially remained genetically undiagnosed after whole-exome sequencing (WES).

**Methods:** Raw WES data from unsolved CAKUT-patients were reanalyzed with our current pipeline prioritizing rare coding variants, near-coding variants (Squirls/SpliceAI), predicting digenic inheritance (ORVAL/DiGePred), and searching for mobile element insertions and mosaicism. To identify or verify structural variants,

high molecular weight DNA was assessed by optical genome mapping (OGM).

**Results:** In eight patients, we identified rare variants in known CAKUT-associated genes undetected by previous WES analysis, e.g. heterozygous variants in *HNF1B*, *SALL1*, *TBX6, ZMYM2*, or biallelic variants in *BBS9* or *GREB1L*. Combinations of variants of uncertain significance (*CRKL_TBC1D1*, *SLC26A4_SLC12A3*) were predicted as digenic disease-causing in two patients. By OGM, copy number aberrations encompassing CAKUT-associated genes were detected in six patients, e.g. chr17q12 loss (*HNF1B*-locus), chr22q11.21 loss (*CRKL*-locus), chr16p11.2 gain (*TBX6*-locus), an intragenic *TBC1D1* insertion, and an unbalanced structural rearrangement. Five new candidate genes were identified. The search for mobile element insertions and mosaicism, and reverse phenotyping efforts are ongoing.

**Conclusion:** New technologies, improvements in bioinformatic tools, updates in databases, literature, and patients' clinical phenotype were most important for increasing the diagnostic yield in CAKUT patients.

**Grants:** Deutsche Forschungsgemeinschaft (MA9606/1-1)

**Conflict of Interest:** None declared

### C05.5 Exome reanalysis in a cohort of patients with inborn errors of immunity

***Emil Vorsteveld***[1;2], *Caspar van der Made*[1;2;3], *Annet Simons*[1;2], *Sanne Smeekens*[1;2], *Mihai Gheorghe Netea*[3], *Alexander Hoischen*[1;2;3]

[1]*Radboudumc, Department of Human Genetics, Nijmegen, Netherlands;* [2]*Academic Alliance for Genetics, Radboud University Medical Center Nijmegen, and MUMC Maastricht, the Netherlands, Nijmegen, Netherlands;* [3]*Radboudumc, Department of Internal Medicine, Nijmegen, Netherlands*

**Background/Objectives:** Inborn errors of immunity (IEI) comprise a group of immune defects that display significant clinical and genetic heterogeneity. Exome sequencing has been fundamental in the understanding of these diseases and has led to a sharp increase in the number of genes associated with these disorders. The diagnostic yield of IEI remains low compared to other rare monogenic disorders. We aimed to improve the diagnostic yield of exome sequencing through a systematic reanalysis.

**Methods:** We have collected exome data of 1316 patients with (suspected) IEI that were sequenced and analysed between 2011 and 2021 at the Radboud University Medical Center. We filtered for rare variants in 484 genes comprising the latest in silico gene panel for IEI.

**Results:** Exome sequencing with standard-of-case analysis provided a diagnostic yield of 10.7% in our patient cohort. Through the systematic reanalysis of the exome data, we identified variants in genes added to the panel after the initial genetic analysis ($n = 15$), and reinterpreted data based on new insight in known IEI genes ($n = 3$) and variants ($n = 11$), and through the addition of CNV analysis ($n = 1$). This yielded new diagnoses in 30 patients (2.3%).

**Conclusion:** We have performed a systematic reanalysis of exome sequencing data from 1316 patients with IEI. We show additional disease causing variants in 2.3% of patients, increasing the diagnostic yield to 13%. There remains continued value in diagnosing individuals with rare (immune) diseases through systematic re-analysis of exome data.

**Grant References:** -

**Conflict of Interest:** None declared

### C05.6 Single cell expression, surface proteins, and T and B cell receptor usage in a multi ancestry systemic lupus erythematosus cohort

***Niek de Klein***[1], *Tarran Rupall*[1], *Bess Chau*[1], *Wanseon Lee*[1], *Haerin Jang*[1], *Simon Eastham*[1], *Norzawani Buang*[2], *Magdalena West*[3], *Emily Holzinger*[4], *Sarah Middleton*[5], *Virginia Savova*[6], *Soren Beinke*[7], *Giorgio Gaglia*[6], *Matthew Pickering*[2], *Marina Botto*[2], *Carla Jones*[1], *James Peters*[2], *Timothy Vyse*[3], *Gosia Trynka*[1], *Emma Davenport*[1]

[1]*Wellcome Sanger Institute, Cambridge, United Kingdom;* [2]*Imperial College London, London, United Kingdom;* [3]*King's College London, London, United Kingdom;* [4]*Bristol Myers Squibb, Cambridge, United States;* [5]*GSK, Collegeville, United States;* [6]*Sanofi, Cambridge, United States;* [7]*GSK, Heidelberg, Germany*

**Background:** Systemic lupus erythematosus (SLE) is an autoimmune disease affecting any organ system in the body. The clinical heterogeneity makes development of new drugs challenging. Additionally, there are stark differences in prevalence depending on ancestry. Here, we create a multi-ancestry study of expression data, surface protein levels, and T and B cell receptor usage to better understand the molecular mechanisms contributing to the pathophysiology of the disease.

**Methods:** 159 SLE patients of European, Afro-Carribean, and South-Asian ancestry have been recruited from two sites in London. For these individuals, we profiled transcription levels, surface proteins, and T and B cell receptor usage using single cell RNA-seq, CITE-seq and VDJ-seq, and they are being genotyped.

**Results:** We have created a dataset of 364,084 cells (average 2289 per individual) with both expression levels and protein surface levels for 130 surface proteins. This dataset can help explain some of the clinical variability of SLE. For example, we observed that for the top 10% of individuals with highest expression for known interferon genes monocyte levels differ from other SLE patients. Additionally, we see that monocyte marker proteins, such as CD14 and CD16, are less abundant on monocytes of these individuals. We will combine genotyping data to identify genetic variants driving heterogeneity in expression and protein levels.

**Conclusion:** We have created a multi-ancestry single cell RNA-seq, CITE-seq, and VDJ-seq dataset to understand clinical heterogeneity in SLE.

**Conflict of Interest:** Niek de Klein Genome Research Limited, Funding: Open Targets, Tarran Rupall Genome Research Limited, Funding: Open Targets, Bess Chau Genome Research Limited, Funding: Open Targets, Wanseon Lee Genome Research Limited, Haerin Jang Genome Research Limited, Simon Eastham: None declared, Norzawani Buang: None declared, Magdalena West: None declared, Emily Holzinger Bristol Myers Squibb (BMS), Sarah Middleton Employed at GSK, Virginia Savova Sanofi, Soren Beinke GSK, Giorgio Gaglia Sanofi, Matthew Pickering: None declared, Marina Botto: None declared, Carla Jones Genome Research Limited, James Peters Imperial College London, Medical Research Foundation grant MRF-057-0003-RG-PETE-C0799, Wellcome Data Sciences, Tools and Technology Discovery Award shortlisting panel.

UK Biobank -omics working group, Timothy Vyse: None declared, Gosia Trynka Genome Research Limited, 220540/Z/20/A, 'Wellcome Sanger Institute Quinquennial Review 2021-2026', In the past 3 years Gosia Received honoraria from Japanese Society of Rheumatology, In the past 3 years Gosia consulted for Relation Therapeutics, is an active member of scientific advisory board for Variant Bio, As a member of Open Targets consortium Gosia is cofounded and actively interacts with: GSK, Sanofi, Pfizer, BMS and Genentech, Emma Davenport Genome Research Limited

## C06 INHERITED METABOLIC DISORDERS

### C06.1 Loss of function variant in SMIM1 is associated with reduced energy expenditure and weight gain

***Mattia Frontini***[1]

[1]*University of Exeter, Department of Clinical and Biomedical Sciences, Exeter, United Kingdom*

Blood group antigens are the archetypal example of human genetic variation. Here, we characterised the functional metabolic consequences in individuals homozygous for a 17bp deletion in *SMIM1* (rs566629828; minor allele frequency 0.0147) and thus lacking the protein defining the Vel blood group. Our analysis, in separate cohorts of *SMIM1*-/- individuals (UK Biobank, NHS Blood and Transplant, Danish Blood Donor Study, Copenhagen Hospital Biobank) and a mouse model, identified an increase in body weight accompanied by a range of metabolic differences, including dyslipidemia, changes in the leptin-adiponectin ratio, increased liver enzymes and lower total thyroid hormone levels. These changes in the metabolic state were at least in part due to a reduction in resting energy expenditure, as assessed during an in-depth clinical assessment of *SMIM1*−/− individuals. Additionally, electronic health records suggest that individuals lacking this 78-amino-acid type II transmembrane protein may be more prone to cerebral bleeds and thrombotic stroke.

**Conflict of Interest:** None declared

### C06.2 Pathological variants in TOP3A cause distinct disorders of mitochondrial and nuclear genome stability

***Robert Taylor***[1], *Direnis Erdinc*[2], *Alejandro Rodriguez-Luis*[1], *Mahmoud Fassad*[1], *Sarah MacKenzie*[3], *Christopher Watson*[4], *Sebastian Valenzuela*[2], *Xie Xie*[2], *Katja Menger*[1], *Kate Sergeant*[5], *Kate Craig*[3], *Sila Hopton*[3], *Gavin Falkous*[3], *Joanna Poulton*[6], *Hector Garcia-Moreno*[7], *Paola Giunti*[7], *Carlos de Moura Aschoff*[8], *Jonas Morales Saute*[8], *Amelia Kirby*[9], *Camilo Toro*[10], *Lynne Wolfe*[10], *Danica Novacic*[10], *Lior Greenbaum*[11], *Aviva Eliyahu*[11], *Ortal Barel Barel*[11], *Yair Anikster*[11], *Robert McFarland*[1], *Grainne Gorman*[1], *Andrew Schaefer*[3], *Claes Gustafsson*[2], *Maria Falkenberg*[2], *Thomas Nicholls*[1]

[1]*Newcastle University, Newcastle upon Tyne, United Kingdom;* [2]*University of Gothenburg, Gothenburg, Sweden;* [3]*Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom;* [4]*North East and Yorkshire Genomic Laboratory Hub, Leeds, United Kingdom;* [5]*Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom;* [6]*University of Oxford, Oxford, United Kingdom;* [7]*UCL Institute of Neurology, London, United Kingdom;* [8]*Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil;* [9]*Wake Forest School of Medicine, Winston-Salem, United States;* [10]*National Human Genome Research Institute, National Institutes of Health, Bethesda, United States;* [11]*Sheba Medical Center, Tel Hashomer, Israel*

**Background**: Topoisomerase 3-alpha (TOP3A) is an enzyme that removes torsional strain and interlinks between DNA molecules. TOP3A localises to the nucleus and mitochondria, with both isoforms playing specialised roles in DNA recombination and the decatenation of replication products, respectively. Pathogenic variants in *TOP3A* have been reported to cause a disorder like Bloom syndrome, which results from segregating pathogenic variants in BLM, a nuclear binding partner of TOP3A. In support of a crucial role for mitochondrially-targeted TOP3A in mitochondrial DNA (mtDNA) replication and separation, we previously characterised a mitochondrial disease (CPEO+) phenotype in a patient with bi-allelic *TOP3A* variants and multiple mtDNA deletions.

**Aims**: To delineate the phenotypic spectrum of pathogenic *TOP3A* variants and characterise the associated molecular defects.

**Methods:** *TOP3A* variants were identified in ten individuals from eight families, including four families identified through targeted re-analysis of Genomics England 100,000 genomes data, all with adult-onset mitochondrial disease. Functional studies used patient-derived material and an in vitro model system.

**Results:** Affected individuals shared a consistent phenotype comprising bilateral ptosis, CPEO, myopathy and axonal sensory motor neuropathy; sensorineural hearing impairment and ataxia were prominent findings. To delineate the biochemical mechanisms underlying the clinical heterogeneity of TOP3A-related pathology, recombinant TOP3A variants with model substrates were used to characterise the molecular defects associated with mutated enzyme.

**Conclusion:** Our data indicate that milder *TOP3A* variants cause adult-onset mitochondrial disease, whereas more severe variants result in a Bloom-like disorder with mitochondrial dysfunction in childhood.

**Grant References:** Wellcome (203105/Z/16/Z and 213464/Z/18/Z).

**Conflict of Interest:** Robert Taylor Full time, Wellcome (203105/Z/16/Z), Direnis Erdinc: None declared, Alejandro Rodriguez-Luis: None declared, Mahmoud Fassad Full time, Sarah MacKenzie Full time, Christopher Watson Full time, Sebastian Valenzuela Full time, Xie Xie Full time, Katja Menger Full time, Kate Sergeant Full time, Kate Craig Full time, Sila Hopton Full time, Gavin Falkous Full time, Joanna Poulton Full time, Hector Garcia-Moreno Full time, Paola Giunti Full time, Carlos de Moura Aschoff Full time, Jonas Morales Saute Full time, Amelia Kirby Full time, Camilo Toro Full time, Lynne Wolfe Full time, Danica Novacic Full time, Lior Greenbaum Full time, Aviva Eliyahu Full time, Ortal Barel Barel Full time, Yair Anikster Full time, Robert McFarland Full time, Grainne Gorman Full time, Andrew Schaefer Full time, Claes Gustafsson Full time, Maria Falkenberg Full time, Thomas Nicholls Full time, Wellcome (213464/Z/18/Z)

### C06.3 Mitochondrial DNA variant detection in over 4000 rare disease families by the systematic analysis of exome, genome, and RNA sequencing data

***Sarah Stenton***[1;2;3], *Kristen M. Laricchia*[1], *Sushma Chaluvadi*[1], *Nicole J. Lake*[4], *Monkol Lek*[4], *Mark Fleming*[5], *Dean Campagna*[5], *Eric Pierce*[6], *Kinga Bujakowska*[6], *Emily Place*[6], *Heidi Rehm*[1;3], *Anne O'Donnell-Luria*[1;2;3]

[1]*Broad Institute of MIT and Harvard, Program in Medical and Population Genetics, Cambridge, United States;* [2]*Boston Children's Hospital, Division of Genetics and Genomics, Boston, United States;* [3]*Massachusetts General Hospital, Center for Genomic Medicine, Boston, United States;* [4]*Yale School of Medicine, Department of Genetics, New Haven, United States;* [5]*Boston Children's Hospital, Department of Pathology, Boston, United States;* [6]*Massachusetts Eye and Ear Infirmary, Ocular Genomics Institute, Boston, United States*

**Background:** Pathogenic variants in the mitochondrial genome (mtDNA) lead to a diverse collection of mitochondrial diseases and may underlie seemingly Mendelian disease given extensive phenotypic overlap.

**Methods:** Using dedicated pipelines addressing technical challenges posed by the mtDNA (circular genome, heteroplasmy, and nuclear misalignment), we called single nucleotide variants, small indels, and single large deletions ≥1% heteroplasmy level (HL) from exome, genome, and RNA-sequencing (7910 samples) from 4057 rare disease families (3399 undiagnosed).

**Results:** Pathogenic variants from MITOMAP and/or ClinVar were identified in 253 samples (87 at ≥10% HL), at a significantly higher rate than observed in the gnomAD reference population ($p < 0.001$). Analysis reidentified six known diagnoses, established three new diagnoses (MT-TA in a metabolic myopathy proband, MT-TL1 in two retinal disease probands), and identified three strong candidates. Next, variants passing allele frequency and in silico pathogenicity prediction filtering based on mtDNA-specifications of the ACMG/AMP guidelines were identified in 2,068 samples (41 at ≥10% HL), of which nine, considered high priority, are being pursued by segregation analyses in families with Leigh syndrome, myopathy, syndromic congenital sideroblastic anemia, and retinitis pigmentosa. Additionally, in one outlier sample, >850 heteroplasmic variants exposed a damaging de novo variant in the nuclear gene *POLG* (DNA polymerase gamma), responsible for mtDNA replication and repair.

**Conclusion:** mtDNA variant calling from genomic data led to a median of one variant per sample for analysis and identified candidate diagnoses in 0.4% (15/3,399) of phenotypically diverse undiagnosed rare disease families at minimal added cost.

**Grant references:** GREGoR consortium.

**Conflict of Interest:** None declared

### C06.4 Biallelic variants in DAP3, encoding the mitochondrial ribosomal protein MRPS29, cause a Perrault syndrome spectrum phenotype

***Thomas Smith***[1;2], *Leigh Demain*[1;2], *Robert Kopajtich*[3;4], *Alessandro Rea*[1;2], *Manuel Schiff*[5;6], *Christian Beetz*[7], *Huw Thomas*[1;2], *Shelagh Joss*[8], *Gerard Conway*[9], *Kyle Thompson*[10], *Monika Olahova*[10], *Ruth Glasgow*[10], *Emma Jenkinson*[1;2], *Amir Jahic*[11], *Inna Belyantseva*[12], *Melanie Barzik*[12], *Jill Urquhart*[1;2], *James O'Sullivan*[1;2], *Wyatt Yue*[13], *Jamie Ellingford*[1;2;14], *Henry Houlden*[15], *Kevin Munro*[16], *Thomas Friedman*[12], *Robert Taylor*[10], *Holger Prokisch*[3;4], *Raymond O'Keefe*[1;2], *William Newman*[1;2]

[1]*University of Manchester, Manchester, United Kingdom;* [2]*Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [3]*Institute Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany;* [4]*Institute of Human Genetics, Technical University of Munich, Munich, Germany;* [5]*Reference Center for Inborn Error of Metabolism, Necker-Enfants Malades Hospital, Paris, France;* [6]*Institut Imagine, Université Paris Cité, Paris, France;* [7]*Centogene GmbH, Rostock, Germany;* [8]*West of Scotland Centre for Genomic Medicine, Glasgow, United Kingdom;* [9]*Institute for Women's Health, UCL, London, United Kingdom;* [10]*Wellcome Centre for Mitochondrial Research, Newcastle, United Kingdom;* [11]*Institute of Laboratory Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany;* [12]*National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, United States;* [13]*Newcastle University Biosciences Institute, Newcastle, United Kingdom;* [14]*Genomics England, London, United Kingdom;* [15]*Department of Molecular Neuroscience, UCL, London, United Kingdom;* [16]*Manchester Centre for Audiology and Deafness (ManCAD), Manchester, United Kingdom*

Perrault syndrome is a genetically heterogeneous, autosomal recessive disorder characterised by sensorineural hearing loss and primary ovarian insufficiency. To date, biallelic variants in nine genes have been associated with Perrault syndrome with neurological phenotypes of leukodystrophy, developmental delay, and seizures present in some affected individuals.

Exome and genome sequencing were performed on affected individuals and unaffected family members. Respiratory chain complex and proteomic analyses were undertaken on fibroblasts from affected individuals. In vitro GTPase assays were conducted to determine the effect of the disease variants.

Biallelic variants in *DAP3* were identified in four unrelated females. In two females with presentations of Perrault syndrome, deletions of 135kb were identified in trans to missense variants p.Cys395Tyr and p.Thr132Ile, respectively. In the other two affected women, homozygosity for p.Glu392Lys was present in a female with classical features of Perrault syndrome and in a female with a presentation of seizures, hearing loss, and raised serum lactate. Proteomic analysis revealed reduced levels of DAP3 (also known as MRPS29) and other members of the small mitochondrial ribosomal subunit, alongside various proteins comprising the respiratory chain complexes COXI and COXIV. Protein modelling suggested that the disease associated missense variants would impact upon GTP binding. An in vitro assay demonstrated that the variants were associated with reduced GTPase activity.

We present the first genetic and functional evidence that biallelic variants in *DAP3*, encoding the small mitochondrial ribosomal subunit, MRPS29, result in a multisystem disorder of combined oxidative phosphorylation deficiency with features overlapping Perrault syndrome.

**Conflict of Interest:** None declared

### C06.5 Non-coding variants in HK1 and hyperinsulinism: genotype-phenotype associations

***Jasmin Hopkins***[1], *Nick Owens*[1], *Bianca Neumann*[2], *Jean-baptiste Arnoux*[3], *Susann Empting*[2], *Jonathan Locke*[1], *Thomas Laver*[1], *Matthew Johnson*[1], *Christine Bellanne-Chantelot*[4], *Klaus Mohnike*[2], *Martin Zenker*[2], *CECILE SAINT MARTIN*[4], *Sarah Flanagan*[1]

[1]*University of Exeter, Department of Clinical and Biomedical Sciences, Exeter, United Kingdom;* [2]*Otto-von-Guericke University Magdeburg, Institute of Human Genetics, Magdeburg, Germany;* [3]*Necker-Enfants-Malades University Hospital, Reference Center for Inherited Metabolic Diseases, Paris, France;* [4]*Pitié-Salpêtrière Hospital, Department of Medical Genetics, Paris, France*

**Background/objectives:** Congenital hyperinsulinism (HI) is characterised by inappropriate insulin secretion by pancreatic beta-cells causing hypoglycaemia. We recently showed that variants within a regulatory element of *HK1* cause severe, persistent HI. These non-coding variants cause *HK1* to be aberrantly expressed in beta-cells, where it is normally silenced. These variants are predicted to disrupt binding sites for three transcription factor families (NFAT, NKX2 and FOX). We investigated the phenotypic variability within *HK1*-HI and assessed if any observed differences could be explained by variant position.

**Methods:** We screened the *HK1* regulatory region in 1658 probands with HI referred to three European Genomics Laboratories. Clinical features were analysed when a variant was identified.

**Results:** We identified disease-causing variants in 111 individuals from 93 families. Analysis of clinical features revealed variation in age at diagnosis (IQR: 2–274 days), birth weight (z-score IQR: −0.39–1.37) and treatment (diazoxide dose IQR: 6.5–15 mg/kg/d, 9% underwent pancreatectomy). Individuals with variants disrupting the NKX2 binding site ($n = 36/111$) were diagnosed later (median 319 vs 2 days, $P < 0.0001$), had a lower birth weight (median z-score −0.17 vs 1.24, $P < 0.0001$) and were less likely to undergo a pancreatectomy (0% vs 21%, $P = 0.005$) than those with variants disrupting either NFAT, FOX, or all three binding sites.

**Conclusions:** We have shown that phenotypic variability exists in *HK1*-HI with the severity of disease correlating to the variants position within the regulatory element. To our knowledge, this is the first genotype-phenotype association described for a monogenic disorder caused by non-coding variants.

**Grant references:** Wellcome trust (223187/Z/21/Z).

**Conflict of Interest:** None declared

### C06.6 Clinical, neuroradiological and molecular characterization of mitochondrial threonyl-tRNA-synthetase (TARS2)-related disorder

*Reza Maroofian[1], **Gaurav Varshney**[1], Andrea Accogli[1], Sheng-Jia Lin[1], MariaSavina Severino[1], Marie Sissler[1], Kim Sunghoon[1], TARS2-related disorders study group[1]*

*[1]UCL Queen Square Institute of Neurology, london, United Kingdom*

**Background:** Biallelic variants in *TARS2*, encoding the mitochondrial threonyl-tRNA-synthetase, have been reported in a small group of individuals displaying a neurodevelopmental phenotype, but with limited neuroradiological data and insufficient evidence for causality of the variants.

**Methods**: Exome or genome sequencing was carried out in 14 families. Clinical and neuroradiological evaluation was performed for all affected individuals, including review of 10 previously reported individuals. The pathogenicity of *TARS2* variants was evaluated using in vitro assays, and a zebrafish model.

**Results:** We report 17 new individuals harboring biallelic *TARS2* variants. Phenotypically, these individuals show developmental delay/intellectual disability, regression, cerebellar and cerebral atrophy, basal ganglia signal alterations, hypotonia, cerebellar signs and increased blood lactate. In vitro studies showed that variants within the TARS2$^{301\text{-}381}$ region had decreased binding to Rag GTPases, likely impairing mTORC1 activity. The zebrafish model recapitulated key features of the human phenotype and unraveled dysregulation of downstream targets of mTORC1 signaling. Functional testing of the variants confirmed the pathogenicity in a zebrafish model.

**Conclusion:** We define the clinico-radiological spectrum of *TARS2*-related mitochondrial disease, unveil the likely involvement of the mTORC1 signaling pathway as a distinct molecular mechanism, and establish a *TARS2* zebrafish model as an important tool to study variant pathogenicity.

**Conflict of Interest:** None declared

## C07 EVOLUTIONARY GENETICS & GENETICS OF DIVERSE POPULATIONS

### C07.2 The DNA of 33 Ashkenazi Jews from 14th-century Erfurt, Germany

*Shai Carmi[1], **Shamam Waldman**[1], Daniel Backenroth[1], Eadaoin Harney[2], Stefan Flohr[3], Nadia Neff[4], Gina Buckley[5], Hila Fridman[1], Ali Akbari[2], Nadin Rohland[2], Swapan Mallick[2], Inigo Olalde[2], Leo Cooper[6], Ariel Lomes[1], Joshua Lipson[7], Jorge Cano Nistal[8], Jin Yu[9], Nir Barzilai[10], Inga Peter[11], Gil Atzmon[12], Harry Ostrer[10], Todd Lencz[9], Yosef Maruvka[8], Maike Laemmerhirt[13], Alexander Beider[14], Leonard Rutgers[15], Virginie Renson[5], Keith Prufer[4], Stephan Schiffels[16], Harald Ringbauer[16], Karin Sczech[17], David Reich[2]*

*[1]The Hebrew University of Jerusalem, Braun School of Public Health and Community Medicine, Jerusalem, Israel; [2]Harvard University, Cambridge, MA, United States; [3]University of Hildesheim, Hildesheim, Germany; [4]University of New Mexico, Albuquerque, NM, United States; [5]University of Missouri, Columbia, MO, United States; [6]Independent Scholar, Kalamazoo, MI, United States; [7]Independent Scholar, New York, NY, United States; [8]Technion – Israel Institute of Technology, Haifa, Israel; [9]The Zucker Hillside Hospital Division of Northwell Health, Glen Oaks, NY, United States; [10]Albert Einstein College of Medicine, Bronx, NY, United States; [11]Icahn School of Medicine at Mount Sinai, New York, NY, United States; [12]University of Haifa, Haifa, Israel; [13]University of Erfurt, Erfurt, Germany; [14]Independent Scholar, Chaville, France; [15]Utrecht University, Utrecht, Netherlands; [16]Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany; [17]City of Erfurt, Erfurt, Germany*

**Background:** The first Ashkenazi Jews (AJ) lived in the Rhineland in the 10th century CE. In the following centuries, AJ migrated to Eastern Europe and expanded rapidly. In the absence of DNA from historical individuals, little is known about the origin and demography of medieval AJ.

**Methods:** Remains of 47 individuals were recovered in a salvage excavation in the medieval Jewish cemetery of Erfurt, Germany. The local Jewish community approved a DNA study based on detached teeth. We sequenced 38 teeth and obtained genome-wide data for 33 individuals.

**Results:** The mean number of covered SNPs per individual was 400k, and ten teeth were dated to the 14th century. Erfurt Jews were genetically similar to modern AJ, with ancestry resembling modern Middle-Eastern, Southern-European, and Eastern-European populations. Erfurt Jews showed all hallmarks of the Ashkenazi founder event: a third of the Erfurt individuals descended from a single maternal lineage, eight carried pathogenic variants known to affect AJ today, and 15 carried long runs of homozygosity. We estimated that the effective size of the Erfurt population was under 1000 for the entire second half of the Middle Ages. Surprisingly, Erfurt Jews showed more variability in their Eastern-European-related ancestry than modern AJ, possibly reflecting a west/east divide in medieval AJ no longer present today.

**Conclusions:** By the 14th century, AJ have already experienced the main admixture and drift events that have shaped their gene pool. However, medieval AJ were more structured than present-day AJ.

**Conflict of Interest:** Shai Carmi Holds stock options at MyHeritage, A paid consultant at MyHeritage, Shamam Waldman: None declared, Daniel Backenroth An employee at The Janssen Pharmaceutical Companies of Johnson & Johnson, A shareholder at The Janssen Pharmaceutical Companies of Johnson & Johnson, Eadaoin Harney An employee of 23andMe, Stefan Flohr: None declared, Nadia Neff: None declared, Gina Buckley: None declared, Hila Fridman: None declared, Ali Akbari: None declared, Nadin Rohland: None declared, Swapan Mallick: None declared, Inigo Olalde: None declared, Leo Cooper: None declared, Ariel Lomes: None declared, Joshua Lipson: None declared, Jorge Cano Nistal: None declared, Jin Yu: None declared, Nir Barzilai: None declared, Inga Peter: None declared, Gil Atzmon: None declared, Harry Ostrer: None declared, Todd Lencz: None declared, Yosef Maruvka: None declared, Maike Laemmerhirt: None declared, Alexander Beider: None declared, Leonard Rutgers: None declared, Virginie Renson: None declared, Keith Prufer: None declared, Stephan Schiffels: None declared, Harald Ringbauer: None declared, Karin Sczech: None declared, David Reich: None declared

### C07.3 Widespread natural selection on metabolite levels in humans

***Yanina Timasheva**[1;2;3], Kaido Lepik[3;4;5], Orsolya Liska[6;7;8], Balázs Papp[6;7;8], Zoltan Kutalik[3;4;5]*

*[1]Institute of Biochemistry and Genetics UFRC RAS, Ufa, Russian Federation; [2]Bashkir State Medical University, Ufa, Russian Federation; [3]University of Lausanne, Department of Computational Biology, Lausanne, Switzerland; [4]Swiss Institute of Bioinformatics, Lausanne, Switzerland; [5]University of Lausanne, Center for Primary Care and Public Health, Lausanne, Switzerland; [6]HCEMM-BRC Metabolic Systems Biology Lab, Szeged, Hungary; [7]Institute of Biochemistry, Biological Research Centre, Eötvös Loránd Research Network (ELKH), Synthetic and Systems Biology Unit, Szeged, Hungary; [8]University of Szeged, Doctoral School of Biology, Szeged, Hungary*

Complex human traits are influenced by natural selection constraining the occurrence of extreme phenotypes (stabilising selection) and affecting the frequencies of the genetic variants associated with traits under selection. The selection signatures can be detected by assessing the relationship between effect size estimates from genetic association studies and corresponding allele frequencies. We estimated the action of natural selection on genetic variants associated with metabolite levels by leveraging summary statistics of published genome-wide association studies with sample size varying between 9363 and 86,507. The analysis provided strong evidence ($P < 5.15e\text{-}5$) of stabilising selection for 15 out of 97 studied plasma metabolites, particularly amino acids. Moreover, we found that metabolites under strong stabilizing selection in humans are more conserved in their concentrations among diverse mammalian species ($r = 0.37$), suggesting shared selective forces across micro and macroevolutionary time scales. Furthermore, Mendelian randomisation analysis revealed that metabolites displaying larger causal effects on low density lipoproteins are under stronger stabilising selection ($r = 0.21$). Similar observed pattern for other cardiometabolic traits suggests that maintaining a healthy cardiometabolic profile may be an important source of selective constraints on the metabolome. Finally, we also found evidence for both disruptive (e.g., citrulline) and directional selection (e.g. arginine) on specific lipid metabolites, potentially indicating ongoing evolutionary adaptation in humans. Overall, our results suggest that variation in metabolite levels among humans is frequently shaped by natural selection and thus may be acting indirectly through maintaining cardiometabolic fitness.

The research was partially supported by the Swiss National Science Foundation (IZSEZ0_194773) and Megagrant (075-15-2021-595).

**Conflict of Interest:** None declared

### C07.4 Using 858,635 individuals' haplotype-sharing between British and Danish populations to infer the North Sea migration history

***Xiaolei Zhang***[1], *Tomas Fitzgerald*[1], *Karina Banasik*[2], *Søren Brunak*[2], *Ewan Birney*[1]

[1]*European Bioinformatics Institute, Cambridge, United Kingdom;* [2]*University of Copenhagen, Novo Nordisk Foundation Center for Protein Research, Copenhagen, Denmark*

The North Sea has been a strategically important area with an extensive history of people and goods flowing through and around. However, it remains unclear how the historical migration events across the North Sea have affected the genetic structure of the modern human population in its coastal nations. Here we use the pairwise haplotype sharing from 858,635 British and Danes, to assemble population genetic evidence and infer the migration history between British and Danish populations within the past 1000 years.

Using nation-wide healthcare registries in Denmark, we analyze the haplotype sharing from 370,259 Danes. Compared with previous studies that reveal the Danish population as highly genetically homogeneous, we identify discernible population structure based on haplotype sharing, which reconstructs the geography of Denmark. We use the haplotype sharing between 370,259 Danes and 488,376 British from UK Biobank to detect regions with excess haplotype sharing across the two populations. We find individuals from South Jutland share the most of their genome with British while English from Eastern coast spanning from Yorkshire to the South East share the most of their genome with Danes. Finally, we characterize the change of the haplotype-sharing patterns over time and estimate the time periods when the patterns emerge.

Our work provides a novel fine-scale view on haplotype sharing patterns within and across Denmark and Britain, identifying regions of interests to population history, and demonstrating the use of multiple nationwide biobanks as a powerful tool to study history across populations.

The study is funded by Novo Nordisk Foundation.

**Conflict of Interest:** Xiaolei Zhang: None declared, Tomas Fitzgerald: None declared, Karina Banasik: None declared, Søren Brunak S. Brunak has ownerships in Intomics A/S, Hoba Therapeutics Aps, Novo Nordisk A/S, Lundbeck A/S, ALK abello A/S and managing board memberships in Intomics A/S and Proscion A/S outside the submitted work., Ewan Birney E.Birney is a paid consultant of Oxford Nanopore.

### C07.5 Use of personalized reference sets for polygenic risk scores negates need for arbitrary groupings

***Ambra Sartori***[1], *Steven Buyske*[2], *Chris Gignoux*[3], *Christopher Haiman*[4], *Eimear Kenny*[5], *Charles Kooperberg*[6], *Kari North*[7], *Genevieve L. Wojcik*[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology, Baltimore, MD, United States;* [2]*Rutgers University, Department of Statistics, Piscataway, NJ, United States;* [3]*Colorado Center for Personalized Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States;* [4]*Keck School of Medicine, University of Southern California, Department of Population and Public Health Sciences, Los Angeles, CA, United States;* [5]*The Institute for Genomic Health, Icahn School of Medicine at Mount Sinai, Department of Medicine, Department of Genetics and Genomic Sciences, New York, NY, United States;* [6]*Fred Hutchinson Cancer Center, Division of Public Health Sciences, Seattle, WA, United States;* [7]*University of North Carolina at Chapel Hill, Department of Epidemiology, Chapel Hill, NC, United States*

Polygenic risk scores (PRS) helped translating GWAS results into clinical practice but with limited portability across individuals of different genetic backgrounds, especially for admixed populations with multiple recent genetic ancestries. PRS performance estimation often uses a single-aggregate population reference set, ignoring inter-individual variability. Moreover, the ability to map traits at the variant-level in GWAS depends on allele frequencies and LD, with GWAS effect sizes tending to decrease between populations. A personalized PRS reference set approach can overcome these problems by tailoring to single individuals. We used $PRS_{BMI}$ from 41,586 admixed individuals from the PAGE Study, and trained a *k*-nearest neighbors model ($k = 1000$) on PCA-based genetic distance to assess their relative position in the genetic ancestry continuum. For each index individual, we defined new reference clusters which do not reflect continental-level genetic ancestry, nor racial/ethnic groupings, thus capturing inter-individual variation. We re-scaled $PRS_{BMI}$ scores within this new reference set and extracted the individual's *k*-ranking. When comparing all individuals to those at the top 10th percentile of the *k*-distribution, effect size increased ($B = 0.514$ to $B = 0.520$), suggesting that individual PRS accuracy fits with genetic distance. Within the Hispanic/Latino group, we observed the same pattern ($B = 0.518$ to $B = 0.520$), while the correlation between Hispanic/Latinos $PRS_{BMI}$ and AMR admixture proportions decreased ($r = 0.309$ to $r = 0.197$). This approach shows that individualized reference sets for PRS estimation can be generated without classification into discrete genetic ancestry groups, holding the potential to translate clinically useful information into

personalized medicine without the need to create borders where none exist.

**Conflict of Interest:** None declared

### C07.6 Structural variation discovery across diverse global populations

***Emma Pierce-Hoffman***[1;2], *Xuefang Zhao*[1;3;4], *Ryan Collins*[1;5;6], *Jack Fu*[1;3;4], *Shadi Zaheri*[1;2], *Isaac Wong*[1;2], *Chris Whelan*[1;2], *Nehir Kurtas*[1;6], *Yulia Mostovoy Mueller*[1;6], *Kiran Garimella*[7], *Mark Walker*[1;2], *Harrison Brand*[1;3;4], *Michael Talkowski*[1;3;4]

[1]*Massachusetts General Hospital, Center for Genomic Medicine, Boston, United States;* [2]*Broad Institute of MIT and Harvard, Program in Medical and Population Genetics and Data Sciences Platform, Cambridge, United States;* [3]*Harvard Medical School, Department of Neurology, Boston, United States;* [4]*Broad Institute of MIT and Harvard, Program in Medical and Population Genetics and Stanley Center for Psychiatric Research, Cambridge, United States;* [5]*Harvard Medical School, Division of Medical Sciences and Department of Medicine, Boston, United States;* [6]*Broad Institute of MIT and Harvard, Program in Medical and Population Genetics, Cambridge, United States;* [7]*Broad Institute of MIT and Harvard, Data Sciences Platform, Cambridge, United States*

**Background/Objectives:** Structural variants (SVs) represent a major source of genetic divergence between individuals. To date, scalable SV discovery has presented significant technical hurdles and the availability of SV references across diverse human populations is limited.

**Methods:** Here, we present and apply a suite of cloud-enabled algorithms for CNV and SV discovery, operable in Terra. GATK-gCNV leverages read-depth for scalable discovery of rare coding CNVs from exomes. From genomes, GATK-SV integrates variant discovery from five algorithms and applies variant filtering, joint genotyping, complex SV discovery, and functional annotation.

**Results:** We applied GATK-gCNV to >650K individuals across diverse populations and studies, including the UK Biobank, the Genome Aggregation Database (gnomAD), and neuropsychiatric disorder cohorts, discovering ~1 rare coding CNV per individual. From short-read genome sequencing, we applied GATK-SV to create the largest and most diverse SV references in the field, including the 1,000 Genomes Project and Human Genome Diversity Panel, gnomAD, and the All of Us Research Program (AoU). In gnomAD-v3, we constructed a reference atlas of 1,785,506 SVs from 141,311 samples spanning all major continental populations. In AoU we generated a pilot set of 515,427 SVs from 11,390 short-read samples and are processing >1000 African-American samples with long-read data, with the goal of scaling to >10K long-read and 1M short-read samples with matched phenotype data.

**Conclusion:** Collectively, these callsets comprising >800K individuals have the potential to redefine our maps of global variation for population genetics, disease-association studies, and diagnostic screening.

**Grant References:**

**Conflict of Interest:** Emma Pierce-Hoffman: None declared, Xuefang Zhao: None declared, Ryan Collins: None declared, Jack Fu: None declared, Shadi Zaheri: None declared, Isaac Wong: None declared, Chris Whelan: None declared, Nehir Kurtas: None declared, Yulia Mostovoy Mueller: None declared, Kiran Garimella: None declared, Mark Walker: None declared, Harrison Brand: None declared, Michael Talkowski M.E.T. receives reagents and research support from Microsoft, Illumina, and Pacific Biosciences.

## C08 REPRODUCTIVE GENETICS

### C08.1 Spatiotemporal transcriptome atlas of human embryos after gastrulation

***Jiexue Pan***[1], *Yuejiao Li*[2], *Zhongliang Lin*[3], *Qing Lan*[2], *Huixi Chen*[4], *Man Zhai*[2], *Shengwei Sui*[2], *Gaochen Zhang*[4], *Yunhui Tang*[4], *Yue Xu*[4], *Hongbo Yang*[4], *Ya Gao*[2], *Guolian Ding*[4], *Xun Xu*[2], *Hefeng Huang*[3;4]

[1]*Obstetrics and Gynecology Hospital, Institute of Reproduction and Development, Fudan University, Shanghai, China;* [2]*BGI-Shenzhen, Shenzhen, China;* [3]*Key Laboratory of Reproductive Genetics (Ministry of Education), Department of Reproductive Endocrinology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China;* [1]*Obstetrics and Gynecology Hospital, Institute of Reproduction and Development, Fudan University, Shanghai, China*

**Background/Objectives:** The spatial and temporal atlas of gene expression during human embryogenesis plays a pivotal role in Human Developmental Cell Atlas (HDCA). As many defects and disorders may trace back to embryonic stage which is the susceptible window of development, understanding spatial and temporal expression of genes during embryonic stage is crucial in deciphering etiology for many pathological conditions.

**Methods:** Ninety sagittal sections from 16 normal human embryos collected from routine termination of pregnancies at Obstetrics and Gynecology Hospital, ranging from post-conception week (PCW) 3 to PCW8 were sequenced by Stereo-seq to obtain spatial-temporal transcriptome atlas at 1-week temporal resolution.

**Results:** Spatial transcriptomics identifies gene expression profiles at known positional addresses in whole human embryos, exploring the cellular heterogeneity underlying organ-specific specializations during embryogenesis. The development regulatory profiling of 53 organs and potential tissue-identity regulators were established. Particularly, previously uncharacterized gene functions in heart and brain development were identified. The atlas also provides evidence and refinement of existing knowledge during human organ development and key organs/cell types that are vulnerable to virus infection and genetic disorders. Finally, we found dynamic changes of imprinting gene expression in specific organs at different stages. This work has enabled the collation of a genome-wide profile of the gene expression in each location-defined cell population, which can be rendered into a spatial display of the transcriptional architecture for the whole embryo.

**Conclusion:** Our work, for the first time, revealed the time-lapse and spatial transcriptome dynamics of human embryogenesis after gastrulation.

**Grant:** NSFC (82088102).

**Conflict of Interest:** Jiexue Pan Full time, National Natural Science Foundation of China (No. 82171688), Yuejiao Li Full time, Zhongliang Lin: None declared, Qing Lan full time, Huixi Chen: None declared, Man Zhai Full time, Shengwei Sui Full time, Gaochen Zhang: None declared, Yunhui Tang Full time, Yue Xu: None declared, Hongbo Yang Full time, Ya Gao Full time, Guolian Ding Full time, National Natural Science Foundation of China (No. 82271722), Xun Xu Full time, Hefeng Huang: None declared

### C08.2 The nature and prevalence of chromosomal dynamics in early spontaneous pregnancy loss

***Rick Essers***[1;2], *Igor Lebedev*[3], *Ants Kurg*[4], *Elizaveta Fonova*[3], *Servi Stevens*[1;2], *Ulrike von Rango*[5], *Spyridon Panagiotis Deligiannis*[6], *Tatyana Nikitina*[3], *Elena Sazhenova*[3], *Ekaterina Tolmacheva*[3], *Anna Kashevarova*[3], *Rebekka Koeck*[1;2], *Salwan Al-Nasiry*[2;7], *Jos C.M.F.*

*Dreessen[1], Aimee D.C. Paulussen[1;2], Merryn Macville[1;2], Alexander Hoischen[8;9;10], Ganesh Acharya[11], Han Brunner[1;2;8], Andres Salumets[6;11], Masoud Zamani Esteki[1;2;11]*

*[1]Maastricht University Medical Centre (MUMC+), Department of Clinical Genetics, Maastricht, Netherlands; [2]GROW School for Oncology and Reproduction, Department of Genetics and Cell Biology, Maastricht, Netherlands; [3]Tomsk National Research Medical Center, Institute of Medical Genetics, Tomsk, Russian Federation; [4]Institute of Molecular and Cell Biology, University of Tartu, Department of Biotechnology, Tartu, Estonia; [5]Care and Public Health Research Institute (CAPHRI), FHML, Department of Anatomy & Embryology, Maastricht, Netherlands; [6]Institute of Clinical Medicine, University of Tartu, Department of Obstetrics and Gynecology, Tartu, Estonia; [7]Maastricht University Medical Center (MUMC+), Departement of Obstetrics and Gynaecology, Maastricht, Netherlands; [8]Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands; [9]Center for Infectious Disease (RCI), Radboud University Medical Center, Department of Internal Medicine, Nijmegen, Netherlands; [10]Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands; [11]Karolinski Institutet, Division of Obstetrics and Gynecology- Department of Clinical Medicine, Stockholm, Sweden*

**Background/Objectives:** Pregnancy loss (PL) occurs in 10–15% of clinically recognized pregnancies, mostly by chromosomal abnormalities of the conceptus. However, the nature and prevalence of these abnormalities and the allocation of (ab) normal cells in embryonic and placental compartments during intrauterine development remains elusive.

**Methods:** We analyzed 1745 spontaneous PLs and found that ~50% were karyotypically normal. We applied genome haplarithmisis to 91 PL families with normal karyotypes, using whole-genome genotypes of the parents as well as of the extraembryonic mesoderm (EM) and chorionic villi (CV), representing embryonic and placental lineages, of the product of conception (POC) to generate detailed chromosomal profiles.

**Results:** We find that 36.4% of PLs have chromosomal aberrations not previously detected by karyotyping. Of the 33 genetically aberrant POCs, 9 POCs show >10% difference in the proportion of cells with aberrant genomes in mosaic EM and CV. Most of these cases (8/9) had higher level of mosaicism in EM as compared to CV .

**Conclusion:** In contrast to viable pregnancies where mosaic chromosomal abnormalities are often restricted to the CV, we find that in spontaneous abortions, the situation is reversed with a higher degree of mosaic chromosomal imbalances in EM rather than in CV. This is compatible with a selective scenario in which chromosomal aberrations derived only from the ICM (or with a higher mosaicism percentage) confer strong detrimental effect on the pregnancy outcome and may explain low fecundity in humans.

**Grant References:**

EVA (KP111513), MUMC+
Horizon 2020 innovation (ERIN) (EU952516), European Commission
RFBR (20-315-90111)

**Conflict of Interest:** None declared

### C08.3 SEMA6A revealed as novel gene controlling vascular permeability and puberty onset

***Antonella Lettieri*[1;2], *Roberto Oleari[2], Marieke Verhagen[3], Carlotta Tacconi[4], Alyssa Paganoni[2], Roberta Azzarelli[5], Luca Palazzolo[2], Ivano Eberini[2], Leo Dunkel[6], Sasha Howard[6], Alessandro Fantin[2], Jeroen Pasterkamp[3], Anna Cariboni[2]***

*[1]University of Milan, Health sciences, Milan, Italy; [2]University of Milan, Pharmacological and Biomolecular Sciences, Milan, Italy; [3]University Medical Center Utrecht Brain Center, Department of Translational Neuroscience, Utrecth, Netherlands; [4]University of Milan, Department of Biosciences, Milan, Italy; [5]University of Cambridge, Wellcome-MRC Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Cambridge, United Kingdom; [6]Queen Mary University of London, Centre for Endocrinology William Harvey Research Institute Barts and the London School of Medicine and Dentistry, London, United Kingdom*

Puberty onset is driven by the activation of gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus at the level of the median eminence (ME) whose innervation by GnRH neurons is vital to ensure gonadal development and successful reproduction. Genetic defects occurring along the reproductive axis can alter pubertal timing thus leading to delayed puberty (DP), which affects up to 2% of adolescents. Unfortunately, the genetic etiologies that underlie DP are still poorly understood.

Here, we applied exome sequencing to identify novel genetic determinants of DP in a cohort of 67 precisely-phenotyped multigenerational families affected by DP. This approach yielded the discovery of a novel variant in the Semaphorin 6A (SEMA6A) gene, which segregated with DP in 4 family members. In silico and in vitro studies revealed that the mutation affects protein stability, synthesis, and localization. Furthermore, functional studies in mice revealed that Sema6a is controlling puberty onset, via the regulation of ME vascular permeability, which is required to maintain neuroendocrine homeostasis.

Overall, our results provide insight into the pathogenic mechanisms linked to a novel DP-associated SEMA6A variant and define a previously unexplored role for SEMA6A in the control of the reproductive axis.

**Grant references:** Italian Ministry of Health (GR-2016-02362389); the Netherlands Organization for Scientific Research (ENW-VICI); the National Institute for Health Research (CL-2017-19-002); Wellcome Trust (222049/Z/20/Z); Barts Charity [MGU0552]; the Rosetrees Trust [M222-F1].

**Conflict of Interest:** None declared

### C08.4 Dynamic transcriptomic changes in endometrial tissue and its association with endometriosis and related infertility

***Fei Yang*[1], *Ting Qi[2;3], Allan F McRae[1], Peter A.W. Rogers[4], Sally Mortlock[1], Grant W. Montgomery[1]***

*[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia; [2]School of Life Sciences, Westlake University, Hangzhou, China; [3]Westlake Laboratory of Life Sciences and Biomedicine, Hangzhou, China; [4]University of Melbourne Department of Obstetrics and Gynaecology, and Gynaecology Research Centre, Royal Women's Hospital, Melbourne, Australia*

**Background/Objects:** Endometrium is a vital tissue for reproduction and undergoes shedding, remodeling, and regeneration throughout the menstrual cycle, all of which are associated with substantial molecular changes. However, a comprehensive understanding of transcriptional and genetic regulation of endometrium in health and disease remains limited.

**Methods:** We integrated genotypes and RNA sequencing from endometrium samples from 206 women of European ancestry, the largest endometrial transcriptomic dataset to date.

**Results:** We firstly demonstrate the dynamic and variable nature of transcriptomic changes in endometrium throughout the

menstrual cycle at gene-, isoform- and splicing-levels as well as their predictable functional consequences on downstream protein isoforms. Subsequently, both network and individual transcriptome analyses revealed transcriptomic changes between women with ($n = 143$) and without endometriosis ($n = 63$), a common gynecological disorder. Larger differences were identified in the mid-secretory phase (MS) of the menstrual cycle, with significant candidate genes related to endometriosis and infertility. We further revealed genetic effects on splicing within endometrium in the form of 3,296 splicing QTLs (sQTLs) and demonstrated that splicing may be an important mediator of genetic effects on endometriosis. Integration of transcriptomic and GWAS data suggested endometriosis risk is, in part, mediated by cis effects on exon 15 skipping of *GREB1* in endometrium.

**Conclusion:** This transcriptome-wide study provides novel insights and evidence into the dynamic changes in endometrium and its association with endometriosis and related infertility. The endometrial sQTL dataset is a valuable resource to investigate functional effects of genetic variants associated with reproductive diseases.

**Grant References:** National health and medical research council

**Conflict of Interest:** None declared

### C08.5 Genome sequencing reveals copy number variants in known and novel candidate genes for idiopathic non-obstructive azoospermia

***Oguzhan KALYON***[1], *Bilal Alobaidi*[1], *Lois Batty*[1], *Jonathan Coxhead*[2], *Kevin Mceleny*[3], *Godfried van der Heijden*[4], *Liliana Ramos*[4], *Manon Oud*[5], *Miguel Xavier*[1], *Aneta Mikulasova*[1], *Giles Holt*[1], *Joris Veltman*[1]

[1]*Biosciences Institute, Faculty of Medical Sciences. Newcastle University, Newcastle Upon Tyne, United Kingdom;* [2]*Genomics Core Facility, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, United Kingdom;* [3]*Newcastle Fertility Centre, The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, United Kingdom;* [4]*Department of Obstetrics and Gynaecology, Radboudumc, Nijmegen, Netherlands;* [5]*Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboudumc, Nijmegen, Netherlands*

**Background/Objectives:** Chromosomal abnormalities are a common cause of male infertility (MI), but little is known about the role of copy number variants (CNVs). Here, we identify CNVs affecting known and novel MI candidate genes by performing whole genome sequencing (WGS) in 200 patient-parent trios with quantitative sperm defects.

**Methods:** WGS of 200 patients with idiopathic azoospermia and their parents was performed. CNVs were detected using CNVRobot and dysgu-SV. Rare de novo and maternally inherited CNVs were prioritised for further studies. Whole exome sequencing (WES) was performed on 235 additional patients with azoospermia to be used as a replication cohort.

**Results:** Approximately 212,000 CNVs were detected in 200 trios with an average of 1083 per proband. A total of 12 rare de novo CNVs were identified ranging from 2 kb to 256 kb, mostly affecting non-coding DNA. One of these de novo deletions affects the *CTNNA3* gene, which was also affected by a duplication CNV in an unrelated azospermic patient. Disruption of *CTNNA3* has been previously described in an azospermic patient (PMID:35017386). In addition, 1863 rare maternally inherited CNVs were identified. A STRING analysis revealed a significant enrichment in the number of interacting genes ($p < 4.99e\text{-}10$) affected by these CNVs, many of which are known to play a role in spermatogenesis.

**Conclusion:** In this WGS study, we discovered many CNVs affecting genes involved in spermatogenesis, potentially explaining MI. Large-scale replication studies are ongoing to further determine their relevance and further our understanding of MI genetics.

**Grants References:** Wellcome Trust (209451)

**Conflict of Interest:** None declared

### C08.6 A map of genetic and phenotypic associations across female reproductive phenotypes

*Natàlia Pujol Gualdo*[1], *Jelisaveta Džigurski*[1], *Valentina Rukins*[1], *Reedik Mägi*[1], ***Triin Laisk***[1]

[1]*Institute of Genomics, University of Tartu, Tartu, Estonia*

**Background/Objectives:** Women's health has been understudied for years. Recently, huge progress has been made with clarifying the genetic architecture of some more common gynecological and obstetrical diagnoses. However, the potential genetic background of other diagnoses remains enigmatic. Here, we carry out the most extensive effort to map the genetic background of phenotypes related to female reproductive health and provide an atlas of genetic and phenotypic correlations.

**Methods:** We used data from Estonian Biobank and FinnGen and performed a genome-wide association study (GWAS) meta-analysis of up to 300,553 women across 48 disease endpoints defined using ICD10 classification and encompassing diagnoses related to endocrine dysfunction, cancers of the reproductive tract, diseases of the genitourinary system, and diagnoses characterizing complications of pregnancy and childbirth. We annotated the GWAS findings, calculated heritabilities, and explored the genetic correlations across phenotypes using the LDScore framework.

**Results:** We identified 43,888 genome-wide significant ($p < 5 \times 10^{-8}$) variants, including numerous novel findings, many of which tag coding variants. We provide the first map of shared genetic architecture for female reproductive health phenotypes with a total of 840 estimated pairwise genetic correlations. Overall, we found the genetic and phenotypic correlations are quite similar and reflect shared biological background. Finally, we characterized pleiotropic associations on gene and variant level and mapped the genetic association "hotspots" that play a central role in women's health.

**Conclusion:** Our work represents the largest effort to comprehensively characterize and map the genetic determinants of different female reproductive health-associated diagnoses.

**Grant references:** Estonian Research Council grants PSG776, PRG1911.

**Conflict of Interest:** None declared

## C09 GENOME VARIATION: FROM SMALL TO LARGE

### C09.1 Cluster analysis identifies genes with distinct patterns of loss of function variants

***Robin Beaumont***[1], *Caroline Wright*[1]

[1]*University of Exeter, Exeter, United Kingdom*

**Background:** Genetic variants that severely alter protein products (e.g., stop-gain, start-loss) are often associated with disease. For some genes, these predicted loss-of-function variants (pLoFs) are observed throughout the gene, while in others they only occur only at specific locations. We investigated whether pLoF location could explain instances of incomplete penetrance of variants expected to be pathogenic for Mendelian conditions.

**Methods:** We used exome sequence data in 454,773 individuals in UK Biobank (UKB) to investigate locations of pLoFs in a population cohort. We counted numbers of unique pLoF, missense, and synonymous variants in UKB in each quintile of the gene, and clustered the variants using Gaussian mixture models. We limited analyses to genes with coding sequence >1000bp and ≥5 variants of each type (11,050 genes). We compared locations of pLoFs in UKB with all theoretically possible pLoFs and pathogenic pLoFs from ClinVar.

**Results:** For most genes, synonymous, missense and all possible pLoF variants fell into clusters representing uniform variant distributions. Of these, pLoFs were not uniformly distributed for 625 genes. Notably, genes causing developmental disorders via haploinsufficiency were less likely to have uniform pLoF distribution than other genes. For two such genes, *GATA6* and *ARID1B*, pathogenic pLoFs were located approximately uniformly across the gene, whilst likely benign pLoFs were clustered in specific locations, suggesting rescue via translation reinitiation, alternative splicing or other mechanisms.

**Conclusions:** Our results suggest potential benefits of localised constraint metrics and that location of pLoF variants should be considered when interpreting variants.

**Grants:** MR/T00200X/1

**Conflict of Interest:** None declared

### C09.2 Assessing missense mutation effects at scale using CRISPR-Cas9 base editors

***Uyen Linh Ho***[1], *René Dreos*[2], *Ronay Çetin*[3], *Martin Wegner*[3], *Manuel Kaulich*[3], *Jolanda van Leeuwen*[2]

[1]*Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland;* [1]*Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland;* [3]*Institute of Biochemistry II, University Hospital Frankfurt, Goethe University, Frankfurt, Germany, Frankfurt, Germany*

Advances in sequencing technology have led to the identification of millions of missense mutations in the human genome. However, the pathogenicity or functional consequence of >99% of these mutations remains unknown. We used CRISPR-Cas9 base editor screens to assess the effects of missense mutations on cell proliferation in a high-throughput manner. We performed a pooled CRISPR-Cas9 cytosine base editor screen targeting 100,000 different sites in 680 core essential genes that are required for viability across cell lines. We tracked the change in abundance of mutants in cell pools over time under both standard conditions and conditions that affect protein stability. These latter conditions are expected to identify partial loss-of-function variants that are buffered under optimal proliferation conditions. Reassuringly, gRNAs predicted to introduce nonsense mutations tended to be depleted under all conditions, whereas levels of gRNAs predicted to introduce synonymous mutations were largely unchanged in our screens. Overall, we found that ~20% of missense alleles led to a proliferation defect in at least one of the tested conditions. Deleterious mutations tended to cluster together, highlighting functionally important residues, domains, and interaction interfaces. Furthermore, highly pleiotropic genes were enriched for detrimental mutations, highlighting the general importance of the encoded multifunctional proteins. Finally, we validated the deleterious effects of several missense mutations by recreating these mutants individually. To conclude, we show that base editor screens can be used to study variant effects, identify hypermorphic mutations, and highlight critical residues and domains within core essential genes.

**Conflict of Interest:** None declared

### C09.3 Shining light on the dark genome: accurate quantification of tandem repeats for translational and basic research

***Egor Dolzhenko***[1], *Adam English*[2], *Harriet Dashnow*[3], *William Rowell*[1], *Zev Kronenberg*[1], *Dalia Kasperaviciute*[4], *Henry Houlden*[5;6], *Roisin Sullivan*[5;6], *Jana Vandrovcova*[5;6], *Arianna Tucci*[4], *Aaron Wenger*[1], *Aaron Quinlan*[3], *Fritz Sedlazeck*[2;7], *Michael Eberle*[1]

[1]*Pacific Biosciences of California, San Diego, United States;* [2]*Baylor College of Medicine, Houston, United States;* [3]*University of Utah, Salt Lake City, United States;* [4]*Genomics England, London, United Kingdom;* [5]*University College London, London, United Kingdom;* [6]*National Hospital for Neurology and Neurosurgery, London, United Kingdom;* [7]*Rice University, Houston, United States*

**Background/Objectives:** Tandem repeats (TRs) are genomic regions consisting of repetitions of a DNA sequence. TRs are associated with many genetic disorders and harbor up to 80% of structural variants. TRs are difficult to analyze with short-read sequencing data due to their repetitiveness and structural complexity. The standard computational methods for variant calling often do not correctly resolve variation in TR regions.

**Methods:** Here we describe the first method for accurate genome-wide characterization of length, sequence, and methylation status of tandem repeats from long-read sequencing data called the Tandem Repeat Genotyping Tool (TRGT). TRGT uses a flexible probabilistic model representing the population structure of a repeat region to accurately resolve each repeat allele in each sample. TRGT can handle simple tandem repeats that consist of repetition of a single fixed-sized sequence as well as more complex regions containing inexact or nested repeats.

**Results:** Benchmarking over one million TRs revealed high accuracy of TRGT with over 99% of genotyped repeats being Mendelian concordant in family trios and also consistent with existing genome assemblies. TRGT accurately detects known repeat expansions in *FMR1*, *HTT*, *ATXN8*, *RFC1*, and other genes. To demonstrate the utility of our method, we used it to create a database with haplotype resolved sequences and methylation levels of TRs across 100 PacBio HiFi whole-genome samples.

**Conclusion:** We developed a method to resolve the structural complexity and population variation of TRs across the genome. Accurate TR analysis will improve our ability to detect known pathogenic expansions and discover novel functional repeats.

**Conflict of Interest:** Egor Dolzhenko Pacific Biosciences of California, Adam English: None declared, Harriet Dashnow: None declared, William Rowell Pacific Biosciences of California, Zev Kronenberg Pacific Biosciences of California, Phase Genomics, Dalia Kasperaviciute Genomics England, Henry Houlden: None declared, Roisin Sullivan: None declared, Jana Vandrovcova: None declared, Arianna Tucci Genomics England, Aaron Wenger Pacific Biosciences of California, Aaron Quinlan: None declared, Fritz Sedlazeck Pacific Biosciences, Oxford Nanopore Technologies, and Illumina, Michael Eberle Pacific Biosciences of California

### C09.4 Misexpression of inactive genes is associated with nearby rare structural variants

***Thomas Vanderstichele***[1], *Katie L Burnham*[1], *Niek de Klein*[1], *Brittany Howell*[1], *Klaudia Walter*[1], *Kousik Kundu*[1;2], *Artika Nath*[2;3], *Elodie Persyn*[2], *Jonathan Marten*[2], *David Roberts*[4], *Emanuele Di Angelantonio*[2], *John Danesh*[2], *Alix Berton*[5], *Adam Platt*[5], *Adam Butterworth*[2], *Nicole Soranzo*[1], *Leopold Parts*[1], *Michael Inouye*[2;3], *Dirk Paul*[2;6], *Emma Davenport*[1]

[1]*Wellcome Sanger Institute, Cambridge, United Kingdom;* [2]*University of Cambridge, Cambridge, United Kingdom;* [3]*Baker Heart and*

*Diabetes Institute, Melbourne, Australia; [4]University of Oxford, Oxford, United Kingdom; [5]AstraZeneca, Translational Science and Experimental Medicine, Research and Early Development, Respiratory and Immunology, Cambridge, United Kingdom; [6]AstraZeneca, Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, Cambridge, United Kingdom*

**Background/Objectives**: Gene misexpression is the unexpected transcription of a gene in a context where it is usually inactive. In this study, we aimed to establish how widespread gene misexpression is in humans, whether it is associated with a specific class of genetic variation and, if so, the mechanisms by which these variants act.

**Methods**: We developed a pipeline to identify misexpression of inactive genes from 4731 whole blood RNA sequencing samples. Using 2804 paired RNA and whole-genome sequencing samples, we conducted genetic variant enrichment tests across different variant classes and allele frequencies to establish whether genetic variation is associated with gene misexpression. Misexpression-associated variants were tested for enrichment across various genomic and regulatory features.

**Results**: We established that gene misexpression occurs frequently, identifying 349,705 misexpression events across 8610 inactive genes with a median of 64 misexpression events per individual. We found that rare (MAF < 1%) structural variants were enriched in *cis* to misexpressed genes. When stratifying structural variants by VEP consequence, transcript amplifications, deletions leading to stop codon loss and regulatory region ablation were most strongly enriched. Misexpression-associated structural variants occurred in more constrained and conserved regions of the genome, and were predicted to be more deleterious by CADD-SV. Misexpression-associated deletions were enriched for transcribed regions, CTCF-binding sites and TAD boundaries, while duplications were enriched for enhancers, CTCF-binding sites, TAD boundaries and active promoters.

**Conclusion**: We demonstrate that gene misexpression occurs frequently and is associated with rare structural variants acting via distinct mechanisms.

**Conflict of Interest:** Thomas Vanderstichele: None declared, Katie L Burnham: None declared, Niek de Klein: None declared, Brittany Howell: None declared, Klaudia Walter: None declared, Kousik Kundu: None declared, Artika Nath: None declared, Elodie Persyn: None declared, Jonathan Marten: None declared, David Roberts: None declared, Emanuele Di Angelantonio: None declared, John Danesh AstraZeneca, Novartis, and UK Biobank, Alix Berton AstraZeneca plc, Adam Platt AstraZeneca plc, Adam Butterworth Institutional grants from AstraZeneca, Bayer, Biogen, BioMarin, Bioverativ, Novartis, Regeneron and Sanofi, Nicole Soranzo: None declared, Leopold Parts: None declared, Michael Inouye: None declared, Dirk Paul AstraZeneca plc, Emma Davenport: None declared

### C09.5 Population analyses of mosaic X chromosome loss identify genetic drivers and widespread signatures of cellular selection

***Aoxing Liu***[1;2], *giulio genovese*[2;3], *yajie zhao*[4], *Matti Pirinen*[1], *Chikashi Terao*[5], *Po-Ru Loh*[2;3], *Andrea Ganna*[1;2;6], *john perry*[4], *mitchell machiela*[7]

[1]*Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland;* [2]*Broad Institute of MIT and Harvard, Cambridge, United States;* [3]*Harvard Medical School, Boston, United States;* [4]*University of Cambridge, Cambridge, United Kingdom;* [5]*RIKEN Center for Integrative Medical Sciences,, Yokohama, Japan;* [6]*Massachusetts General Hospital, Boston, United States;* [7]*National Cancer Institute, Rockville, United States*

Mosaic loss of the X chromosome (mLOX) is the most commonly occurring clonal somatic alteration detected in the leukocytes of women, yet little is known about its genetic determinants or phenotypic consequences. To address this, we estimated mLOX in >900,000 women across eight biobanks, identifying 10% of women with detectable X loss in approximately 2% of their leukocytes. Out of 1253 diseases examined, women with mLOX had an elevated risk of myeloid and lymphoid leukemias and pneumonia. Genetic analyses identified 49 common variants influencing mLOX, implicating genes with established roles in chromosomal missegregation, cancer predisposition, and autoimmune diseases. Complementary exome-sequence analyses identified rare missense variants in *FBXO10* which confer a two-fold increased risk of mLOX. A small fraction of these associations were shared with mosaic Y chromosome loss in men, suggesting different biological processes drive the formation and clonal expansion of sex chromosome missegregation events. Allelic shift analyses identified alleles on the X chromosome which are preferentially retained, demonstrating that variation at many loci across the X chromosome is under cellular selection. A novel polygenic score including 44 independent X chromosome allelic shift loci correctly inferred the retained X chromosomes in 80.7% of mLOX cases in the top decile. Collectively our results support a model where germline variants predispose women to acquiring mLOX, with the allelic content of the X chromosome possibly shaping the magnitude of subsequent clonal expansion.

**Conflict of Interest:** Aoxing Liu Full Employment of Institute for Molecular Medicine Finland (FIMM), giulio genovese Fully employed by Broad institute., G.G. was supported by NIH grants R01 MH104964 and R01 MH123451., G.G., P.-R.L., and S.A.M. declare competing interests: patent application PCT/WO2019/079493 has been filed on the mosaic chromosomal alterations detection method used in this work., yajie zhao Fully employed by Cambridge University., Matti Pirinen Fully employed by Helsinki University., Chikashi Terao Fully employed by RIKEN Center for Integrative Medical Sciences., C.T. was supported by Japan Agency for Medical Research and Development (AMED) grants JP21kk0305013, JP21tm0424220, and JP21ck0106642, and Japan Society for the Promotion of Science (JSPS) KAKENHI grant JP20H00462., Po-Ru Loh Fully employed by Broad Institute and Havard medical school., P.-R.L. was supported by NIH grant DP2 ES030554, a Burroughs Wellcome Fund Career Award at the Scientific Interfaces, the Next Generation Fund at the Broad Institute of MIT and Harvard, and a Sloan Research Fellowship., G.G., P.-R.L., and S.A.M. declare competing interests: patent application PCT/WO2019/079493 has been filed on the mosaic chromosomal alterations detection method used in this work., Andrea Ganna Fully employed by the University of Helsinki., A.G. was supported by the Academy of Finland (grant no. 323116) and by the European Research Council under the European Union's Horizon 2020 Research and Innovation Programme (grant no. 945733)., john perry J.R.B.P is employee of and hold shares in Adrestia Therapeutics., The Medical Research Council (unit programs: MC_UU_12015/2, MC_UU_00006/2)., mitchell machiela Fully employed by National Cancer Institute., The Intramural Research Program of the National Cancer Institute, National Institutes of Health.

### C09.6 Advances in long-read sequencing and telomere-to-telomere assembly enable breakpoint discovery in ring chromosomes and rearrangements involving acrocentric p-arms

***Yulia Mostovoy***[1;2;3], *Phillip Boone*[1;2;4], *Yongqing Huang*[5], *Kiran Garimella*[5], *Bianca Russell*[6], *Monica Salani*[1], *Benjamin Currall*[1;2;3], *Tera Bowers*[7], *Tim DeSmet*[7], *Stacey Gabriel*[7], *James Gusella*[1;2;3;8],

*Fabiola Quintero-Rivera[9], Harrison Brand[1;2;3;10], Michael Talkowski[1;2;3;11]*

*[1]Massachusetts General Hospital, Center for Genomic Medicine, Boston, United States; [2]Broad Institute of MIT and Harvard, Program in Medical and Population Genetics, Cambridge, United States; [3]Massachusetts General Hospital and Harvard Medical School, Department of Neurology, Boston, United States; [4]Boston Children's Hospital, Division of Genetics and Genomics, Boston, United States; [5]Broad Institute of MIT and Harvard, Data Sciences Platform, Cambridge, United States; [6]University of California, Los Angeles, Division of Genetics, Department of Pediatrics, Los Angeles, United States; [7]Broad Institute of MIT and Harvard, Genomics Platform, Cambridge, United States; [8]Harvard Medical School, Department of Genetics, Boston, United States; [9]University of California, Irvine, Division of Genetic and Genomic Medicine, Departments of Pathology, Laboratory Medicine, and Pediatrics, Irvine, United States; [10]Massachusetts General Hospital, Pediatric Surgery Research Laboratory, Department of Pediatrics, Boston, United States; [11]Broad Institute of MIT and Harvard, Stanley Center for Psychiatric Research, Cambridge, United States*

Resolving genomic variation within complex, highly repetitive regions of the human genome has been largely intractable with conventional genomic tools. As a consequence, there is a dearth of sequence-based insights into entire classes of genomic structural variation including ring chromosomes, Robertsonian translocations, and other repeat-associated rearrangements. We sought to access these events at sequence resolution and explore their mechanisms of formation and functional impact by leveraging two recent technical milestones: (1) maturation of long-read sequencing technologies; and (2) the Telomere-to-Telomere (T2T) gapless genome assembly.

We performed Oxford Nanopore long-read sequencing on 13 patient-derived cell lines harboring seven ring chromosomes, three Robertsonian translocations, two inter-chromosomal translocations, and one pericentric inversion. Among these rearrangements, 9/13 involved the highly repetitive acrocentric p-arms. We aligned reads to the T2T assembly and reconstructed breakpoints using custom scripts and copy-number variant detection (GATK-gCNV). We resolved 10/13 events, including all ring chromosomes, one Robertsonian translocation, and 6/9 samples involving acrocentric p-arms. Strikingly, two samples had breakpoints inside ribosomal DNA arrays. Two ring breakpoints included telomeric repeats, while four rings involved complex structures such as inversions and dispersed duplications. One Robertsonian translocation had a dispersed duplication from chr19 mediating the fusion of 14p and 15p.

In summary, integrating long-reads with the T2T assembly enabled precise characterization of rearrangements in previously intractable genomic regions and illuminated a wide range of structural diversity and rearrangement mechanisms. The approaches presented here can be used to probe a variety of complex structural variants with important implications for molecular diagnosis and genome biology.

**Conflict of Interest:** None declared

## C10 NEUROGENETICS: AN UPDATE

### C10.1 The annotation and function of the Parkinson's and Gaucher disease linked gene GBA1 has been concealed by its protein coding pseudogene GBAP1

***Emil Gustavsson**[1], Siddharth Sethi[2], Yujing Gao[2], David Zhang[1], Jonathan Brenton[1], Sonia García-Ruiz[1], Raquel Garza[3], Rhys Dore[1], Regina H. Reynolds[1], Charlie Arber[4], James Evans[5;6], Selina Wray[4], Johan Jakobsson[3], Sonia Gandhi[5;6], Henry Houlden[4], Harpreet Saini[2], Carla Bento[2], John J. Hardy[6;7;8], Mina Ryten[1;9]*

*[1]UCL GOS Institute of Child Health, Genetics and Genomic Medicine, London, United Kingdom; [2]Astex Pharmaceuticals, Milton, United Kingdom; [3]Lund University, Department of Experimental Medical Science, Lund, Sweden; [4]UCL Queen Square Institute of Neurology, Department of Neuromuscular Disease, London, United Kingdom; [5]Francis Crick Institute, London, United Kingdom; [6]UCL Queen Square Institute of Neurology, Department of Neurodegenerative Disease, London, United Kingdom; [7]UCL Queen Square Institute of Neurology, Reta Lila Weston Institute, London, United Kingdom; [8]UCL, UK Dementia Research Institute, London, United Kingdom; [9]UCL, NIHR Great Ormond Street Hospital Biomedical Research Centre, London, United Kingdom*

The human genome contains numerous duplicated and highly similar regions, such as parent-pseudogene pairs, causing short sequencing reads to align equally well to either gene - a phenomenon known as multimapping. While multimapping has been shown to complicate genomic analyses, to what extent this ambiguity complicates transcriptomic analyses remains unknown. This is concerning as 734 (20.0%) parent genes have been linked to disease, including *GBA1* in Parkinson's and Gaucher disease. We find that *GBA1* and its pseudogene *GBAP1* share 96% sequence similarity. This results in 58% of short-read RNA sequencing (RNA-seq) reads that map to *GBA1* also map to *GBAP1*. To overcome this problem, we performed long-read RNA-seq, where reads map without ambiguity, and found that quantification of *GBA1* and *GBAP1* is 2–3-fold different when compared to short reads across human brain regions. Furthermore, using targeted, and highly accurate (accuracy >99%), long-read RNA-seq across 12 human brain regions we identified numerous novel transcripts from both genes. This includes 18 novel protein coding transcripts from *GBA1* and 7 protein coding transcripts from *GBAP1* that are transcribed across all brain regions and are translated in vitro. This accounts for a mean of 38.4 ± 7.6% of total transcription at the GBA1 locus. By combining single-cell long-read and single-nuclear RNA-seq we demonstrate that transcript expression varies by brain region with cell-type-selectivity. Furthermore, we demonstrate that inaccuracies in annotation are widespread among parent genes, with implications for many human diseases.

**Grant References:** Aligning Science Across Parkinson's and BrightFocus Foundation

**Conflict of Interest:** None declared

### C10.2 Contrasting patterns of somatic mutations in neurons and glia reveal differential predisposition to disease in the aging human brain

***Sara Bizzotto**[1;2;3], Javier Ganz[1;2], Lovelace J. Luquette[4], Craig L. Bohrson[4], Hu Jin[4], Micheal B. Miller[1;2;5], Zinan Zhou[1;2], Alon Galor[4], Peter Park[4;6], Chris Walsh[1;2]*

*[1]Boston Children's Hospital, Genetics and Genomics, Boston (MA), United States; [2]Harvard Medical School, Pediatrics and Neurology, Boston (MA), United States; [3]Sorbonne Université, Institut du Cerveau (Paris Brain Institute) ICM, Inserm, CNRS, Hôpital de la Pitié Salpêtrière, Paris, France; [4]Harvard Medical School, Biomedical Informatics, Boston (MA), United States; [5]Brigham and Women's Hospital, Pathology and Neurology, Boston (MA), United States; [6]Brigham and Women's Hospital, Genetics, Boston (MA), United States*

**Background**: Somatic mutations accumulate in human tissues during life with rates and patterns that depend on intrinsic tissue and cell type properties and on environmental exposures.

Characterizing the mechanisms of somatic mutations in the brain is important for understanding aging and disease, but little is known about the mutational patterns of different cell types.

**Methods**: We performed single-cell whole-genome sequencing of 71 oligodendrocytes and 51 neurons from neurotypical individuals (0.4 to 104 years old) and identified >67,000 somatic single nucleotide variants (sSNVs) and small insertions and deletions (indels) using SCAN2. In order to study cell type-specific mutation distribution across the genome with respect to several covariates, we used single-cell RNA and chromatin accessibility from the same brains, complemented by additional available data modalities.

**Results**: We found that while both cell types accumulate mutations linearly with age, oligodendrocytes accumulate sSNVs 69% faster than neurons (27/year versus 16/year) whereas indels accumulate 42% slower (1.8/year versus 3.1/year). Signature analysis revealed cell type-specific mutational mechanisms, with neuronal mutations associated with transcription, and oligodendrocytes mutations with cell divisions at the precursor stage, coherent with oligodendrocyte turnover during adult life. Correlation with genomic covariates showed that oligodendrocyte mutations are enriched in inactive genomic regions and are distributed similarly to mutations in brain cancers. In contrast, neuronal mutations are enriched in open, transcriptionally active chromatin.

**Conclusion**: Our results highlight differences in the mutagenic processes in glia and neurons, and suggest cell type-specific, age-related contributions to neurodegeneration and oncogenesis.

**Grants**: National Institute on Aging (NIA) R01AG070921 and R01AG078929

**Conflict of Interest:** None declared

### C10.3 Variants leading to impaired N-terminal acetylation capacity: a new cause of autosomal recessive primary familial brain calcifications

***Viorica Chelban***[1], *Henriette Aksnes*[2], *Reza Maroofian*[1], *Lauren LaMonica*[3], *Luis Seabra*[4], *Rita Horvath*[5], *Nicholas Wood*[1], *Jana Vandrovcova*[1], *Andreas Ernst*[3], *Alistair Pagnamenta*[6], *Yanick Crow*[7], *Gaël Nicolas*[8], *James Rothman*[3], *Thomas Arnesen*[2], *Henry Houlden*[1]

[1]*Queen Square Institute of Neurology, London, United Kingdom;* [2]*University of Bergen, Department of Biomedicine, Bergen, Norway;* [3]*Yale School of Medicine, Department of Cell Biology, New Haven, United States;* [4]*Université Paris Cité INSERM, Laboratory of Neurogenetics and Neuroinflammation, Paris, France;* [5]*University of Cambridge, Department of Clinical Neurosciences, Cambridge, United Kingdom;* [6]*Oxford NIHR Biomedical Research Centre, Oxford, United Kingdom;* [7]*Institute of Genetics and Cancer, University of Edinburgh, MRC Human Genetics Unit, Edinburgh, United Kingdom;* [8]*Univ Rouen Normandie, Inserm, Department of Genetics and CNRMAJ, Rouen, France*

**Background/Objectives:** Primary familial brain calcification (PFBC) is characterized by calcium deposition in the brain, causing progressive movement disorders, psychiatric symptoms, and cognitive decline. PFBC is a heterogeneous disorder currently linked to variants in six different genes, but most patients remain genetically undiagnosed. Our aim was to identify novel pathogenic variants underlying PFBC

**Methods:** We performed genome-wide sequencing, homozygosity mapping, segregation analysis and in-depth clinical and neuroimaging phenotyping for novel disease-causing gene discovery. Pathogenicity was confirmed by immunofluorescence and fluorescence microscopy to assess subcellular distribution and characterization of disease variants, in vitro carbon [14-C]-Nt-acetylation assay on immunoprecipitated variants to investigate their enzymatic activity, surface biotinylation and extracellular free phosphate quantification on patient-derived fibroblasts. We used Crispr genomic editing in zebrafish model and examined the functional and morphological characteristics.

**Results:** We identified biallelic *NAA60* variants in seven individuals from four families with autosomal recessive PFBC. The *NAA60* variants lead to loss-of-function with lack of protein N-terminal (Nt)-acetylation activity. We show that the phosphate importer SLC20A2 is a substrate of NAA60 in vitro. In cells, loss of NAA60 caused reduced surface levels of SLC20A2 and a reduction in extracellular phosphate uptake. A zebrafish disease model of NAA60 deficiency displayed a motor deficit with altered phosphate homeostasis.

**Conclusion:** This study establishes *NAA60* as a causal gene for PFBC, provides a biochemical explanation of its disease-causing mechanisms and underscores NAA60-mediated Nt-acetylation of transmembrane proteins as a fundamental process for healthy neurobiological functioning.

**Grant References:** The Wellcome Trust (104033), European Research Council (ERC) (772039).

**Conflict of Interest:** None declared

### C10.4 Missense variants in RPH3A cause defects in excitatory synaptic function and are associated with a clinically variable neurodevelopmental disorder

***Lisa Pavinato***[1], *Jennifer Stanic*[2], *Marta Barzasi*[2], *Antonia Gurgone*[3], *Giuseppe Chiantia*[3], *Valentina Cipriani*[4], *Ivano Eberini*[2], *Luca Palazzolo*[2], *Monica Diluca*[2], *Alex Costa*[5;6], *Andrea Marcantoni*[7], *Elisa Biamino*[8], *Marco Spada*[8], *Susan Hiatt*[9], *Whitley V. Kelley*[9], *Letizia Vestito*[4], *Sanjay M. Sisodiya*[10;11], *Alessandro Bruselles*[12], *Simona Cardaropoli*[13], *Stephanie Efthymiou*[14], *Prem Chand*[15], *Rauan Kaiyrzhanov*[16], *Marco Tartaglia*[17], *Silvia De Rubeis*[18;19;20;21], *Joseph Buxbaum*[18;19;20;21;22], *Damian Smedley*[4], *Giovanni Battista Ferrero*[23], *Maurizio Giustetto*[3], *Fabrizio Gardoni*[2], *Alfredo Brusco*[1;24]

[1]*University of Turin, Department of Medical Sciences, Turin, Italy;* [2]*University of Milan, Department of Pharmacological and Biomolecular Sciences, DiSFeB, Milan, Italy;* [3]*University of Turin, Department of Neuroscience, Turin, Italy;* [4]*Queen Mary University of London, William Harvey Research Institute, Clinical Pharmacology Precision Medicine, London, United Kingdom;* [5]*University of Milan, Department of Biosciences, Milan, Italy;* [6]*Consiglio Nazionale delle Ricerche, Institute of Biophysics, Milan, Italy;* [7]*University of Turin, Department of Drug Science and Technology, Turin, Italy;* [8]*Regina Margherita Children Hospital, Department of Pediatrics, Turin, Italy;* [9]*HudsonAlpha Institute for Biotechnology, Huntsville, United States;* [10]*UCL Queen Square Institute of Neurology, Department of Clinical and Experimental Epilepsy, London, United Kingdom;* [11]*Chalfont Centre for Epilepsy Bucks, London, United Kingdom;* [12]*Istituto Superiore di Sanità, Department of Oncology and Molecular Medicine, Rome, Italy;* [13]*University of Turin, Department of Public Health and Pediatric Sciences, Turin, Italy;* [14]*UCL Queen Square Institute of Neurology, Department of Neuromuscular diseases, London, United Kingdom;* [15]*Aga Khan University Hospital, Department of Paediatric and Child Health, Karachi, Pakistan;* [16]*University College London, UCL Queen Square Institute of Neurology, London, United Kingdom;* [17]*Ospedale Pediatrico Bambino Gesù, Genetics and Rare Diseases Research Division, Rome, Italy;* [18]*Icahn School of Medicine at Mount Sinai, Seaver Autism Center for Research and Treatment, New York, United States;* [19]*Icahn School of Medicine at Mount Sinai, Department of Psychiatry, New York, United States;* [20]*Icahn School of Medicine at Mount Sinai, The Mindich Child Health and Development Institute, New York, United States;* [21]*Icahn School of Medicine at Mount Sinai, Friedman Brain Institute, New York, United States;* [22]*Icahn School of Medicine at Mount Sinai, Department of Neuroscience, New York, United States;* [23]*University of Turin, Department of Clinical and Biological Sciences, Turin, Italy;* [24]*Città della Salute e della Scienza University Hospital, Medical Genetics Unit, Turin, Italy*

**Background/Objectives:** *RPH3A* encodes for a protein that stabilizes synaptic NMDA glutamatergic receptors at cell surface through the formation of a ternary complex with PSD95 and the GluN2A subunit, a fundamental step for the correct activation of the signal following the long-term potentiation induction, proper synaptic adaptation, and cognitive behaviour.

**Methods:** By trio-based exome sequencing we identified six heterozygous variants in *RPH3A* in patients affected by neurodevelopmental disorders (NDDs). In silico and in vitro models, including rat hippocampal neuronal cultures, have been used to characterize the effect of the variants.

**Results:** Four cases had a NDD with untreatable epileptic seizures [p.(Gln73His)d.n.; p.(Thr450Ser)d.n.; p.(Arg209Lys); p.(Gln508His)], two cases [p.(Asn618Ser)d.n.; p.(Arg235Ser)] showed high functioning autism spectrum disorder (ASD). Using neuronal cultures, we analysed p.(Arg209Lys), p.(Thr450Ser), p.(Gln508His) and p.(Asn618Ser) variants, showing a decreased synaptic localization of GluN2A, while p.(Thr450Ser) and p.(Gln508His) variants also increased GluN2A surface levels. Using a dendritic spines-specific probe, we reported also an alteration of postsynaptic calcium levels at resting state. Electrophysiological recordings and analysis of dendritic spines morphology were also performed for p.(Thr450Ser) and p.(Asn618Ser), showing increased GluN2A-dependent NMDAR currents for both analysed mutants and impairment of dendritic spines morphology for p.(Thr450Ser).

**Conclusion:** Overall, our data show that missense gain-of-function variants in *RPH3A* are involved in onset of NDDs, and their impact on neuronal function can be various and associate with a broad range of disease severity. We propose *RPH3A* as a novel candidate gene for a NDD associated with variable clinical presentation ranging from untreatable epilepsy to ASD.

**Conflict of Interest:** Lisa Pavinato Holder of stock units of REGN, VRTX, LLY, NOVO-B.CO, PACB, AMGN, Jennifer Stanic: None declared, Marta Barzasi: None declared, Antonia Gurgone: None declared, Giuseppe Chiantia: None declared, Valentina Cipriani: None declared, Ivano Eberini: None declared, Luca Palazzolo: None declared, Monica Diluca: None declared, Alex Costa: None declared, Andrea Marcantoni: None declared, Elisa Biamino: None declared, Marco Spada: None declared, Susan Hiatt: None declared, Whitley V. Kelley: None declared, Letizia Vestito: None declared, Sanjay M. Sisodiya: None declared, Alessandro Bruselles: None declared, Simona Cardaropoli: None declared, Stephanie Efthymiou: None declared, Prem Chand: None declared, Rauan Kaiyrzhanov: None declared, Marco Tartaglia: None declared, Silvia De Rubeis: None declared, Joseph Buxbaum: None declared, Damian Smedley: None declared, Giovanni Battista Ferrero: None declared, Maurizio Giustetto: None declared, Fabrizio Gardoni: None declared, Alfredo Brusco: None declared

### C10.5 Genetic prevalence and population diversity of repeat expansion disorders

***Kristina Ibañez***[1], *Bharati Jadhav*[2], *Matteo Zanovello*[3], *Stefano Facchini*[3], *Alejandro Martin Trujillo*[4], *Valentina Galassi Deforie*[3], *Delia Gagliardi*[3], *Scott Gies*[4], *Maryam Shoai*[3], *Genomics England Research Consortium*[5], *Mark Caulfield*[1], *Andrea Cortese*[3], *John J. Hardy*[3], *Henry Houlden*[3], *Andrew Sharp*[4], *Arianna Tucci*[1]

[1]*William Harvey Research Institute, London, United Kingdom;* [2]*Icahn School of Medicine at Mount Sinai, Department of Genetics and Genomic Sciences and Mindich Child Health and Development Institute, New York, United States;* [3]*UCL Queen Square Institute of Neurology, London, United Kingdom;* [4]*Icahn School of Medicine at Mount Sinai, New York, United States;* [5]*Genomics England Research Consortium, London, United Kingdom*

Repeat expansion disorders (REDs) include some of the most common inherited neurological diseases such as myotonic dystrophy. REDs have a worldwide distribution and are estimated to affect ~1 per 3,000 individuals. Prevalence studies of REDs are hampered by heterogeneous clinical presentation leading to under-ascertainment, variable geographic distributions, and by technological limitations for screening large numbers of individuals. Here, we leveraged whole genome sequencing (WGS) data in the 100,000 Genomes Project and TopMed cohorts to estimate RED prevalence globally and among different ancestral groups. RED loci (*AR, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, CACNA1A, C9orf72, DMPK, HTT, FXN, JPH3* and *TBP*) were genotyped in a total of 82,176 unrelated individuals, including 59,516 Europeans, 12,886 Africans, 5,682 Americans, 2,881 South Asians, and 1,265 East Asians.

We found that the overall carrier frequency of REDs is approximately 1 per 300, which is ten times higher than the previously estimated prevalence. Overall, the most frequent pathogenic repeat expansions are those in *C9orf72*, *DMPK* and surprisingly *ATXN2*. In contrast, some REDs like *ATN1* are population-specific. Furthermore, we observed repeat lengths at specific loci (*AR*) are shorter in African genomes compared to other populations. Modelling disease prevalence using carrier frequency, age at onset and survival for each RED, we show a significant increase compared to currently reported figures. This is likely due to underdiagnosis of REDs. These results have important implications for local and global health communities.

**Conflict of Interest:** Kristina Ibañez part-time, MRC, Bharati Jadhav full, Matteo Zanovello full, Stefano Facchini full, Alejandro Martin Trujillo full, Valentina Galassi Deforie full, Delia Gagliardi full, Scott Gies: None declared, Maryam Shoai: None declared, Genomics England Research Consortium: None declared, Mark Caulfield: None declared, Andrea Cortese full, John J. Hardy full, Henry Houlden full, Andrew Sharp full, Arianna Tucci full

### C10.6 Position effects in the 3D genome as the cause of demyelinating leukodystrophy

***Jana Henck***[1;2], *Varun Sreenivasan*[1], *Saranya Balachandran*[1], *Josh Kim*[1;2], *Mai Phan*[2;3], *Konrad Chudzik*[2], *Lars Wittler*[4], *Elisa Giorgio*[5;6], *Malte Spielmann*[1;2]

[1]*Institute of Human Genetics, University Medical Center Schleswig-Holstein, University of Lübeck & Kiel University, Lübeck, Germany;* [2]*Max Planck Institute for Molecular Genetics, RG Development & Disease, Berlin, Germany;* [3]*Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin, Berlin, Germany;* [4]*Max Planck Institute for Molecular Genetics, Berlin, Germany;* [5]*University of Pavia, Department of Molecular Medicine,, Pavia, Italy;* [6]*IRCCS Mondino Foundation, Medical Genetics Unit, Pavia, Italy*

**Background/Objectives:** Structural variants (SVs) can lead to repositioning of topologically associated domain (TAD) boundaries or relocation of regulatory elements, potentially resulting in gene missexpression and disease. These 3D position effects have been described for rare skeletal phenotypes, but have yet to be studied in higher prevalence diseases such as neurodegenerative disorders. Here we chose to study autosomal dominant adult-onset demyelinating leukodystrophy (ADLD) as a model for neurodegenerative disorders. ADLD is caused by duplications of *LMNB1*, but recently we identified patients with deletions downstream of *LMNB1* with a very similar phenotype, potentially caused by disruption of a TAD boundary.

**Method:** Here, we generated several mouse models using the CRISPR/Cas9 systems of the different SVs found in patients,

followed by investigating 3D genome changes using HiC and gene expression changes using single cell RNA sequencing.

**Results:** Analyzing deletion mutant brains we observe increased contact from *Lmnb1* to the neighboring TAD with distinct loops connecting *Lmnb1* with neuron specific regulatory elements, indicating enhancer hijacking as a disease mechanism. Already in embryonic mutant brains we detected cell type changes in neuronal progenitors and the glia trajectory, indicating that ADLD can be caused by misregulating *Lmnb1* through ectopic contacts with regulatory elements.

**Conclusion:** In summary, we show that deletions downstream of *LMNB1* cause ADLD via enhancer hijacking. Our data further highlight that changes in the 3D organization can result in neurodegenerative disorders.

**Conflict of Interest:** None declared

## C11 GENETIC EPIDEMIOLOGY OF MOLECULAR TRAITS

### C11.1 Single-cell consortium for federated PBMC data pipeline for cell-type specific eQTL mapping and downstream analyses

***Dan Kaptijn***[1;2], *Maryna korshevniuk*[1;2], *Roy Oelen*[1;2], *Lude Franke*[1;2], *Monique van der Wijst*[1;2], *Marc Jan Bonder*[1;3]

[1]*University Medical Center Groningen, University of Groningen, Genetics, Groningen, Netherlands;* [2]*Oncode Investigator, Groningen, Netherlands;* [3]*Deutsches Krebsforschungszentrum, Computational Genomics and Systems Genetics, Heidelberg, Germany*

**Background/Objectives:** Over the last decade, single-cell RNA sequencing (scRNA-seq) has grown in popularity as a potent alternative to bulk RNA-seq, as scale, cost and sensitivity have significantly improved. These developments have now paved the way for the generation of large numbers of population-based scRNA-seq datasets that are a valuable resource for studying the effect of genetic variation (through expression quantitative trait locus (eQTL) mapping) on expression of individual cell types. To fully leverage these emerging data resources, we have founded the single-cell eQTLGen consortium (sc-eQTLGen).

**Methods:** We have developed a pipeline that harmonizes the preprocessing, QC, cell type annotation and eQTL mapping of peripheral blood mononuclear cell (PBMC) scRNA-seq data. Every cohort in the consortium has run these pipelines, and shared the non-privacy sensitive summary statistics, which we combine in a federated manner .

**Results:** Here, we present the results of the first data freeze in which we have meta-analyzed scRNA-seq-derived eQTL summary statistics of over 2,000 individuals for each of the major cell types in PBMCs. These results reveal the cell types in which eQTLs are to be identified.

**Conclusion:** Upcoming data freezes will further increase the sample size, cell type resolution, and number of donor characteristics under study, and will expand our analyses to other data modalities. Thereby, we expect that the sc-eQTLGen framework enables us and the community at large to gain a more complete understanding of gene expression and its regulation in health and disease.

**Grant References:** Horizon2020 №860895 (LF)

**Conflict of Interest:** None declared

### C11.2 Causes and consequences of telomere shortening: A Mendelian randomisation study

***Samuel Moix***[1], *Chiara Auwerx*[1;2;3], *Marie Sadler*[1;3;4], *Zoltan Kutalik*[1;3;4]

[1]*University of Lausanne, Department of Computational Biology, Lausanne, Switzerland;* [2]*University of Lausanne, Center for Integrative Genomics, Lausanne, Switzerland;* [3]*Swiss Institute of Bioinformatics, Lausanne, Switzerland;* [4]*University Center for Primary Care and Public Health, Lausanne, Switzerland*

**Background/Objectives:** Telomeres form repeated DNA sequences at the ends of chromosomes, whose length shortens with each cell division. Factors modulating their attrition and the consequences of this phenomenon on human health remain poorly understood.

**Methods:** Using data from 326,363 unrelated white UK Biobank participants, we used linear regression and bidirectional univariable (MR) and multivariable Mendelian randomization (MVMR) to assess the relationships between leukocyte telomere length (TL) and 147 complex traits, including biomarkers, diseases, and lifestyle factors.

**Results:** TL significantly decreases with age ($p < 2.2e-16$), with a stronger ($p_{diff.}=5.5e-28$) decline in males ($\beta_{males} = -0.026$) than in females ($\beta_{females} = -0.021$) that could not be explained by sex-differences in sex hormones or lifestyle factors. After correcting for age, age$^2$ and sex, 95 traits significantly associated with TL, with cardiovascular, pulmonary, and renal conditions showing negative associations, while lipoprotein levels, cancer, and female reproductive traits were positively associated.

Inverse-variance weighted MR identified 27 traits affected by TL, e.g., forced vital capacity ($\alpha_{ivw} = 0.073$; $p = 3.2e-6$), systemic lupus erythematosus ($\alpha_{ivw} = 0.167$; $p = 5.8e-5$), and vessel aneurysm ($\alpha_{ivw} = -0.200$; $p = 1.2e-5$), and 23 traits modulating TL, including drinking ($\alpha_{ivw} = -0.086$; $p = 1.3e-4$), smoking cessation ($\alpha_{ivw} = -0.142$; p = 1.8e-4), BMI ($\alpha_{ivw} = -0.048$; $p = 4.9e-10$), and educational attainment ($\alpha_{ivw} = 0.075$; $p = 2.2e-15$). MVMR identified age at last birth and educational attainment as potential mediators for the MR effect of BMI on TL.

**Conclusion:** We provide new insights into the biology of telomeres, by distinguishing between modulators, mediators and consequences of telomere shortening.

**Grant References:** SNSF 310030-189147

**Conflict of Interest:** None declared

### C11.3 Genetic regulation of the human plasma proteome in children and adolescents

***Lili Niu***[1;2], *Sara Stinson*[3], *Louise Aas Holm*[3;4], *Morten Lund*[4;5], *Cilius Esmann Fonvig*[3;4;6], *Leonardo Cobuccio*[1], *Jonas Meisner*[1], *Helene Bæk Juel*[3], *Jens-Christian Holm*[3;4;6], *Simon Rasmussen*[1], *Torben Hansen*[3], *Matthias Mann*[1;2]

[1]*University of Copenhagen, Novo Nordisk Foundation Center for Protein Research, Copenhagen, Denmark;* [2]*Max Planck Institute of Biochemistry, Department of Proteomics and Signal Transduction, Martinsried, Germany;* [3]*University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research, Copenhagen, Denmark;* [4]*Copenhagen University Hospital Holbæk, Department of Pediatrics, Holbæk, Denmark;* [5]*University of Copenhagen, Department of Biomedical Sciences, Copenhagen, Denmark;* [6]*University of Copenhagen, The Faculty of Health and Medical Sciences, Copenhagen, Denmark*

**Background/Objectives:** Understanding the genetic underpinning of the human plasma proteome is crucial for biomarker research and drug development. A few proof-of-concept studies using mass spectrometry (MS)-based proteomics have identified dozens of protein quantitative trait loci (pQTLs) for plasma proteins. However, these studies are limited by small sample sizes and/or proteome depths. Existing large-scale studies using affinity-based proteomics have focused on adult populations.

We aim to investigate the impact of genetic variation on the human plasma proteome in children and adolescents using MS-based proteomics.

**Methods:** We performed plasma proteomics on a discovery cohort of 2147 children aged 5–20 years and a replication cohort of adults aged 18–82 years ($n = 558$), both recruited from Denmark. We performed genome-wide association analysis between 420 proteins and 5.2 million SNPs using a linear mixed model.

**Results:** We identified more than 1000 primary pQTLs ($p < 5 \times 10^{-8}$) for half of the measured plasma proteins, 30% of which were novel, including pQTLs for 50 proteins not assayed in previous studies. Approximately 90% of the pQTLs were successfully replicated in the validation cohort ($p < 0.05$), with highly concordant effect sizes in direction and strength.

**Conclusion:** This study provides the largest set of pQTLs for plasma proteins identified by MS-based proteomics. Our quantitative data demonstrate broad and, in some instances, drastic genetic effects on plasma protein levels in both children and adults. These findings provide new insights into the molecular underpinnings of the human plasma proteome with great potential in improving biomarker research and drug development.

**Grant References:** Novo Nordisk Fonden

**Conflict of Interest:** None declared

### C11.4 Genome-wide association analysis of plasma lipidome identifies 495 genetic associations

***Linda Ottensmann***[1], *Rubina Tabassum*[1], *Sanni Ruotsalainen*[1], *Mathias Gerl*[2], *Christian Klose*[2], *Elisabeth Widén*[1], *Kai Simons*[2], *Samuli Ripatti*[1;3;4], *Matti Pirinen*[1;3;5]

[1]*Institute for Molecular Medicine Finland, Helsinki, Finland;* [2]*Lipotype GmbH, Dresden, Germany;* [3]*Department of Public Health, Clinicum, Faculty of Medicine, University of Helsinki, Helsinki, Finland;* [4]*Broad Institute of the Massachusetts Institute of Technology and Harvard University, Cambridge, United States;* [5]*Department of Mathematics and Statistics, University of Helsinki, Helsinki, Finland*

**Background/Objectives:** Human plasma lipidome captures risk for cardiovascular diseases (CVD) and beyond. Genome-wide association studies (GWAS) of individual lipid species (univariate analysis) have identified many lipid-associated loci, however the influence of genetic variants on lipid metabolism and CVD risk is still mostly not understood. Multivariate analysis of correlated lipid species improves statistical power and therefore has potential to identify additional lipid-associated loci and identify the causal variants underlying the associations.

**Methods:** We performed univariate GWAS of 179 lipid species in 7174 participants from the Finnish GeneRISK cohort and multivariate analyses for 11 clusters of correlated lipid species using metaCCA software. We further fine-mapped the associated loci with FINEMAP software, prioritized genes, and examined their disease links in 377,277 FinnGen participants.

**Results:** We identified 495 genome-trait associations in 56 genetic loci with a considerable boost provided by multivariate analysis. We identified 9 new loci in or near genes *DTL*, *STK39*, *CDS1*, *AGPAT2*, *SGPL1*, *YPEL2*, *KCNJ12*, *SPHK2*, and *AGPAT3* in multivariate analysis, of which only *AGPAT2* and *SGPL1* reached the threshold in univariate analysis. For 26 loci, fine-mapping identified variants with a high causal probability, including 14 coding variants indicating likely causal genes. Phenome-wide analysis across 953 disease endpoints in FinnGen revealed disease associations for 40 lipid loci. For 11 known coronary artery disease risk variants, we detected strong associations with lipid species.

**Conclusion**: Our study demonstrates the power of multivariate genetic analysis in correlated lipidomics data and reveals genetic links between diseases and detailed lipid measures beyond standard lipids.

**Conflict of Interest:** Linda Ottensmann: None declared, Rubina Tabassum: None declared, Sanni Ruotsalainen: None declared, Mathias Gerl employee of Lipotype GmbH, Christian Klose shareholder of Lipotype GmbH, Elisabeth Widén: None declared, Kai Simons CEO of Lipotype GmbH, shareholder of Lipotype GmbH, Samuli Ripatti: None declared, Matti Pirinen: None declared

### C11.5 Spatial transcriptomics data identifies disease-relevant tissue structures from genetically implicated GWAS genes and drug targets

***Linda Kvastad***[1], *Aaron Kollotzek*[1], *Chih-Fan Chang*[1], *Tuuli Lappalainen*[1;2;3]

[1]*Science for Life Laboratory, KTH Royal Institute of Technology, Gene Technology, Stockholm, Sweden;* [2]*New York Genome Center, New York, United States;* [3]*Columbia University, Department of Systems Biology, New York, United States*

Pinpointing the causes and discovering effective treatment targets for complex diseases remains challenging. However, it has been estimated that selecting genetically supported drug targets could increase new drugs' clinical development success rate. The Open Targets platform provides a systematic drug-target identification and prioritization resource, integrating publicly available datasets and scoring target-disease associations. In this study, we integrate these data with published spatial transcriptomics (ST) datasets of healthy tissues from humans and mice to identify and prioritize spatial structures of interest for complex disease traits. Comparing disease-associated genes per trait to a random set of genes, we found that in the brain, genes associated with Alzheimer's disease were enriched for expression in the thalamus and the white matter fiber tracts detected in ST data. In the colon, genes associated with inflammatory bowel disease had an enriched expression in cells located at the top of the villus and in submucosal lymphoid follicles. These enrichments were particularly pronounced for top-ranked genes based on the strength of genetic evidence. Furthermore, schizophrenia-associated genes indicate enrichment in the cortex and hippocampus, with ongoing analyses of diverse diseases and traits linked to the heart and liver. Interestingly, drug target genes often showed a different spatial tissue enrichment pattern than genetically implicated disease genes, pinpointing novel opportunities for drug development. Altogether, in addition to discovery of tissue structures where causal complex disease processes take place, this work highlights the potential of integration of emerging spatial transcriptomics and human genetics data.

**Conflict of Interest:** Linda Kvastad: None declared, Aaron Kollotzek: None declared, Chih-Fan Chang: None declared, Tuuli Lappalainen Equity in Variant Bio., Paid advisor to GSK, Pfizer, Goldfinch Bio and Variant Bio

### C11.6 Proteome-wide analysis in 124,000 individuals identifies novel putative drug targets for chronic kidney disease

***Joao Fadista***[1], *Sile Hu*[1], *Vivek Das*[2], *Anil Karihaloo*[3], *Joanna Howson*[1], *Dmitry Shungin*[1]

[1]*Human Genetics Centre of Excellence, Novo Nordisk, Måløv, Denmark, Oxford, United Kingdom;* [2]*Bioinformatics and Data Mining, Digital Science and Innovation, Novo Nordisk, Måløv, Denmark;* [3]*T1D & Kidney Disease, Novo Nordisk Research Centre, Seattle, United States*

**Background/Objectives:** Chronic kidney disease (CKD) affects ~800 million individuals globally yet has a large unmet therapeutic

need. Given proteins represent ~80% of therapeutic targets, we integrated proteomics with genetics to discover new therapeutic targets for CKD, in the largest study to date.

**Methods:** We performed GWAS of ~1500 plasma proteins in 35,571 participants from the UK Biobank Pharma Proteomics Project and derived 1008 cis- protein quantitative trait loci (pQTL), which were used together with previously published cis-pQTLs for 2363 proteins (from deCODE, ARIC, Fenland, INTERVAL, EPIC-Norfolk, SCALLOP) to perform systematic Mendelian Randomization (MR) in 124,463 individuals to derive per study effects on estimated glomerular filtration rate (eGFR), a key clinical indicator of renal function. To assess tissue specificity, we combined results with published transcriptome-wide eQTLs in tubular and glomerular kidney tissues.

**Results:** We found genetically predicted levels of 98 proteins associated with eGFR ($P_{MR} < 0.05/20{,}000 = 2.5 \times 10^{-6}$) with strong genetic colocalization. Integration with kidney eQTLs intersected 27 proteins prioritized in both sources, representing a set of potential causal proteins of high therapeutic value. Of these proteins, 16 were consistent with inhibition and 11 with activation as drug target modality. Genetically predicted kidney gene expression of an additional 166 genes were also causally associated with eGFR with strong genetic colocalization. Among prioritized proteins several had been reported as relevant for CKD (UMOD, MANBA, DPEP1 and CHMP1A) while others are novel representing new therapeutic opportunities.

**Conclusion:** This study elucidated novel biological underpinnings behind kidney function and prioritized 264 putative drug targets for CKD.

**Grant References:**NA

**Conflict of Interest:** Joao Fadista Novo Nordisk, Sile Hu Novo Nordisk, Vivek Das Novo Nordisk, Anil Karihaloo Novo Nordisk, Joanna Howson Novo Nordisk, Dmitry Shungin Novo Nordisk

## C12 CANCER GENETIC SERVICES

### C12.1 Evaluation of the breast cancer mainstream genetic testing program at the Parkville Familial Cancer Centre, Australia: Patients and clinicians' experiences and health service outcomes

*Katrina Monohan[1;2], Catherine Beard[1;2;3], **Tiffany O'Brien**[4], Linda Cicciarelli[1], Nitzan Lang[5], Kirsten Allan[6], Geoff Lindeman[2;3;7], Gregory Bruce Mann[8], Paul James[1;2;9], Laura Forrest[1;2;9]*

*[1]Parkville Familial Cancer Centre, Peter MacCallum Cancer Centre, Melbourne, Australia; [2]The Royal Melbourne Hospital, Parkville Familial Cancer Centre and Genomic Medicine, Parkville, Australia; [3]The University of Melbourne, Department of Medicine, Parkville, Australia; [4]Peter MacCallum Cancer Centre, Melbourne, Australia; [5]University of Technology Sydney, Graduate School of Health, Chippendale, Australia; [6]The University of Melbourne, Department of Paediatrics, Parkville, Australia; [7]The Walter and Eliza Hall Institute, Breast Cancer Laboratory, Parkville, Australia; [8]The Royal Melbourne Hospital, Department of Surgery, Parkville, Australia; [9]The University of Melbourne, Sir Peter MacCallum Department of Oncology, Parkville, Australia*

**Background**: Increasing demand for genetic testing for breast cancer patients has necessitated new models of care. To improve accessibility, the Parkville Familial Cancer Centre (PFCC) established a mainstream breast cancer genetic testing program in oncology clinics. A program evaluation was undertaken after 2 years aiming to evaluate patient experiences and outcomes, clinician impact, and health service implications.

**Methods**: Data were collected via clinical audit, patient survey and semi-structured interviews, and breast specialist survey. Descriptive analyses were undertaken for quantitative measures and content analysis for qualitative data.

**Results***:* Between 2017 and 2019, 72 breast specialists from nine hospitals facilitated genetic testing for 230 patients, resulting in treatment changes for 87% patients. Forty-seven patients (20.4%) attended a PFCC appointment after mainstream testing, with 413 PFCC appointments saved. Sixty-eight patients (30%) completed the survey with most satisfied with the information provided by their breast specialist before testing (94%) and after results (86%). Twenty patients were interviewed and most preferred mainstream testing due to the existing relationship with their breast specialist and feeling overwhelmed by many treatment-related appointments. Forty-five breast specialists responded (63%); most had discussed (87%) and consented (80%) patients for mainstream genetic testing. The majority (89%) believed mainstream genetic testing should be part of their role and felt well supported by the PFCC (90%).

**Conclusion**: The PFCC mainstreaming model increased access to genetic testing, is acceptable, and introduced health service efficiencies. This evidence supports scalability of this model to other sites and other cancer types.

**Conflict of Interest:** None declared

### C12.2 The efficacy of genetic counselling for familial colorectal cancer: findings from a randomised controlled trial

***Andrada Ciuca***[1], *Adriana Baban[1], Tara Clancy[2;3], Ramona Moldovan[1;2;3]*

*[1]Babes-Bolyai University, Cluj-Napoca, Romania; [2]Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom; [3]Division of Evolution and Genomic Sciences, School of Biological Science, University of Manchester, Manchester, United Kingdom*

**Background:** Genetic counselling (GC) for familial colorectal cancer (fCRC) has been previously shown to improve outcomes such as emotional distress and screening adherence. This is the first randomised clinical trial to evaluate the efficacy of GC for fCRC.

**Method:** We included individuals affected or at-risk for fCRC (Lynch syndrome, APC-associated polyposis, other risk-associated pathogenic variants and clinically defined fCRC). Participants were randomised to (1) standard care or (2) standard care and genetic counselling. Measures include empowerment, anxiety, depression, knowledge, risk perception, emotional distress, screening/surveillance behaviours, perceived social support, decisional conflict and quality of life.

**Results:** We recruited 82 participants. The average age was 44.81 years old, with 52.4% females. There was a significant effect in the counselling group (42/82) on post-intervention empowerment scores compared to control group (40/82) after controlling for pre-intervention empowerment scores ($p = 0.0043$, $d = 0.60$), and depression scores ($p = 0.025$, $d = 0.17$). Further analysis showed that participants in the counselling group had significantly improved post-intervention anxiety ($p = 0.04$, $d = 0.54$), depression ($p = 0.03$, $d = 0.55$), emotional distress ($p = 0.03$, $d = 0.44$), and knowledge ($p = 0.025$, $d = 0.45$) scores compared to pre-intervention scores. Exploratory analysis showed that emotional distress had a moderating effect, individuals with lower initial emotional distress benefit more in terms of empowerment at post-intervention ($\beta = -0.26$, $t(59) = -2.49$, $p = 0.016$).

**Conclusions**: Our data show significant improvements for both primary endpoint (empowerment) and secondary endpoints (knowledge, depression, anxiety, emotional distress). Genetic

counselling is an effective intervention for fCRC both when the diagnosis is part of a syndrome or clinically defined, and both for affected or at-risk individuals.

**Conflict of Interest:** None declared

### C12.3 How well do cancer genetics clinicians report discussing psychosocial implications during cancer genetic counselling appointments? Results from the first 51 participant appointments in the PersOnalising gEneTIc Counselling (POETIC) trial, a hybrid effectiveness-implementation randomised clinical trial

***Laura Forrest***[1;2], *Rebecca Purvis*[1;2], *Amy Ruscigno*[1], *Erin Tutty*[1], *Paul James*[1;2]

[1]*Peter MacCallum Cancer Centre, Parkville Familial Cancer Centre, Melbourne, Australia;* [2]*The University of Melbourne, Sir Peter MacCallum Department of Oncology, Parkville, Australia*

**Background:** As genomic medicine becomes mainstream the psychosocial implications will remain and the need for genetic counselling will persist. POETIC will evidence whether using a screening tool, Genetic Psychosocial Risk Instrument (GPRI), ensures patient-centred care prevails irrespective of provider. While the trial is in progress, we aimed to examine clinicians' identification of psychosocial implications during cancer genetic counselling appointments.

**Methods:** POETIC participants consent to have their Parkville Familial Cancer Centre appointments audio-recorded. A checklist of 16 psychosocial implications is completed twice for every appointment, once by the clinicians after the appointment and independently by researchers using appointment audio-recordings. Descriptive and inferential statistics were used to compare the paired samples; $p < 0.05$ considered significant.

**Results:** From May 2022 to February 2023, 204 patients were invited, 67 consented and tilized, and 51 attended their first appointment. Twenty-five participants were consulted by genetic counsellors, 21 by medical practitioners, and 5 by genetics fellows. Psychosocial implications were most frequently discussed by genetics fellows ($\mu = 7.6$, 95%CI 5.0–10.1) and genetic counsellors ($\mu = 7.2$, 95%CI 6.2–8.2) with no differences identified by the researchers. Medical practitioners reported discussing a greater number of psychosocial implications ($\mu = 7.9$, 95%CI 6.6–9.2) compared to the number identified by researchers ($\mu = 5.7$, 95%CI 3.5–7.8, $p = 0.01$). Overall, risks to children and relatives, and family communication were most frequently discussed implications.

**Discussion:** Differences in genetic counselling practice are emerging. The impact of the GPRI on genetic counselling practice will be elucidated after 246 patients have been tilized and data collection completed (ANZCTR no.12621001582842p; Victorian Cancer Agency MCRF20012).

**Conflict of Interest:** None declared

### C12.4 MyCanRisk: Development of a public-facing app for CanRisk through user-centred design

***Stephanie Archer***[1;2], *Timothy Carver*[1], *Francisca Stutzin Donoso*[1], *Soh Yon Park*[3], *Nichola Fennell*[4], *Jonathan Roberts*[5;6], *Lorenzo Ficorella*[1], *Fiona Walter*[1;7], *Juliet Usher-Smith*[1], *marc tischkowitz*[4], *Douglas F. Easton*[1], *Antonis C. Antoniou*[1]

[1]*University of Cambridge, Department of Public Health and Primary Care, Cambridge, United Kingdom;* [2]*University of Cambridge, Department of Psychology, Cambridge, United Kingdom;* [3]*Imperial College London, Institute of Global Health Innovation, Department of Surgery and Cancer, London, United Kingdom;* [4]*University of Cambridge, Department of Medical Genetics, Cambridge, United Kingdom;* [5]*Cambridge University Hospitals NHS Foundation Trust, Clinical Genetics, Cambridge, United Kingdom;* [6]*Genomics England, London, United Kingdom;* [7]*Queen Mary University of London, Wolfson Institute of Population Health, Barts and the London School of Medicine and Dentistry, London, United Kingdom*

**Background/Objectives:** The CanRisk tool calculates personalised future risks of breast and ovarian cancer based on genetic and other risk factors. Since gaining CE marking in 2020, it has been used over 1.3 million times worldwide. CanRisk is acceptable for use in a variety of healthcare settings, but the time required to collect the risk factor information can be a barrier to its implementation. To address this issue, we aimed to develop a public-facing app for CanRisk to support the collection of risk factor information.

**Methods:** We conducted a six-month long user-centred design (UCD) process consisting of four testing workshops (three face-to-face, one online) that were supplemented with additional design/feedback activities. Participants involved in the UCD process included patients and members of the public, software designers, healthcare professionals and researchers.

**Results:** The UCD process resulted in the development of a platform independent app called MyCanRisk. The app supports on- and off-line collection of personal information about lifestyle, reproductive history and women's health, alongside detailed cancer family history in first and second degree relatives. Users can check the information added to MyCanRisk before sending the encrypted data to their healthcare professional, where further data, including genetic testing results, can be added/updated before the calculation is completed.

**Conclusion:** The prototype MyCanRisk app, developed by a multi-stakeholder group through UCD, has the potential to support the implementation of CanRisk through time-efficient collection of key risk information required to conduct multi-factorial breast and ovarian cancer risk assessments.

Grant References: Cancer Research UK (PPRPGM-Nov20\100002).

**Conflict of Interest:** Stephanie Archer: None declared, Timothy Carver Is a named inventor of BOADICEA v5 commercialised by Cambridge Enterprise, Francisca Stutzin Donoso: None declared, Soh Yon Park: None declared, Nichola Fennell: None declared, Jonathan Roberts: None declared, Lorenzo Ficorella: None declared, Fiona Walter: None declared, Juliet Usher-Smith: None declared, marc tischkowitz: None declared, Douglas F. Easton Is a named inventor of BOADICEA v5 commercialised by Cambridge Enterprise, Antonis C. Antoniou Is a named inventor of BOADICEA v5 commercialised by Cambridge Enterprise

### C12.5 Developing evidence for a polygenic breast cancer risk report: Consumers and clinicians' perspectives

*Sharne Limb*[1;2], ***Rebecca Purvis***[1;2], *Jack Wheeler*[2;3], *Matilda Hilton*[4], *Yuhan Shen*[5], *Christina Wade*[2;3], *Rowan Forbes Shepherd*[2], *Sibel Saya*[6], *Jon Emery*[6;7], *Paul James*[1;2], *Laura Forrest*[1;2]

[1]*The University of Melbourne, The Sir Peter MacCallum Department of Oncology, Melbourne, Australia;* [2]*Peter MacCallum Cancer Centre, Parkville Familial Cancer Centre, Melbourne, Australia;* [3]*The University of Melbourne, Department of Paediatrics, Melbourne, Australia;* [4]*University of Technology Sydney, Sydney, Australia;* [5]*The University of Melbourne, Melbourne Medical School, Melbourne, Australia;* [6]*The University of Melbourne, Centre for Cancer Research, Melbourne, Australia;* [7]*The University of Melbourne, Department of General Practice, Melbourne, Australia*

**Background:** Translating polygenic breast cancer information into health outcomes relies on effective communication to enable risk-

appropriate decision-making. Effective communication is a key implementation challenge and evidence-based communication resources are lacking.

***Aim:*** To examine stakeholders' perspectives about communicating polygenic breast cancer risk to inform the development of a clinical report.

**Methods:** The Theoretical Framework of Acceptability guided the study design. Data were collected using a mixed-methods approach examining stakeholders' perspectives on communicating polygenic breast cancer risk, including a comparison of four visual risk communication tools. Stakeholders included consumers and clinicians from genetics, oncology, and primary care disciplines.

**Results:** Surveyed consumers ($n = 165/504$, response rate 33%) reported an icon array and a graph of cumulative breast cancer risk over age impacted their affective attitude but were less burdensome to understand and more effective at communicating risk compared to a skewed normal distribution curve ($p < 0.05$). Eleven consumers were subsequently interviewed and reported they would want to know their polygenic breast cancer risk if it were offered. They also wanted written information after a verbal discussion with a clinician. Six genetics, nine oncology, and ten primary care clinicians were interviewed. Most thought reports should be tailored to the recipients' genomic literacy, with separate reports for patients and clinicians. Suggested report content included a plain language summary explaining the polygenic breast cancer risk result, numerical risk with risk stratification categories, cancer risk management recommendations, and references to sources.

**Conclusion:** This evidence will inform the development of a polygenic breast cancer report that will subsequently require evaluation.

**Conflict of Interest:** None declared

### C12.6 UK consensus guidance on prenatal diagnosis and preimplantation genetic testing for germline cancer susceptibility gene variants (gCSGV) by the CCG and FGG for the BSGM

*Anjana Kulkarni[1], Tazeen Ashraf[2], Carol Gardiner[3], Alison Hall[4], Mark Kilby[5], Alison Male[2], Alec McEwan[6], Rhianna Rakhra[1], Amy Taylor[7],* ***charlotte tomlinson***[1]

*[1]Guy's and St. Thomas' NHS Trust, London, United Kingdom; [2]Great Ormond Street Hospital, London, United Kingdom; [3]West of Scotland Clinical Genetics Service, Glasgow, United Kingdom; [4]PHG Foundation, Cambridge, United Kingdom; [5]Birmingham Women's & Children's Foundation Trust, Birmingham, United Kingdom; [6]Nottingham University Hospital, Nottingham, United Kingdom; [7]Cambridge University Hospital, Cambridge, United Kingdom*

**Background:** Testing for germline cancer susceptibility gene variants (gCSGV) is common practice. For some high risk gCSGV, prenatal diagnosis (PND) and pre-implantation testing for monogenic disorders (PGT-M) has been advocated by healthcare professionals, legal bodies and the public. However, use of these reproductive options for genes of moderate penetrance or those with variable penetrance, depending on whether the gCSGV carrier is male or female, has been more contentious. These factors and the resulting complexity have led to discrepancy in clinical practice to PND and PGT-M for families with gCSGV in the UK.

**Methods:** A multi-disciplinary working group of delegates from across the UK in fields of fetal medicine, prenatal genetics, cancer genetics, PGT-M, ethics & patient groups were involved in a workshop held in November 2020 which discussed areas of consensus and contention regarding PND and PGT-M for gCSGV. Subsequently three workstreams then collaboratively produced best practice guidance which was ratified through stakeholder consultation.

**Results:** The guidance describes how individuals and couples at risk of having a child with a gCSGV should always have the chance to discuss their reproductive choices. It includes a genetic counselling framework and patient information leaflet to support healthcare professionals (HCP) and individuals with a gCSGV.

**Conclusion:** All individuals and couples at risk of having a child with a gCSGV should be made aware of their reproductive choices through their genetic testing process. Those considering PND and PGT-M should have counselling from an appropriately trained genetic/genomic healthcare professional using the framework provided.

**Grant References:** None

**Conflict of Interest:** None declared

## C13 DEVELOPMENTAL ANOMALIES

### C13.1 Variants in SART3 cause a novel spliceosomopathy characterised by failure of testis development and neuronal defects

*Katie Ayers[1;2], Stefanie Eggers[3], Ben Rollo[4], Katherine Smith[5], Nadia Davidson[5], Nicole Siddall[6], Liang Zhao[7], Josephine Bowles[8], Karin Weiss[9], Ginevra Zanni[10], lydie BURGLEN[11;12], Shay Ben Shachar[13], Jenny Rosensaft[14], annick raas-rothschild[15;16], Anne Jorgensen[17], Ralf Schittenhelm[18], Cheng Huang[18], Gorjana Robevska[1], Jocelyn van den Bergen[1], Franca Casagranda[6], Justyna Cyza[1], Svenja Pachernegg[1;2], David Wright[4], Melanie Bahlo[5], Alicia Oshlack[19;20], Terence O'Brien[4], Patrick Kwan[4], Peter Koopman[7], Gary Hime[6], Nadine Girard[21], Chen Hoffmann[22], Yuval Shilon[23], Amnon Zung[24;25], Enrico Bertini[10], Mathieu MILH[21], Bochra Ben Rhouma[26;27], Neila Belguith[27;28], anu bashamboo[29], Ken McElreavey[29], Ehud Banne[14;30], Naomi Weintrob[16;31], Bruria Ben Zeev[32],* ***Andrew Sinclair***[1;2;3]

*[1]Murdoch Children's Research Institute, Melbourne, Australia; [2]University of Melbourne, Department of Paediatrics, Melbourne, Australia; [3]Victorian Clinical Genetics Services, Melbourne, Australia; [4]Monash University, Alfred Centre, Department of Neuroscience, Central Clinical School, Melbourne, Australia; [5]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; [6]University of Melbourne, Department of Anatomy and Physiology,, Melbourne, Australia; [7]The University of Queensland, Brisbane, Australia; [8]The University of Queensland, School of Biomedical Sciences, Brisbane, Australia; [9]Technion-Israel Institute of Technology, The Genetics Institute Rambam Health Care Campus, Haifa, Israel; [10]Bambino Gesù Children's Hospital, IRCCS, Department of Neurosciences, Rome, Italy; [11]Sorbonne Université, Hôpital Trousseau, Centre de Référence des Malformations et Maladies Congénitales du Cervelet, Et Laboratoire de Neurogénétique Moléculaire, Département de Génétique et Embryologie Médicale, APHP, Paris, France; [12]Imagine Institute, Developmental Brain Disorders Laboratory, Paris, France; [13]Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; [14]Hebrew University Hadassah Medical School, Genetics Institute, Kaplan Medical Center,, Rehovot, Israel; [15]Chaim Sheba Medical Center, Edmond and Lily Safra Children's Hospital,, Ramat Gan, Israel; [16]Tel Aviv University,, Sackler School of Medicine, Tel Aviv, Israel; [17]Copenhagen University Hospital (Rigshospitalet), Department of Growth and Reproduction,, Copenhagen, Denmark; [18]Monash University, Monash Proteomics & Metabolomics Facility, Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology,, Clayton, Australia; [19]The Peter Maccallum Cancer Centre,, Melbourne, Australia; [20]The University of Melbourne, Department of BioScience, Melbourne, Australia; [21]Aix-Marseille Univ. APHM., Department of Neuroradiology, Timone Hospital, Marseille, France; [22]Sheba medical Centre, Radiology department, Tel Aviv, Israel;*

[23]Hebrew University Hadassah Medical School,, Kaplan Medical Center, Rehovot, Israel; [24]Kaplan Medical Center, Pediatrics Department,, Rehovot, Israel; [25]Hadassah Medical School, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel; [26]University of Gabes, Higher Institute of Nursing Sciences of Gabes, Gabes, Tunisia; [27]Sfax University, Laboratory of Human Molecular Genetics, Faculty of Medicine of Sfax,, Sfax, Tunisia; [28]Charles Nicolle Hospital,, Department of Congenital and Hereditary Diseases, Tunis, Tunisia; [29]Institut Pasteur, Paris, France; [30]Wolfson Medical Center, The Rina Mor Genetic Institute, Holon, Israel; [31]Dana-Dwek Children's Hospital, Tel Aviv Medical Center, Pediatric Endocrinology Unit,, Tel Aviv, Israel; [32]Sheba Medical Center, Tel Aviv, Israel

**Background:** Squamous cell carcinoma antigen recognized by T cells 3 (SART3) is an RNA-binding protein with numerous biological functions including recycling small nuclear RNAs to the spliceosome. Here, we have identified recessive variants in the *SART3* gene in nine individuals from six families.

**Methods:** An extensive range of methods were used including: detailed clinical phenotyping of affected patients and exome sequencing. SART3 variant RNA expression were analysed by qPCR and proteins were visualised in cells by immunoflurorecence and Western blots used to assess protein levels. *Drosophilia* knockdown of the *SART3* orthologue and mouse transgenics with *SART3* variants were produced and analysed. Human iPSCs carrying *SART3* variants were differentiated into neural and testicular cells and analysed by RNA sequencing and liquid chromatography-mass spectrometry.

**Results:** All affected individuals presented with intellectual disability, global developmental delay and a subset of brain anomalies, together with gonadal dysgenesis in those with a 46,XY chromosome complement. Knockdown of the *Drosophila* orthologue of *SART3* revealed a conserved role in testicular and neuronal development. Human induced pluripotent stem cells (iPSCs) carrying patient variants in *SART3* showed disruption to multiple signalling pathways, upregulation of spliceosome components and demonstrated aberrant gonadal and neuronal differentiation in vitro.

**Conclusions:** Collectively, these findings suggest that bi-allelic *SART3* variants underlie a new spliceosomopathy. We tentatively propose this condition be termed INDYGON syndrome (Intellectual disability, Neurodevelopmental defects and Developmental delay with 46,XY GONadal dysgenesis). These findings will enable additional diagnoses and improved outcomes for individuals with syndromic differences of sex development.

NHMRC Program Grant APP1074258

**Conflict of Interest:** None declared

### C13.2 Characterising clinically relevant complex structural variants in craniosynostosis using long-range technologies

***YANG PEI***[1], *Eduardo Calpena*[1], *Dagmara Korona*[1], *Louise Wilson*[2], *Wendy Jones*[2], *Tazeen Ashraf*[3], *Francesca Forzano*[3], *Deborah Shears*[4], *David Johnson*[5], *Gregory Thomas*[5], *Jill Clayton-Smith*[6], *Ajoy Sarkar*[7], *Urvi Savania*[8], *Mélanie Tanguy*[9], *Adam Giess*[9], *Greg Elgar*[9], *Genomics England Research Consortium*[9], *Richard Gibbons*[10], *Stephen Twigg*[1], *Andrew Wilkie*[1]

[1]*The MRC Weatherall Institute of Molecular Medicine, Clinical Genetics Group, Oxford, United Kingdom;* [2]*Great Ormond Street Hospital for Children, Clinical Genetics Service, London, United Kingdom;* [3]*Guy's and St Thomas' NHS Foundation Trust, Clinical Genetics Department, London, United Kingdom;* [4]*Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom;* [5]*John Radcliffe Hospital, Oxford Craniofacial Unit, Oxford, United Kingdom;* [6]*University of Manchester, Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [7]*Nottingham University Hospitals NHS Trust – City Hospital Campus, Nottingham Regional Genetics Service, Nottingham, United Kingdom;* [8]*Northampton General Hospital, Leicestershire-Northamptonshire–Rutland Clinical Genomics Service, Northampton, United Kingdom;* [9]*Genomics England, London, United Kingdom;* [10]*The MRC Weatherall Institute of Molecular Medicine, MRC Molecular Haematology Unit, Oxford, United Kingdom*

**Background/Objectives:** Complex structural variants (SVs) comprising multiple breakpoints are often detectable using short-read technologies, but their full characterisation is impossible when the informative segments are longer than single reads can span. We attempted to resolve clinically relevant complex SVs using long-range approaches to aid assessment of pathogenicity.

**Methods:** Using craniosynostosis as a test disease, we interrogated patient data from the 100,000 Genomes Project and local cases, seeking clinically relevant SVs. We then used orthogonal technologies, Oxford Nanopore (ONT) sequencing and Bionano Optical Genome Mapping (OGM), to seek and characterise complex SVs in 19 families. We used Deep-C to predict computationally the effect on topologically associating domains, and supplemented this where relevant with functional analysis using Capture-C, ATAC-seq, and RNA-seq.

**Results:** We identified 9 SVs that had eluded previous research/clinical investigations. Long-range technologies successfully resolved some of the large complex SVs, including pathogenic rearrangements in the *TWIST1* and *HOXC* regions, allowing improved assessment of clinical relevance. Comparison of Illumina, ONT, and OGM technologies showed clear advantages of each in specific areas of SV detection, but also highlighted current limitations.

**Conclusion:** Complex SVs account for some of the missing genetic diagnoses in craniosynostosis. Routine diagnostic approaches and short-read technologies are insufficient to fully assess the clinical relevance of such SVs. Long-range technologies successfully resolved previously occult rearrangements and illuminate their clinical significance. This work provides insights into missing clinical diagnoses and the rapidly developing approaches to understand complex SVs

**Grant References:** MRC MR/T031670/1

**Conflict of Interest:** None declared

### C13.3 Episignatures in practice: independent evaluation of the predictive abilities of ten published episignatures for the molecular diagnostics of neuro-developmental disorders

*Thomas Husson*[1], *François Lecoquierre*[2], *Gaël Nicolas*[2], *anne-claire richard*[2], *Anne Boland*[3], *Jean-François Deleuze*[3], *Alice Goldenberg*[2], *Anne-Marie Guerrot*[2], *Gabriella Vera*[2], *Pascale Saugier-Veber*[2], *AnDDI-Rares collaborators*[4], *Olivier Guillin*[1], *Dominique Campion*[1], ***Charbonnier Camille***[5]

[1]*Univ Rouen Normandie, Inserm U1245 and Centre Hospitalier du Rouvray, Sotteville-les-Rouen, France;* [2]*Univ Rouen Normandie, INSERM U1245 and CHU Rouen, Department of Genetics and reference center for developmental abnormalities, Rouen, France;* [3]*Centre National de Recherche en Génomique Humaine, Université Paris-Saclay, CEA, Evry, France;* [4]*AnDDI-Rares network, France, France;* [5]*Univ Rouen Normandie, INSERM U1245 and CHU Rouen, Department of Biostatistics, Rouen, France*

**Background/Objectives**: Variants of uncertain significance (VUS) are a constant challenge for the molecular diagnostic testing of rare diseases. The publication of episignatures as effective biomarkers of certain neurodevelopmental disorders has raised hopes in that area. However, prediction abilities of most published episignatures have not been independently investigated yet,

which is a prerequisite for an informed and rigorous use in a diagnostic setting.

**Methods**: We generated DNA methylation data from 102 carriers of (likely) pathogenic variants in ten different genes, 58 VUS carriers, and 25 negative controls. Combining published episignature information and new validation data with a k-nearest-neighbour classifier within a leave-one-out scheme, we provide unbiased specificity and sensitivity estimates for each of the signatures.

**Results**: We show that our procedure guarantees 100% specificity, including against other syndromes. However, the sensitivities unexpectedly span a very large spectrum. While *ATRX, DNMT3A, KMT2D*, and *NSD1* signatures displayed a 100% sensitivity, *CREBBP-RSTS* and the most recent *CHD8* signature reached less than 40% sensitivity on our dataset. Remaining Cornelia de Lange syndrome, *KMT2A*, *KDM5C* and *CHD7* signatures reached fair but unstable sensitivity, suffering from heterogeneous methylation profiles among cases and rare discordant samples.

**Conclusion**: Our results call for cautiousness and demonstrate that episignatures do not perform equally well. Some signatures are ready for confident use in a diagnostic setting. Yet, it is imperative that we should characterize the actual validity perimeter and interpretation of each episignature with the help of larger validation sample sizes and in a broader set of episignatures.

**Conflict of Interest:** None declared

### C13.4 ZFTRAF1 deficiency causes syndromic microcephaly in humans and gross defects in zebrafish

***Maria Asif***[1;2], *Arwa Ishaq A. Khayyat*[3], *Hans-Martin Pogoda*[4], *Salem Alawbethani*[5], *Anne Sanner*[6], *Theodoros Georgomanolis*[7], *Judith Haasters*[8], *Birgit Budde*[1], *Christian Becker*[1], *Holger Thiele*[1], *Dominic Winter*[6], *Hilario Gomez Martin*[8], *Matthias Hammerschmidt*[4], *Matias Wagner*[9;10;11], *Peter Nürnberg*[1;2], *Muhammad Sajid Hussain*[1;2]

[1]*CCG – Cologne Center for Genomics, Köln, Germany;* [2]*Center for Molecular Medicine Cologne, Köln, Germany;* [3]*Biochemistry department, King Saud University, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia;* [4]*Institute of Zoology, Developmental Biology Unit, University of Cologne, Cologne, Germany;* [5]*GenAlive Lab, Riyadh, Saudi Arabia;* [6]*Institute for Biochemistry and Molecular Biology, Medical Faculty, University of Bonn, Bonn, Germany;* [7]*Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany, Cologne, Germany;* [8]*Hospital Universitario de Salamanca, Salamanca, Spain;* [9]*Department of Pediatric Neurology, Developmental Medicine and Social Pediatrics, Dr. von Hauner's Children's Hospital, University of Munich, Munich, Germany;* [10]*Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany;* [11]*Institute for Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany*

**Background/objectives:** Deciphering genetic determinants of congenital microcephaly had been instrumental to reveal the complexities of human brain development. Here, we investigated five patients afflicted with microcephaly syndrome to identify the genetic component(s) and to reveal the pathomechanism.

**Methods:** The genetic investigation was carried out using high-throughput next-generation sequencing. Functional analyses were conducted by immunoreactivity, transcriptome-profiling, mass spectrometry and zebrafish morpholinos knockdown.

**Results:** We recruited five patients from three unrelated families from Yemen, Germany and Spain manifesting global developmental delay, variable degrees of microcephaly and hypotonia. We identified two homozygous frameshift variants, in four patients from two independent families and an intronic variant NM_138496.1:c.486-2A > C;p.(?) in a patient from the third family in *ZFTRAF1*, encoding zinc finger TRAF-type-containing protein 1 of unknown function. The intronic variant impairs the canonical splice donor site and results in the formation of an aberrant transcript (p.(Val205Glyfs*18)). ZFTRAF1 showed predominantly nuclear and weak cytosolic expression observed in several cell lines and absent in patients-derived primary fibroblasts. A similar pattern was observed for the transiently expressed mutant proteins recovered by treatment with cycloheximide. Moreover, 110 interacting partners of ZFTRAF1 were identified, and involved in mRNA processing and autophagy-related pathways. Intriguingly irregularities of both pathways were found in patients-derived fibroblasts. Knockdown of *zftraf1* in *zebrafish* revealed a completely necrotic and/or dysmorphic brain. Surprisingly, iPSCs colonies generated from patient fibroblasts could only achieve the pre-iPSCs phase.

**Conclusion:** Our findings suggest that loss-of-function variants of *ZFTRAF1* cause microcephaly syndrome thus likely to be a novel key factor in human brain development.

**Conflict of Interest:** None declared

### C13.5 Truncating ASXL1 mutations in Bohring-Opitz Syndrome cause epigenetically driven upregulation of Wnt signaling

***Isabella Lin***[1;2;3], *Angela Wei*[1;3;4], *Zain Awamleh*[5], *Meghna Singh*[1;3;4], *Aileen Ning*[1;3;4], *Analeyla Herrera*[1;3;4], *Bianca Russell*[6], *Rosanna Weksberg*[5;7;8], *Valerie Arboleda*[1;3;4]

[1]*University of California Los Angeles, Pathology and Laboratory Medicine, Los Angeles, United States;* [2]*University of California Los Angeles, Medical Scientist Training Program, Los Angeles, United States;* [3]*University of California Los Angeles, Human Genetics, Los Angeles, United States;* [4]*University of California Los Angeles, Computational Medicine, Los Angeles, United States;* [5]*The Hospital for Sick Children, Genetics and Genome Biology, Toronto, Canada;* [6]*University of California Los Angeles, Medical Genetics, Los Angeles, United States;* [7]*University of Toronto, Medical Sciences and Molecular Genetics, Toronto, Canada;* [8]*The Hospital for Sick Children, Clinical & Metabolic Genetics, Toronto, Canada*

**Background/Objectives:** *ASXL1* (Additional Sex Combs Like 1) is a gene that regulates development of hematopoietic, neural crest and cardiac systems. De novo heterozygous, truncating mutations in *ASXL1* cause Bohring-Opitz Syndrome (BOS, OMIM#605039), characterized by profound intellectual disability, seizures, heart defects, and 'BOS posture'. *ASXL1* mediates epigenetic silencing and transcriptional regulation through targeting of the epigenome. Therefore, we explored how BOS-patient *ASXL1* mutations dysregulate epigenome and transcriptome across multiple cell types.

**Methods:** We assayed the epigenome and transcriptome in peripheral blood and dermal fibroblasts from BOS ($n = 18$) and normotypic controls ($n = 51$). The epigenome was assessed by ATAC-seq, DNA methylation, and histone methylation (H3K4me3, H3K27me3), and transcriptome by RNA-seq.

**Results:** Our integrative analyses identified activation of canonical and non-canonical Wnt signalling pathways in patient cells harbouring *ASXL1* mutations. One of the strongest dysregulations was the epigenetically-mediated 14-fold and 6-fold upregulation of non-canonical Wnt planar cell polarity protein *VANGL2* in blood and fibroblasts respectively. *VANGL2* plays an important role in directing embryonic tissue patterning and migration, and neural crest development. Patient samples were hypomethylated at *VANGL2*, with increased histone H3K4 trimethylation, and 2.3-fold increased chromatin accessibility at TSS. Epigenetic and transcriptomic dysregulation in 39 other Wnt pathway genes drive

aberrant pathway activation. This is consistent with BOS phenotypes such as rapid hair and nail growth, 'BOS posture' contractures, and increased Wilm's tumour risk.

**Conclusion:** We identified epigenetically-driven and novel upregulation of Wnt signalling pathways in BOS patient samples which paves the way for targeted therapeutic options in BOS patients.

**Conflict of Interest:** None declared

### C13.6 Al Kaissi syndrome : a novel cohort of 15 patients with biallelic variations in the CDK10 gene : functional analysis, phenotypic description and review of the literature

***Manon Chretien***[1;2], *Adella Karam*[1], *Theresa Brunet*[3], *Perrine Charles*[4], *Claude Darcha*[5], *Evren Gümüs*[6], *Bertrand Isidor*[7], *Kimia Kahrizi*[8], *Vincent Michaud*[9], *Renske Oegema*[10], *Juliette Piard*[11], *Davut Pehlivan*[12], *Laure Raymond*[13], *Marcia Rodrigues*[14], *Hélène Dollfus*[1;2], *Elise Schaefer*[2], *Pierre Colas*[15], *Jean Muller*[1;16]

[1]*Institut de génétique médicale d'Alsace, Laboratoire de génétique médicale UMR_S 1112, Strasbourg, France;* [2]*Institut de génétique médicale d'Alsace, Service de génétique médicale, Strasbourg, France;* [3]*Technical University of Munich, Institute of Human Genetics, Munich, Germany;* [4]*Hôpital La Pitié Salpêtrière, Service de Génétique Clinique et Médicale, Paris, France;* [5]*CHU Estaing, Service d'Anatomopathologie, Clermont-Ferrand, France;* [6]*Mugla Sitki Kocman University, Medical Genetics Department, Mugla, Turkey;* [7]*CHU de Nantes, Service de Génétique Médicale, Nantes, France;* [8]*University of Social Welfare and Rehabilitation Sciences, Medical Genetics Research Center, Evin Theran, Iran;* [9]*CHU de Bordeaux, Service de Génétique Médicale, Bordeaux, France;* [10]*University Medical Center, Clinical Genetics, Universitair Medisch Centrum, Utrecht, Netherlands;* [11]*CHU Saint-Jacques, Service de Génétique Médicale, Besançon, France;* [12]*Baylor College of Medicine, Texas Children's Hospital, Pediatrics and Neurology, Houston, Texas, United States;* [13]*Laboratoire Eurofins Biomnis, Lyon, France;* [14]*Hospital de Santa Maria, CHU Lisboa Norte, Serviço de Genética Médica, Lisboa, Portugal;* [15]*Sorbonne Université, Station Biologique UMR8227 CNRS, Roscoff, France;* [16]*Laboratoires de Diagnostic Génétique, Strasbourg, France*

**Background** : Al Kaissi syndrome (#MIM617694) is an autosomal recessive disorder linked to the *CDK10* gene, combining growth delay, intellectual disability, thin corpus callosum, morphological features and vertebral segmentation defects. The *CDK10* gene encodes a serine-threonine kinase that is thought to be involved in ciliogenesis, making Al Kaissi syndrome a ciliopathy. Since 2017, only 14 individuals have been reported in the literature, mostly carrying loss-of-function variations.

**Methods** : We report on the clinical, molecular and functional aspects of an international cohort of 15 patients including 9 new cases, carrying variants in the *CDK10* gene. Functional characterizations of the variants were performed using skin fibroblasts and cell models.

**Results** : Clinically, all our patients have a mild to severe intellectual disability (10/10). Some patients (9/14) had postnatal growth retardation. Eight of them (8/15) have hypoplasia of the corpus callosum. Finally, four individuals (4/9) have vertebral segmentation defects. At the molecular level, the pathogenicity of 3 missense variants, one in frame deletion, and three splice variants was characterized using functional assays. As previously described (only on cell models and murine cells), we confirm in patients an elongation of primary cilia on human cells (skin fibroblasts).

**Conclusion** : This study describing the largest cohort of patients with Al Kaissi syndrome allows to broaden the clinical and molecular spectrum and finally allows a better understanding of its natural history and its membership in the heterogeneous group of ciliopathies.

**Conflict of Interest:** None declared

## C14 EYE GENETICS

### C14.1 Role of LRP5 in hereditary retinal vasculopathies: Zebrafish disease model

***Fulya Yaylacioglu Tuncay***[1;2], *sairah yousaf*[1], *harold antony burgess*[3], *robert b. hufnagel*[1]

[1]*National Institutes of Health (NIH), National Eye Institute (NEI), Ophthalmic Genetics and Visual Function Branch, Bethesda, MD, United States;* [2]*University of Health Sciences, Gulhane Medical Faculty, Medical Biology, Ankara, Turkey;* [3]*Eunice Kennedy Shriver National Institute of Child Health and Human Development, Division of Developmental Biology, Bethesda, MD, United States*

**Background:** The *LRP5* (LDL receptor related protein 5) gene encodes a transmembrane protein involved in Norrin/β-catenin signaling pathway which has a fundamental role in the retinal vascular development. Some *LRP5* variations have been linked to eye conditions in humans such as Familial Exudative Vitreoretinopathy and Osteoporosis Pseudoglioma Syndrome. The purpose of this study was to evaluate whether zebrafish can be used as a model organism for investigating the mechanisms underlying *LRP5*-related eye diseases.

**Methods:** *Lrp5* knock-out (KO) zebrafish which had a premature stop codon in exon 5, wild-type (WT) zebrafish, and (Tg[fli1:EGFP] AB) zebrafish line which had a reporter gene to visualize blood vessels were used. The survival rate, gross morphology, the structural and functional integrity of the retina and retinal vasculature were compared between *lrp5* KO and WT zebrafish.

**Results:** *Lrp5* KO zebrafish larvae showed decreased survival rates. During the larval period only head size was significantly smaller in KO ($p = 0.0407$). However, *lrp5* KO adult zebrafish demonstrated significantly smaller size both in head and eye measurements ($p = 0.0094$ and $p < 0.0001$). The analysis of retinal vasculature showed closer and more branched retinal vessels in *lrp5* KO adult zebrafish. The dark-flash response assay showed no detectable functional visual defect in *lrp5* KO larvae.

**Conclusion:** This was the first study to discover *Lrp5*-related eye pathologies in zebrafish. *Lrp5* mutant zebrafish could be studied further to learn more about *LRP5's* role in retinal vascular pathologies.

**Grant references:** This study was conducted by the support of the ICO- NEI 1-Year Fellowship in Ocular Genetics.

**Conflict of Interest:** None declared

### C14.2 Regulation of non-canonical expression of ABCA4 by an RPE-specific enhancer with implications in ABCA4-associated disease

***Víctor López Soriano***[1], *Alfredo Dueñas Rey*[1], *Giulia Ascari*[1], *Munevver Cicekdal*[1;2], *Eva D'haene*[1], *Quinten Mahieu*[1], *Hanna De Saffel*[1], *Andy Willaert*[1], *Kris Vleminckx*[1;2], *Juan Jesus Tena-Aguilar*[3], *Juan Ramón Martínez Morales Martínez-Morales*[3], *Miriam Bauwens*[1], *Elfride De Baere*[1]

[1]*Center for Medical Genetics, Department of Biomolecular Medicine (GE31), Ghent University and Ghent University Hospital, Ghent, Belgium;* [2]*Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium;* [3]*Centro Andaluz de Biología del Desarrollo (CABD), Consejo Superior de Investigaciones Científicas (CSIC), Sevilla, Spain*

**Purpose:** *ABCA4*-associated Stargardt disease is the most frequent recessive blinding disorder. A major part of its missing heritability, particularly in monoallelic cases, has been solved by non-coding deep-intronic mutations that affect splicing of the canonical *ABCA4* transcript, predominantly expressed in photoreceptor cells. Given minor expression in the supporting retinal pigment epithelium (RPE), we aimed to identify the regulators of RPE expression and non-canonical RPE-expressed transcripts.

**Methods:** To identify candidate *cis*-regulatory elements (cCREs) in human RPE, we integrated epigenomic data from human RPE, iPSC-RPE and primary RPE cell cultures, such as Dnase-seq, ATAC-seq, ChIP-seq for active enhancer marks; and RPE transcriptomic data. To functionally validate the cCREs, in vitro reporter assays using pGL4.23 vectors were performed in hTERT RPE-1 cells. Chromatin interaction profiling to map enhancer-promoter interactions was done on RPE by UMI-4C. A novel RPE-expressed ABCA4 isoform was validated by cDNA sequencing, Western blotting and immunoprecipitation.

**Results:** From four cCREs displaying reporter activity, one displayed active enhancer mark H3K27ac and high chromatin accessibility exclusively in the RPE-derived datasets, pointing toward a cell type-specific role in RPE. Next, UMI-4C revealed a distal (300 kb) enhancer-promoter interaction for the RPE-specific cCRE, mediated by CTCFs. The transcriptomics data pointed toward a role for this specific RPE-cCRE as an alternative promoter for one particular isoform that constitutes half of the ABCA4 transporter.

**Conclusions:** Mapping the regulatory landscape of *ABCA4* in the uncharted RPE revealed one cCRE regulating a specific ABCA4 isoform, which could have implications for *ABCA4*-associated disease.

**Funding:** StarT-H2020-MSCA-ITN-2018-N°81349, FWO1802220

**Conflict of Interest:** None declared

### C14.3 Pharmacological cAMP synthesis stimulation improves cilia formation in CEP290-deficient fibroblasts and retinal response to light in CEP290 transgenic mice

***France de Malglaive***[1;2], *Iris Barny*[2], *Isabelle Perrault*[1], *Shahd Machroub*[2], *Ema Cano*[1], *Tania Attie-Bitach*[3], *Josseline Kaplan*[1], *Luis Briseño-Roa*[2], *Jean-Michel Rozet*[1], *Jean-Philippe Annereau*[2]

[1]*Laboratory of ophtalmologic genetics, Imagine Institute, paris, France;* [2]*Medetia, Paris, France;* [3]*Institut Imagine, Paris, France*

**Background and objectives:** The prostaglandin-E2 receptor agonist MDT-110 has recently been shown to stimulate cAMP synthesis and rescue ciliogenesis in fibroblasts homozygous for *NPHP1* deletion, as well as to alleviate *Nphp1*-/- mouse retinopathy. NPHP6, also known as CEP290, is another NPHP component, which mutations cause severe cilia formation defects and a wide range of ciliopathies from non-syndromic Leber congenital amaurosis (LCA10) to Meckel syndrome (MKS). Here, we investigated in vitro and in vivo rescue of NPHP6/CEP290-associated phenotypes by MDT-110.

**Methods:** EP2 receptor expression in fibroblasts was assessed by RT-PCR. Serum-starved fibroblasts from 5 homozygous LCA10 individuals (c.2991+1655A > G, $n = 3$; p.Lys1575*, $n = 2$), 1 MKS embryo (p.Met407Glufs*14/Ile1372Lysfs*5) and 2 controls were treated with 0.2µM MDT110 or sham for 24h and ciliogenesis was analyzed. For in-vivo analysis, 6-days old mice homozygous for CEP290 exon 36 deletion were treated by intraperitoneal injections every 3 days at 18 and 30 mg/kg for 1 month and the retina function was analyzed (ERG).

**Results:** We showed EP2 expression and MDT-110-mediated increase of cAMP intracellular concentration (>10 fold) in fibroblasts, which led to improved ciliogenesis in all cell lines while CEP290 expression was unchanged. In the mouse, MDT-110 was well tolerated and elicited a highly significant dose-response increase of rod (but not cone)-response to light.

**Conclusion:** The improvement of ciliogenesis in cells from patients carrying various CEP290 mutations and rescue of the retinal response upon MDT-110 treatment hold great promises for systemic treatment of CEP290-related ciliopathies to alleviate retinal, renal and cerebral lesions.

**Grant References:** Medetia, ANRT– RHU Cil'lico

**Conflict of Interest:** France de Malglaive Medetia Pharmaceuticals, Iris Barny Medetia Pharmaceuticals, RHU Cil'lico, Isabelle Perrault: None declared, Shahd Machroub Medetia Pharmaceuticals, RHU Cil'lico, Ema Cano: None declared, Tania Attie-Bitach: None declared, Josseline Kaplan: None declared, Luis Briseño-Roa Medetia Pharmaceuticals, RHU Cil'lico

European grant Theracil, Shareholder Medetia, Co-founder & consultant for AtmosR, CPO & Co-founder of Medetia, Jean-Michel Rozet Medetia, Intergalactic, Jean-Philippe Annereau Medetia Pharmaceuticals, European Grant Theracil

RHU Cil'lico, Shareholder of Medetia, Co-founder and consultant for AtmosR, CEO & Co-founder of Medetia

### C14.4 Impairment of light-induced stress response in the Retinitis Pigmentosa mouse model CerklKD/KO

***Rocío García-Arroyo***[1;2;3], *Elena B. Domènech*[1;2;3], *Carlos Herrera-Úbeda*[1], *Miguel Ángel Asensi*[4], *F.V Pallardó*[4], *Jordi Garcia-Fernàndez*[1], *Serena Mirra*[1;2;3], *Gemma Marfany*[1;2;3]

[1]*Universitat de Barcelona, Department of Genetics, Microbiology and Statistics, Barcelona, Spain;* [2]*Institut de Biomedicina de la Universitat de Barcelona – Institut de Recerca Sant Joan de Déu (IBUB-IRSJD), Barcelona, Spain;* [3]*Centro de Investigación Biomédica En Red (CIBER) de Enfermedades Raras (CIBERER), Madrid, Spain;* [4]*University of Valencia, Department of Physiology, Valencia, Spain*

The retina, the specialized organ that transduces light into neural signals, is particularly vulnerable to genetic and environmental alterations that generate reactive oxygen species (ROS). Light and oxidative stress damage photoreceptors and other retinal neurons, eventually triggering cell death. Mutations in *CERKL* (*CERamide Kinase-Like*) cause Retinitis Pigmentosa in humans, a rare disease characterized by photoreceptor degeneration and progressive vision loss. *CERKL* is described as a resilience gene against oxidative stress, and its overexpression protects cells from oxidative stress-induced apoptosis. Besides, preliminary evidences indicate that CERKL contributes to RNA stress granule formation and regulates mitochondrial dynamics in the retina.

Using *Cerkl*$^{KD/KO}$ mouse models, we aimed to study the impact of *Cerkl* depletion on stress response and photoreceptor death mechanisms in response to light/oxidative stress. Thus, we assessed the alteration of retinas from *Cerkl*$^{KD/KO}$ albino mice to acute light stress, as an immediate (early) or after two weeks (late) response, using RNA-Seq, immunostaining, Western blot, and glutathione determination assays. Our results show that the depletion of *Cerkl* causes an aberrant early response to stress by means of altered RNA stress granules production and glutathione metabolism, as well as increased levels of ROS. In addition, we found activation of cell death mechanisms in light exposed *Cerkl*$^{KD/KO}$ retinas.

Overall, our studies indicate that *Cerkl* gene is a novel player in regulating light-challenged retinal homeostasis and shed light on how mutations in *CERKL* cause blindness by impairing the oxidative stress response in the retina.

Grants: FI-DGR 2021 (2021 FI_B 00099) Generalitat de Catalunya, PID2019-108578RB-I00 (MICINN).

**Conflict of Interest:** None declared

### C14.5 Whole genome sequencing delineates novel non-coding variants and candidate genes in inherited retinal diseases

***Marta del Pozo Valero***[1], *Miriam Bauwens*[1], *Marieke De Bruyne*[1], *Filip Van den Broeck*[2], *Stephanie Dulst*[1], *Quinten Mahieu*[1], *Audrey Meunier*[3], *Thomy de Ravel*[4], *Joke Ruys*[5], *Mattias Van Heetvelde*[1], *Alfredo Dueñas Rey*[1], *Irina Balikova*[6], *Sascha Vermeer*[6], *Julie De Zaeytijd*[2], *Bart Leroy*[2], *Elfride De Baere*[1]

[1]*Center for Medical Genetics, Ghent University Hospital, Department of Biomolecular Medicine, Ghent, Belgium;* [2]*Ghent University Hospital, Department of Ophthalmology, Department of Head & Skin, Ghent, Belgium;* [3]*University Hospital Saint-Pierre, Université Libre de Bruxelles (ULB), Department of Ophthalmology, Brussels, Belgium;* [4]*Center for Medical Genetics, University Hospital Brussels, Brussels, Belgium;* [5]*ZNA Middelheim, Department of Ophthalmology, Antwerp, Belgium;* [6]*University Hospitals Leuven, Department of Ophthalmology,, Leuven, Belgium*

**Background/Objectives:** Non-coding regulatory variants and variants in candidate genes have been under investigated in inherited retinal diseases (IRD). Here, we leveraged whole genome sequencing (WGS) in a prescreened exome-tested IRD cohort to provide insight into their contribution to disease.

**Methods:** Short-read WGS (Illumina) was performed in 77 IRD probands. Coding and non-coding regions of IRD and candidate genes were analyzed using the in-house Seqplorer tool and Franklin (Genoox). Structural variants (SVs) were called by ExomeDepth and Manta. Intronic variants were prioritized using SpliceAI and AlamutVisual. Candidate variants were assessed in silico using multi-omics and retinal single-cell datasets and when applicable, in vitro assays, segregation analysis and clinical reassessment.

**Results:** Novel (likely) pathogenic regulatory variants were found in 4% (3/77), one of which is a promoter variant of *RPGRIP1* in a monoallelic case predicted to disrupt an OTX2 binding site. Two 5'UTR variants in *BBS12* and *ELOVL4* were identified in a monoallelic and sporadic case, respectively. Coding variants in novel candidate genes represent 6.5% (5/77), such as *ACACB*, encoding a player in lipid metabolism. Variants in syndromic genes or recent IRD-associated genes, such as *ALPK1* and *ITM2B*, facilitated a genotype-driven diagnosis in 9% (7/77). We pinpointed deep-intronic variants in 16% (12/77), illustrated by the first *ALMS1* deep-intronic variant. Finally, variants in well-established IRD genes were found in 12% (9/77), including two SVs.

**Conclusion:** WGS allowed to solve approximately 47% of our IRD cohort, with 25% cases explained by non-coding variants or novel candidate genes.

**Grant References:** EJPRD19-234, FAME, BOF20-GOA-023, H2020-MSCA-ITN-2018-N°81349, FWO1802220N

**Conflict of Interest:** None declared

### C14.6 Rescue of complex splicing defects in ABCA4 employing antisense oligonucleotides

*Laura De Rooij*[1,2], *Rebekkah Hitti-Malin*[1,2], *Alejandro Garanto*[1,3], *Rob W.J. Collin*[1], *Frans P.M. Cremers*[1,2]

[1]*Radboud University Medical Center, Department of Human genetics, Nijmegen, Netherlands;* [2]*Academic Alliance Genetics, Radboud University Medical Center and Maastricht University Medical Center +, Nijmegen and Maastricht, Netherlands;* [3]*Radboud University Medical Center, Department of Pediatrics, Nijmegen, Netherlands*

**Background:** The *ABCA4* gene, implicated in Stargardt disease, has a high percentage of pathogenic variants altering splicing, some of which lead to complex defects. Antisense oligonucleotides (AONs) have shown promising results in targeting these variants. Here, we performed AON-based rescue studies in four *ABCA4* variants which lead to complex splicing defects.

**Methods:** Three variants were selected in intron 13: c.1938-619A > G and c.1938-621G > A lead to inclusion of a pseudoexon (PE2) alone or together with a second upstream PE (PE1), while c.1938-514A > G resulted in the inclusion of PE3 or PE3 + PE1 together. Variant c.6148-84A > T in intron 44 causes the inclusion of a short PE4 or of a longer PE5 + exon 44 skipping. Five novel AONs were designed, in addition to the previously tested AON1. All AONs have phosphorothioate backbones and 2′-*O*-methoxyethyl sugar modifications. AON efficacy was assessed using in vitro splice assays in HEK293T cells using midigene constructs.

**Results:** Treatment with AON1 for all intron 13 variants successfully promoted PE1 exclusion. AON2 and AON3 efficiently corrected the splicing defects caused by variants c.1938-619A > G and c.1938-621G > A, including the PE1 insertion. Aberrant splicing due to c.1938-514A > G was completely corrected by AON3 and partially (~80%) by AON4. Surprisingly, AON2, which did not target PE3 directly, was also able to partially correct PE3 insertion. For intron 44 splicing defects, AON6 completely restored normal splicing.

**Conclusions:** AON-based splicing modulation is a promising approach to target complex splicing defects in *ABCA4*. Different variants localized in specific regions can be efficiently targeted by a single AON.

**Conflict of Interest:** Laura De Rooij: None declared, Rebekkah Hitti-Malin: None declared, Alejandro Garanto: None declared, Rob W.J. Collin RWJC is a founder and chief scientific officer of Astherna, a spin-off company of RadboudUMC that develops RNA therapies targeting blinding diseases., Patents EP16203864, EP18184432.5, EP18210107.1 and EP19190673.4, related to Stargardt-disease have been licensed out of Astherna., Frans P.M. Cremers: None declared

## C16 PRENATAL GENETICS

### C16.1 Application of RNA sequencing in genetic diagnosis of fetal structural abnormalities – virtual

***Sze Shing Fan***[1], *Ziheng Zhou*[2], *Mengmeng Shi*[2], *Yu ZHENG*[2], *Yilin Zhao*[2], *Shuwen Xue*[2], *Yvonne Kwok*[2], *T. Y. Leung*[2], *Zirui Dong*[2], *Kwong Wai Choy*[2], *Ye Cao*[2]

[1]*The Chinese University of Hong Kong, Department of Paediatrics, Hong Kong, Hong Kong;* [2]*The Chinese University of Hong Kong, Department of Obstetrics and Gynaecology, Hong Kong, Hong Kong*

**Background**: RNA sequencing (RNA-seq) has been shown to improve the diagnostic rate by 8–36% for patients with suspected Mendelian disorders but inconclusive genetic investigations. However, there were only limited studies regarding the application of RNA-seq in prenatal diagnosis. This study aims to determine the utility of RNA-seq supplementing genome sequencing (GS) analysis in genetic diagnosis of fetal structural abnormalities.

**Methods**: This study recruited 32 trios with GS (30X) performed after various fetal structural abnormalities detected by antenatal ultrasound. Subsequently, mRNA sequencing was performed using amniotic fluid (AF) cells. The transcriptome of AF cells was tilized to detect aberrant expression and splicing, which might indicate pathogenic genomic variants previously unrecognized.

**Results**: On average, 67% of genes in a fetal anomalies panel (1940 genes) were expressed in AF cells with transcripts per million (TPM) level >1. We observed in RNA-seq data that rare canonical splicing variants result in fewer abnormal splicing events than expected, while other coding and non-coding variants with SpliceAI>0.8 cause a higher percentage of such events. In a case with known homozygous deletion involving exon 3 of *PIEZO2* gene, RNA-seq not only showed in-frame exon 3 skipping in all transcripts, but also with additional exon 2 skipping (in-frame) in 29% of the transcripts, which could possibly be due to another variant in intron 1.

**Conclusion**: RNA-seq of AF cells could facilitate variant prioritization and interpretation in GS analysis, hence supplementing GS to uncover the cryptic genetic etiologies of fetal structural abnormalities in prenatal diagnosis.

**Conflict of Interest:** None declared

### C16.2 Biallellic variants in SNAPIN are associated with a novel foetal neuroanatomical phenotype

***Maayke de Koning***[1], *Hammad Yousaf*[2], *Kelly Gilmore*[3], *Mariette Hoffer*[1], *Georgios Kellaris*[2], *Phebe Adama van Scheltema*[4], *Monique Haak*[4], *Cacha Peeters-Scholte*[5], *Delphine Heron*[6], *Boris Keren*[6], *Karen El Akouri*[7;8], *Sophie Lanone*[9], *Maylis Dagouassat*[9], *Binnaz YALCIN*[10], *Neeta Vora*[3], *Manon Suerink*[1], *Erica Davis*[2], *Gijs Santen*[1]

[1]*Leiden University Medical Center, Clinical Genetics, Leiden, Netherlands;* [2]*Ann & Robert H Lurie Children's Hospital of Chicago, Stanly Manne Children's Research Institute, Chicago, United States;* [3]*The University of North Carolina School of Medicine, Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Chapel Hill, United States;* [4]*Leiden University Medical Center, Obstetrics, Leiden, Netherlands;* [5]*Leiden University Medical Center, Neurology, Leiden, Netherlands;* [6]*University Hospitals Pitié Salpêtrière – Charles Foix, Genetics, Paris, France;* [7]*Hamad Medical Corporation, Adult and Pediatric Medical Genetics, Doha, Qatar;* [8]*Sidra Medicine, Division of Genetic and Genomic Medicine, Ar Rayyan, Qatar;* [9]*University of Paris-Est, INSERM Institut Mondor de Recherche Biomédicale, Champs-sur-Marne, France;* [10]*University Bourgogne Franche-Comté, Inserm Unit 1231, Besançon, France*

Prenatal whole exome sequencing (WES) has emerged as a comprehensive diagnostic tool for pregnancies with foetal malformations, with a diagnostic rate of 10–20%. Due to this widespread implementation, novel prenatal phenotype-genotype correlations are increasingly encountered. We identified a foetus presenting with structural anomalies in which trio-WES detected a homozygous truncating variant in *SNAPIN*, encoding the ubiquitously expressed SNARE-associated protein. SNAPIN is an important component in several autophagy-lysosomal pathways and enhances retrograde axonal transport. Through GeneMatcher, we identified six additional cases from four unrelated families with biallelic variants in *SNAPIN*. Prenatal imaging showed overlapping neuroanatomical phenotypes including ventriculomegaly (6/7), cerebellar hypoplasia or atrophy (6/7), corpus callosum agenesis or hypoplasia (4/7), abnormal head size (4/7), and lissencephaly (3/7). Most prevalent additional anatomical features included talipes equinovarus (5/7) and flexion contractures (4/7). All six cases with truncating variants did not survive beyond the perinatal period either due to intrauterine demise (1 case), elective termination (3 cases), or neonatal death (1 case). One case homozygous for a missense variant survived but has severe neurological impairment. To investigate the role of SNAPIN in development of anterior structures, we targeted the single ortholog in zebrafish. We generated F0 mutants using CRISPR/Cas9 and performed transient knockdown using morpholinos. Both *snapin* loss-of-function models recapitulated human-relevant disease phenotypes including cerebellar hypoplasia, reduced intertectal neuron count, diminished optic tectum size, and aberrant facial patterning with concomitant apoptosis in the head compared to wild type. These findings suggest that biallelic variants in *SNAPIN* are likely causative of a severe neuroanatomical disorder.

**Conflict of Interest:** None declared

### C16.3 Prenatal exome sequencing in corpus callosum anomalies: lessons from a cohort of 209 fetuses

***Anna Gerasimenko***[1], *Solveig Heide*[1], *Lisa Frugere*[1], *Stéphanie Valence*[2], *Jean-Marie Jouannic*[3], *Tania Attie-Bitach*[4], *Catherine Garel*[5], *Mathilde Nizon*[6], *Audrey Putoux*[7], *Cecile Prudhomme*[8], *Jade Ducourneau*[8], *Thomas Courtin*[9], *Daphné Lehalle*[1], *Jean-Madeleine de Sainte Agathe*[10], *des portes vincent*[11], *Olivier Patat*[12], *Mathieu MILH*[13], *Sebastien Moutton*[14], *Myrtille Spentchian*[15], *Cyril Mignot*[1;8], *Boris Keren*[10], *Delphine Heron*[1]

[1]*APHP Sorbonne Université, Hôpital La Pitié Salpetrière, UF de Génétique Médicale et CRMR "Déficience intellectuelle, Département de Génétique Clinique, Paris, France;* [2]*Hôpital Armand Trousseau, APHP Sorbonne Université, Service de Neurologie Pédiatrique, Paris, France;* [3]*Hôpital Armand Trousseau, APHP, Sorbonne Université, Fetal Medecine Department, Paris, France;* [4]*Hôpital Necker-Enfants Malades, APHP, Paris, France, Embryofoetopathologie, Service Histologie-Embryologie-Cytogénétique, Paris, France;* [5]*Hôpital Armand Trousseau, Service de Radiologie Pédiatrique, Paris, France;* [6]*CHU de Nantes, Service de Génétique Clinique, Nantes, France;* [7]*CHU de Lyon, HCL de Lyon, Service de Génétique Clinique, Lyon, France;* [8]*Hôpital Armand-Trousseau, APHP Sorbonne Université, Service de Génétique Clinique, Paris, France;* [9]*Hôpital Necker-Enfants Malades, Service de Génétique Clinique, Paris, France;* [10]*Groupe hospitalier la Pitié Salpêtrière, APHP Sorbonne Université, UF de Génomique du Développement, Département de Génétique, Paris, France;* [11]*Hospices Civils de Lyon, Service de Neurologie Pédiatrique, Bron, France;* [12]*CHU de Toulouse, Service de Génétique Clinique, Toulouse, France;* [13]*Hôpital La Timone, APHM, Aix-Marseille Université, Service de Neurologie Pédiatrique, Marseille, France;* [14]*CHU de Bordeaux, Service de Génétique Clinique, Bordeaux, France;* [15]*Hôpital Andre Mignot, Génétique Médicale, Versailles, France*

**Background/Objectives:** Anomalies of the corpus callosum (ACC) are most often diagnosed antenatally, at the 2nd trimester ultrasound, which leads to difficulties in terms of prenatal and genetic counseling. Indeed, the associated neurodevelopment prognosis is very large, from normal development to severe intellectual disability (ID) and depends mainly on the underlying etiology of the AnCC.

**Methods:** We report the results of prenatal trio exome sequencing (pES) in 209 fetuses with ACC.

**Results:** The overall diagnosis yield was 23.5%, 31.3% for ACC associated with other malformations and 22.5% for isolated ACC. Pathogenic variants in the DCC gene were the most recurrent etiology (12.8%) and are not associated with ID. Other etiological diagnoses involved 36 genes, most of which being associated with ID (including ARID1B, ZEB2 and ARX). After pES, the parents requested termination of pregnancy in 75% of cases with an etiological diagnosis, versus 20.2% in the absence of a diagnosis. The pregnancy was continued to term for all cases of ACC related to DCC.

**Conclusion:** These results confirm the major interest of pES during pregnancy after diagnosis of ACC, tilized accurate information regarding the ND prognosis. Further prospective cohort studies with long-term follow-up of the born children will be needed to provide accurate prenatal counceling after a negative pES result.

**Conflict of Interest:** None declared

### C16.4 Prenatal-onset hypertrophic cardiomyopathy in 47 patients with RASopathies: understanding phenotype-genotype correlations for risk stratification, medical management and targeted therapies assessment through an international cohort study

***Guillaume Jouret***[1;2], *Dominik Brado*[3], *Jeroen Breckpot*[4], *Atilano Carcavilla*[5], *Melanie FRADIN*[6], *Irene Valenzuela Palafoll*[7], *Paula Fernandez*[7], *Núria Martínez-Gil*[7], *Fabio Sirchia*[8], *Alessandro Mussa*[9;10], *Federico Fornari*[9;10], *Charlotte Dubucs*[11;12], *Jacqueline Aziza*[12], *Maria Isis Atallah Gonzalez*[13], *Fanny Laffargue*[14], *Michèle Gueneret*[15], *Adela Chirita-Emandi*[16;17], *Mathilde Nizon*[18], *Philippe Khau Van Kien*[19], *Francesca Mari*[20;21], *Anna Carrer*[20;21], *Maria Haanpää*[22], *Caroline Rooryck-Thambo*[23], *Sophie Naudion*[23], *Claire Beneteau*[23], *Jean-Baptiste Ichtertz*[24], *Marie Bournez*[24], *Jean-Paul Hermand*[25], *Didier Menzies*[26], *Barbara Klink*[27]

[1]*National Center of Genetics, Clinical Genetics, Dudelange, Luxembourg;* [2]*ERN ITHACA, National Hub of European Reference Networks (ERN), Center Hospitalier de Luxembourg, Luxembourg, Luxembourg;* [1]*National Center of Genetics, Clinical Genetics, Dudelange, Luxembourg;* [4]*University Hospitals Leuven, Center for Human Genetics, Leuven, Belgium;* [5]*Atilano Carcavilla, Endocrinología Pediátrica, Hospital Universitario La Paz, Paseo de la Castellana, Madrid, Spain;* [6]*Service de Génétique Médicale, CLAD Ouest, CHU Rennes, Rennes, France;* [7]*Department of Clinical and Molecular Genetics and Rare Disease Unit. Medicine Genetics Group, Vall Hebron Research Institute, Barcelona, Spain;* [8]*Medical Genetics Unit, IRCCS San Matteo Foundation, Pavia, Italy. / Department of Molecular Medicine, University of Pavia, Pavia, Italy;* [9]*Department of Public Health and Pediatric Sciences, University of Torino, Torino, Italy;* [10]*Clinical Pediatric Genetics Unit, Regina Margherita Children's Hospital, Città Della Salute e Della Scienza di Torino, Torino, Italy;* [11]*Service de Génétique Médicale CHU Toulouse, Toulouse, France;* [12]*Département de pathologie, Institut Universitaire du Cancer – Oncopole, Toujouse, France;* [13]*Division of Genetic Medicine, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland;* [14]*Service de génétique médicale et centre de référence des anomalies du développement et des déficits intellectuels rares, CHU de Clermont-Ferrand, Clermont-Ferrand, France;* [15]*Service d'anatomopathologie, CHU Martinique, Martinique, Martinique;* [16]*Genetic Discipline, Center of Genomic Medicine, University of Medicine and Pharmacy "Victor Babes" Timisoara, Timis, Romania;* [17]*Regional Center of Medical Genetics Timis, Clinical Emergency Hospital for Children "Louis Turcanu" Timisoara, Timis, Romania;* [18]*CHU Nantes, Service de Génétique Médicale, Nantes, France;* [19]*Génétique Médicale, Centre de Compétences Anomalies du Développement et Syndromes Malformatifs, Hôpital Carémeau, CHU de Nîmes, Nîmes, France;* [20]*Medical Genetics, University of Siena, Italy, Siena, Italy;* [21]*Genetica Medica, Azienda Ospedaliero – Universitaria Senese, Senese, Italy;* [22]*Department of Genomics, Turku University Hospital, Turku, Finland;* [23]*CHU de Bordeaux, Service de génétique médicale, Bordeaux, France;* [24]*Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, Hôpital d'Enfants, CHU Dijon, Dijon, France;* [25]*Centre Hospitalier de Luxembourg, Maternité Grande Duchesse Charlotte, Luxembourg, Luxembourg;* [26]*National Center of Pathology, Laboratoire national de santé, Dudelange, Luxembourg;* [27]*National Center of Genetics (NCG), Laboratoire national de santé (LNS), Dudelange, Luxembourg*

**Background/Objectives:** The medical management of patients with RASopathies is challenging, especially when presenting with hypertrophic cardiomyopathy (HCM). Prenatal-onset HCM is rare and potentially severe, with clinical presentations ranging from stable conditions to rapidly progressing and fatal courses. Early genotyping and precise understanding of the phenotype-genotype correlations is crucial for risk stratification of HCM progression, and may enable patients to benefit from MEK or mTOR inhibitors. Our aim is to provide new insights into the understanding of genotype-phenotype correlations of prenatal-onset HCM in patients with RASopathies, to better delineate high-risk genotypes, guide the medical management and treatment indications, as well as facilitate a transition to future clinical trials.

**Methods/Results:** Thanks to the ERN-ITHACA network, we report the first cohort of patients with prenatal-onset HCM and molecular diagnosis of RASopathy, consisting of 47 patients with causative variants in the genes *PTPN11* (15 patients), *RAF1* (13 patients), *RIT1* (9 patients), *BRAF* (5 patients), *MRAS*, *HRAS*, *RRAS2*, *MAP2K1* and *SHOC2*. We performed phenotype-genotype correlation analysis by cross-linking molecular data, anatomical description of the hypertrophic myocardium, HCM progression follow-up, medical management, treatment including MEK and mTOR inhibitors, surgical outcome, prognosis and survival.

**Conclusion:** For the first time, we report a large cohort of patients with RASopathies and prenatal onset HCM. We contribute to the stratification of genotypes into risk categories, to delineate high-risk genotypes of HCM progression in infancy. In addition, early genotyping may reveal addressable targets, advocating for a prenatal molecular diagnosis.

**Conflict of Interest:** None declared

### C16.5 Maternal age-independent human aneuploidies are common and associated with mitochondrial disfunctions: analysis of 11,610 genomes of human embryos – virtual

***Natalia Ree***[1], *Maxim Ri*[1], *Jonathan Tilly*[2], *Ilya Mazunin*[3;4], *Konstantin Popadin*[1;5]

[1]*Center for Mitochondrial Functional Genomics, Institute of Living Systems, Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation;* [2]*Laboratory for Aging and Infertility Research, Department of Biology, Northeastern University,, Boston, United States;* [3]*Center for Molecular and Cellular Biology, Skolkovo Institute of Science and Technology (Skoltech),, Skolkovo, Russian Federation;* [4]*Medical Genomics LLC, Moscow, Russian Federation;* [5]*Swiss Institute of Bioinformatics, Lausanne, Swaziland*

Association between mtDNA content and embryo aneuploidy was seriously debated due to contradictory results on different datasets.

We used the biggest dataset of low-coverage WGS of trophectoderm biopsies from 11,610 human blastocyst-stage embryos to analyze the relationship between chromosomal abnormalities and mitochondrial mtDNA dynamics. For WGS data analysis we used BWA (version 0.7.17), samtools, RtN tools and R scripts for statistical analysis.

Comparing 6208 aneuploid (NRFT) and 5402 euploid embryos (RFT) in cohort studies, we found that mtDNA content in aneuploid embryos was significantly higher than that in euploid embryos for two different platforms (corrected *P*-values for VeriSeq PGS and AB-PGT were 0.0008 and 0.0262 correspondingly). This trend was confirmed by intrafamilial analysis of sibling emryos for 1827 families ($P < 2E\text{-}16$). Additional analyses uncovered a higher abundance of ultra-rare mtDNA variants located in never-altered positions in NRFT versus RFT embryos in both cohort- and family-based analyses. (https://www.biorxiv.org/content/10.1101/2022.10.14.512116v2.full.pdf).

We described the maternal age-independent association between increased mtDNA content and aneuploidy in human embryos and proposed a mechanism based on de novo germline deleterious events (nucDNA or mtDNA variants), which lead to mitochondrial dysfunctionality, compensatory increase in mtDNA content and aneuploidy.

**Conflict of Interest:** None declared

### C16.6 Prenatal and lethal phenotypes of Mendelian disorders: a cross-species comparison

***Pilar Cacheiro***[1], *Arun Retnakumar*[1], *Peter N. Robinson*[2], *Damian Smedley*[1]

[1]*William Harvey Research Institute, Queen Mary University of London, London, United Kingdom;* [2]*The Jackson Laboratory for Genomic Medicine, Farmington, United States*

The Human Phenotype Ontology has recently expanded the representation of prenatal phenotypes. Additionally, by directly querying the OMIM database, we can retrieve records of lethality and earliest age of death reported. These annotations can be mapped to embryonic and viability phenotypes in the ortholog mouse knockout from the IMPC and MGI resources to perform cross-species comparisons.

In the knockout mice, up to 38% of genes show lethality between the embryonic and weaning stages, while 17% of all genes have an abnormal embryo phenotype, mainly reported in those lethal lines (95%). In humans, 23% of OMIM genes (898) are mapped to abnormal prenatal development phenotypes, and 912 to early lethality (186 prenatal, 282 neonatal, 444 infant), with 60%, 48%, and 32% respectively being also associated to prenatal phenotypes.

Human disease genes are known to be enriched for mouse lethal genes. With the current data available, 56% of OMIM disease genes are lethal in the mouse (OR, 2.8; $P < 2.2e\text{-}16$), rising to 72% for the subset of OMIM genes with prenatal phenotypes reported, and 74% for those with pre-infant death annotations.

Prenatal lethal disorders are likely underrepresented in current disease databases, and identifying those genes essential for human development is key to understanding the spectrum of intolerance to loss-of-function variation and facilitating prenatal diagnosis. Mouse lethal genes constitute compelling candidates for Mendelian phenotypes with prenatal manifestations including death. We are currently building a Lethality Gene Portal to catalogue and display human and mouse lethal genes and support fetal sequencing studies. [NIH-grant-U54-HG006370]

**Conflict of Interest:** None declared

## C17 FUNCTIONAL GENOMICS

### C17.1 Aberrant phase separation and nucleolar dysfunction in rare genetic diseases

*Martin Atta Mensah*[1], ***Henri Niskanen***[2], *Alexandre Magalhaes*[2], *Shaon Basu*[2], *Martin Kircher*[3], *Henrike Sczakiel*[1], *Alisa MV Reiter*[1], *Jonas Elsner*[1], *Peter Meinecke*[4], *Saskia Biskup*[5], *Brian Hon Yin Chung*[6], *Gregor Dombrowsky*[7], *Christel Eckmann-Scholz*[8], *Marc Phillip Hitz*[7], *Alexander Hoischen*[9], *Paul-Martin Holterhus*[10], *Wiebke Hülsemann*[11], *Kimia Kahrizi*[12], *Vera Kalscheuer*[2], *Anita Kan*[13], *Mandy Krumbiegel*[14], *Ingo Kurth*[15], *Jonas Leubner*[16], *Ann Carolin Longardt*[17], *Jörg Detlev Moritz*[18], *Hossein Najmabadi*[19], *Karolina Skipalova*[1], *Lot Snijders Blok*[20], *Andreas Tzschach*[21], *Martin Zenker*[22], *Eberhard Wiedersberg*[23], *Carla Garcia-Cabau*[24], *René Buschow*[2], *Xavier Salvatella*[24], *Matthew Kraushar*[2], *Stefan Mundlos*[1], *Almuth Caliebe*[25], *Malte Spielmann*[25], *Denise Horn*[1], *Denes Hnisz*[2]

[1]*Institute of Medical Genetics and Human Genetics, Charité–Universitätsmedizin Berlin, Berlin, Germany;* [2]*Max Planck Institute for Molecular Genetics, Berlin, Germany;* [3]*Exploratory Diagnostic Sciences, Berlin Institute of Health at Charité, Universitätsmedizin Berlin, Berlin, Germany;* [4]*Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [5]*Center for Genomics and Transcriptomics (CeGaT), Tübingen, Germany;* [6]*School of Clinical Medicine, LKS Faculty of Medicine, The University of Hong Kong, Department of Pediatrics and Adolescent Medicine, Hong Kong, Hong Kong;* [7]*Department of Congenital Heart Disease and Pediatric Cardiology, University Hospital Schleswig-Holstein, Kiel, Germany;* [8]*Department of Obstetrics and Gynecology, University Hospital Schleswig-Holstein, Kiel, Germany;* [9]*Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Department of Internal Medicine,, Nimjegen, Netherlands;* [10]*University Hospital Schleswig-Holstein, Department of Pediatrics, Schleswig-Holstein, Germany;* [11]*Handchirurgie, Katholisches Kinderkrankenhaus Wilhelmstift, Hamburg, Germany;* [12]*Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran;* [13]*Department of Obstetrics and Gynaecology, Queen Mary Hospital, Hong Kong, Hong Kong;* [14]*Institute of Human Genetics, Universitätsklinikum Erlangen, Erlangen, Germany;* [15]*Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University Hospital, Aachen, Germany;* [16]*Department of Pediatric Neurology, Charité–Universitätsmedizin Berlin, Berlin, Germany;* [17]*Department of Pediatrics, University Hospital Center Schleswig-Holstein, Kiel, Germany;* [18]*Department of Radiology and Neuroradiology, Pediatric Radiology, University Hospital Schleswig-Holstein, Kiel, Germany;* [19]*Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran;* [20]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands;* [21]*Institute of Human Genetics, Medical Center, University of Freiburg, Freiburg, Germany;* [22]*Institute of Human Genetics, University Hospital, Otto-von-Guericke University, Madgeburg, Germany;* [23]*Zentrum für Kinder-und Jugendmedizin, Helios Kliniken Schwerin, Schwerin, Germany;* [24]*Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain;* [25]*Institute of Human Genetics, University Hospitals Schleswig-Holstein, University of Lübeck and Kiel University, Lübeck, Kiel, Germany, Lübeck, Germany*

**Background:** Brachyphalangy, polydactyly and tibial aplasia syndrome (BPTAS) is an ultra-rare complex multi-organ malformation syndrome, with an unknown cause. A BPTAS-patient featured a novel de novo frameshift at the beginning of the C-terminal acidic intrinsically disordered region (IDR) of HMGB1 (usually associated with a neurodevelopmental phenotype). The etiological impact of mutations within such IDRs is unknown. IDRs can mediate phase separation and the formation of biomolecular condensates but have overall poorly defined functions.

**Objective:** To evaluate the role of IDR mutations in BPTAS and to investigate if similar mutations generally alter condensate properties and function.

**Materials and Methods:** Genome, exome and targeted sequencing of 5 unrelated individuals with BPTAS. Droplet formation assays with recombinant wt and mutant HMGB1. Microscopy and FRAP with U2OS cells expressing HMGB1 and other selected protein variants. Search of pathogenic variants within IDRs using ClinVar, COSMIC, 1000 Genomes project and dbSNP databases.

**Results:** BPTAS-patients featured de novo frameshifts in HMGB1 replacing its acidic IDR with an arginine-rich basic tail. This alters HMGB1 phase separation and enhances its partitioning into the nucleolus causing nucleolar dysfunction. We identified 200,000 reported variants in C-terminal IDRs, including 624 frameshifts that create arginine-rich tails. Enhanced partitioning into the nucleolus occured in 12 of 13 tested variants, and several altered rRNA biogenesis.

**Conclusion:** Frameshift-induced IDR-swapping is a novel disease mechanism and the cause of BPTAS. It also likely elucidates the functional impact of numerous reported pathogenic – yet functionally unexplained – variants.

**Reference:** Mensah*, Niskanen*, et al. Nature 2023, PMID:36755093

**Conflict of Interest:** Martin Atta Mensah: None declared, Henri Niskanen: None declared, Alexandre Magalhaes: None declared, Shaon Basu: None declared, Martin Kircher: None declared, Henrike Sczakiel: None declared, Alisa MV Reiter: None declared, Jonas Elsner: None declared, Peter Meinecke: None declared, Saskia Biskup: None declared, Brian Hon Yin Chung: None declared, Gregor Dombrowsky: None declared, Christel Eckmann-Scholz: None declared, Marc Phillip Hitz: None declared, Alexander Hoischen: None declared, Paul-Martin Holterhus: None declared, Wiebke Hülsemann: None declared, Kimia Kahrizi: None declared, Vera Kalscheuer: None declared, Anita Kan: None declared, Mandy Krumbiegel: None declared, Ingo Kurth: None declared, Jonas Leubner: None declared, Ann Carolin Longardt: None declared, Jörg Detlev Moritz: None declared, Hossein Najmabadi: None declared, Karolina Skipalova: None declared, Lot Snijders Blok: None declared, Andreas Tzschach: None declared, Martin Zenker: None declared, Eberhard Wiedersberg: None declared, Carla Garcia-Cabau: None declared, René Buschow: None declared, Xavier Salvatella X. Salvatella is founder of Nuage Therapeutics, X. Salvatella is a scientific advisor of Nuage Therapeutics, Matthew Kraushar: None declared, Stefan Mundlos: None declared, Almuth Caliebe: None declared, Malte Spielmann: None declared, Denise Horn: None declared, Denes Hnisz D. Hnisz is founder of Nuage Therapeutics, D. Hnisz is scientific advisor of Nuage Therapeutics

### C17.2 Exome copy number variant (CNV) detection, analysis, and curation from 6,678 families with undiagnosed rare genetic disease

***Gabrielle Lemire***[1;2;3], *Kathryn Russell*[1], *Samantha Baxter*[1;3], *Katherine Chao*[1;3], *Emily Evangelista*[1], *Moriel Singer-Berk*[1], *Alba Sanchis-Juan*[1;3], *Emily Groopman*[1;2], *Isaac Wong*[1;3], *Stephanie DiTroia*[1;3], *Eleina England*[1;3], *Julia Goodrich*[1;3], *Lynn Pais*[1;2;3], *Christina Austin-Tse*[1;3], *Emily O'Heir*[1;3], *Hana Snow*[1], *Ikeoluwa A. Osei-Owusu*[1;3], *Jack Fu*[1;3], *Daniel MacArthur*[1;4;5], *Heidi Rehm*[1;3], *Michael Talkowski*[1;3], *Harrison Brand*[1;3], *Anne O'Donnell-Luria*[1;2;3]

[1]*Broad Institute of MIT and Harvard, Cambridge, United States;* [2]*Boston Children's Hospital, Division of Genetics and Genomics, Boston, United States;* [3]*Massachusetts General Hospital, Center for Genomic Medicine and Analytical and Translational Genetics Unit, Boston, United States;* [4]*Garvan Institute, Center for Population Genomics, Sydney, Australia;* [5]*Murdoch Children's Research Institute, Centre for Population Genomics, Melbourne, Australia*

**Background/objectives:** CNVs are a significant contribution to pathogenic variation in rare genetic diseases and can be identified from exome sequencing. Accurate classification of CNV pathogenicity requires a consistent and evidence-based method.

**Methods:** CNV calling using the GATK-gCNV algorithm was performed on exomes from the Broad Center for Mendelian Genomics (member of the GREGoR consortium), a cohort of 6678 families with heterogeneous phenotypes and variable prior genetic testing. Each family's CNV data was analyzed using the *seqr* platform, and orthogonal validation by another method is in progress. We classified CNVs using the 2020 ACMG/ClinGen CNV interpretation standards and developed additional evidence criteria to address limitations of these standards.

**Results:** While analysis is ongoing, we have solved 169 previously undiagnosed families to date. The estimated sizes of CNVs ranged from 293 bp to 80 Mb, and consisted of 140 deletions, 14 duplications, 3 insertions and 12 complex structural variants (SV). Comparing the original result with the orthogonal validation for the first 77 CNVs, 19 showed differences in gene/exon content or in SV type (25%). Our approach refined multiple aspects of CNV curation: we classified 146 CNVs as likely pathogenic/pathogenic and 23 CNVs as VUS.

**Conclusion:** Calling CNVs from existing exome data increases the diagnostic yield for individuals undiagnosed after standard testing approaches, providing a higher resolution alternative to arrays and at a fraction of the cost of genome sequencing. Our improvements to the curation approach offers a systematic framework to assess the pathogenicity of CNVs.

**Grant references:** UM1HG008900, U01HG011755

**Conflict of Interest:** None declared

### C17.3 Saturation mutagenesis data facilitate the interpretation of noncoding variants in the IRF6 enhancer associated with nonsyndromic cleft-lip w/o cleft palate

***Ronja Hollstein***[1], *Frederic Thieme*[1], *Felicitas Lauber*[1], *Angelika Biedermann*[1], *Julia Fazaal*[1], *Max Schubach*[2], *Martin Kircher*[2;3], *Kerstin Ludwig*[1]

[1]*University Hospital Bonn, Institute of Human Genetics, Bonn, Germany;* [2]*Universitätsmedizin Berlin, Berlin Institute of Health at Charité, Berlin, Germany;* [3]*University Hospital Schleswig Holstein, Institute of Human Genetics, Lübeck, Germany*

Nonsyndromic cleft lip with/without cleft palate (nsCL/P) is a common, multifactorial birth defect with a strong genetic component, which is only partially understood. So far, the contribution of rare noncoding variants has been rarely investigated, largely due to limitations in attributing pathogenic effects. Here, we combined two sequencing-based high-throughput methods to screen an enhancer of IRF6, a well-known nsCL/P locus, for rare candidate variants. We used molecular inversion probes (MIPs) to sequence 699 nsCL/P cases and 511 population controls. In total, we found 11 variants in cases: three common single nucleotide variants (SNV), two 4- and 9-bp indels, and six rare SNVs.

In parallel, we performed a massively parallel reporter assay (MPRA) to facilitate variant interpretation. We transfected HaCaT, GMSM-K and HEKT293T cells with a previously established saturation mutagenesis library of the IRF6 enhancer to analyse the effects of all possible single nucleotide exchanges within the enhancer. Integrating both datasets revealed one rare and one common SNV (rs76145088) with a significant decrease of enhancer activity that was specific to HaCaT cells. Interestingly, the other five rare variants affected the enhancer activity in either HaCat or GMSM-K at a nominal significance threshold ($0.05 > p > 1e\text{-}5$). The two common variants had no effect ($p > 0.05$).

In conclusion, the combination of MPRA data together with large-scale identification of risk variants is a promising tool for the interpretation of non-coding variants in orofacial clefting, and possibly other human disorders with a suspected contribution of rare non-coding variants.

**Conflict of Interest:** Ronja Hollstein: None declared, Frederic Thieme: None declared, Felicitas Lauber: None declared, Angelika Biedermann: None declared, Julia Fazaal: None declared, Max Schubach: None declared, Martin Kircher: None declared, Kerstin Ludwig Speaker at trainee workshops by Hans-Riegel Foundation, Co-founder and stake holder in LAMPseq Diagnostics GmbH

### C17.4 Assessing tissue-specific effects of rare and structural variants towards gene regulation with the EN-Tex personal genome resource

***Matthew Jensen***[1;2], *Tai Michaels*[2], *Anna Su*[1], *Timur Galeev*[1;2], *Sushant Kumar*[1;2], *Kun Xiong*[1;2], *Beatrice Borsari*[1;2], *Joel Rozowsky*[1;2], *Mark Gerstein*[1;2]

[1]Yale University, Program in Computational Biology and Bioinformatics, New Haven, United States; [2]Yale University, Department of Molecular Biophysics and Biochemistry, New Haven, United States

**Background:** Comprehensive tissue-specific analyses of how variants alter molecular phenotypes have greatly improved our understanding of genomic mechanisms for complex traits. For example, the EN-Tex resource, consisting of long read-based personal genomes and a full battery of functional assays across 25 tissues in four donors, allowed for systematic assessment of allelic activity of common SNVs. The EN-Tex resource is also ideal for studying the functional effects of a full spectrum of genomic variants, in particular rare SNVs and all types of structural variants (SVs), which are often under-represented in functional genomics studies.

**Methods:** We aligned 381 tissue-specific and single-cell ATAC-Seq, DNAse-Seq, and ChIP-Seq datasets within EN-Tex onto personal genomes using alternate-aware methods, and prioritized functional signals within variant regions adjacent to genes with altered expression.

**Results:** We found ~293 SV-eQTLs per individual, and linked key functional signals within these variants to genes with altered expression; for instance, deletion of an upstream H3K27Ac peak led to reduced expression of *ZFAND2A*. We also identified ~152 functional elements within novel genomic insertions per individual, and found that 16% of these variants could contribute to perturbed expression patterns. For example, the tumor suppressor *ASMTL-AS1* showed 4.2-fold increased expression when coupled with an upstream duplication spanning a novel ATAC-Seq peak. Finally, we identified ~620 rare SNVs per individual that disrupted candidate *cis*-regulatory elements (cCREs) near protein-coding genes, and prioritized variants in cCREs near genes with altered expression.

**Conclusion:** Our study emphasizes the broad effects of both rare variants and SVs towards tissue-specific gene regulatory patterns.

**Conflict of Interest:** None declared

### C17.5 eQTLGen phase 2: Genome-wide trans-eQTL analysis in blood in over 30,000 individuals provides insight into the genetic architecture of molecular traits

***Robert Warmerdam***[1;2], *Tõnu Esko*[3], *Harm-Jan Westra*[1;2], *Lude Franke*[1;2], *Urmo Võsa*[3]

[1]University Medical Center Groningen, Department of Genetics, Groningen, Netherlands; [2]Oncode Institute, Utrecht, Netherlands; [3]Estonian Genome Centre, Institute of Genomics, Tartu, Estonia

**Background**: Gene expression quantitative trait loci (eQTL) analysis has become an essential tool for understanding complex disease mechanisms. However, previous eQTL studies have been limited by sample size and therefore mainly focused on cis-regulatory effects. We recently performed a trans-eQTL analysis in 30,000 samples (eQTLGen), but were only able to study 10,000 genetic variants, due to computational challenges. Here, we present interim results from eQTLGen phase 2, the largest-scale genome-wide trans-eQTL analysis to date.

**Methods**: We have developed robust pipelines to perform automated, comprehensive data quality control and genotype imputation in individual cohorts. An efficient pipeline adapted from the HASE framework allows us to perform genome-wide meta-analyses for thousands of phenotypes and samples, while limiting data transfer sizes and ensuring participant privacy. We have successfully piloted this method on 13 cohorts, reflecting 6569 samples, and are currently expanding to over 30,000 samples.

**Results**: We present results on a genome-wide trans-eQTL analysis in over 6500 samples and are expanding our approach to over 30,000 samples. This will provide us with a more complete understanding of gene expression regulation, demonstrate the importance of considering trans-effects, and will enable analyses to reveal gene-to-gene interactions in gene regulatory networks. Moreover, our findings provide a resource for the genetics research community and offer a foundation for future functional genomics studies.

**Conclusion**: In conclusion, we highlight the importance of large-scale trans-eQTL analysis in understanding gene expression regulation and its implications for complex disease biology.

**Grant References**: NWO VICI 09150182010019, Oncode Investigator; PRG1291, Estonian Research Council Grant

**Conflict of Interest:** Robert Warmerdam: None declared, Tõnu Esko: None declared, Harm-Jan Westra: None declared, Lude Franke I VICI 09150182010019, Oncode Senior Investigator, Urmo Võsa: None declared

### C17.6 Genome-wide evaluation of the effect of short tandem repeat variation on local DNA methylation

***Alejandro Martin Trujillo***[1], *Paras Garg*[1], *Bharati Jadhav*[2], *Nihir Patel*[1;3], *Andrew Sharp*[1]

[1]Icahn School of Medicine at Mount Sinai, New York, United States; [2]Icahn School of Medicine at Mount Sinai, New York, United States; [3]Admera Health, Plainfield, United States

Short tandem repeats (STRs) contribute significantly to genetic diversity in humans, including disease-causing variation. While the effect of STR variation on gene expression has been extensively assessed, their impact on epigenetics has been poorly studied and limited to specific genomic regions. Here, we investigated the hypothesis that some STRs act as independent regulators of local DNA methylation in the human genome, and modify risk of common human traits. To address these questions, we first analyzed two independent datasets comprising PCR-free whole genome sequencing (WGS) and genome-wide DNA methylation levels derived from whole blood samples in 245 (discovery cohort) and 484 individuals (replication cohort). Utilizing genotypes for 131,635 polymorphic STRs derived from WGS using HipSTR, we identified 11,870 STRs that associated with DNA methylation levels (mSTRs) of 11,774 CpGs (Bonferroni $p < 0.001$) in our discovery cohort, with 90% successfully replicating in our second cohort. Subsequently, through fine-mapping using CAVIAR we defined 585 of these mSTRs as the likely causal variants underlying the observed associations (fm-mSTRs), and linked a fraction of these to previously reported genome-wide association study signals, providing insights into the mechanisms underlying complex human traits. Furthermore, by integrating gene expression data, we observed that 12.5% of the tested fm-mSTRs also modulate expression levels of nearby genes, reinforcing their regulatory potential. Overall, our findings expand the catalog of functional sequence variants that affect genome regulation, highlighting the importance of incorporating STRs in future genetic association analysis and epigenetics data for the interpretation of trait-associated variants.

**Conflict of Interest:** Alejandro Martin Trujillo Full time employee at Icahn School of Medicine at Mount Sinai, Post-doctoral Fellowship from the American Heart Association to Martin-Trujillo A

Early career fellowship to Martin-Trujillo A., Paras Garg full time Employee, Bharati Jadhav Full time employee, Nihir Patel Full time employee at Admera Health, Andrew Sharp Full time employee at Icahn School of Medicine at Mount Sinai, NIH grant R01NS105781 to Sharp AJ

## C18 NEURODEVELOPMENTAL DISORDERS: NEW GENES

### C18.1 Biallelic inactivating variants in DMAP1 underlie a syndromic neurodevelopmental disorder

***Dong Li***[1], *Qin Wang*[1], *Christian Tirrito*[1], *Carolyn Applegate*[2], *Fowzan Alkuraya*[3], *Elizabeth Bhoj*[1], *Nathalie Couque*[4], *Noor Giesbertz*[5], *Hakon Hakonarson*[1], *Ada Hamosh*[2], *Tina Duelund Hjortshoej*[6], *Mari Mori*[7], *Konrad Platzer*[8], *Silke Redler*[9], *Raashda Sulaiman*[3], *Alain Verloes*[4], *Andrew Sobering*[10], *Yuanquan Song*[11]

[1]*Children's Hospital of Philadelphia, Philadelphia, United States;* [2]*Johns Hopkins University School of Medicine, Baltimore, United States;* [3]*King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia;* [4]*APHP-Robert DEBRE University Hospital, Paris, France;* [5]*University Medical Center Utrecht, Utrecht, Netherlands;* [6]*University Hospital of Copenhagen, Copenhagen, Denmark;* [7]*Nationwide Children's Hospital, Columbus, United States;* [8]*Heinrich Heine University, Düsseldorf, Germany;* [9]*University of Leipzig Medical Center, Leipzig, Germany;* [10]*University of Georgia, Athens, United States;* [11]*Children's Hospital of Philadelphia, Philadelphia, United States*

*DNA Methyltransferase 1 Associated Protein 1* (*DMAP1*) encodes a versatile protein involved in different complexes responsible for maintenance of DNA methylation, regulation of histone acetylation and catalysis of histone H2A.Z deposition. Despite DMAP1's essential roles in multiple transcriptional processes, it has not been implicated in human disease. By exome sequencing and international matchmaking, we identified nine individuals from eight families with a syndromic neurodevelopmental disorder carrying homozygous or compound heterozygous variants in *DMAP1*. We identified three truncating variants and seven missense variants residing in or around the SANT domain, suggesting they may affect its interaction with DNA and/or histone. All nine individuals have global developmental delay, intellectual disability, hypotonia, and craniofacial dysmorphisms, although the reported findings varied. Neural-specific knockdown of the *Drosophila* ortholog *Dmap1* led to pupal lethality and structural defects of the mushroom body (MB), highlighting an underappreciated role of Dmap1 in MB development. These phenotypes can be rescued by WT and the two missense variants of human *DMAP1*, allowing us to conduct the social space assay to further query the high order effect in social tilized. We found that WT DMAP1 restored proper fly distribution, whereas the two missense variants caused clustering and reduced inter fly distance, reflecting impaired social avoidance and conforming the pathogenicity of these variants. Furthermore, WT DAMP1 but not the two missense variants partially rescued the locomotion defects and the seizure induced by mechanical stimulation in a sex dependent manner. We demonstrate that biallelic variants in *DMAP1* are associated with a novel neurodevelopmental disorder.

**Conflict of Interest:** None declared

### C18.2 De novo variants in DENND5B perturb intracellular vesicular trafficking and cause neurodevelopmental disorders with epilepsy and white matter abnormalities

***Marcello Scala***[1;2;3], *Valeria Tomati*[3], *Julie Cohen*[4;5], *Ali Fatemi*[4;5], *Elly Brokamp*[6], *John Phillips*[6], *Mary Koziura*[6], *Michael Nicouleau*[7], *Marlene Rio*[8;9], *Nathalie Boddaert*[10], *Michele Iacomino*[3], *Ilaria Musante*[3], *Paolo Scudieri*[3], *Simona Baldassari*[3], *Jill A. Rosenfeld*[11;12], *Jon Bernstein*[13], *Gary Bellus*[14], *Sara Reed*[14], *Hind Al Saif*[15], *Rossana Sanchez*[16], *Matthew Walsh*[16], *Vincent Cantagrel*[17], *Pasquale Striano*[1;2], *Vincenzo Salpietro*[1;2], *Nicoletta Pedemonte*[3], *Federico Zara*[3]

[1]*University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Genoa, Italy;* [2]*IRCCS Istituto Giannina Gaslini, Pediatric Neurology and Muscular Diseases Unit, Genoa, Italy;* [3]*IRCCS Giannina Gaslini, UOC Genetica Medica, Genoa, Italy;* [4]*Kennedy Krieger Institute, Department of Neurology and Developmental Medicine, Baltimore, United States;* [5]*Johns Hopkins University School of Medicine, Department of Neurology, Baltimore, United States;* [6]*Vanderbilt University Medical Center, Nashville, Department of Pediatrics, Tennessee, United States;* [7]*INSERM, Laboratory of Molecular and Pathophysiological Bases of Cognitive Disorders, Paris, France;* [8]*Université de Paris, Developmental Brain Disorders Laboratory, INSERM, Paris, France;* [9]*Necker Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris, Service de Génétique, Paris, France;* [10]*INSERM, AP-HP, Hôpital Necker-Enfants Malades, Paris, Département de Radiologie Pédiatrique, Paris, France;* [11]*Baylor College of Medicine, Department of Molecular & Human Genetics, Houston, United States;* [12]*Baylor Genetics Laboratories, Houston, United States;* [13]*Stanford University School of Medicine, Department of Pediatrics, Stanford, United States;* [14]*Geisinger Medical Center, Clinical Genetics, Danville, United States;* [15]*Virginia Commonwealth University School of Medicine, Department of Human and Molecular Genetics, Division of Clinical Genetics,, Richmond, United States;* [16]*Emory University, Department of Human Genetics, Atlanta, United States;* [17]*Université de Paris, Developmental Brain Disorders Laboratory, Imagine Institute, INSERM, Paris, France*

The Rab family of guanosine triphosphatases (GTPases) include key regulators of intracellular transport and membrane trafficking. DENND5b (Rab6-interacting Protein 1B – R6IP1B), is the longest isoform of DENND5, an evolutionarily conserved DENN domain-containing GEF that is highly expressed in the brain and has been proposed to form a molecular link between Golgi and Rab11-recycling endosome compartment. In mice, *Dennd5b* knockdown promotes epileptic seizures through increased chronic spontaneous epileptic seizures and discharges. Through exome sequencing and international matchmaking platforms, we identified a range of de novo variants in *DENND5B* in a cohort of 8 unrelated individuals with neurodevelopmental disorders and epilepsy. We used different techniques based on biochemical assays and confocal microscopy to assess DENND5B variant proteins expression and distribution in various cell models. Exploiting fluorescent lipid cargoes coupled to high-content imaging and analysis in living cells, we investigated the organization and dynamics of membrane transport. Autophagic pathway was also investigated using a monodansyl-cadaverine-based functional assay. All patients harbored rare and predicted damaging de novo missense or splicing variants in *DENND5B*, affecting RUN1 and RUN2 domains. The core phenotype consisted of psychomotor delay, moderate-to-severe intellectual disability, abnormal behavior, infantile-onset epilepsy, and white matter hyperintensities. *DENND5B* variants resulted in decreased protein expression possibly due to protein misfolding and premature degradation. Additionally, specific *DENND5B* variants perturbed intracellular membrane trafficking pathways and the endosomal system. Our study identifies de novo *DENND5B* missense variants in association with a novel neurodevelopmental disorder with epilepsy and white matter involvement.

**Conflict of Interest:** None declared

### C18.3 Loss-of-function of the Zinc Finger Homeobox 4 (ZFHX4) gene causes a novel neurodevelopmental disorder

***María del Rocío Pérez Baca***[1;2], *María Palomares Bralo*[3;4], *Michiel Vanhooydonck*[1;2], *Eva Z. Jacobs*[1;2], *Pontus LeBlanc*[1;2], *Lies Vantomme*[1;2], *Fernando Santos*[5], *Roser Lleuger-Pujol*[6], *Sixto García-Miñaur*[3;4], *Itsaso Losantos-García*[7], *Björn Menten*[1;2], *Ananilia Silva*[8;9],

*Sadegheh Haghshenas[9], Michael Levy[9], Jennifer Kerkhof[9], Haley McConkey[9], Zfhx4 consortium[1], Bekim Sadikovic[8;9], Bart Dermaut[1;2], Elke Bogaert[1;2], Sarah Vergult[1;2], Bert Callewaert[1;2]*

*[1]Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium; [2]Department of Biomolecular Medicine, Ghent University, Ghent, Belgium; [3]CIBERER-ISCIII and INGEMM, Institute of Medical and Molecular Genetics, Hospital Universitario La Paz, Madrid, Spain; [4]ITHACA-European Reference Network, Madrid, Spain; [5]Unit of Molecular Diagnostics and Clinical Genetics, Hospital Universitari Son Espases, Health Research Institute of the Balearic Islands (IdiSBa), Palma, Spain; [6]Hereditary Cancer Program, Catalan Institute of Oncology IDIGBI, Girona, Spain; [7]Hospital Universitario La Paz, Department of Biostatistics, Madrid, Spain; [8]Western University, Department of Pathology and Laboratory Medicine, London, Canada; [9]Verspeeten Clinical Genome Centre, London Health Sciences Centre, London, Canada*

Neurodevelopmental disorders (NDDs) result from impaired development and functioning of the brain. Here, we identify a novel NDD caused by loss-of-function variation in *ZFHX4*, encoding a zinc-finger homeodomain transcription factor. In 2011, *ZFHX4* haploinsufficiency was suggested as (one of) the underlying mechanism(s) in 8q21.11 microdeletions. Moreover, in 2020, *ZFHX4* was reported as a novel NDD candidate gene in a large-scale exome-sequencing study.

Through an international collaboration, we gathered data on 47 individuals with protein truncating variants or (micro)deletions affecting *ZFHX4*. Loss-of-function of *ZFHX4* consistently associates with ID, morphological abnormalities of the central nervous system, short stature, hypotonia and distinctive facial characteristics as supported by artificial intelligence (Face2Gene), and, occasionally, cleft palate and anterior segment dysgenesis. We identified a preliminary mild common DNA methylation profile in leukocyte-derived DNA of patients with truncating variants and with (micro)deletions affecting *ZFHX4*. Via data-mining and multiple in vitro models we identified ZFHX4 as a nuclear protein and found increasing expression during human brain development and neuronal differentiation. First-generation (F0) *zfhx4* crispant zebrafish – (mosaic) mutant for *zfhx4* loss-of-function variants – have significantly smaller Meckel's cartilages and ethmoid plates in comparison with control zebrafish upon Alcian blue staining.

To get a better understanding of its role during neurodevelopment, we are currently assessing the interaction partners and downstream targets of ZFHX4 in human neuronal cell types.

In conclusion, ZFHX4 appears to be essential for neural and craniofacial development and *ZFHX4* loss-of-function variants are associated with a novel form of syndromic ID.

Marguerite_Marie_Delacroix_fellowship_BOFSTG2019010301_-Marguerite_Marie_Delacroix_fellowship_STI.DIV.2023.0005.01_Research_Foundation_Flanders_G055422N

**Conflict of Interest:** None declared

### C18.4 A novel neurodevelopment syndrome caused by recessive variants in the FSD1L gene

***Valentina Serpieri[1]***, *Alessia Orsi[1], Silvia Cavan[1], Concetta Mazzotta[1], Ludovica Celli[2], Roberta De Mori[3], Tommaso Biagini[4], Marta Romani[5], Anna Garbelli[6], Noor Smal[7], Tommaso Mazza[4], Simone Sabbioneda[6], Silvia Bione[6], Claudia Condoluci[8], Sarah Weckhuysen[7], Enza Maria Valente[1;9]*

*[1]University of Pavia, Department of moelcular Medicine, Pavia, Italy; [2]Sapienza University of Rome, Department of Molecular Medicine, Rome, Italy; [3]IRCCS Santa Lucia Foundation, Neurogenetics Unit, Rome, Italy; [4]Fondazione IRCCS Casa Sollievo della Sofferenza, S. Giovanni Rotondo, Bioinformatics Unit, S. Giovanni Rotondo, Italy; [5]Eurofins Genoma Group, Molecular Genetics Laboratory, Rome, Italy; [6]IGM CNR "Luigi Luca Cavalli-Sforza", Institute of Molecular Genetics, Pavia, Italy; [7]VIB Centre for Molecular Neurology, Applied and Translational Neurogenomics Group, Antwerp, Belgium; [8]IRCCS San Raffaele Pisana, Rome, Italy; [9]IRCCS Mondino Foundation, Neurogenetics Research Center, Pavia, Italy*

**Background:** Through exome sequencing and subsequent GeneMatcher search, we identified 7 patients from 3 unrelated families carrying biallelic variants (either missense, splice or nonsense) in a novel gene, *FSD1L*. Patients phenotype is characterized by intellectual disability, spasticity, corpus callosum hypoplasia, periventricular white matter reduction and mild hydrocephalus. FSD1L is highly expressed in the developing and mature brain, and it shares three conserved domains (CC-COS, FnIII and B30.2/SPRY) and an intrinsically disordered region, with its paralog *FSD1*.

**Methods:** Fibroblasts from three patients and three controls were reprogrammed into induced pluripotent stem cells and differentiated towards neural stem cells (NSCs) and neurons. Control and mutant cells underwent a preliminary set of experiments, including expression profiling of isoforms along neuronal differentiation, whole transcriptome studies on NSCs and neurons and neuronal differentiation and migration assays.

**Results:** *FSD1L* isoforms were increasingly expressed along neuronal differentiation in control cells, while expression appeared largely deregulated in all patients, especially in mature neurons. RNAseq analysis showed a massive downregulation of several genes encoding transcription factors and proteins implicated in neuronal migration and axon guidance. Mutant neurospheres were smaller and prone to disaggregation, and premature neurons showed markedly reduced migration ability compared to controls. Finally, mutant NSCs showed impaired neuronal differentiating ability and increased apoptotic death.

**Conclusion:** Recessive mutations of *FSD1L* cause a novel neurodevelopment syndrome. FSD1L seems to play a key role in controlling neuronal migration and differentiation and in regulating transcription of key embryonic developmental pathways. The molecular mechanisms underlying these cellular phenotypes are currently being investigated.

**Conflict of Interest:** None declared

### C18.5 LHX2 haploinsufficiency causes a variable neurodevelopmental disorder

*Cosima Schmid[1;2], Anne Gregor[1;2], Gregory Costain[3], Chantal Morel[4], Lauren Massingham[5], Jennifer Schwab[5], Chloe Quelin[6], Marie Faoucher[7;8], Julie Kaplan[9], Rebecca Procopio[9], Carol J Sauders[10], Ana S. A. Cohen[10], Gabrielle Lemire[11;12], Stephanie Sacharow[12], Anne O'Donnell-Luria[11;12], Ranit Jaron[13], Jessica Shamshoni[14], Daniela Schweitzer[14], Darius Ebrahimi-Fakhari[15], Kristin Monaghan[16], Timothy Palculict[16], Melanie Napier[16], Alice Tao[17], Bertrand Isidor[18], Kamran Moradkhani[18], André Reis[19;20], Heinrich Sticht[21], Wendy Chung[22], **Christiane Zweier**[1;2]*

*[1]Inselspital Bern, University of Bern, Department of Human Genetics, Bern, Switzerland; [2]University of Bern, Department for Biomedical Research (DBMR), Bern, Switzerland; [3]The Hospital for Sick Children, Division of Clinical and Metabolic Genetics, Toronto, Canada; [4]University Health Network and Mount Sinai Hospital, The Fred A. Litwin Family Centre in Genetic Medicine, Toronto, Canada; [5]Warren Alpert Medical School of Brown University, Division of Human Genetics, Department of Pediatrics, Providence, United States; [6]CHU Hôspital Sud, Clinical Genetics Department, Rennes, France; [7]CHU, Service de Génétique Moléculaire et Génomique, Rennes, France; [8]Univ Rennes, CNRS, IGDR, UMR 6290, Rennes, France; [9]Nemours/*

*Alfred I. DuPont Hospital for Children, Division of Genetics, Department of Pediatrics, Wilmington, United States; [10]Children's Mercy Kansas City, Genomic Medicine Center, Department of Pathology and Laboratory Medicine, Kansas City, United States; [11]Broad Institute of MIT and Harvard, Broad Center for Mendelian Genomics, Cambridge, United States; [12]Boston Children's Hospital, Harvard Medical School, Division of Genetics and Genomics, Boston, United States; [13]Schneider Children's Medical Center of Israel, Petach Tikvah, Israel; [14]David Geffen School of Medicine, UCLA, Division of Medical Genetics, Department of Pediatrics, Los Angeles, United States; [15]Boston Children's Hospital, Harvard Medical School, Movement Disorders Program, Department of Neurology, Boston, United States; [16]GeneDx, LLC, Gaithersburg, United States; [17]Columbia University Vagelos College of Physicians and Surgeons, New York, United States; [18]CHU Nantes, Department of Medical Genetics, Nantes, France; [19]Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute of Human Genetics, Erlangen, Germany; [20]University Hospital Erlangen, Friedrich-Alexander University of Erlangen-Nürnberg (FAU), Centre for Rare Diseases Erlangen (ZSEER), Erlangen, Germany; [21]Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut für Biochemie, Erlangen, Germany; [22]Columbia University, Departments of Pediatrics and Medicine, New York, United States*

**Background/Objectives:** *LHX2* encodes the LIM homeobox 2 transcription factor (LHX2), which is highly expressed in brain and well conserved across species, but has not been clearly linked to neurodevelopmental disorders (NDD) to date.

**Methods:** Through international collaboration, we identified 19 individuals from 18 families with variable neurodevelopmental phenotypes, carrying a small chromosomal deletion, likely gene-disrupting or missense variants in *LHX2*. Functional consequences of missense variants were investigated in cellular systems, and impact of *LHX2* loss on neuronal function was investigated in *Drosophila melanogaster*.

**Results:** Affected individuals presented with developmental and/or behavioral abnormalities, autism-spectrum disorder, variable intellectual disability, and microcephaly. We observed nucleolar accumulation for two missense variants located within the DNA-binding HOX domain, impaired interaction with co-factor LDB1 for another variant located in the protein-protein interaction mediating LIM domain, and impaired transcriptional activation by luciferase assay for four missense variants. In *Drosophila*, we observed impaired basic locomotor ability as well as reduced daily activity upon pan-neuronal knockdown of the fly ortholog *apterous*.

**Conclusion:** We implicate *LHX2* haploinsufficiency as causative for a variable NDD. Our findings suggest a loss-of-function mechanism also for *LHX2* missense variants. Together, our observations underscore the importance of *LHX2* in nervous system and for variable neurodevelopmental phenotypes.

**Conflict of Interest:** Cosima Schmid: None declared, Anne Gregor: None declared, Gregory Costain: None declared, Chantal Morel: None declared, Lauren Massingham: None declared, Jennifer Schwab: None declared, Chloe Quelin: None declared, Marie Faoucher: None declared, Julie Kaplan: None declared, Rebecca Procopio: None declared, Carol J Sauders: None declared, Ana S. A. Cohen: None declared, Gabrielle Lemire: None declared, Stephanie Sacharow: None declared, Anne O'Donnell-Luria AODL is a paid member of the Scientific Advisory Board of SPARK for Autism, Ranit Jaron: None declared, Jessica Shamshoni: None declared, Daniela Schweitzer: None declared, Darius Ebrahimi-Fakhari: None declared, Kristin Monaghan employee of GeneDx, LLC, Timothy Palculict employee of GeneDx, LLC, Melanie Napier employee of GeneDx, Alice Tao: None declared, Bertrand Isidor: None declared, Kamran Moradkhani: None declared, André Reis: None declared, Heinrich Sticht: None declared, Wendy Chung: None declared, Christiane Zweier: None declared

### C18.6 De novo variants in KCNA3 cause developmental and epileptic encephalopathy

*Maria Virginia Soldovieri[1], Paolo Ambrosino[1], Ilaria Mosca[1], Ilenio Servettini[1], Francesca Pietrunti[1], Georgio Belperio[2], Thomas Bast[3], Paul Benke[4], Tobias Dietel[3], Sian Ellard[5], Alice Gardham[6], Susan Hughes[7], Vain Jain[8], Angie Lichty[9], Raymond Louie[9], Sarju Mehta[10], Sandra Moore[5], Konrad Platzer[11], Eloise Prijoles[9], Carol J Sauders[7], Jolanda Schieving[12], Bonnie R. Sullivan[7], Brian Udell[13], Bregje van Bon[12], Judith S Verhoeven[14], Steffen Syrbe[15], Maurizio Taglialatela[16],* ***Johannes Lemke***[11]

*[1]University of Molise – Department of Medicine and Health Sciences Vincenzo Tiberio, Naples, Italy; [2]University of Sannio RCOST, Benevento, Italy; [3]Diakonie Kork Epilepsiezentrum, Kehl, Germany; [4]Joe Di Maggio Children's Hospital, Hollywood, United States; [5]Royal Devon & Exeter Hospital, Exeter, United Kingdom; [6]Northwick Park Hospital, Harrow, United Kingdom; [7]Children's Mercy Adele Hall Campus, Kansas City, United States; [8]University Hospital of Wales, Cardiff, United Kingdom; [9]Greenwood Genetic Center, Greenwood, United States; [10]Addenbrooke's Hospital, Cambridge, United Kingdom; [11]Institute of Human Genetics, Leipzig, Germany; [12]Radboud University Medical Center, Nijmegen, Netherlands; [13]Child Development Center, Bethesda, United States; [14]Kempenhaeghe, Loc. Hans Berger Kliniek, Oosterhout, Netherlands; [15]University Hospital Heidelberg, Heidelberg, Germany; [16]University of Naples Federico II, Napoli, Italy*

Developmental and Epileptic Encephalopathies (DEE) are rare conditions frequently associated with drug-resistant epilepsy and developmental delay. We describe ten individuals with a novel type of DEE, each carrying a de novo heterozygous variant in *KCNA3* encoding the Kv1.3 voltage-gated, shaker-related potassium channel subunit. Affected patients showed marked speech delay with or even without intellectual disability, epilepsy, and autism spectrum disorder. Except for A361T, electrophysiological recordings from heterologously expressed Kv1.3 channels carrying each of the variant found in our cohort revealed significant functional changes, supporting their role in disease pathogenesis. These ranged from faster inactivation kinetics (P11R), to reduced currents with (T443I, G468F, and P477H) or without (I431N) dominant-negative effects indicative of "pure" loss-of-function (LoF), to mixed loss- and gain-of-function (GoF) effects (A357V, I455V, V460M, and V478M). Kv1.3 currents in lymphoblasts from the proband carrying the V478M variant displayed functional changes qualitatively similar to those observed upon heterologous expression of mutant channels compared to controls. Furthermore, we identifyed that the antidepressant drug fluoxetine blocked Kv1.3 and Kv1.3 V478M channels in lymphoblasts. Thus, we associate variants in *KCNA3* with a human phenotype for the first time and show that affected patients present with DEE sharing similarities with other shaker-related channelopathies. Our results moreover suggest a potential personalized treatment approach for individuals carrying *KCNA3* variants with significant GoF effects.

**Conflict of Interest:** None declared

## C19 INNOVATIVE METHODS IN STATISTICAL GENETICS

### C19.1 Inferring comprehensive disease signatures with machine learning from UK Biobank biomarkers and other quantitative traits to enhance PheWAS analyses

***Manik Garg***[1], *Marcin Karpinski[1], Ryan Dhindsa[2;3;4], Dorota Matelska[1], Amanda O'Neill[1], Quanli Wang[4], Andrew Harper[1], Slavé Petrovski[1], Dimitrios Vitsios[1]*

[1]AstraZeneca, Centre for Genomics Research, Melbourn, United Kingdom; [2]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, United States; [3]Jan and Dan Duncan Neurological Research Institute, Houston, United States; [4]Astra Zeneca, Centre for Genomics Research, Waltham, United States

Refined patient stratification and sufficient sample sizes are critical factors in identifying confident genetic signals from phenome-wide association studies (PheWAS). Characterising patient cohorts with high confidence can suffer from incompleteness of electronic health records, inaccuracies in self-reported diseases, undiagnosed conditions due to an early stage of the disease at the time of assessment and other factors. Leveraging the wealth of information from UK Biobank (UKB), we present MILTON: a framework for phenome-wide inference of disease signatures using machine learning. The learnt models infer the signature of each phenotype or disease based on a set of 60 quantitative traits available in UKB, taking into account the time-lag between biomarker collection and diagnosis. We then extract novel cases from the rest of the UKB cohort, predicted to have similar biomarker profile with the original disease cohorts. We applied MILTON to 4,152 ICD10-based cohorts with at least 122 known cases, achieving an AUC > 0.80 for 405 of those phenotypes. By performing binary PheWAS analysis on MILTON extended cohorts, we were able to pick up 1268 out of 18,502 significant associations ($p < 5 \times 10^{-8}$), previously missed by binary PheWAS collapsing analysis but picked up by quantitative PheWAS on 450K UKB WES samples reported on AstraZeneca PheWAS Portal (https://azphewas.com/, Wang et al., 2021). We also found 396 completely novel hits of higher confidence (MILTON AUC > 0.90) as well as thousands more that require further investigation.

**Conflict of Interest:** Manik Garg Author is an employee of AstraZeneca., Marcin Karpinski Author is an employee of AstraZeneca., Ryan Dhindsa Author is an employee of AstraZeneca., Dorota Matelska Author is an employee of AstraZeneca., Amanda O'Neill Author is an employee of AstraZeneca., Quanli Wang Author is an employee of AstraZeneca., Andrew Harper Author is an employee of AstraZeneca., Slavé Petrovski Author is an employee of AstraZeneca., Dimitrios Vitsios Author is an employee of AstraZeneca.

### C19.2 Imputation of low-coverage sequencing data from 150,119 UK Biobank genomes

***Simone Rubinacci***[1;2;3], *Robin J. Hofmeister*[3], *Bárbara Sousa da Mota*[3], *Olivier Delaneau*[3]

[1]Brigham and Women's Hospital and Harvard Medical School, Division of Genetics, Department of Medicine, Boston, United States; [2]Broad Institute of MIT and Harvard, Cambridge, United States; [3]University of Lausanne, Department of Computational Biology, Lausanne, Switzerland

**Background**: Recent work highlights low-coverage whole-genome sequencing (LCWGS), as a cost-effective genotyping technology for statistical and population genetics. However, imputation methods cannot cope with the size of recent high-coverage reference panels.

**Methods**: We introduce GLIMPSE2, a LCWGS imputation method designed to handle millions of reference samples. We applied GLIMPSE2 to impute LCWGS genomes from a reference panel of 150,119 high-coverage UK-Biobank (UKB) samples and compared genotyping accuracy and downstream performances to high-coverage and imputed Axiom-array data.

**Results**: Using the UKB reference panel, GLIMPSE2 imputes a genome for less than 0.10$, a decrease of two-to-three orders of magnitude compared to existing methods. LCWGS benefits from the UKB panel mainly for very low coverage settings and rare variants: for 0.25x and 0.5x, GLIMPSE2 imputes variants at 0.01% MAF with r2 = 0.8 and r2 = 0.89 respectively, compared to r2 = 0.71 for the Axiom-array. We quantify the impact of this increase in accuracy for disease association by performing GWAS with 10,000 individuals across 100 traits and show that the Axiom-array performs similarly to 0.25x (Beta r2 = 0.9, p-val r2 = 0.87), and is inferior to 1x data (Beta r2 = 0.97, *p*-val r2 = 0.95). Finally, we looked at the power of imputed data in burden-test analysis and show that 1x data significantly outperforms the Axiom-array for each annotation ($p < 2e\text{-}16$).

**Conclusions**: GLIMPSE2 is an efficient LCWGS imputation method designed for the most recent high-coverage biobanks. Our results show that LCWGS increases the accuracy of associations compared to SNP-arrays, proposing an better-suited alternative for future large-scale genetic studies.

**Grant References**: Swiss National Science Foundation PP00P3_176977.

**Conflict of Interest:** None declared

### C19.3 AduLT: Fast and powerful alternative to time-to-event GWAS

***Emil Pedersen***[1], *Esben Agerbo*[1], *Oleguer Plana-Ripoll*[1], *Jette Steinbach*[1], *Morten Krebs*[2], *David Michael Hougaard*[3], *Thomas Werge*[2], *merete nordentoft*[4], *Anders Børglum*[4], *Katherine Musliner*[1], *Andrea Ganna*[5], *andrew schork*[2], *Preben Bo Mortensen*[1], *John J. McGrath*[1], *Florian Privé*[1], *Bjarni Vilhjalmsson*[1]

[1]national center for register-based research, Aarhus, Denmark; [2]Institute of biological psychiatry, mental health center – sct Hans, Copenhagen University Hospital, Copenhagen, Denmark; [3]Department for Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark; [4]Lundbeck Foundation Initiative for Integrative Psychiatric Research, iPSYCH, Aarhus, Denmark; [5]Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland

**Background:** Cox proportional hazards (PH) models have been proposed to analyse time-to-event phenotypes in GWAS data. Recently, SPACox, a computationally efficient Cox PH method, was proposed to handle large GWAS data sets. While PH models have many useful properties, their ability to identify genetic associations has not been benchmarked under different generative models and case ascertainment, which is common in GWAS data.

**Methods:** We propose using the age-dependent liability threshold model (AduLT), first introduced with the LT-FH++ method, as an alternative to using Cox PH models for time-to-event phenotypes in GWAS data. AduLT first estimates an individual genetic liability, based on liability-scale heritability and population-representative cumulative incidence proportions stratified by sex and birth year. The AduLT phenotype can then be used with any continuous GWAS method, e.g. linear (mixed) models.

**Results:** We benchmarked GWAS with AduLT, SPACox, and case-control status in simulated and iPSYCH2015 study. In simulations with case ascertainment, we found AduLT and case-control status had a higher power than SPACox in almost all simulations. We analysed four ascertained psychiatric disorders in the iPSYCH cohort and AduLT found 20 independent genome-wide significant associations, while case-control found 17 and SPACox found 8, consistent with simulation results.

**Conclusion:** We find that AduLT performs on par with or better than SPACox in simulations and four of the psychiatric disorders considered in the iPSYCH2015 cohort, especially when cases are ascertained. As electronic health records linked to genetic data become more common, we expect robust time-to-event models, like AduLT, to become widely used.

**Conflict of Interest:** None declared

### C19.4 Fine-mapping genome-wide association studies with missing genotype data

***Joonas Kartau***[1;2], *Matti Pirinen*[1;2;3]

[1]*Department of Mathematics and Statistics, University of Helsinki, Helsinki, Finland;* [2]*Institute for Molecular Medicine Finland (FIMM), Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki, Finland;* [3]*Department of Public Health, University of Helsinki, Helsinki, Finland*

Fine-mapping is a statistical approach to identify causal variants from the summary data of a Genome-Wide Association Study (GWAS). To improve statistical power, data from many GWAS cohorts are routinely combined in a meta-analysis before fine-mapping. This can result in sample size mismatches between variants, as some studies might not include certain variants. Current methods, such as FINEMAP, use computational approximations that ignore missing information and may produce unreliable results. We have extended the FINEMAP algorithm to properly handle missing information and have implemented the algorithm in an efficient way.

We initially tested our method in a comprehensive simulation study using UK biobank data, and then applied it to real data by fine-mapping the association between the GCKR locus and triglyceride levels. Using a sample of 30,000 individuals, we identified the missense variant rs1260326 as a causal variant (probability > 0.999) in accordance with existing literature. Next, we modeled a meta-analysis setting by splitting the data into subcohorts and induced cohort-dependent missingness among the variants. We show that when the missense variant was not observed in full data, then the standard FINEMAP algorithm failed to identify the missense variant (probability < 0.08), whereas our method still succeeded (probability > 0.98).

In conclusion, our extension of the FINEMAP algorithm allows reliable fine-mapping in a typical meta-analysis setting where different variants may have varying sample sizes.

**Conflict of Interest:** Joonas Kartau University of Helsinki, Matti Pirinen University of Helsinki, Academy of Finland (338507, 352795), Sigrid Jusélius Foundation project grant

### C19.5 Detection of assortative mating on complex traits in the absence of spousal information

***Yuanxiang Zhang***[1], *Peter Visscher*[1], *Loic Yengo*[1]

[1]*Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia*

**Background/Objectives:** Assortative mating (AM), occurs when mate choice is driven by phenotypic (dis)similarities among mates. This phenomenon is commonly observed in humans and is known to increase the heritability of complex traits and contribute to increasing disease prevalence (if AM involves disease risk factors). Current statistical methods for detecting AM rely on the availability of large association studies of traits potentially driving AM, or large collections of genotyped spouses or close relatives.

**Methods:** We introduce the Disequilibrium Genome-based Restricted Maximum Likelihood (DGREML) method, a new method to quantify the contribution of AM to the SNP-based heritability of complex traits only using unrelated individuals. We show through theory and simulations that DREML can detect and quantify AM, and reliably predict the underlying phenotypic correlations between mates.

**Results:** We applied DREML to 26 complex traits in UK Biobank (UKB). We detected AM for height, educational attainment (EA), income, fluid intelligence, self-reported health status, and time spent watching TV. Our method predicts that BMI similarity between spouses is largely attributable to (geographically stratified) environments. DREML analyses conditioned on EA show that signals of AM detected for income, fluid intelligence, self-reported health status, and time spent watching TV can be explained by AM on EA.

**Conclusion:** Our findings show that DREML is capable of quantifying AM patterns using genotypes and phenotypes of unrelated individuals, which opens new opportunities to study mating choice in populations currently under-represented in worldwide genetic studies.

**Grant references:** This research was supported by the Australian Research Council (Grant number: DE200100425).

**Conflict of Interest:** Yuanxiang Zhang Full-time PhD Student at the University of Queensland

Casual academic staff at the University of Queensland, Peter Visscher Professor at the University of Queensland, Loic Yengo Associate professor at the University of Queensland, ARC grant number DE200100425

### C19.6 GestaltMatcher supports lumping and splitting decision-making by facial phenotype descriptors

***Hannah Klinkhammer***[1;2], *Hellen Lesmann*[1;3], *Shahida Moosa*[4], *Alexander Hustinx*[1], *Behnam Javanmardi*[1], *Jing-Mei Li*[1], *Christopher Chun Yu Mak*[5], *Luisa Averdunk*[6], *Felix Distelmaier*[6], *Brian Hon Yin Chung*[5], *Peter Krawitz*[1], *Tzung-Chien Hsieh*[1]

[1]*University Hospital Bonn, Institute for Genomic Statistics and Bioinformatics, Bonn, Germany;* [2]*University Hospital Bonn, Institute for Medical Biometry, Informatics and Epidemiology, Bonn, Germany;* [3]*University Hospital Bonn, Institute of Human Genetics, Bonn, Germany;* [4]*Stellenbosch University, Division of Molecular Biology and Human Genetics, Stellenbosch, South Africa;* [5]*LKS Faculty of Medicine, University of Hong Kong, Department of Paediatrics and Adolescent Medicine, School of Clinical Medicine, Hong Kong, Hong Kong;* [6]*University Children's Hospital Duesseldorf, Department of General Pediatrics, Neonatology and Pediatric Cardiology, Duesseldorf, Germany*

**Background:** Lumping and splitting is a longstanding discussion in clinical genetics. Accurately categorizing the disorders will help patients receive the appropriate treatment earlier. Because many pleiotropic genes cause more than one disorder, it is crucial to split different phenotypes. Since next-generation phenotyping (NGP), such as GestaltMatcher, can quantify the facial syndromic similarities among patients, it can help to lump and split phenotypes. Therefore, we demonstrate how GestaltMatcher splits two phenotypes caused by mutations in the same gene.

**Methods:** We compiled a dataset of 7459 images from 5995 patients diagnosed with 449 rare disorders. We first utilized GestaltMatcher to encode each photo to a facial phenotype descriptor in the Clinical Face Phenotype Space (CFPS), where distances between images define facial syndromic similarity. We further developed a statistical method based on the pairwise distances of images to validate if two given cohorts stem from the same disorder or different disorders. We presented a cohort with disease-causing mutations in *Gene-X* for the lumper and splitter analysis.

**Results:** With clustering analysis in the CFPS, we demonstrated how GestaltMatcher can split *Gene-X* into two different phenotypes. On the molecular level, the pathogenic mutations of *Gene-X* are subgrouped into two different exons differing in nonsense-mediated decay (NMD), and the two subgroups also showed

different methylation signatures. Additionally, combining NGP and methylation data yielded evidence to split subgroups of three known pleiotropic genes (*SRCAP, NFIX, SMARCA2*).

**Conclusion:** The combination of NGP and the analysis of methylation signatures facilitates the lumping and splitting decision-making.

**Conflict of Interest:** Hannah Klinkhammer: None declared, Hellen Lesmann: None declared, Shahida Moosa: None declared, Alexander Hustinx: None declared, Behnam Javanmardi: None declared, Jing-Mei Li: None declared, Christopher Chun Yu Mak This work was supported by grants from the Society for the Relief of Disabled Children, The Health and Medical Research Fund (PR-HKU-4), the Agence Nationale de la Recherche "Investissements d'Avenir" program (ANR-10-IAHU-01), MSDAvenir (Devo-Decode project) and AXA ("Tête et Cœur" project)., Luisa Averdunk: None declared, Felix Distelmaier: None declared, Brian Hon Yin Chung This work was supported by grants from the Society for the Relief of Disabled Children, The Health and Medical Research Fund (PR-HKU-4), the Agence Nationale de la Recherche "Investissements d'Avenir" program (ANR-10-IAHU-01), MSDAvenir (Devo-Decode project) and AXA ("Tête et Cœur" project)., Peter Krawitz: None declared, Tzung-Chien Hsieh: None declared

## C20 HIGH- AND LOW-PENETRANCE INHERITED CANCER RISK

### C20.1 Diagnostic yield and short-term clinical implications of nationwide germline whole genome sequencing in 280 children with solid tumors

***Bianca Tesi***[1;2;3], *Fulya Taylan*[1;3], *Kristina Lagerstedt-Robinson*[1;3], *Frida Abel*[4;5], *Maria Hellberg*[6], *Teresita Diaz de Stahl*[7;8], *Sandra Wessman*[7;9], *Sofie Samuelsson*[6;10], *Karin Henning*[11;12], *Tony Frisk*[11;12], *Magnus Sabel*[13;14], *Hartmut Vogt*[15], *Per Nyman*[16], *Geraldine Giraud*[17;18], *Joakim Wille*[19;20], *Cornelis Jan Pronk*[19;20], *Ulrika Norén Nyström*[21], *Magnus Borssén*[22], *Maria Fili*[23], *Gustav Stålhammar*[24;25], *Nikolas Herold*[26;27], *Sara Orrsjö*[5;28], *Anna Poluha*[3;29], *Carolina Maya Gonzalez*[3], *Linda Arvidsson*[6;10], *Anna Rosén*[30], *Katja Ekholm*[1;31], *Ekaterina Kuchinskaya*[32], *Anna-Lotta Hallbeck*[33], *Alia Shamikh*[7;9], *Fredrik Mertens*[10], *Richard Rosenquist Brandell*[1;31], *Emma Tham*[1;3], *Pernilla Grillner*[27], *Johanna Sandgren*[7;9], *Gustaf Ljungman*[18], *David Gisselsson Nord*[10;34], *Ann Nordgren*[1;3;35]

[1]*Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden;* [2]*Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden;* [3]*Department of Molecular Medicine and Surgery, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden;* [4]*Departmentof Clinical Genetics and Genomics, Sahlgrenska University Hospital, Gothenburg, Sweden;* [5]*Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden;* [6]*Department of Clinical Genetics, Pathology, and Molecular Diagnostics, Office for Medical Services, Region Skåne, Lund, Sweden;* [7]*Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden;* [8]*Clinical Pathology and Cancer Diagnostics, Karolinska University Hospital, Stockholm, Sweden;* [9]*Department of Pathology and Cancer Diagnostics, Karolinska University Hospital, Stockholm, Sweden;* [10]*Division of Clinical Genetics, Department of Laboratory Medicine, Lund University, Lund, Sweden;* [11]*Department of Pediatric Oncology, Karolinska University Hospital, Stockholm, Sweden;* [12]*Childhood Cancer Unit, Department of Women and Children´s health, Karolinska Institutet, Stockholm, Sweden;* [13]*Childhood Cancer Centre, Queen Silvia Children´s Hospital, Sahlgrenska University Hospital, Gothenburg, Sweden;* [14]*Institute for clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden;* [15]*H.R.H. Crown Princess Victoria Children's Hospital, and Division of Children's and Women's Health, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden;* [16]*H.R.H. Crown Princess Victoria Children's Hospital, and Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden;* [17]*Department of Immunology, Genetic and Pathology, Uppsala University Hospital, Uppsala, Sweden;* [18]*Department of Woman and child's heath, Uppsala University Hospital, Uppsala, Sweden;* [19]*Childhood Cancer Center, Skåne University Hospital, Lund, Sweden;* [20]*Div Molecular Hematology/Wallenberg Center Molecular Medicine, Lund University, Lund, Sweden;* [21]*Departement of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden;* [22]*Department of Pediatrics, Umeå University Hospital, Umeå, Sweden;* [23]*Department of Ocular Oncology, St Erik Eye Hospital, Stockholm, Sweden;* [24]*St. Erik Eye Hospital, Stockholm, Sweden;* [25]*Department of Clinical Neuroscience, Division of Ophthalmology and Vision, Unit of Ocular Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden;* [26]*Division of Paediatric Oncology and Surgery, Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden;* [27]*Paediatric Oncology, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden;* [28]*Department of Clinical Genetics and Genomics, Sahlgrenska University Hospital, Gothenburg, Sweden;* [29]*4Department of Clinical Genetics, Uppsala University Hospital, Uppsala, Sweden;* [30]*Department of Radiation sciences, Umeå university, Umeå, Sweden;* [31]*Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden;* [32]*Department of Clinical Genetics, Linköping University Hospital, Linköping, Sweden;* [33]*Department of Clinical Clinical Genetics, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden;* [34]*Medical Services, Section for pathology, Region Skåne, Lund, Sweden;* [35]*"Department of Laboratory Medicine, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden*

**Background/Objectives:** To present the results from the nationwide and prospective Childhood Cancer Predisposition (ChiCaP) study that investigates a universal germline testing strategy for children with cancer.

**Methods:** Germline whole genome sequencing (gWGS) was performed in 280 children consecutively diagnosed with a solid malignant tumor including central nervous system (CNS) tumors from May 2021 until December 2022 in Sweden. gWGS data was analyzed for germline variants in either 189 ($n = 234$) or 55 ($n = 46$) genes based on phenotype. Clinical information was collected through a questionnaire.

**Results:** Overall, 35 children (12,5%) had a germline finding consistent with a ChiCaP syndrome, of which 25 (71%) were unknown at cancer diagnosis. Sixteen different genes were found mutated, the most common being *RB1* ($n = 7$), *NF1* ($n = 6$), *MSH2* ($n = 4$), and *WT1* ($n = 3$). Paired tumor sequencing data was available for 24 (68%) of the 35 children with ChiCaP findings and showed a somatic profile concordant with the germline finding in 20 (80%) cases. ChiCaP findings were discussed at multidisciplinary rounds and reported to the treating physicians. Further analyses on potential ChiCaP indicators, such as family history and other phenotypes, as well as analysis of short-term clinical implications from ChiCaP findings are ongoing.

**Conclusions:** Overall, germline testing finds a heterogeneous group of ChiCaP diagnoses in 12.5% of children with solid tumors. Our results support the implementation of universal germline testing for children with cancer in clinical routine and highlight the strength of paired germline-tumor analysis for interpretation of germline variants.

**Grant References:** The Swedish Childhood Cancer Fund.

**Conflict of Interest:** None declared

### C20.2 Genome-wide polygenic risk scores substantially impact colorectal neoplasm risk with implications for stratified screening

***Max Tamlander***[1], *Bradley Jermy*[1], *Elisabeth Widén*[1], *Samuli Ripatti*[1;2;3], *Nina Mars*[1;3]

[1]*Institute for Molecular Medicine Finland, FIMM, HiLIFE, University of Helsinki, Helsinki, Finland;* [2]*Clinicum, Department of Public Health, University of Helsinki, Helsinki, Finland;* [3]*Broad Institute of MIT and Harvard, Cambridge, MA, United States*

**Background/Objectives:** Polygenic risk scores (PRS) show promise in colorectal cancer (CRC) prediction, but data on how PRSs impact risk-based screening and the evolution of CRC risk in men and women are lacking.

**Methods:** We studied 412,180 individuals (6847 CRC cases) in the FinnGen study, combining longitudinal health and genomics data. After careful sex- and population-specific calibration and development of a genome-wide CRC PRS, we evaluate optimal PRS-informed CRC screening initiation ages through modeling absolute and relative disease risks using Cox model. We further evaluate sex-specific impacts of the PRS on CRC evolution and heterogeneity (e.g., precursor benign colorectal neoplasms, tumor location).

**Results:** Compared to the current screening onset at age 60 in Finland, a comparable cumulative risk to the average population is reached by individuals with high PRS (>99th percentile) at age 48.7 (women) and 49.8 (men), and correspondingly at ages 65.1 and 66.0 with protective PRS (<20th percentile). Prediction accuracy was highest for men, distal CRC, and adenocarcinoma, with PRS improving clinical risk prediction. The PRS predicted 10-year CRC risk after negative index colorectal endoscopy among healthy, average-risk individuals (>80th vs <20th percentile adjusted HR 3.2 (95%CI 2.1–4.9), absolute risks at 0.2% (0.01–0.3) vs 0.8% (0.6–1.0)), suggesting benefits of earlier surveillance for high-risk individuals.

**Conclusions:** We demonstrate utility of PRS-informed CRC screening, and similar approaches may be useful also in other cancers. Sex- and site-specific disparities of the PRS require attention when applying it to CRC prediction.

**Grant References**: Academy of Finland;EU Horizon 2020;Sigrid Jusélius Foundation;Univ.Helsinki

**Conflict of Interest:** None declared

### C20.3 Large scale case-control analyses of rare variant data; application to BRCA1 and BRCA2

***Denise O'Mahony***[1], *Maria Zanti*[2], *Michael T. Parsons*[3], *Joe Dennis*[4], *Cristina Fortuno*[5], *Maaike Vreeswijk*[6], *Miguel de la Hoya*[7], *Logan Walker*[8], *Melissa C. Southey*[9;10;11], *Peter Devilee*[6;12], *Paul James*[13], *David E. Goldgar*[14], *Douglas F. Easton*[4;15], *Amanda B. Spurdle*[5], *Kyriaki Michailidou*[2], *Evidence-based Network for the Interpretation of Germline Mutant Alleles*[16], *Breast Cancer Association Consortium*[17]

[1]*The Cyprus Institute of Neurology and Genetics, Biostatistics Unit, Nicosia, Cyprus;* [1]*The Cyprus Institute of Neurology and Genetics, Biostatistics Unit, Nicosia, Cyprus;* [3]*QIMR Berghofer Medical Research Institute, Population Health Program, Queensland, Australia;* [4]*University of Cambridge, Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, Cambridge, United Kingdom;* [3]*QIMR Berghofer Medical Research Institute, Population Health Program, Queensland, Australia;* [6]*Leiden University Medical Center, Department of Human Genetics, Leiden, Netherlands;* [7]*Hospital Clinico San Carlos, IdISSC (Instituto de Investigación Sanitaria del Hospital Clínico San Carlos), Molecular Oncology Laboratory, CIBERONC, Madrid, Spain;* [8]*University of Otago, Department of Pathology and Biomedical Science, Chirstchurch, New Zealand;* [9]*Monash University, Precision Medicine, School of Clinical Sciences at Monash Health, Victoria, Australia;* [10]*University of Melbourne, Department of Clinical Pathology, Melbourne, Australia;* [11]*Cancer Council Victoria, Cancer Epidemiology Division, Melbourne, Australia;* [12]*Leiden University Medical Center, Department of Pathology, Leiden, Netherlands;* [13]*The Royal Melbourne Hospital and Peter MacCallum Cancer Centre, Parkville Familial Cancer Centre, Melbourne, Australia;* [14]*Huntsman Cancer Institute, University of Utah School of Medicine, Department of Dermatology, Utah, United States;* [15]*University of Cambridge, Centre for Cancer Genetic Epidemiology, Department of Oncology, Cambridge, United Kingdom;* [16]*Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) Consortium, Queensland, Australia;* [17]*Breast Cancer Association Consortium (BCAC), Cambridge, United Kingdom*

**Background/Objectives**. Clinical genetic testing of high-risk cancer predisposition genes often leads to the identification of variants of uncertain significance, which complicate management of carriers and families. Case-control data can be used to inform variant interpretation. We developed a novel rare variant case-control likelihood ratio (ccLR) method, that incorporates gene- and age-specific penetrance, which we applied to a large breast cancer case-control dataset.

**Methods**. Our method was used for the analysis of 1553 rare *BRCA1* and *BRCA2* variants (with ≥2 carriers and MAF < 0.001), identified by panel sequencing of 35,674 breast cancer cases and 32,532 controls from the Breast Cancer Association Consortium (BCAC). Resulting LRs were categorised as weights for (LR ≥ 18.70) or against (LR < 0.48) pathogenicity following recommendations of the American College of Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/AMP) variant classification framework.

**Results**. Approximately 10% of variants (PVS1 ACMG/AMP criterion) were predicted pathogenic. For the remainder 1,399 variants with unknown consequence (missense, intronic, UTRs, in-frame indels, synonymous), LRs provided evidence in favour of pathogenicity for 16 variants (5 intronic and 11 missense) and evidence against pathogenicity for 952 variants. For a set of missense variants with (likely) benign/pathogenic ClinVar class, we observed a 97.8% consistency with the calculated case-control LRs.

**Conclusion**. Our analysis provided evidence relevant for variant classification for 70% of *BRCA1* and *BRCA2* rare variants of unknown consequence. We demonstrate the utility of the ccLR method to provide weighted information to aid interpretation of a large proportion of rare variants identified by sequencing of case-control datasets.

**Conflict of Interest:** None declared

### C20.4 Clinical implications of incorporating genetic and non-genetic risk factors in CanRisk-based breast cancer risk prediction: results from an international multicenter-study

***Anja Tüchler***[1], *Antoine De Pauw*[2;3], *Corinna Ernst*[1], *Amélie Anota*[4;5;6], *Inge Lakeman*[7], *Julia Dick*[1], *Kerstin Rhiem*[1], *Cristi J. van Asperen*[7], *Monika Maringa*[1], *Natalie Herold*[1], *Britta Bluemcke*[1], *Robert Remy*[1], *Anke Westerhoff genannt Hestenberg*[1], *Lisa Richters*[1], *Nadine Kütting*[1], *Barbara Wappenschmidt*[1], *Peter Devilee*[8;9], *Dominique Stoppa-Lyonnet*[10;11], *Rita Katharina Schmutzler*[1], *Eric Hahnen*[1]

[1]*Center for Familial Breast and Ovarian and Cancer, Center for Integrated Oncology (CIO), University of Cologne, Cologne, Germany;* [2]*Institut Curie, Paris, France;* [3]*PSL Research University, Paris, France;* [4]*Léon Bérard Center, Department of Clinical Research and Innovation, Lyon, France;* [5]*Léon Bérard Center, Human and Social Sciences Department, Lyon, France;* [6]*Léon Bérard Center, French National*

*Platform Quality of Life and Cancer, Lyon, France; [7]Leiden University Medical Center (LUMC), Department of Clinical Genetics, Leiden, Netherlands; [8]Leiden University Medical Center (LUMC), Department of Human Genetics, Leiden, Netherlands; [9]Leiden University Medical Center (LUMC), Department of Pathology, Leiden, Netherlands; [10]Université Paris Cité, Paris, France; [11]Institut Curie, Inserm U830, Paris, France*

**Objective:** To determine changes in intensified breast surveillance (IBS) recommendations by including polygenic risk scores (PRSs) and non-genetic risk factors (NGRFs) in addition to cancer family history (FH) and germline pathogenic variant (PVs) status in CanRisk-based breast cancer (BC) risk prediction according to country-specific IBS thresholds.

**Methods:** CanRisk-based BC risks (estimated lifetime risk, eLTR; estimated 10 year risk, e10YR) were calculated using FH and germline PV status alone and complemented with NGRFs (excluding breast density), and a 306 SNP-based $PRS_{306}$. Thresholds for IBS were 20% eLTR (France), 30% eLTR (Netherlands) and 5% e10YR (Germany).

**Results:** 425 women (mean age 40.6 years, range 21-74) were included. For women who tested non-informatively, $PRS_{306}$ and NGRFs changed clinical recommendations for 31.0%, (57/184, 20% eLTR), 15.8% (29/184, 30% eLTR) and 22.4% (41/183, 5% e10YR) compared with using only FH and PV status. For women tested negatively for PVs observed in the respective index patients, risk category changed for 16.7% (25/150, 20% eLTR), 1.3% (2/150 (30% eLTR) and 9.5% (14/147, 5% e10YR). For 82 women with PVs in highly penetrant risk genes (*BRCA1*, *BRCA2*, *PALB2*), no eLTR category change was observed.

**Conclusion:** Irrespective of country guideline, PRS and NGRFs have considerable impact on individual IBS recommendations for women tested non-informatively/negatively.

**Grant References:** EU Horizon 2020 (634935), German Cancer Aid (110837, 70114178), German Federal Ministry of Education and Research (01GY1901), Köln Fortune, Faculty of Medicine, University of Cologne, Germany.

**Conflict of Interest:** Anja Tüchler: None declared, Antoine De Pauw AstraZeneca, Corinna Ernst: None declared, Amélie Anota: None declared, Inge Lakeman: None declared, Julia Dick: None declared, Kerstin Rhiem: None declared, Cristi J. van Asperen: None declared, Monika Maringa: None declared, Natalie Herold: None declared, Britta Bluemcke: None declared, Robert Remy: None declared, Anke Westerhoff genannt Hestenberg: None declared, Lisa Richters: None declared, Nadine Kütting: None declared, Barbara Wappenschmidt: None declared, Peter Devilee: None declared, Dominique Stoppa-Lyonnet: None declared, Rita Katharina Schmutzler: None declared, Eric Hahnen: None declared

### C20.5 Data from the SWEP53 study: Whole-body MRI surveillance in gTP53 carriers is perceived as beneficial with no increase in cancer worry regardless of previous cancer

***Meis Omran***[1;2], *Hemming Johansson*[1], *Claudia Lundgren*[3], *Gustav Silander*[4], *Marie Stenmark Askmalm*[5], *Niklas Loman*[6;7], *Annika Baan*[8], *Jamila Adra*[8], *Ekaterina Kuchinskaya*[9], *Lennart Blomqvist*[10;11], *Emma Tham*[10;12], *Svetlana Bajlica Lagercrantz*[1;2;12], *Yvonne Brandberg*[1]

*[1]Karolinska Institutet, Oncology-Pathology, Stockholm, Sweden; [2]Karolinska University Hospital, Cancer Theme, Stockholm, Sweden; [3]Uppsala University Hospital, Department of Immunology, Genetics and Pathology, Uppsala, Sweden; [4]Umeå University, Department of Radiation Sciences, Umeå, Sweden; [5]Skåne University Hospital, 5Division of Clinical Genetics, Department of Laboratory Medicine, Office for Medical Services, Lund, Sweden; [6]Lund University, Division of Oncology and Pathology, Department of Clinical Sciences, Lund, Sweden; [7]Skåne University Hospital, Department of Hematology, Oncology and Radiation Physics, Lund, Sweden; [8]Sahlgrenska University Hospital, Department of Oncology, Gothenburg, Sweden; [9]Linköping University Hospital, Department of Clinical Genetics, Linköping, Sweden; [10]Karolinska Institutet, Department of Molecular Medicine and Surgery, Stockholm, Sweden; [11]Karolinska University Hospital, Department of Imaging and Physiology, Stockholm, Sweden; [12]Karolinska University Hospital, Department of Clinical Genetics, Stockholm, Sweden*

**Background:** To evaluate the psychosocial consequences of surveillance with whole-body MRI (WB-MRI) in individuals with the heritable *TP53* related cancer (h*TP53*rc) syndrome, also known as the Li-Fraumeni syndrome, with regards to cancer worry, perceived benefits and risks to surveillance and overall health.

**Patients and methods:** Since 2016, the national Swedish *TP53* Study (SWEP53) has offered surveillance with WB-MRI to all individuals with h*TP53*rc syndrome. Sixty participants fulfilled a baseline evaluation and an evaluation after 1 year with structured questionnaires; the Cancer Worry Scale (CWS), perceived benefits and risks of surveillance, and the 36-item Short Form Survey (SF-36). Individuals with or without previous personal cancer diagnosis were enrolled and results at baseline and after 1 year of surveillance were compared. For SF-36, a comparison with the normal population was also made.

**Results:** Participants with previous cancer tend to worry more about cancer, but both individuals with and without cancer had a positive attitude towards surveillance with no differences regarding perceived benefits and barriers to surveillance. Participants with a previous cancer scored significantly lower on some of the SF-36 subscales, as expected.

**Conclusions:** Surveillance with WB-MRI is feasible from a psychosocial point of view among *TP53* carriers with as well as without a previous history of cancer and does not increase cancer worry in any of the groups.

**Grant references:** The Cancer Research KI at Karolinska Institutet, The Cancer Research Funds of Radiumhemmet, The Rare Disease Research Foundation, Stockholm County Council, The Swedish Cancer Society, and The Swedish Childhood Cancer Fund.

**Conflict of Interest:** Meis Omran The Cancer Research Funds of Radiumhemmet, The Rare Disease Research Foundation, The Swedish Childhood Cancer Fund., Hemming Johansson: None declared, Claudia Lundgren: None declared, Gustav Silander: None declared, Marie Stenmark Askmalm: None declared, Niklas Loman: None declared, Annika Baan: None declared, Jamila Adra: None declared, Ekaterina Kuchinskaya: None declared, Lennart Blomqvist Lennart Blomqvist is co-founder of Collective Minds Radiology., Lennart Blomqvist is co-founder of Collective Minds Radiology., Emma Tham The Swedish Cancer Society, and The Swedish Childhood Cancer Fund., Svetlana Bajlica Lagercrantz The Cancer Research KI at Karolinska Institutet, The Cancer Research Funds of Radiumhemmet, Stockholm County Council., Yvonne Brandberg: None declared

### C20.6 Integrating Polygenic Risk Scores into clinical breast cancer models improves prediction in diverse cohorts

*Alessandro Bolli*[1], *Scott Kulm*[1], *Jen Kintzle*[1], *Paolo Di Domenico*[1], *Giordano Bottà*[1], ***George Busby***[1]

*[1]Allelica Inc, New York, United States*

**Background:** Breast cancer (BC) is the second leading cause of cancer death in women worldwide. Periodic mammography screening has been shown to reduce breast cancer mortality by around 20% in average-risk women and several BC risk models are currently used to identify women at higher risk who can be targeted with increased or earlier screening. However, despite

their broad use, these models display only moderate discrimination performance. Here we explore the potential of integrating PRS into BC risk models using a testing dataset comprising over 175,000 women of diverse ethnicity in the UK Biobank and Women Health's Initiative Cohorts.

**Methods:** We assessed genetic ancestry from 5 continental level ancestry groups across all individuals. We built, validated, and applied novel ancestry-specific BC PRSs, utilizing novel methodology, to our testing dataset and assessed the clinical implications of adding these PRSs to the Tyrer-Cuzick (TC) breast cancer clinical risk model.

**Results:** The PRSs displayed high risk stratification (Hazard Ratios: 1.36 (1.22–1.52; Afro-American) to 1.72 (1.39–2.13; Admixed-American) which were comparable or better than benchmarking comparisons with previously published PRS panels. The Net Reclassification Improvement comparing TC + PRS and TC-only model was around 12% for East-Asian (0.001–0.254), European (0.111–0.133), and Admixed American ancestries (0.002–0.228), 17% (0.079–0.266) for South-Asians, and 5% (0.008–0.094) for African ancestry individuals.

**Conclusions:** Our results demonstrate that optimizing PRSs for genetic ancestries and integrating them into BC risk models can lead to a significant improvement in risk stratification. This can enable more targeted use of enhanced screening/prevention strategies improving their cost/benefit ratio.

**Conflict of Interest:** Alessandro Bolli Allelica Inc, Scott Kulm Allelica Inc, Jen Kintzle Allelica Inc, Paolo Di Domenico Allelica Inc, Allelica Inc, Giordano Bottà Allelica Inc, Allelica Inc, George Busby Allelica Inc, Allelica Inc

## C21 LATE BREAKING ABSTRACTS

### C21.1 Accurate rare variant phasing of 200,031 UK Biobank whole-genomes

*Robin J. Hofmeister[1], Diogo Ribeiro[1], Simone Rubinacci[1], **Olivier Delaneau**[1]*

*[1]University of Lausanne, Lausanne, Switzerland*

**Background :** Investigating the impact of rare variations on complex traits requires large collections of samples with whole-genome or whole-exome sequencing (WGS/WES). Recently, the UK Biobank released sequence data for over 200,000 samples, with more than 750 million variant sites. The availability of haplotypes for this dataset offers vast opportunities for disease and population genetics, but phasing this large amount of data implies significant computational and statistical challenges.

**Results :** To address these challenges, we present SHAPEIT5, to efficiently and accurately phase large-scale sequencing datasets, accounting for family data when available. On the UK Biobank's WGS and WES data, we phase variants found in one individual out of 100,000 with 95% accuracy. Using the resulting haplotypes as a reference panel for genotype imputation, we greatly enhance imputation accuracy compared to the Haplotype Reference Consortium panel. Variants with a minor allele frequency of 0.01% see a four-fold increase in imputation accuracy, which in turn enhances the power of downstream GWAS. In addition, these haplotypes allow for the detection of 549 genes with loss-of-function compound heterozygotes (i.e, complete gene knockouts), which we show to complement current knowledge of gene essentiality in the human genome.

**Conclusion :** We showcase how to efficiently infer and leverage haplotypes in large sequencing datasets. Phased haplotypes for the 200,031 UKB samples and pipelines to impute SNP arrays and low-coverage sequencing data will be included in an upcoming UK Biobank data release.

**Grant references:** Swiss National Science Foundation PP00P3_176977

**Conflict of Interest:** None declared

### C21.2 Functional multi-omic studies unveil ER stress and proteasomal dysfunction in early-onset neurodegeneration in XP

*Cherif Badja[1], **Sophie Momen**[1], Serena Nik-Zainal[1]*

*[1]University of Cambridge, Cambridge, United Kingdom*

**Background:** Xeroderma pigmentosum (XP) is a genetic disorder that leads to impaired DNA repair in response to ultraviolet radiation (UVR). XP patients have UVR sensitivity and a 1000–10,000 fold increased risk of skin cancer. Early, effective photoprotection has helped reduce childhood cancer-related deaths, only to reveal adolescence-onset neurodegeneration, driven by mechanisms that are currently unknown.

**Methods:** To decipher the underlying mechanisms of XP neurodegeneration, we induced pluripotent stem cells from XP patients and healthy relatives, performing functional multi-omics on samples taken through directed neuronal differentiation. A total of 24 cases were explored altogether: 8 XP with neurodegeneration, 8 without and 8 heterozygous relatives as controls. Various validation assays were employed: Western blotting, functional assays, immunoprecipitation and quantitative immunofluorescence.

**Results and impact:** We uncover how Endoplasmic Reticulum stress is strongly upregulated in XP neurons and show that this is preceded by marked oxidative stress. We also reveal for the first time, the DNA damage culprits in XP neurons: 5',8-cyclopurines and 8-oxopurines, byproducts of oxidative stress. Interestingly, we found that neurons from XP neurodegeneration patients exhibited inappropriate downregulation of the protein clearance Ubiquitin-Proteasome System (UPS). Our attempt to enhance UPS activity using small molecule inhibitors (rolipram), improved neuronal phenotypes albeit inadequately, suggesting that early detection and prevention strategies will be necessary to achieve meaningful clinical outcomes. We developed an early detection assay for predicting neurodegeneration in at-risk patients, through machine-learning on proteomic data. This assay could help identify patients that require early intervention to prevent or decelerate neurodegeneration, ultimately improving patient outcomes.

**Conflict of Interest:** Cherif Badja university of cambridge, Sophie Momen NHS, wellcome trust clinical phd 216339/z/19z, Serena Nik-Zainal university of cambridge, Cancer Research UK (CRUK) Advanced Clinician Scientist Award (C60100/A23916), Dr Josef Steiner Cancer Research Award 2019, Basser Gray Prime Award 2020, CRUK Pioneer Award (C60100/A23433), CRUK Grand Challenge Award (C60100/A25274), CRUK Early Detection Project Award (C60100/A27815), and the National Institute of Health Research (NIHR) Research Professorship (NIHR301627). This work wasalso supported by the NIHR Cambridge Biomedical Research Centre (BRC-1215-20014). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care., mutational signature based algorithms not applicable to this work

### C21.3 Federated analysis of 30,168 patients quantifies the recessive contribution to developmental disorders across diverse populations and identifies new genes

***Kartik Chundru**[1], Zhancheng Zhang[2], Klaudia Walter[1], Petr Danecek[1], Ruth Eberhardt[1], Eugene Gardner[3], Rebecca Torene[2], Kyle*

*Retterer[2], Caroline Wright[4], Eamonn Sheridan[5], Helen Firth[1], Matthew Hurles[1], Kaitlin Samocha[6], Vincent Ustach[2], Hilary Martin[1]*

*[1]Wellcome Sanger Institute, Hinxton, United Kingdom; [2]GeneDx, Gaithersburg, MD, United States; [3]University of Cambridge, MRC Epidemiology Unit, Cambridge, United Kingdom; [4]University of Exeter Medical School, Exeter, United Kingdom; [5]University of Leeds, Leeds, United Kingdom; [6]Massachusetts General Hospital, Boston, United States*

**Background:** Previous work on 6040 developmental disorder (DD) patients from the Deciphering Developmental Disorders (DDD) study estimated that autosomal recessive (AR) coding variants explained 3.6% of patients with European ancestries but a higher fraction (31%) in those with Pakistani ancestries due to elevated consanguinity (Martin et al., Science, 2018). Forty-eight percent of this exome-wide burden was explained by the AR DD-associated (ARDD) genes known at the time.

**Methods:** We tilized the AR burden exome-wide and per gene in 30,168 DD trios from DDD and the genetic diagnostics company GeneDx, comprising individuals from twenty-two genetically-inferred sub-populations of whom 21.5% have non-European ancestries.

**Results:** The estimated fraction of patients attributable to AR coding variants ranged from ~2% to ~17% across sub-populations, and was significantly correlated with the average autozygosity ($r^2 = 0.52$; $p = 8.6 \times 10^{-6}$). Established ARDD genes explained 87.4% of the total AR coding burden in probands with European ancestries and 80.8% in those with non-European ancestries (difference not significant). By testing for statistical enrichment of damaging biallelic genotypes, we identified two novel ARDD genes that pass Bonferroni correction, *KBTBD2* ($p = 2.1 \times 10^{-7}$) and *CRELD1* ($p = 1.2 \times 10^{-7}$). Several other novel or recently-reported genes were identified at a more lenient 5% false-discovery rate threshold: *ZDHHC16*, *HECTD4* and *LSM7*.

**Conclusions:** We quantified the AR contribution to DDs across diverse global populations, demonstrated that most DD patients with an AR coding cause can be explained by the known disease genes, and identified several novel ARDD genes.

**Conflict of Interest:** Kartik Chundru: None declared, Zhancheng Zhang GeneDx, Klaudia Walter: None declared, Petr Danecek: None declared, Ruth Eberhardt: None declared, Eugene Gardner Adrestia Therapeutics, Rebecca Torene Geisinger, Kyle Retterer Geisinger, OKPO, Caroline Wright: None declared, Eamonn Sheridan: None declared, Helen Firth: None declared, Matthew Hurles Congenica, Congenica, Kaitlin Samocha: None declared, Vincent Ustach GeneDx, Hilary Martin: None declared

## C21.4 GPATCH11 variants cause mis-splicing and early onset retinal dystrophy with neurological impairment

***Andrea Zanetti***[1], *Lucas Fares Taie[1], Jeanne Amiel[1], Pierre David[1], Stéphanie Moriceau[2], Nicolas Goudin[2], Jerome Roger[3], Chiara Guerrera[2], Isabelle Audo[4], Robert Matthieu[5], Sabine Sigaudy[6], Josseline Kaplan[1], Jean-Michel Rozet[1], Isabelle Perrault[1]*

*[1]Institut Imagine, Paris, France; [2]Structure Fédérative de Recherche Necker, Paris, France; [3]Paris-Saclay Institut of Neuroscience, Paris, France; [4]Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France; [5]University Hospital Necker-Enfants Malades, Paris, France; [6]Hospital Timone Enfant, Marseille, France*

*GPATCH11* encodes a protein of unknown function, which contains a G-Patch domain found in RNA binding proteins playing various roles in RNA biology. We report a *GPATCH11* exon4-skipping variant in homozygosity or compound heterozygosity with a nonsense change, causing early-onset severe retinal dystrophy, neurodevelopmental anomalies and dysmorphic features in 3 families. Studying fibroblasts, we show that GPATCH11 displays a nuclear as well as a centriolar localization. We report expression of a G-patch domain-truncated protein in cells from homozygous patients, displaying normal abundance and subcellular localization, as were cilia formation and SHH signaling; whether GPATCH11 has a role in cilia, it seems independent of the G-patch domain. In wildtype and mutant (homozygous orthologous exon deletion) mice, we show expression of GPATCH11 and the G-patch domain-truncated isoform in all retina cell types, respectively. We describe altered ERG-responses as of Day-15 (eye opening), consistent with RNAseq analysis at the same age, showing deregulation of expression and splicing (160 and 178 genes, respectively), mainly involving visual perception. Three genes combined differential splicing with downregulation of mRNA and protein expression (proteomic analysis): *Arr3*, *Mpp4* and *Tulp1*, involved in retinal degenerations. Additionally, we show that mutant mice display photoreceptor degeneration (complete at 6 months), as well as learning and memorization anomalies ($p < 0.001$), associated with hippocampal expression of GPATCH11 and the truncated isoform. This study supports the role of GPATCH11 in RNA splicing and its importance for retina biology and development (including brain), while, hitherto, global splicing defects have been involved in retina- or brain-specific diseases.

**Conflict of Interest:** None declared

## C21.5 Structural elements facilitate 3D locus topology and regulation of a human disease gene

***Hannah Long***[1], *Liang-Fu Chen[2], Alistair Boettiger[2], Joanna Wysocka[2]*

*[1]University of Edinburgh, Edinburgh, United Kingdom; [2]Stanford University, Stanford, United States*

**Background/Objectives:** Non-coding mutations are increasingly associated with Mendelian developmental disorders. We previously demonstrated that enhancer clusters overlapping disease-associated mutations in Robin sequence (RS) patients regulate *SOX9* expression at genomic distances over 1.25 Mb. However, how gene regulation occurs across such enormous genomic distances is still poorly understood. We therefore set out to explore mechanisms facilitating extreme long-range regulation at the *SOX9* locus relevant to human developmental disease.

**Methods:** We tilized directed differentiation to derive human cranial neural crest cells (CNCCs), progenitor cells that give rise to the majority of the vertebrate face, and targeted genome editing to delete regions with predicted 3D topological roles. We then leveraged optical reconstruction of chromatin architecture (ORCA), a multiplexed chromosomal imaging approach, to trace 3D locus topology during RS enhancer activation.

**Results:** We observed pronounced changes in locus topology during CNCC differentiation from pluripotent stem cells. Analysis of single-chromatin fiber traces revealed that these ensemble-average differences arise through changes in the frequency of commonly sampled topologies across development. We further identified two CTCF-bound elements within the *SOX9* topologically associating domain, which are positioned near the domain's 3D geometric core, bridge enhancer-promoter contacts and when ablated diminish *SOX9* expression.

**Conclusion:** Together, we provide mechanistic insights into gene regulation over ultra-long genomic ranges, involving both distal enhancer clusters and structural elements. Identification of this novel class of regulatory element will have wide-reaching implications for our interpretation of non-coding variants in human gene regulation and disease.

**References:** Chen, Long et al., Mol Cell, 2023

**Grants:** 106051/Z/14/Z, MC_UU_00007/18
**Conflict of Interest:** None declared

### C21.6 Prevalence, causes and impact of TP53-loss phenocopying events in human tumors

*Bruno Fito-Lopez[1], Marina Salvadores[1], Miguel-Martin Alvarez[1], **Fran Supek**[1]*

*[1]IRB Barcelona, Barcelona, Spain*

**Background:** *TP53* is a master tumor suppressor gene, mutated in approximately half of all human cancers. It is possible to infer loss of p53 activity – which may occur due to alterations in *trans* – from gene expression patterns. Several alterations that phenocopy p53 loss are known, however additional ones may exist, but their identity and prevalence are unclear.

**Results:** We perform a large-scale statistical analysis on transcriptomes of ~7000 tumors and ~1000 cell lines, estimating that 12% and 8% thereof, respectively, are deficient in the activity of the p53 pathway, while not bearing obvious *TP53* inactivating mutations. While some of these cases are explained by amplifications in the known phenocopying genes *MDM2*, *MDM4* and *PPM1D*, many are not. An association analysis of cancer transcriptome scores jointly with CRISPR/RNAi genetic screening data identified an additional common *TP53*-loss phenocopying gene, *USP28*. Deletions in *USP28* associate with a TP53 functional impairment in 2.9–7.6% of breast, bladder, lung, liver and stomach tumors, and have comparable effect size to *MDM4* amplifications. Analysis of cell line screens using phenocopy scores suggests that *TP53* (in)activity commonly modulates associations between anticancer drug effects and *PIK3CA* and *PTEN* mutations, and should thus be considered as a drug activity modifying factor in precision medicine. As a resource, we provide all drug-genetic marker associations that depend on *TP53* functional status.

**Conclusions:** Human tumors that do not bear obvious *TP53* genetic alterations but that phenocopy p53 activity loss are common, with *USP28* deletions being a salient example.

**Grant References:** ERC StG 757700.
**Conflict of Interest:** None declared

## C22 CARDIOVASCULAR AND MUSCULAR GENOMICS

### C22.1 Long-read sequencing reveals novel transcripts induced by misexpression of DUX4 in FSHD muscle

***Dongxu Zheng**[1], Anita van den Heuvel[2], Ahmed Mahfouz[1;3], Silvère van der Maarel[1]*

*[1]Leiden University Medical Center, Human Genetics, Leiden, Netherlands; [2]Utrecht University, Department Biomolecular Health Sciences —Cell Biology, Metabolism & Cancer, Utrecht, Netherlands; [3]Delft University of Technology, Delft Bioinformatics Lab, Delft, Netherlands*

Double homeobox 4 (DUX4) encodes for a transcription factor DUX4 that is physiologically expressed during early embryogenesis and subsequently silenced in most somatic tissues. Misexpression of DUX4 in skeletal muscle is considered the major cause of Facioscapulohumeral muscular dystrophy (FSHD). DUX4 activates a cascade of downstream events leading to muscle wasting. DUX4 is known to regulate not only protein-coding genes, but also several classes of repetitive elements, and is known to affect RNA processing. Only using data from short-read sequencers limits our understanding of the complex transcriptional events provoked by DUX4. We combined Pacbio Iso-Seq with short-read RNA-seq of DUX4-inducible myoblasts to investigate the full-length transcriptome landscape inflicted by DUX4. DUX4 overexpression caused a more complex transcriptome than anticipated with novel isoforms of known genes. In addition, DUX4-dependent transcriptional activation of 777 novel intergenic loci was identified, which was verified by corresponding short-read RNA-seq data, bulk RNA-seq data of primary myotubes, and embryonic scRNA-seq data. Analysis of public DUX4 ChIP-seq data revealed DUX4 binding sites upstream of 234 intergenic loci. Intergenic loci with predicted coding transcripts could be confirmed in Ribo-seq data of DUX4-expressing myoblasts, indicating that these novel intergenic transcripts are translated into novel polypeptides. Taken together, our study elaborates on the transcriptional features induced by DUX4 and reveals unannotated transcripts at transcriptome and translatome levels. These can be considered potential biomarkers for disease diagnosis, progression, and therapeutic intervention in FSHD, as well as being explored for their role in cleavage-stage embryos.

**Grant references:** Prinses Beatrix Spierfonds [W.OR19-06].
**Conflict of Interest:** None declared

### C22.2 D4Z4 methylation analysis combined with machine learning pipelines: a novel tool for the rapid identification of FSHD subjects

***Valerio Caputo**[1;2], Giulia Trastulli[1], Domenica Megalizzi[1;2], Carlo Fabrizio[3], Andrea Termine[3], Claudia Strafella[1;2], Luca Colantoni[1], Juliette Gimenez[4], Mauro Monforte[5], Carlo Caltagirone[6], Enzo Ricci[5;7], Giorgio Tasca[5;8], Raffaella Cascella[1;2], Emiliano Giardina[1;2]*

*[1]Santa Lucia Foundation IRCCS, Genomic Medicine Laboratory-UILDM, Rome, Italy; [2]University of Rome Tor Vergata, Department of Biomedicine and Prevention, Rome, Italy; [3]Santa Lucia Foundation IRCCS, Data Science Unit, Rome, Italy; [4]Santa Lucia Foundation IRCCS, Epigenetics and Genome Reprogramming Laboratory, Rome, Italy; [5]Fondazione Policlinico Universitario A. Gemelli IRCCS, Unità Operativa Complessa di Neurologia, Rome, Italy; [6]Santa Lucia Foundation IRCCS, Department of Clinical and Behavorial Neurology, Rome, Italy; [7]Università Cattolica del Sacro Cuore, Istituto di Neurologia, Rome, Italy; [8]Newcastle University and Newcastle Hospitals NHS Foundation Trusts, John Walton Muscular Dystrophy Research Centre, Newcastle Upon Tyne, United Kingdom*

**Objectives:** The present study was thought to tackle the challenges related to the heterogeneity and diagnosis of Facio-Scapulo-Humeral Dystrophy (FSHD). To this purpose, a workflow combining *D4Z4* methylation assessment with Machine Learning (ML) approaches was developed with the aim of identifying FSHD subjects.

**Methods:** The study included two independent cohorts, a training group of 133 patients with clinical signs of FSHD and 150 healthy controls (CTRL) and a testing set of 27 FSHD patients and 25 CTRL. The DNA methylation levels of two *D4Z4* regions (DR1 and *DUX4*-PAS) were assessed by an in-house protocol based on bisulfite sequencing (BSS) and capillary electrophoresis with the Amplification Fragment Length Polymorphisms (AFLP) module, followed by statistical and ML analyses.

**Results:** FSHD patients showed significantly reduced methylation levels compared to CTRL (FDR $p < 0.001$). Notably, single CpG sites were utilized to develop a ML pipeline able to discriminate FSHD subjects. The model identified four CpG sites as the most relevant for the identification of FSHD patients, reporting high metrics values (accuracy: 0.94, sensitivity: 0.93, specificity: 0.96). Two additional models were developed for differentiating patients with reduced *D4Z4* size and predicting patients who may carry pathogenic variants in FSHD genes, respectively.

**Conclusion:** Overall, the developed tool enables an accurate classification of FSHD patients, providing additional evidence for

DNA methylation as a powerful biomarker for prioritizing subjects to be tested for FSHD. Moreover, the workflow proved to be cost-effective and rapid, further supporting its application into the clinical practice.

**Grant References:** FSHD Society Research Grant #Winter2021-0992658837.

**Conflict of Interest:** Valerio Caputo Post-Doc Researcher, Giulia Trastulli Research Biologist, Domenica Megalizzi PhD student, Carlo Fabrizio Data scientist, Andrea Termine Data Scientist, Claudia Strafella Research Biologist, FSHD Society Research Grant #Winter2021-0992658837, Luca Colantoni Research Biologist, Juliette Gimenez Research Biologist, Mauro Monforte Neurologist, Carlo Caltagirone Full professor, Enzo Ricci Associate professor, Giorgio Tasca Neurologist, Raffaella Cascella Post-Doc Researcher, Emiliano Giardina Associate professor/Laboratory Director

### C22.3 Establishment of the first reported zebrafish model for thoracic aortic dissection and rupture

***Michiel Vanhooydonck***[1], *Lore Pottie*[1], *Annekatrien Boel*[2], *Piyanoot Tapaneeyaphan*[1], *Marta Santana Silva*[1], *Adelbert De Clerq*[3], *Lisa Caboor*[1], *Matthias Van Impe*[4], *Patrick Segers*[4], *Delfien Syx*[1], *Andy Willaert*[1], *Patrick Sips*[1], *Bert Callewaert*[1]

[1]*Ghent University, Biomolecular Medicine, Ghent, Belgium;* [2]*Ghent University, Reproductive Medicine, Ghent, Belgium;* [3]*Ghent University, Biology, Ghent, Belgium;* [4]*Ghent University, Institute of Biomedical Engineering and Technology (IbiTech), Ghent, Belgium*

With 3–4 cases per 100,000 person-years, thoracic aortic dissection (TAD) is a relatively rare but devastating disease that associates with a high mortality rate. Weakening of the vessel wall and progressive dilatation of the thoracic aorta may precede TAD, but often remains undetected. Treatment options are limited and consist of surgical repair at the critical diameter as there is currently no pharmacological intervention available. Despite the existence of different mouse models for TAD, the underlying disease mechanisms remain largely elusive.

We developed a zebrafish model for aortic dissection/rupture targeting 2 genes involved in angiogenesis, *SMAD3* and *SMAD6*. In humans, loss of function (LOF) of *SMAD3* results in thoracic aortic aneurysm and dissection (TAAD), arterial tortuosity and early onset osteoarthritis. *SMAD6* LOF mutations increase the risk for a bicuspid aortic valve and TAAD. In zebrafish, both *SMAD3* and *SMAD6* have 2 paralogues. Using CRISPR/Cas9 gene-editing technology, we developed a quadruple knockout (KO): *smad3a*$^{-/-}$;*smad3b*$^{-/-}$;*smad6a*$^{-/-}$;*smad6b*$^{-/-}$. Survival of adult quadruple KO zebrafish is severely decreased (<1 year). A stress-inducing protocol causes sudden death in 60% of the mutant zebrafish. Histochemical investigation of consecutive sections of the ventral aorta in quadruple mutants stained for elastin shows medial elastolysis, intramural hematomas, false lumens and aortic dissections and ruptures at sites with high hemodynamic stress, as supported by 3D-reconstructions. RNA-sequencing reveals upregulation of melanogenesis and the transcription factor *mitfa*, a previously unaddressed pathway.

Hence, we successfully developed the first-ever reported zebrafish model for TAD that reveals unexpected novel mechanistic insights in TAD, targetable for therapy.

BOF.GOA.2021.0004.02
BOF.BAS.2020.0021.01

**Conflict of Interest:** Michiel Vanhooydonck full-time (Ghent university), BOF, GOA, FWO (collaborator), Lore Pottie full-time (Ghent University Hospital), BOF, GOA, FWO (collaborator), Annekatrien Boel full-time (Ghent University), BOF, GOA, FWO, Piyanoot Tapaneeyaphan full-time (Ghent University hospital), BOF, GOA, FWO (collaborator), Marta Santana Silva full-time (Ghent University), BOF, GOA, FWO (collaborator), Adelbert De Clerq part-time (Ghent university), Lisa Caboor full-time (Ghent University), BOF, GOA, FWO (collaborator), Matthias Van Impe full-time (Ghent University), BOF, GOA, FWO (collaborator), Patrick Segers full-time (Ghent University), BOF, GOA, FWO (principal investigator), Delfien Syx full-time (Ghent university), FWO (collaborator), Andy Willaert full-time (Ghent university), BOF, GOA, FWO (collaborator), Patrick Sips full-time (Ghent university), BOF, GOA, FWO (principal investigator), Bert Callewaert full-time (Ghent university/Ghent university hospital), BOF, GOA, FWO (Principal investigator)

### C22.4 Long-read RNA-sequencing identifies novel protein coding transcripts of genes with implications for inherited and complex cardiac disease

***Rhys Dore***[1], *Sonia García-Ruiz*[1], *Emil Gustavsson*[1;2], *Mina Ryten*[1;2]

[1]*UCL GOS Institute of Child Health, Department of Genetics and Genomic Medicine Research & Teaching, London, United Kingdom;* [2]*NIHR Great Ormond Street Hospital Biomedical Research Centre, University College London, London, United Kingdom*

There has been considerable progress in the identification of genes causing inherited cardiac diseases, yet many individuals remain undiagnosed. This could be explained by incomplete annotation of genes causally linked to these disorders leading to inaccurate variant interpretation. To test this hypothesis, we analysed long-read RNA-sequencing data originating from 16 individuals and nine cardiac regions (GENCODE v29), focusing on 105 genes implicated in monogenic cardiac disease defined by PanelApp (retrieved 02/12/22). Of these genes, we found that 67 met a conservative threshold for expression of RPM ≥ 20 in at least 80% of samples. After filtering for transcripts mapping to these 67 genes and with a minimum of two full length supporting reads per sample, we identified 245 unique transcripts. 57.1% of these transcripts were novel (Ensembl v105). We identified 12 novel transcripts which were predicted to be protein coding from six genes, including three transcripts generated through novel combinations of known splice junctions, and nine transcripts that included novel splice sites including *DES* and *TNNC1*. Notably, annotation of a novel *CASQ2* exon with proteomic support contains a common variant predicted to alter the novel protein sequence and significantly associated with left ventricular strain, left ventricular thickness and atrial fibrillation. In fact, *CASQ2* is already a candidate target for arrhythmia treatments. Despite the small sample set, through the example of *CASQ2*, this project demonstrates that long-read transcriptomics improves pathogenic variant interpretation and provides key insights into complex human disease.

**Conflict of Interest:** None declared

### C22.5 Loss-of-function variants in POPDC2 cause a novel autosomal recessive syndrome with sinus node disease and AV conduction defects in combination with hypertrophic cardiomyopathy

*Michele Nicastro*[1], *Alexa Vermeer*[2], *Pieter Postema*[1], *Forrest Bowling*[3], *Rafik Tadros*[4], *Alex Postma*[5], ***Elisabeth Lodder***[1], *Karel van duijvenboden*[5], *Rob Zwart*[2], *Leander Beekman*[1], *Paul van der Zwaag*[6], *Yasmine Aguib*[7], *Mona Allouba*[8], *Luis Santome*[9], *David Deuna*[9], *Lorenzo Monserrat*[10], *Francesco Fortunato*[11], *Giacomo Comi*[11], *Dario Ronchi*[11], *Peter van Tintelen*[12], *Michael Airola*[3], *Imke Christiaans*[6], *Arthur Wilde*[1], *Ronald Wilders*[5], *Sally-ann Clur*[13], *Arie Verkerk*[5], *Connie R. Bezzina*[1], *Najim Lahrouchi*[1]

[1]Amsterdam UMC, Department of Clinical and Experimental Cardiology, Amsterdam, Netherlands; [2]Amsterdam, Department of Human Genetics, Amsterdam, Netherlands; [3]Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY, USA, New York, United States; [4]Montreal Heart Institute and Faculty of Medicine, Université de Montréal, Cardiovascular Genetics Center, montreal, Canada; [5]Amsterdam UMC, Department of Medical Biology, Amsterdam, Netherlands; [6]University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands; [7]Aswan Heart Centre, Magdi Yacoub Foundation, Aswan, Egypt; [8]NHLI, Imperial College, London, United Kingdom; [9]Health in Code, Coruna, Spain; [10]Dilemma Solutions SL, Medical Department, Corona, Spain; [11]Dino Ferrari Center, University of Milan, Department of Pathophysiology and Transplantation, Milan, Italy; [12]University Medical Center Utrecht, Department of Clinical Genetics, Utrecht, Netherlands; [13]Emma Children's Hospital, Amsterdam UMC, Department of Pediatric Cardiology, Amsterdam, Netherlands

**Background:** Cardiac conduction defects in the young should raise suspicion of a genetic disorder. Yet, a large number of patients with early-onset conduction defects remain genetically elusive. We conducted genetic studies to identify the causal genetic defect for a cardiac disorder presenting with sinus node dysfunction and atrioventricular (AV) conduction defects, in combination with hypertrophic cardiomyopathy (HCM).

**Methods:** Genetic analyses were performed using whole-exome sequencing and Sanger-sequencing. A homology model of the POPDC2 Popeye domain was generated to rationalize the effects of the identified *POPDC2* variants. Expression of POPDC2 in the cardiac conduction system was assessed through single-cell RNA sequencing from embryonic mouse hearts. Functional effects of modulation of the TREK-1 potassium current by POPDC2 were assessed through co-expression studies in HEK-293 cells.

**Results**: We report recessive variants in *POPDC2* causing an autosomal-recessive syndrome in two unrelated consanguineous families with sinus node disease and AV conduction defects in combination with HCM. Both variants are expected to significantly alter POPDC2 protein structure. Consistent with the phenotype observed in patients, POPDC2 was expressed in cardiomyocytes, sinus node, atrioventricular node and His region cells. While co-expression of wild-type POPDC2 with TREK-1 increased TREK-1 current density, both homozygous *POPDC2* variants in patients did not increase TREK-1 current density.

**Conclusion**: *POPDC2* loss-of-function causes a Mendelian autosomal-recessive cardiac syndrome characterized by sinus node dysfunction and atrioventricular conduction defects, in combination with HCM. Our findings are consistent with previous work showing that mice and zebrafish deficient in functional *POPDC2* display sinus node dysfunction and AV conduction defects.

**Conflict of Interest:** None declared

### C22.6 Functional analysis of LDLR variants using automated systems to improve rare-variant association studies and risk assessment in hypercholesterolemia

*Mohammad Majharul Isalm[1], Iryna Hlushchenko[1], Max Tamlander[2], Samuli Ripatti[2],* ***Simon Pfisterer***[1]

[1]University of Helsinki, Anatomy, Helsinki, Finland; [2]Institute for Molecular Medicine FinaInd, Helsinki, Finland

**Background:** Lack of functional information for low-density lipoprotein receptor (LDLR) mutations limits the use of genetics for early diagnosis of familial hypercholesterolemia (FH) and risk assessment in cardiovascular disease (CVD). The goal of this study was an in-depth functional characterization of LDLR variants at large-scale, to improve rare-variant association studies and decision-making in the treatment of FH.

**Methods:** We combined open-source robotics with multiplexed high-content imaging and python-based image and data analysis to establish a semi-automated analysis pipeline, enabling large-scale functional characterization of LDLR variants regarding LDL uptake, LDLR expression and subcellular localization. LDLR variants were expressed in a LDLR deficient liver cell line using CRISPR technology. The functional data was then integrated with genetic and health data from UK Biobank.

**Results:** We performed functional characterization of more than 250 LDLR variants, shedding light on more than 60 variants of unknown significance and enabling reclassification of 100 likely-pathogenic and 30 likely-benign LDLR variants. Only 39% of predicted loss-of-function LDLR variants displayed a severe functional impairment, deflating previous genetic studies which utilized such tools. Functional activity groups of LDLR variants showed large differences in regard to circulating lipids and cardiovascular risk and outperformed existing variant classification methods. Moreover, functional groups allowed us to link ultrarare ($MAF < 1 \times 10^{-5}$) LDLR variants with cardiovascular outcomes.

**Conclusion:** Systematic functional data for LDLR variants improves risk assessment of hypercholesterolemia and cardiovascular disease and paves the way for better characterization and treatment of FH patients.

**Grants:** Academy of Finland 328861 and 32504

**Conflict of Interest:** None declared

## C24 INTELLECTUAL DISABILITY: AN UPDATE

### C24.1 Clinical, genetic and molecular delineation of KPTN-related disorder in humans and mice identifies mTOR inhibition as a candidate therapeutic approach

***Lettie Rawlins***[1;2], *Maria Levitin[3;4], Philip Iffland[5], Sanchez-Andrade Gabriela[3], James Tonks[6], James Fasham[2], Stephan Collins[7], Joseph Leslie[1], Stephen Sawiak[8], Conny Van Ravenswaaij-Arts[9], Caleb Bupp[10], Sarina Kant[11], Olivia Wenger[12], Ethan Scott[12], Helen Stewart[13], Esther Nibbeling[11], Eva Schwaibold[14], Andrew Shuen[15;16], Andrea Seeley[17], Sabine Rudnik[18], Adila Al-Kindi[19;20], Andreas Hahn[21], Almundher Al-Maawali[19;20], Saskia Biskup[21], Inger-Lise Mero[22], Martin Heier[23], Johanna Herkert[11], Thorkild Terkelsen[24], Tobias Haack[14], Kwame Anyane-Yeboa[25], Heather Rocha[17], Logan Jory[6], Ian Ellis[26], Michael Wright[27], Pradeep Vasudevan[28], John Schreiber[29], Gaurav Harlalka[1;30], Christopher Lelliott[3], Binnaz YALCIN[7], Matthew Hurles[3], Darren Logan[31], Peter Crino[5], Sebastian Gerety[3], Andrew Crosby[1], Emma Baple[1;2]*

[1]University of Exeter, Clinical and Biomedical Sciences, Exeter, United Kingdom; [2]Royal Devon University NHS Foundation Trust, Peninsula Clinical Genetics Service, Exeter, United Kingdom; [3]Wellcome Sanger Institute, Wellcome Genome Campus, Cambridge, United Kingdom; [4]Evox Therapeutics Limited, Oxford, United Kingdom; [5]University of Maryland School of Medicine, Department of Neurology, Baltimore, United States; [6]Haven Clinical Psychology Practice Limited, Bude, Cornwall, United Kingdom; [7]Université de Bourgogne Franche-Comté, INSERM Unit1231, Dijon, France; [8]University of Cambridge, Behavioural and Clinical Neuroscience Institute, Cambridge, United Kingdom; [9]Universitary Medical Centre Groningen, Department of Genetics, Groningen, Netherlands; [10]Spectrum Health Medical Group, Michigan, United States; [11]Leiden University Medical Center, Department of Clinical Genetics, Leiden, Netherlands; [12]New Leaf Centre, Clinic for Special Children, Mount Eaton, Ohio, United States; [13]Oxford Regional Genetics Service, Department of Clinical Genetics, Oxford, United Kingdom; [14]University of Tuebingen, Institute of

*Medical Genetics and Applied Genomics, Tuebingen, Germany; [15]University of Toronto, Toronto, Canada; [16]The Hospital for Sick Children, Toronto, Canada; [17]Geisinger Genomic Medicine Institute, Weis Center for Research, Danville, Pennsylvania, United States; [18]Innsbruck Medical University, Institute of Human Genetics, Innsbruck, Austria; [19]Sultan Qaboos University, Department of Genetics, Muscat, Oman; [20]Sultan Qaboos University Hospital, Genetic and Developmental Medicine Clinic, Muscat, Oman; [21]Tuebingen Centre for Human Genetics, Tuebingen, Germany; [22]University of Oslo, Department of Medical Genetics, Oslo, Norway; [23]University of Oslo, Department of Neurology, Oslo, Norway; [24]Aarhus University Hospital, Department of Clinical Genetics, Aarhus, Denmark; [25]Morgan Stanley Children's Hospital of New York, Division of Clinical Genetics, New York, United States; [26]Liverpool Women's NHS Foundation Trust, Department of Clinical Genetics, Liverpool, United Kingdom; [27]International Centre for Life, Institute of Human Genetics, Newcastle upon Tyne, United Kingdom; [28]University Hospital of Leicester, Department of Clinical Genetics, Leicester, United Kingdom; [29]Children's National Medical Center, Department of Neurology, Washington, United States; [30]Rajarshi Shahu College of Pharmacy, Department of Pharmacy, Malvihir, India; [31]Waltham Petcare Science Institute, Waltham on the Wolds, United Kingdom*

*KPTN*-related disorder is an autosomal recessive condition associated with pathogenic biallelic germline variants in *KPTN* encoding kaptin, a component of the mTOR regulatory complex KICSTOR. We identified 30 new patients with a confirmed molecular diagnosis, revisited the nine Amish patients that we originally described, and reviewed data for twelve patients in previously published studies, to further delineate the clinical phenotypic and genotypic spectrum of this condition. To gain further pathomechanistic insights into this disorder, we undertook protein modelling studies and analysed mouse and human iPSC *KPTN* loss-of-function models. $Kptn^{-/-}$ mice display many of the key phenotypic features seen in the human disorder, including megalencephaly, behavioural abnormalities, and cognitive deficits. This led us to re-evaluate these aspects in humans, revealing concordant specificity of cognitive deficits with relative sparing of hippocampal-independent memory, postnatal progressive brain overgrowth, and a previously unrecognised dosage-sensitivity to loss of KPTN, affecting head circumference of heterozygous carriers. Molecular and structural analysis of mouse brain tissue revealed pathological changes contributing to the mouse phenotypes, including differences in brain size, shape, and cell numbers primarily due to abnormal postnatal brain development. Both the mouse and differentiated iPSC models display transcriptional and biochemical evidence for altered mTOR pathway signalling, supporting the role for KPTN in regulating mTORC1 and place *KPTN*-related disorder in the broader group of "mTORopathies". Increased mTOR signalling downstream of KPTN is rapamycin sensitive, highlighting possible therapeutic avenues with currently available mTOR inhibitors. Finally, we propose clinical management guidelines for patients with *KPTN*-related disorder.

**Conflict of Interest:** None declared

### C24.2 PhenomAD-NDD: the Phenomics Aggregation Database of comorbidities in 42,411 pediatric individuals with NeuroDevelopmental Disorders

***Alexander Dingemans***[1], *Sandra Jansen*[1], *Jeroen van Reeuwijk*[1], *Claudia Hellebrekers*[1], *Nicole de Leeuw*[1], *Rolph Pfundt*[1], *Janneke Schuurs-Hoeijmakers*[1], *Bregje van Bon*[1], *Charlotte Ockeloen*[1], *Carlo Marcelis*[1], *Marjolein Willemsen*[1], *Eline van der Sluijs*[2], *Gijs Santen*[2], *Frank Kooy*[3], *Anneke Vulto-van Silfhout*[1], *Tjitske Kleefstra*[1], *David Koolen*[1], *Lisenka Vissers*[1], *Bert B.A. de Vries*[1]

*[1]Radboud University Medical Center, Nijmegen, Netherlands; [2]Leiden University Medical Center (LUMC), Leiden, Netherlands; [3]University of Antwerp, Antwerpen, Belgium*

**Background/Objectives:** The prevalence of comorbidities in individuals with neurodevelopmental disorders (NDD) is not well understood, while these are important for accurate diagnosis and prognosis in routine care and for characterizing the clinical spectrum of NDD syndromes. We aimed to establish a phenomics-based aggregation database (PhenomAD-NDD, https://humandiseasegenes.nl/phenomad-ndd) to define the baseline prevalence of NDD comorbidities and to showcase its use for well-established NDDs.

**Methods:** We deep-phenotyped 1477 children with NDD (between 2009–2020) using Human Phenotype Ontology (HPO) and supplemented these with phenotypes of 40,934 individuals from literature. EUROCAT, reporting congenital anomalies in the general population, was used as control.

**Results:** PhenomAD-NDD contains 42,411 patients with in total 3041 unique HPO-terms (average 12.7 per patient including parent-HPO-nodes) reported: epilepsy (21.5%), behavioural problems (37.0%), and microcephaly (23.5%) are common whereas kidney anomalies (2.3%), heart defects (7.6%), macrocephaly (2.8%), craniosynostosis (0.5%) and neoplasms (3.8%) are relatively rare. Sex differences were noted for autistic behaviour (39%/21%, $p < 0.00001$) and urogenital problems (22%/10%, $p < 0.00001$). Almost all of the congenital anomalies (40/41), reported in both PhenomAD-NDD and EUROCAT, were more prevalent in PhenomAD-NDD – such as congenital heart defects (7.6%/0.7%, $p < 0.00001$) and craniosynostosis (0.5%/0.02%, $p < 0.00001$). The NDD baseline prevalence allowed for approximation of specificity of symptoms such as hypertrichosis for Coffin-Siris syndrome ($RR = 9.0$, $p = 1.2*10^{-81}$) or hypotonia for Helsmoortel-van der Aa syndrome ($RR = 7.7$, $p = 7.7*10^{-29}$), demonstrating the power in detecting characteristically phenotypic features.

**Conclusion:** Using PhenomAD-NDD (https://humandiseasegenes.nl/phenomad-ndd), professionals in the NDD field can determine the baseline prevalence of every clinical feature and possible enrichment of symptoms in a particular genetic disorder.

**Conflict of Interest:** None declared

### C24.3 Biallelic variants in ITFG2, a subunit of the KICSTOR complex, affect mTOR signaling pathway and lead to a syndromic neurodevelopmental disorder

***Elisa Cali***[1], *Gifty Bhat*[2], *Sahar Da'as*[3], *yamna kriouile*[4], *Florence Petit*[5], *Karine Poirier*[6], *Stephanie Efthymiou*[1], *Rauan Kaiyrzhanov*[1], *Gijs Santen*[7], *Hoda Tomoum*[8], *Aisha Mohamed Saeed Mohamed Al Shamsi*[9], *Huma Cheema*[10], *Boris Keren*[11], *Cyril Mignot*[11], *Kristen Park*[12], *John B. Vincent*[13], *Vincenzo Salpietro*[1], *Salvatore Mangano*[14], *Fowzan Alkuraya*[15], *Reza Maroofian*[1], *Khalid Fakhro*[3], *Henry Houlden*[1]

*[1]University College London, Department of Neuromuscular Diseases, London, United Kingdom; [2]Illinois Hospital, Chicago, United States; [3]Sidra Medicine, Qatar, Qatar; [4]Hôpital d'enfants de Rabat, Rabat, Morocco; [5]CHU Lille, Lille, France; [6]Inserm, France, France; [7]Leiden University Medical Center, Leiden, Netherlands; [8]Ain Shams University, Cairo, Egypt; [9]Tawam Hospital, Al ain, United Arab Emirates; [10]the Children's Hospital, Lahore, Pakistan; [11]AP-HP.Sorbonne Université, Paris, France; [12]Children's Hospital Colorado, Aurora, United States; [13]University of Toronto, Toronto, Canada; [14]University of Palermo, Palermo, Italy; [15]King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia*

**Background/Objectives:** The KICSTOR complex is a four-membered protein complex (SZT2, KPTN, ITFG2, C12orf66) that acts as a lysosome-associated negative regulator of the mTORC1 signaling pathway. Variants in several genes in the mTOR signalling network have been associated with an heterogeneous group of neurological diseases including epilepsy and autism. Among these, two members of the KICSTOR complex (SZT2, KPTN) have already been linked with global developmental delay, macrocephaly, and seizures. We have identified 40 affected individuals from 25 unrelated families carrying 16 different biallelic variants in *ITFG2*.

**Methods:** The affected individuals were identified by screening genomic datasets from several diagnostic and research genetic laboratories internationally, as well as using GeneMatcher. Knock-out and knock-in zebrafish models were generated using Crispr-Cas9 gene editing. Cellular studies on patient-derived fibroblast cell lines and zebrafish mutants were performed to investigate the functional consequence of the variants.

**Results:** All the patients presented with global developmental delay/intellectual disability, ranging from mild to moderate, postnatal macrocephaly and abnormal facial features. Seizures occurred in half of the affected individuals. Brain MRI was unremarkable in most of the patients, or showed mild changes in the others. Knock-out and knock-in zebrafish mutants presented abnormal craniofacial features and major changes in locomotion. Functional studies revealed abnormal mTOR signaling in patients and zebrafish mutants.

**Conclusions:** With our study, we established a novel neurodevelopmental disorder characterized by global developmental delay/intellectual disability, macrocephaly and distinctive facial features, caused by biallelic variants in *ITFG2*, a subunit of the KICSTOR complex.

**Grant References:** None.

**Conflict of Interest:** None declared

### C24.4 Biallelic variants in BRF2 are associated with craniofacial anomalies and cognitive impairment

*Anne Hebert[1], **Sissy Bassani**[1], Francesca Mattioli[1], Nazia Ibrahim[1;2], Flavia Palombo[3], Tommaso Pippucci[4], Nayereh Nouri[5], Marco seri[4], Emily Farrow[6], Carol J Sauders[6], Muhammad Ansar[7], Shagufta Naz[2], Alexandre Reymond[1]*

*[1]Center for Integrative Genomics – UNIL, Lausanne, Switzerland; [2]Lahore College for Women University, Department of zoology, Lahore, Pakistan; [3]Istituto delle Scienze Neurologiche, Bologna, Italy; [4]IRCCS Azienda Ospedaliero-Universitaria di Bologna, U.O. Genetica Medica, Bologna, Italy; [5]Isfahan University of Medical Sciences, Isfahan, Iran; [6]University of Missouri-Kansas City, Kansas City, United States; [7]University of Lausanne, Department of Ophthalmology, Lausanne, Switzerland*

**Background/Objectives:**Monogenic forms of intellectual disability(ID) are characterized by extreme heterogeneity. While NGS allowed identifying many ID genes, many patients remain without diagnosis. Multiple subunits of the RNA polymerase III that synthesizes rRNAs, tRNAs and other small RNAs were associated with neurological disorders, such as syndromic hypomyelinating leukodystrophies and/or Treacher-Collins syndrome, e.g., *BRF1* was linked to autosomal recessive cerebellar-facial-dental syndrome

**Methods**:We combined exome sequencing with functional characterization to identify elusive ID variants.

**Results**:We describe seven individuals from three families carrying bi-allelic variants in *BRF2*, the paralog of *BRF1*, encoding a TFIIB-like factor that recruits the RNA polymerase III complex to type 3 promoters to initiate transcription of U6, RnaseP, 7SK and others RNAs. Affected individuals present a variable phenotype ranging from Treacher-Collins syndrome to severe ID with motor and speech development delays depending on the combination of variants, i.e., homozygous stop-gain, truncation paired with missense in the DNA binding domain and homozygous missense in the zinc-finger domain that brings polymerase III subunits to type 3 promoters, respectively. In silico 3D modelling and cellular assays show functional impairment of the missense and truncation variants. Zebrafish knocked down for the orthologous *brf2* presented with impaired escape responses and thigmotaxis, reduced swimming velocities and morphological anomalies. These phenotypes could be complemented by the human *BRF2* mRNA confirming that they were associated with loss of function of this TFIIB-like factor.

**Conclusion**:Overall, our results support the pathogenicity of the identified *BRF2* variants and provide another link between brain anomalies and RNA polymerase III subunits and its targets.

**Conflict of Interest:** None declared

### C24.5 Utility of DNA methylation episignatures in neurodevelopmental disorders: from variant classification to new diagnoses and novel episignature discovery

***Slavica Trajkova***[1], *Jennifer Kerkhof[2;3], Lisa Pavinato[1], Chiara Giovenino[1], Giovanni Battista Ferrero[4], Barbara Pasini[5], Eleonora Di Gregorio[5], Verdiana Pullano[1], Silvia Carestiato[1], Antonina Rinninella[1], Alessandro Mussa[6], Diana Carli[1], Simona Cardaropoli[6], Enza Ferrero[1], Elena Sukarova-Angelovska[7], Dragica Nestoroska[7], Aleksandar Petlichkovski[8], Tommaso Pippucci[9], Paola Dimartino[10], Silvia De Rubeis[11], Joseph Buxbaum[11;12], Sadegheh Aghshenas[2;3], Kathleen Rooney[2;3], Haley McConkey[2;3], Bekim Sadikovic[2;3], Alfredo Brusco[1;5]*

*[1]University of Turin, Department of Medical Sciences, Turin, Italy; [2]London Health Sciences Centre, Verspeeten Clinical Genome Centre, London, Canada; [3]Western University, Department of Pathology and Laboratory Medicine, London, Canada; [4]University of Turin, Department of Clinical and Biological Sciences, Turin, Italy; [5]Città della Salute e della Scienza, Medical Sciences, Unit of Medical Genetics, Turin, Italy; [6]University of Turin, Department of Public Health and Pediatrics, Turin, Italy; [7]Faculty of Medicine, University Sv. Kiril I Metodij, Department of Endocrinology and Genetics, Skopje, Macedonia; [8]Faculty of Medicine, University Sv. Kiril I Metodij, Department of Immunology and Human Genetics, Skopje, Macedonia; [9]Polyclinic Sant'Orsola-Malpighi University Hospital, Medical Genetics Unit, Bologna, Italy; [10]University of Bologna, Department of Medical and Surgical Sciences, Bologna, Italy; [11]Icahn School of Medicine at Mount Sinai, Seaver Autism Center for Research and Treatment, New York, United States; [12]Icahn School of Medicine at Mount Sinai, Department of Genetics and Genomic Sciences, New York, United States*

**Background:** Unique DNA methylation patterns, known as "episignatures", are associated with specific genes/pathways in neurodevelopmental disorders (NDDs). The use of episignatures as biomarkers to classify ambiguous variants is receiving great attention in clinical settings.

**Material and methods:** In collaboration with the EpiSign consortium, we analyzed episignature profiles (EpiSign™ classifier v.3) in 164 NDD cases from four cohorts: (i) validation ($n = 71$), cases with pathogenic/likely-pathogenic variants in genes with known episignatures; (ii) uncertain ($n = 13$), cases with VoUS; (iii) unsolved ($n = 24$), cases with uninformative CMA/ES; (iv) discovery ($n = 56$), aimed at identifying novel disease-associated episignatures.

**Results:** We found the expected episignature in 65/71 cases in the validation cohort. Exceptions included a *SMARCA2* variant in the Nicolaides-Baraitser syndrome-associated domain, with a methylation profile for the allelic disease Blepharophimosis-

impaired intellectual development syndrome (BIS). The patient was later re-diagnosed with BIS. In a patient with Rubinstein-Taybi syndrome 2 (*EP300*), we found a GNAS methylation pattern suggestive of Pseudohypoparathyroidism 1B, as secondary finding. In the uncertain cohort, 8/13 cases did not match the methylation profile of the reported VoUS, suggesting that these were benign variants. Among these, a VoUS in *ARID1B* (Coffin-Siris 1) showed a Cornelia de Lange episignature, the initial clinical diagnosis. In the unsolved cohort, one case showed an *ATRX*-methylation profile, subsequently revealing a deletion of *ATRX* ex3-4 missed by ES. In the discovery cohort, we defined three robust novel episignatures for NDD genes: *TLK2, CAPRIN1* and *ZMYM3*.

**Conclusion:** Episignature analyses are a powerful tool to improve variant classification/diagnosis and can support novel gene-disease association.

**Conflict of Interest:** None declared

### C24.6 Epileptogenic mosaic brain malformations: a single-cell and spatial transcriptomic landscape

***Sara Baldassari***[1], *Lucía Gomez*[2], *Esther Klingler*[2], *Lina Sami*[1], *Sara Bizzotto*[1], *Marion Doladilhe*[1], *Sarah Ferrand-Sorbets*[3], *George Dorfmuller*[3], *Mathilde Chipaux*[3], *Homa Adle-Biassette*[4], *Denis Jabaudon*[2], *Stéphanie Baulac*[1]

[1]*Institut du Cerveau—Paris Brain Institute—ICM, Sorbonne Université, Inserm, CNRS, Hôpital de la Pitié Salpêtrière, Paris, France;* [2]*Department of Basic Neurosciences, University of Geneva, Geneva, Switzerland;* [3]*Department of Pediatric Neurosurgery, Rothschild Hospital-Foundation, Paris, France;* [4]*Université de Paris, service d'Anatomie Pathologique, AP-HP, Hôpital Lariboisière, DMU DREAM, UMR 1141, INSERM, Paris, France*

**Background/objectives:** Brain somatic mutations in mTOR pathway genes are the primary etiology of focal cortical dysplasia type II (FCDII), a brain malformation characterized by cortical dyslamination and the presence of mTOR-hyperactive dysmorphic neurons and balloon cells. FCDII is a significant cause of drug-resistant epilepsy in children who undergo epilepsy surgery to control seizures. The neurodevelopmental effects of mTOR pathway somatic mutations and the affected cell types remain unclear.

**Methods:** We investigated ten surgical FCDII tissues with somatic mTOR-activating mutations using histological, genetic, single-cell, and spatial transcriptomic approaches.

**Results:** Histopathology measures revealed that dysmorphic neurons and balloon cells, although enriched for the mutations, only represent a minor fraction (<10%) of mutated cells in the dysplastic tissues. Transcriptomic analysis of laser-captured pools of dysmorphic neurons showed their immature principal neurons signature, while balloon cells correlated with the astrocytic cell lineage and displayed transcriptomic signatures usually observed in neuroglial progenitor cells. By combining single-nuclei profiling and long-read sequencing, we showed that FCDII somatic mutations affect multiple brain cell lineages, thus suggesting they occurred at early developmental stages. Moreover, we observed that mTOR-activating mutations in different cell types lead to cell-specific transcriptomic alterations. Finally, using spatial transcriptomics, we correlated histological and gene expression alterations, thus allowing unprecedented insights into FCDII pathology.

**Conclusions:** Our results highlight an unforeseen diversity of FCDII cell types affected by mTOR pathway mutations and represent a significant resource for better understanding FCDII-related epilepsy and identifying biomarkers for targeted therapeutics.

**Grant references:** ERC Consolidator n.682345, Easi-Genomics Access n.7574

**Conflict of Interest:** None declared

## C25 DIAGNOSTICS: LARGE COHORTS AND NOVEL OMICS

### C25.1 Increasing the diagnostic yield in the 100,000 Genomes Project through Diagnostic Discovery

***Susan Walker***[1], *Cassandra Smith*[1], *Helen Brittain*[1], *Emily Cheng*[1], *Alexander Ross*[1], *Ana Lisa Tavares*[1], *Meriel McEntagart*[1], *Mitra Sato*[1], *Catherine Ludden*[1], *Dalia Kasperaviciute*[1], *Liam Abrahams*[1], *Kevin Savage*[1], *Ellen Thomas*[1], *Matt Brown*[1], *Augusto Rendon*[1], *Rachael Mein*[2], *Sue Hill*[2], *Richard Scott*[1]

[1]*Genomics England, London, United Kingdom;* [2]*NHS England & NHS Improvement, Genomics Unit, London, United Kingdom*

**Introduction:** Frequent reanalysis of genomic data increases the diagnostic yield for families with rare diseases but introduces a substantial burden on healthcare systems, particularly when operating at scale. Through the 100,000 Genomes Project, primary clinical interpretation of whole genome sequencing data was performed by NHS clinical teams and data were subsequently shared in a trusted research environment (the National Genomic Research Library) to facilitate identification of new findings.

**Methods:** We are using a combination of cohort wide and family-based analysis approaches on a research basis, and collaborating with the research community, to identify putative new diagnoses – referred to as Diagnostic Discovery. Findings are subsequently evaluated and reported clinically by NHS laboratory scientists.

**Results:** Of the first 1410 putative new diagnoses identified, 35% result from new or expanded gene-phenotype associations and 16% from improved variant-level knowledge, including non-coding variants. 25% are copy number or structural variants, reflecting new analytical approaches, and 18% are variants which do not pass standard variant quality filtering methods, some in challenging loci. Other findings are attributable to scenarios that are challenging to accommodate in high-throughput automated pipelines, such as incomplete penetrance, locus heterogeneity and parental mosaicism. Multiple dual diagnoses have been identified.

**Conclusions:** Research investigations have putatively increased the number of diagnostic findings in the 100,000 Genomes Project by ~20%. Over 85% of variants reported clinically have been classified as pathogenic/likely pathogenic. Ongoing interrogation of genomic data on a research basis efficiently supports clinical services with finding new diagnoses and informs improvements to clinical pipelines.

**Conflict of Interest:** None declared

### C25.2 Systematic Pan-European reanalysis of 6004 genetically undiagnosed rare disease families yields 506 new diagnoses

***Wouter Steyaert***[1], *Steven Laurie*[2], *Elke de Boer*[1], *Kiran Polavarapu*[3], *Nika Schuermans**[4], *Anna Sommer*[5], *Anthony Brookes*[6], *Han Brunner*[1], *Holm Graessner*[7], *Olaf Riess*[7], *Katja Lohmann*[8], *Richarda de Voer*[1], *Ana Töpf*[9], *Lisenka Vissers*[1], *Sergi Beltran*[2], *Alexander Hoischen*[1]

[1]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands;* [2]*CNAG-CRG, The Barcelona Institute of Science and Technology, Barcelona, Spain;* [3]*Children's Hospital of Eastern Ontario Research Institute, Ottawa, Canada;* [4]*Center for Medical Genetics Ghent, University Hospital, Ghent, Belgium;* [5]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany;* [6]*University of Leicester, Department of Genetics and*

*Genome Biology, Leicester, United Kingdom; [7]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany; [8]Institute of Neurogenetics, University of Lübeck, Lübeck, Germany; [9]John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle, United Kingdom*

**Background/Objectives:** Effective diagnosis of rare genetic diseases (RD) depends on the ability to accurately identify and interpret variants from genomic data.

**Methods:** Clinical and molecular scientists from 36 centres of expertise across Europe joined efforts in Solve-RD to design, and apply, a strategy for systematic re-evaluation of genomic data. This approach led to reanalysis of 9874 previously negative genomic datasets for 6449 affected, but undiagnosed, individuals from 6004 RD families.

**Results:** We established a genetic diagnosis in 506 (8.4%) families by systematic reassessment of genomic data. From the 552 (likely) pathogenic variants consistent with the respective phenotypes, 464 (84.1%) were single nucleotide variants or short insertions/deletions. These variants were located in novel disease genes ($n = 67$), had a new or altered variant classification in ClinVar ($n = 117$), were inconclusive before ($n = 70$) or were only classified as disease causing by consensus expert decision within Solve-RD ($n = 210$). Bespoke analysis workflows for novel variant types (e.g., copy number variants, structural variants and mobile element insertions) identified the remaining 15.9% of disease causing variants.

In addition to the systematic reanalysis, 250 (4.2%) families were diagnosed by ad hoc expert-review leading to an overall diagnostic yield of 12.6%.

**Conclusion:** There remains a continued need for reanalysis of genomic data from individuals with RD, even among centres of expertise for these conditions. The Solve-RD approach exemplifies the considerable impact of sharing data and expertise across Europe for genomic analysis and interpretation, providing a template for global RD reanalysis at scale.

**Grant References:** Horizon 2020 grant agreement No.779257.

**Conflict of Interest:** None declared

### C25.3 MANE Select in 2023: expanding the joint NCBI and EMBL-EBI transcript set

***Adam Frankish***[1], *Jane Loveland*[1], *Shashikant Pujar*[2], *Alex Astashyn*[2], *Ruth Bennett*[1], *Andrew Berry*[1], *Eric Cox*[2], *Claire Davidson*[1], *Olga Ermolaeva*[2], *Catherine Farrell*[2], *Reham Fatima*[1], *Tamara Goldfarb*[2], *Jose M Gonzalez*[1], *Diana Haddad*[2], *Matt Hardy*[1], *Toby Hunt*[1], *John Jackson*[2], *Vinita Joardar*[2], *Michael Kay*[1], *Vamsi Kodali*[2], *Kelly McGarvey*[2], *Jonathan Mudge*[1], *Michael Murphy*[2], *Sanjida Rangwala*[2], *Francoise Thibaud-Nissen*[2], *Anjana Vatsan*[2], *Craig Wallin*[2], *David Webb*[2], *Fiona Cunningham*[1], *Terence Murphy*[2]

*[1]EMBL-EBI, Genome Assembly and Annotation, Cambridge, United Kingdom; [2]National Institutes of Health, National Center for Biotechnology Information, Bethesda, United States*

Comprehensive gene annotation is essential for understanding the impact of clinically relevant variants and standards support both consistent interpretation and reporting. The Matched Annotation from NCBI and EMBL-EBI (MANE) collaboration between Ensembl/GENCODE and RefSeq has defined a high-value set of transcripts and corresponding proteins for use as a universal standard for variant reporting. Each MANE transcript represents an exact match between the exonic sequence of an Ensembl/GENCODE transcript and its RefSeq counterpart, allowing identifiers to be used synonymously. The MANE Select set identifies a representative transcript for each human protein-coding gene with the MANE Plus Clinical set providing additional transcripts at loci where the Select alone is not sufficient to report all currently known clinical variants. MANE release 1.0 has MANE Select transcripts for 99.7% of human protein-coding genes, including all ACMG SF v3.1 genes.

The MANE collaboration continues with fewer than 50 protein-coding genes on the human reference genome GRCh38 not represented in the next release, including those on patches and alternative loci. We continue to add new MANE Plus Clinical transcripts and will incorporate transcripts from clinically relevant non-coding genes during 2023. Please give feedback at mane-help@ebi.ac.uk or MANE-help@ncbi.nlm.nih.gov to help us ensure that the MANE collaboration provides maximum benefit to users.

This work is supported by: Wellcome Trust-WT200990/Z/16/Z; EMBL-Core-Funds; NIH-U41HG007234. This work was supported in part by the National Center for Biotechnology Information of the National Library of Medicine (NLM), National Institutes of Health (NIH).

**Conflict of Interest:** None declared

### C25.4 Guideline for interpretation of proteomics data as functional evidence (PS3) in the context of the ACMG/AMP sequence variant interpretation framework

***Fatemeh Peymani***[1;2], *Dmitrii Smirnov*[1;2], *Robert Kopajtich*[1;2], *Riccardo Berutti*[1;2], *Christina Ludwig*[3], *Christiane Neuhofer*[1;2], *Thomas Meitinger*[1], *Holger Prokisch*[1;2]

*[1]Institute of Human Genetics, Technical University of Munich, School of Medicine, Munich, Germany; [2]Institute of Neurogenomics, Computational Health Center, Helmholtz München, Munich, Germany; [3]Bavarian Center for Biomolecular Mass Spectrometry, TUM School of Life Sciences, Technical University of Munich, Freising, Germany*

**Background:** Integrating proteomics data with genomic data has demonstrated a 21% improvement in the diagnostic yield of Mendelian disorders. Detection of reduced protein expression allows the reclassification of variants of uncertain significance to pathogenic. However, there is no defined threshold and no accepted guideline for the integration of the proteomics result into the ACMG/AMP framework. To address this, we applied Odds of Pathogenicity (OddsPath) to determine qualifying thresholds for the strong level of pathogenicity (PS3) for proteomics assay.

**Methods:** We used OUTRIDER2 for normalization and to calculate the z-score of protein expression in proteomics data from 136 fibroblast cell lines from patients with rare disorders. We calculated OddsPath using 1114 rare ClinVar benign and 142 pathogenic variants. The strength level of pathogenicity was assigned, stratified by zygosity and variant type. Additionally, we compared protein z-score with prediction algorithms such as CADD.

**Results:** Protein z-score below -2 provides strong evidence of pathogenicity for homozygous and compound heterozygous variants, whereas protein levels offer less informative value for heterozygous variants. Our results also demonstrate that the accuracy of protein z-score outperforms prediction algorithms based on DNA sequence.

**Conclusion:** This study provides valuable guidance on the interpretation of proteomics data within the context of the ACMG/AMP guidelines and shows the power of proteomics in variant classification.

**Grant References:** 01GM1920A

**Conflict of Interest:** None declared

### C25.5 SEALigHTS: An innovative, high throughput targeted technique to study messenger RNAs and circular RNAs

***Corentin Levacher***[1], *Charbonnier Françoise*[1], *Mathieu Viennot*[2], *Louis Jeanne*[1], *Thorn Hugo*[1], *Camille Charbonnier*[1], *Sophie Coutant*[1], *Théry Jean-Christophe*[2;3], *Gaelle Bougeard*[1], *Stéphanie Baert-Desurmont*[1;4], *Marick Laé*[2;3], *Philippe Ruminy*[2], *Claude Houdayer*[1;4]

[1]*Univ Rouen Normandie, Inserm U1245, Cancer and Brain Genomics, FHU-G4 Génomique, F-76000 Rouen, France, Rouen, France;* [2]*Univ Rouen Normandie, Inserm U1245, Cancer and Brain Genomics, Cancer Center Henri Becquerel, FHU-G4 Génomique, F-76000 Rouen, France, Rouen, France;* [3]*Department of Pathology, Cancer Center Henri Becquerel, Univ Rouen Normandie, F-76000 Rouen, France, Rouen, France;* [4]*Department of Genetics, Univ Rouen Normandie, Inserm U1245 and Rouen University Hospital, Cancer and Brain Genomics, FHU-G4 Génomique, F-76000 Rouen, France, Rouen, France*

**Background:** The study of messenger RNA (mRNA) is essential in the understanding of physiological and pathological processes, in both development and carcinogenesis. Its study is currently limited by the low throughput of targeted approaches (e.g. RT-PCR and Sanger) or the cost and high expertise of high-throughput approaches (RNAseq).

**Method:** We developed the new approach SEALigHTS (Splice and Expression Analyses by exon Ligation and High Throughput Sequencing) allowing the simultaneous exploration of all exon-exon junctions on a panel of genes of interest, thanks to probes designed at exon extremities. Following reverse transcription and probing on cDNA, nearby probes are ligated and the number of ligations quantified using unique molecular identifiers and sequencing. SEALigHTS was first validated on 282 RNA samples carrying various anomalies and previously validated by a reference approach. Then, we explored the physiological and pathological splicing of 45 cancer predisposition genes for 1553 RNA samples from diverse sources (895 Paxgene blood tubes, 107 cultivated peripheral blood mononuclear cells, 55 lymphoblastoid cell lines, 142 frozen fresh blood and 354 formalin-fixed paraffin-embedded breast, ovarian and salpingian tissues).

**Results:** Splicing abnormalities, physiological splicing differences, but also expression levels between genes were characterized. In addition, SEALigHTS allows the analysis of circular RNAs, produced by backsplicing, whose importance is increasingly documented. The splicing, backsplicing and expression study of 45 genes for 80 patients in parallel takes two days with a cost of 28 euros per sample.

**Conclusion:** SEALigHTS, which uses custom panels, can easily be implemented in laboratories, in research and in diagnosis.

**Conflict of Interest:** None declared

### C25.6 Diagnostic analysis of RNA splicing reclassifies 50% of candidate pathogenic splicing variants

***Mark Drost***[1], *Jordy Dekker*[1], *Janna A Hol*[1], *Hannie Douben*[1], *Peter Elfferich*[1], *Esmee Kasteleijn*[1], *Leontine M A van Unen*[1], *Rick van Minkelen*[1], *Marjon van Slegtenhorst*[1], *Martina Wilke*[1], *Lies Hoefsloot*[1], *Jasper J Saris*[1], *Yvette Van Ierland*[1], *Arjan Bouman*[1], *Ingrid MBH van de Laar*[1], *Grazia Mancini*[1], *Marianne Hoogeveen-Westerveld*[1], *Mark Nellist*[1], *Tjakko Van Ham*[1]

[1]*ErasmusMC, Dept of Clinical Genetics, Rotterdam, Netherlands*

**Background/Objectives:** Adverse effects of DNA variants on pre-mRNA splicing are an important cause of genetic disorders. However, effects on pre-mRNA splicing are difficult to predict, and for variant classification the ACMG/AMP guidelines (PVS1 criterion) recommend experimental characterization. We therefore assessed the utility of in vitro RNA analyses in a diagnostic setting.

**Methods:** We investigated 136 clinically relevant variants identified in Dutch diagnostic centers. We performed targeted analysis of pre-mRNA splicing in blood or skin fibroblasts by RT-PCR followed by Sanger sequencing, and performed mini-gene exon trapping experiments. We compared the outcomes to in silico splicing predictions (SpliceAI and predictions integrated in Alamut).

**Results:** An effect on pre-mRNA splicing was demonstrated in 64% (87/136) of tested cases. RNA analyses facilitated reclassification of 50% (59/118) of variants of unknown significance to (likely) pathogenic. SpliceAI predictions were concordant in 90% of cases where an effect on mRNA splicing was predicted (54/60), and in 66% of cases where no splice effect was predicted (33/50). Alamut-based predictions were concordant in 84% of cases where an effect on splicing was predicted (78/93), and in 76% of cases where no effect on mRNA splicing was predicted (22/29).

**Conclusion:** In silico predictions of pre-mRNA splicing often correlate with in vitro analyses, but should be used with caution, confirming existing guidelines. Experimental analysis of pre-mRNA splicing in a diagnostic setting facilitates variant classification, in particular upgrading variants to (likely) pathogenic. We provide a framework for splicing analysis in a molecular diagnostic setting.

**Conflict of Interest:** None declared

## C26 GWAS

### C26.1 Deciphering the genetic architecture of retinal vascular traits and their potential to predict cardiovascular disease risk

***Sven Bergmann***[1]

[1]*University of Lausanne, Department of Computational Biology, Lausanne, Switzerland*

Fundus images allow for non-invasive assessment of the retinal vasculature whose features provide important information on health. The characterisation of vessel parameters, such as their diameter, number and angles of bifurcations or tortuosity allows for studying the genetic architecture and association with cardiovascular diseases.

We analysed 116,639 fundus images of 63,662 participants from three cohorts: the UK Biobank ($n = 62{,}751$), SKIPOGH ($n = 397$), and OphtalmoLaus ($n = 512$). We used a fully automated image processing pipeline to annotate vessels and a deep learning algorithm to determine the vessel type, characterising these subjects in terms of their median values for 17 vessel parameters specific to arteries and to veins. Using these measures as traits, we performed genome-wide association studies (GWAS) of these parameters with unprecedented power. We assessed gene set enrichment for each parameter using the novel high-precision statistical method PascalX.

Correlations analysis revealed the relationships between these parameters and their association with different vascular diseases and their risk factors. Our GWAS identified 175 significantly associated genetic loci for tortuosity alone, and a large number of loci for the other retinal traits. Many genes with significant association were overexpressed in arteries and heart muscle and linked to pathways related to the structural properties of the vasculature. We demonstrated that tortuosity loci served pleiotropic functions as cardiometabolic disease variants and risk factors.

Our results shed new light on the genetics of vascular diseases and their pathomechanisms, and highlight how heritability can be

used to improve phenotype extraction from high-dimensional data, such as images.

**Conflict of Interest:** None declared

### C26.2 Genetic analysis in 1.9 million individuals reveals new insights into genetic variation underlying heart failure susceptibility

***Albert Henry***[1;2;3], *Thomas Lumbers*[2;4]

[1]*University College London, Institute of Cardiovascular Science, London, United Kingdom;* [2]*University College London, Institute of Health Informatics, London, United Kingdom;* [3]*University College London, British Heart Foundation Research Accelerator, London, United Kingdom;* [4]*University College London, Health Data Research UK London, London, United Kingdom*

**Background/Objectives:** Characterising genetic variation underlying susceptibility to heart failure (HF) may inform the development of new therapeutic interventions. To identify effector genes for HF and gain insights into molecular mechanisms of disease, we performed a genome-wide association analysis with detailed functional locus characterisation.

**Methods:** We conducted a multi-ancestry genome-wide association meta-analysis in 1.9 million individuals comprising 145,795 HF cases, stratified by aetiology and left ventricular ejection fraction (LVEF). We undertook a systematic approach combining functionally informed fine-mapping and six in silico gene mapping techniques, to identify putative causal variants and effector genes at each identified locus. Using enrichment and single-cell differential gene expression analyses, we identified disease-relevant pathways, tissues, and cell types. Finally, we characterised the pleiotropic effects of HF loci through phenome-wide association and colocalization analyses.

**Results:** We identified 66, including 37 novel, independent genomic loci associated with HF. We generated a ranked list of 133 putative effector genes for HF, of which 50 were differentially expressed in failing heart cells. The mapped genes were enriched for terms related to Mendelian cardiovascular diseases, cellular senescence, and sarcomere components. Heritability was enriched for renal and pancreatic tissues in HF with preserved LVEF; and cardiac tissues in HF with reduced LVEF. Associations across 88 diseases were identified for 44 sentinel variants at FDR < 1%. Colocalization of causal variants were identified in 39 HF loci across 32/35 tested traits.

**Conclusion:** These findings extend our understanding of the genetic basis and molecular aetiology of HF.

**Grant References:** British Heart Foundation PhD Studentship.

**Conflict of Interest:** Albert Henry AH was supported by the British Heart Foundation PhD studentship and has received funding from Pfizer for an unrelated work, Thomas Lumbers TL is supported by the UKRI Innovation Fellowship and has received funding from Pfizer for an unrelated work

### C26.3 Copy-number variants as modulators of common disease susceptibility

***Chiara Auwerx***[1;2;3], *Maarja Jõeloo*[4;5], *Nicolò Tesio*[1], *Sven Erik Ojavee*[2;3], *Marie Sadler*[2;3;6], *Charlie Clark*[1], *Alexandre Reymond*[1], *Zoltan Kutalik*[2;3;6]

[1]*University of Lausanne, Center for Integrative Genomics, Lausanne, Switzerland;* [2]*University of Lausanne, Department of Computational Biology, Lausanne, Switzerland;* [3]*Swiss Institute of Bioinformatics, Lausanne, Switzerland;* [4]*University of Tartu, Institute of Molecular and Cell Biology, Tartu, Estonia;* [5]*University of Tartu, Estonian Genome Centre, Tartu, Estonia;* [6]*University Center for Primary Care and Public Health, Lausanne, Switzerland*

**Background/Objectives:** Copy-number variations (CNVs) cause rare genomic syndromes but their impact on common diseases remains understudied.

**Methods:** We performed genome-wide association scans (GWASs) between the copy-number of CNV-proxy probes and 60 clinical diagnoses in 331'522 unrelated white UK Biobank participants.

**Results:** Logistic regression identified 70 signals across 40 diseases, which were confirmed by Fisher test (40%), residual regression (32%), and time-to-event analysis (100%), the latter suggesting that CNVs cause earlier disease onset. We replicated four of 33 testable associations in the Estonian Biobank, with 5.5-fold enrichment for nominally significant signals ($p = 2.5e\text{-}5$).

We recapitulated know associations, e.g., between the deletion of *BRCA1* and ovarian cancer (OR > 24.6; $p = 6.1e\text{-}6$) or the LDL-binding domain of *LDLR* and ischemic heart disease (IHD; OR > 7.1; $p = 5.6e\text{-}6$). Others were supported by colocalization with SNP-GWAS signals (39%), overlap with relevant OMIM genes (21%), or CNV-biomarker associations (52%), e.g., deletion of autosomal recessive pseudoxanthoma elasticum-associated *ABCC6* increased kidney stone risk (OR > 2.9; $p = 7.3e\text{-}5$), while 22q11.2 CNVs, which are linked to congenital heart diseases, increased IHD (OR > 1.6; $p = 1.5e\text{-}7$) and aneurysm (OR > 10.0; $p = 3.2e\text{-}7$) risk.

Genes encompassed by disease-associated CNVs were under stronger evolutionary constraint ($p_{pLI} = 1.0e\text{-}4$; $p_{LOEUF} = 1.9e\text{-}7$). While pleiotropy was infrequent (16% of CNVs) and dependent on the number of affected genes (+0.2 associations/gene; $p = 1.5e\text{-}5$), 16p11.2 BP4-5 stood out with 15 associations. Finally, even after correcting for GWAS signals, a higher CNV burden increased risk for 18 disorders, mainly through the number of deleted genes ($p = 1.3e\text{-}6$).

**Conclusion:** These results shed light on the prominent role of CNVs in common diseases within the general population.

**Conflict of Interest:** None declared

### C26.4 Whole genome burden testing in 333,100 individuals identifies novel rare non-coding associations with height

***Gareth Hawkes***[1], *Robin Beaumont*[1], *Zilin Li*[2], *Ravi Mandla*[3], *Xihao Li*[4], *Alisa Manning*[3], *Xihong Lin*[4], *Caroline Wright*[1], *Andrew Wood*[1], *Timothy M. Frayling*[1], *Michael Weedon*[1]

[1]*University of Exeter, Exeter, United Kingdom;* [2]*Indiana University, Department of Biostatistics and Health Data Science, West Lafayette, United States;* [3]*MGH Clinical and Translational Epidemiology Unit, Boston, United States;* [4]*Harvard T.H. Chan School of Public Health, Boston, United States*

**Background/Objectives:** Most sequence-based association studies for common human phenotypes have focussed on rare variants that reside in the coding regions of the genome. However, the recent release of whole-genome-sequence (WGS) data in 100,000s of individuals from several studies provides an unprecedented opportunity to examine rare, non-coding variants and their contribution towards the genetic architecture of common traits.

**Methods:** We performed the largest WGS-based analysis for height to-date using 333,100 individuals from three studies: UK Biobank (N = 200,003), TOPMed ($N = 87{,}652$) and AllOfUs ($N = 45{,}445$). We developed a generalised analytical pipeline with the aim of finding novel rare (<0.1% minor-allele frequency) non-coding genetic associations. We tested 75,311,546 variants which had at least 20 carriers in the UK Biobank, and performed 52,749,161 genomic aggregates tests split into gene-centric (e.g., proximal) and non-gene-centric (e.g., regulatory), and grouped by

measures of conservation, constraint and deleteriousness. Finally, we performed a hypothesis-free 2kbp sliding window analysis.

**Results:** We observed 30 independent novel rare variants associated with height at $p < 6E\text{-}10$, after conditioning on more than 13,000 previously reported loci. We observed effect sizes range from −7 cm to +2 cm, and replicated three rare single variant associations. We also observed evidence for non-coding associations proximal to *HMGA1* and *GH1*, and an association downstream of *C17orf49* overlapping *mRNA*. All three burden associations showed evidence of replication.

**Conclusions:** Our approach found novel non-coding associations for height, and provides a template for the analysis of non-coding rare variants for common human phenotypes.

**Conflict of Interest:** None declared

### C26.5 Genetics of endocrine-related brain anatomy using biomedical image derived phenotypes

***Hannah Currant***[1], *Christoph Arthofer*[2], *Stephen Smith*[2], *Teresa Ferreira*[3], *Christoffer Nellaker*[3], *Gwenaëlle Douaud*[2], *Andreas Bartsch*[4], *Jesper Andersson*[2], *Margaret F. Lippincott*[5], *Yee-Ming Chan*[6], *Stephanie B. Seminara*[5], *Thomas E. Nichols*[2;3], *Søren Brunak*[1], *Frederik J. Lange*[2], *Cecilia M. Lindgren*[3;7]

[1]*University of Copenhagen, Novo Nordisk Foundation Center for Protein Research, Copenhagen, Denmark;* [2]*University of Oxford, FMRIB (Oxford Centre for Functional MRI of the Brain), Nuffield Department of Clinical Neuroscience, Oxford, United Kingdom;* [3]*University of Oxford, Big Data Institute at the Li Ka Shing Centre for Health Information and Discovery, Oxford, United Kingdom;* [4]*University of Heidelberg, Department of Neuroradiology, Heidelberg, Germany;* [5]*Massachusetts General Hospital, Reproductive Endocrine Unit, MGH-Harvard Center for Reproductive Medicine, Boston, MA, United States;* [6]*Boston Children's Hospital, Division of Endocrinology, Department of Pediatrics, Boston, MA, United States;* [7]*University of Oxford, Wellcome Centre for Human Genetics, Nuffield Department of Medicine, Oxford, United Kingdom*

**Background:** Several brain structures have roles in regulating the endocrine system, as reflected in their morphology: pituitary gland volume correlates with sex-steroid concentrations, whilst olfactory-bulb hypoplasia is a phenotype of Kallmann syndrome, which causes infertility. Magnetic resonance imaging (MRI) can provide rich data describing brain structures. Here, we elucidate genetics underlying these brain structures and thus further our knowledge of the endocrine system.

**Methods:** We derived volumetric and intensity measures of the hypothalamus, pituitary gland, and olfactory bulbs from UK Biobank brain MRI ($n = 34{,}834$, 53% female), segmented using multimodal registration to a manually-labelled atlas. We conducted the largest genome- and exome-wide association studies (GWAS and EWAS) to date of these measures. Sexual-dimorphic effects were assessed by comparing weighted-effect sizes in males and females.

**Results:** We discovered 24, 16, 4 and 6 loci ($p < 5 \times 10^{-8}$) associated with volume of hypothalamus, pituitary gland, left and right olfactory bulb, respectively. Additionally, 13 loci were associated with one or more intensity measures of the four brain structures. Across GWASs of all phenotypes, 8 of the identified loci were sex-dimorphic ($p_{diff} < 5.5 \times 10^{-4}$). In the EWAS, 23 genes were associated with one or more brain structure volumes ($p < 5 \times 10^{-5}$). Annotating loci using ENSEMBL and OpenTargets-Genetics found prior associations to endocrine phenotypes including testosterone levels and ages at menopause and menarche.

**Conclusion:** Empowered by its unparalleled size, our study furthers our understanding of genetics underlying brain structure and shows links between brain structures and endocrine biology.

**Grant References:** HC-MSCA(101064250,RMCmplxPheno); CML-NIH(1P50HD104224-01), Gates Foundation(INV-024200), Wellcome Trust(221782/Z/20/Z); CA, SS, FL-Wellcome Trust(215573/Z/19/Z,203141/Z/16/Z,203139/Z/16/Z).

**Conflict of Interest:** Hannah Currant: None declared, Christoph Arthofer: None declared, Stephen Smith: None declared, Teresa Ferreira: None declared, Christoffer Nellaker: None declared, Gwenaëlle Douaud: None declared, Andreas Bartsch: None declared, Jesper Andersson: None declared, Margaret F. Lippincott: None declared, Yee-Ming Chan: None declared, Stephanie B. Seminara: None declared, Thomas E. Nichols: None declared, Søren Brunak Novo Nordisk Foundation, Intomics, Hoba Therapeutics, Novo Nordisk, Lundbeck and ALK, Proscion and Intomics, Frederik J. Lange: None declared, Cecilia M. Lindgren Bayer AG, Novo Nordisk, Pfizer, Pfizer, Spouse/partner: Vertex

### C26.6 Large-scale blood mitochondrial genome wide associations study provides novel insights into mitochondrial disease related traits

*Stuart Cannon*[1;2], *Tim Hall*[1], *Gareth Hawkes*[1], *Kevin Colclough*[3], *Roisin Boggan*[4], *Andrew Hattersley*[1], *Sarah Pickett*[4], ***Michael Weedon***[1;2], *Kashyap Patel*[1;2]

[1]*Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, United Kingdom;* [2]*Translational Research Exchange Exeter, University of Exeter, Exeter, United Kingdom;* [3]*Exeter Genomics Laboratory, Royal Devon University Healthcare, Exeter, United Kingdom;* [4]*Wellcome Centre for Mitochondrial Research, Translational and Clinical Research Institute, Newcastle, United Kingdom*

**Background/Objectives:** We aim to identify the impact of blood mitochondrial DNA variants (common, rare, and pathogenic) on 15 commonly presenting mitochondrial disease-related phenotypes by performing large-scale mitochondrial genome-wide associations and rare-variant aggregation study of 179,862 individuals.

**Methods:** We identified mitochondrial variants from blood whole genome sequencing data on 179,862 multi-ancestry individuals from UK Biobank. We used REGENIE to perform single and rare variant aggregation associations with 15 mitochondrial disease-relevant phenotypes. Our Bonferroni corrected significance threshold was ($p < 4 \times 10^{-7}$). Results were compared to a clinically referred patient cohort.

**Results:** We found 15,274 variants in 179,862 individuals after robust filtering. The variant m.3243A > G (MAF = 0.0002), was associated with diabetes (OR = 6[3–8], $P < 4 \times 10^{-9}$), deafness (OR = 12[10–15], $P = 6 \times 10^{-13}$) and heart failure (OR = 10[7–13], $P < 8 \times 10^{-7}$). The association was stronger with higher heteroplasmy >10% (OR = 25,55,39, respectively). Diabetes penetrance at age 50 was significantly lower (15%) than clinically selected probands (96%) and was further modified by nuclear T2D genetic risk. Other known pathogenic variants ($n = 5$, MAC > 20) showed no or minimal assortation with respective diseases suggesting lower penetrance. We found 9 homoplasmic variants associated with increased aspartate aminotransferase (AST) led by m.15758A > G (MAF = 0.02) and m.13488:T > C (MAF = 0.0022). Variant aggregation testing did not identify any additional associations.

**Conclusion:** In this large study very few mitochondrial variants reached genome wide significance. The known pathogenic variants showed lower penetrance in a population cohort. m.3243A > G was strongly associated with mitochondrial-related traits at higher heteroplasmy and modified by nuclear T2D genetic

risk. Our results support the clinical reporting of incidental identification of m.3243A > G at high heteroplasmy.

**Grant References:** 219606/Z/19/Z;WT105618MA

**Conflict of Interest:** None declared

## C27 NEW TECHNOLOGIES IN CANCER GENETICS

### C27.1 TET1 and TDG suppress inflammatory response in intestinal tumorigenesis: implications for colorectal tumors with the CpG Island Methylator Phenotype - virtual

***Rossella Tricarico***[1;2;3], *Jozef Madzo*[4], *Gabrielle Scher*[1;2], *Maya Cohen*[1;2], *Jaroslav Jelinek*[4], *Shinji Maegawa*[5], *Rajeswari Nagarathinam*[6], *Carly Scher*[1;2], *Wen-Chi Chang*[7], *Emmanuelle Nicolas*[8], *Michael Slifker*[9], *Yan Zhou*[9], *Karthik Devarajan*[9], *Kathy Q. Cai*[10], *Tim Kwok*[11], *Pamela Nakajima*[11], *Jinfei Xu*[1;2], *Pietro Mancuso*[1;2], *Valentina Doneddu*[1;2], *Luigi Bagella*[12;13], *Riley Williams*[8], *Siddharth Balachandran*[8], *Nicholas Maskalenko*[8], *Kerry Campbell*[8], *Xueying Ma*[2], *Israel Cañadas*[1;2], *Julen Viana-Errasti*[14], *Victor Moreno*[15;16;17], *Laura Valle*[14;18], *Sergey Grivennikov*[7;19], *Yulia Peshkova*[8;19], *Natalia Kurilenko*[8;19], *Aleksandra Mazitova*[8;19], *Ekaterina Koltsova*[8;19], *Hayan Lee*[1;2], *Martin Walsh*[1;2], *Reuben Duttweiler Duttweiler*[1;2], *Johnathan Whetstine*[1;2], *Timothy Yen*[1;2], *Jean-Pierre Issa*[4], *Alfonso Bellacosa*[1;2]

[1]*Institute for Cancer Research Fox Chase Cancer Center, Cancer Epigenetics Institute, Philadelphia, United States;* [2]*Institute for Cancer Research Fox Chase Cancer Center, Nuclear Dynamics and Cancer Program, Institute for Cancer Research, Philadelphia, United States;* [3]*Present address: University of Pavia, Department of Biology and Biotechnology, Pavia, Italy;* [4]*Coriell Institute for Medical Research, Camden, United States;* [5]*University of Texas, M.D. Anderson Cancer Center, Houston, United States;* [6]*Institute for Cancer Research Fox Chase Cancer Center, Department of Pathology, PHILADELPHIA, United States;* [7]*Institute for Cancer Research Fox Chase Cancer Center, Cancer Prevention and Control Program, PHILADELPHIA, United States;* [8]*Institute for Cancer Research Fox Chase Cancer Center, Cancer Signaling and Microenvironment Program, PHILADELPHIA, United States;* [9]*Institute for Cancer Research Fox Chase Cancer Center, Department of Biostatistics, PHILADELPHIA, United States;* [10]*Institute for Cancer Research Fox Chase Cancer Center, Experimental Histopathology, PHILADELPHIA, United States;* [11]*Institute for Cancer Research Fox Chase Cancer Center, Cell Culture Facility, PHILADELPHIA, United States;* [12]*University of Sassari, Department of Biomedical Sciences, Sassari, Italy;* [13]*Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, United States;* [14]*Oncobell Program, IDIBELL, Hospitalet de Llobregat, Hereditary Cancer Program, Barcelona, Spain;* [15]*Oncobell Program, IDIBELL, Hospitalet de Llobregat, Oncology Data Analytics Program, Catalan Institute of Oncology, Barcelona, Spain;* [16]*Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain;* [17]*University of Barcelona, Department of Clinical Sciences, Faculty of Medicine, Barcelona, Spain;* [18]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain;* [19]*Cedars-Sinai Medical Center, Department of Medicine and Department of Biomedical Sciences, Los Angeles, United States*

**Background/Aim:** Aberrant DNA methylation is frequent in colorectal cancer (CRC), but underlying mechanisms and pathological consequences are poorly understood.

**Methods:** We explored the role of active DNA demethylation in intestinal tumorigenesis, by inactivating *Tet1* and/or *Tdg* in *Apc*$^{Min}$ mice and characterizing the methylome and transcriptome of colonic adenomas. Data were compared to human colonic adenocarcinomas (COAD) in TCGA.

**Results:** *Tdg*-mutant *Apc*$^{Min}$ mice developed increased number of small intestinal adenomas, whereas *Tet1*-mutant and *Tet1/Tdg*-double heterozygous *Apc*$^{Min}$ mice showed larger colonic adenomas with features of erosion and invasion. We detected reduction in global DNA hypomethylation in colonic adenomas from *Tet1*- and *Tdg*-mutant *Apc*$^{Min}$ mice, and hypermethylation of CpG islands in *Tet1*-mutant *Apc*$^{Min}$ adenomas, which resembles the CpG island methylator phenotype (CIMP) in human CRC. Upregulation of inflammatory, immune and interferon response genes was present in *Tet1*- and *Tdg*-mutant colonic adenomas compared to control *Apc*$^{Min}$ adenomas, in murine colonic organoids and human CRC lines expressing *TET1* or *TDG* shRNA. A 127-gene inflammatory signature separated COAD into four groups, closely aligned with their microsatellite or chromosomal instability, and characterized by different levels of DNA methylation and *DNMT1* expression that anti-correlated with *TET1* expression. CIMP tumors had concerted high *DNMT1*/low *TET1* expression.

**Conclusions:** Our findings reveal a novel epigenetic regulation, linked to the type of genomic instability, by which TET1-TDG-mediated DNA demethylation decreases methylation and inflammatory/interferon/immune responses. CIMP in CRC is triggered by an imbalance of methylating activities over demethylating activities.

**Grant references:** CA78412, CA191956, CA218133, R35GM144131, CA006927, MRA693670, W81XWH-21-1-0648, William J. Avery Postdoctoral Fellowship.

**Conflict of Interest:** Rossella Tricarico: None declared, Jozef Madzo: None declared, Gabrielle Scher: None declared, Maya Cohen: None declared, Jaroslav Jelinek: None declared, Shinji Maegawa: None declared, Rajeswari Nagarathinam: None declared, Carly Scher: None declared, Wen-Chi Chang: None declared, Emmanuelle Nicolas: None declared, Michael Slifker: None declared, Yan Zhou: None declared, Karthik Devarajan: None declared, Kathy Q. Cai: None declared, Tim Kwok: None declared, Pamela Nakajima: None declared, Jinfei Xu: None declared, Pietro Mancuso: None declared, Valentina Doneddu: None declared, Luigi Bagella: None declared, Riley Williams: None declared, Siddharth Balachandran: None declared, Nicholas Maskalenko: None declared, Kerry Campbell: None declared, Xueying Ma: None declared, Israel Cañadas: None declared, Julen Viana-Errasti: None declared, Victor Moreno: None declared, Laura Valle: None declared, Sergey Grivennikov: None declared, Yulia Peshkova: None declared, Natalia Kurilenko: None declared, Aleksandra Mazitova: None declared, Ekaterina Koltsova: None declared, Hayan Lee: None declared, Martin Walsh: None declared, Reuben Duttweiler Duttweiler: None declared, Johnathan Whetstine Salarius Pharmaceuticals and Oryzon Genomics, Qsonica, Salarius Pharmaceuticals, Daiichi Sankyo, Inc., Vyne Therapeutics and Lily Asia Ventures, Timothy Yen: None declared, Jean-Pierre Issa: None declared, Alfonso Bellacosa: None declared

### C27.2 Merging patient-derived iPSCs and organ-on-a-chip technology to disclose the genesis of Hereditary Diffuse Gastric Cancer

***Daniel A. Ferreira***[1;2], *Rita Barbosa-Matos*[1;2;3], *Luzia Garrido*[4;5], *Ana C. Brito*[1;2;3], *João Fonseca*[1;2;3], *Silvana Lobo*[1;2;3], *Ana Moutinho*[1;2;6], *Alexandre Dias*[1;2;3], *Irene Gullo*[1;4;5], *Ana Mamede*[1;2], *MengWei Yang*[7], *Aldana G. Gojanovich*[7], *Renata Oliveira*[4;5], *João Parente Freixo*[8], *Pedro Louro*[9;10], *Ana Rita Ferreira Pacheco Quental*[4;5], *Ana Grangeia*[4;5], *Hugo Pinheiro*[1;2;11], *Peter Ertl*[12], *Pedro L. Granja*[1], *Susana Fernandes*[5], *Fátima Carneiro*[1;4;5], *Sérgio Castedo*[1;4;5], *Gustavo Mostoslavsky*[7], *Carla Oliveira*[1;2;5]

[1]*i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;* [2]*Ipatimup – Institute of Molecular*

*Pathology and Immunology, Universidade do Porto, Porto, Portugal; [3]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; [4]CHUSJ, Centro Hospitalar e Universitário de São João, Porto, Portugal; [5]Faculty of Medicine of the University of Porto, Porto, Portugal; [6]CHUP, Centro Hospitalar Universitário do Porto, Porto, Portugal; [7]Center for Regenerative Medicine (CReM), Boston University School of Medicine, Boston, United States; [8]Center for Predictive and Preventive Genetics, Institute for Molecular and Cell Biology, Porto, Portugal; [9]Medical Genetics Service, Centro Hospitalar Universitário de São João, Porto, Portugal; [10]Faculty of Health Sciences, Universidade da Beira Interior, Covilhã, Portugal; [11]Department of Internal Medicine, Centro Hospitalar Tâmega e Sousa, Paredes, Portugal; [12]Faculty of Technical Chemistry, Vienna University of Technology (TUW), Vienna, Austria*

**Background/Objectives:** Hereditary Diffuse Gastric Cancer (HDGC), caused by *CDH1* loss of function, can only be prevented with prophylactic removal of stomach and breasts. The growing number of prophylactic surgeries hamper the study of disease initiation. Herein, we present the development of a biomimetic HDGC model combining organ-on-a-chip technology with patient-derived induced pluripotent stem cells (iPSCs) to overcome the lack of patients' target organs.

**Methods:** We developed a microfabrication methodology based on xurography to engineer a functional stomach-on-a-chip, which was tested for biomimetic stretching capability and structural integrity. The biochip was characterized with immunofluorescence, enzymatic, and trans-epithelial transport assays. Patients' blood samples were collected for the establishment of a biobank of HDGC families with peripheral mononuclear blood cells (PBMCs) and its derived iPSCs. iPSCs were obtained using the Sendai virus integration-free method.

**Results:** We designed and fabricated a stomach-on-a-chip device emulating the 3 inner gastric layers and recapitulating gastric peristaltic-like motion, intraluminal-flow, cell polarization, barrier function, pepsin activity, and the gastric lumen. We also established and characterized iPSCs from 9 patient-derived PBMCs, which were validated for pluripotency and further differentiated into stomach organoids. The stomach-on-a-chip is ready to be populated with HDGC patient-derived organoids.

**Conclusion:** We created a functional stomach-on-a-chip that can be produced in a few hours, and a unique infrastructure that will allow recreating organs that no longer exist from HDGC patients submitted to life-saving prophylactic surgery. This is a pioneer model to study early diagnosis and treatment in HDGC.

**Funding:** Researcher/Horizon_Europe/i3S/2311/2022
LEGOHProject/PTDC/BTM-TEC/6706/2020
DETONATEProject/2022.02951.PTDC

**Conflict of Interest:** None declared

### C27.3 Mutation rate variability at the sub-gene scale due to distinct DNA methylation gradients

***David Mas-Ponte***[1], *Fran Supek*[1;2]

[1]*Institute for Research in Biomedicine - IRB, Barcelona, Spain;* [2]*Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain*

The mutation rate of somatic genomes varies extensively among megabase scale replication time domains and, on a finer scale, due to the trinucleotide preference of distinct mutational processes. Beyond these two major factors, a less characterized set of genetic and epigenetic elements may shape the accumulation of mutations at the gene scale, spanning roughly 0.3kb – 100kb.

Here, we systematically analyze a set of ~2800 whole genome sequences from tumors and healthy tissues to identify mutation rate gradients along gene bodies. The major intragenic mutation rate heterogeneity is located at the 5′ end of genes, extending downstream within the gene body for several kilobases. This pervasive trend of mutation coldspots at 5′ ends of gene bodies matches the gradients of DNA methylation. Distinct mutational processes show however opposite interactions with methylation. While the mutagenesis due to spontaneous cytosine deamination is massively depleted in the hypomethylated section, mutations resulting from the APOBEC cytidine deaminases show increased mutagenesis. The width and intensity of this hypomethylated interval is variable across genes and can be attributed to intragenic enhancers, minor promoters, and large Polycomb-marked hypomethylation. We further classify human genes according to differences in DNA methylation gradients and quantified how these modulate local mutation rate, showing reduced mutagenesis in the gene groups with wider intragenic hypomethytlation section.

Overall, we suggest that DNA methylation is an important determinant of sub-gene resolution mutation rate heterogeneity in humans. Modeling sub-gene mutation rate variation can be beneficial to estimate somatic selection.

**Conflict of Interest:** None declared

### C27.4 PBRM1 and KDM5C loss modulate the tumor immune microenvironment of clear cell renal carcinoma

***Carlos Valdivia***[1], *Javier de Nicolás-Hernández*[1], *Javier Lanillos*[1], *Ángel M. Martínez-Montes*[1], *Alicia Arenas*[1], *Sara Mellid*[1], *María Monteagudo*[1], *RAUL TORRES*[2], *Eduardo Jose Caleiras-Pires*[3], *Javier Leandro*[1], *Mercedes Robledo*[1], *Cristina Rodriguez-Antona*[1]

[1]*Spanish National Cancer Research Center, Hereditary Endocrine Cancer Group, Madrid, Spain;* [2]*Spanish National Cancer Research Center, Cytogenetics Unit, Madrid, Spain;* [3]*Spanish National Cancer Research Center, Histopathology Unit, Madrid, Spain*

**Introduction**. Clear cell renal cell carcinoma (ccRCC) is driven by the inactivation of *VHL* plus frequent secondary mutations in chromatin remodelers. Loss of PBRM1, a component of the SWI/SNF chromatin-remodeling complex, and of KDM5C, a histone demethylase, have been shown to modify tumor microenvironment. Furthermore, patient studies support an impact of altered chromatin remodelers on antiangiogenic and immunotherapy treatment response. Here, we extend this notion through a comprehensive transcriptomic characterization of a newly developed ccRCC immunocompetent murine model.

**Material and methods**. To mimic ccRCC patient mutational landscape, single, double and triple knock-out (KO) of *Vhl* (V), *Kdm5c* (K) and *Pbrm1* (P) were generated in the murine renal cancer cell line Renca using CRISPR/Cas9. Subcutaneous allografts were generated in BALB/c mice and molecular features among genetic groups were identified by bulk RNA-seq analyses, including immune deconvolution, and immunohistochemistry.

**Results**. Unsupervised hierarchical clustering grouped in vitro cells and tumor samples according to the genetic groups. Differential expression analysis against V-KO, identified VKP-KO as the most distinct group, followed by VK-KO and VP-KO (with 3597, 1136 and 39 differentially expressed genes, respectively). Pathway enrichment analysis showed increased angiogenesis in *Vhl*-KO groups and alteration of immune-related pathways in VKP-KO. Immune deconvolution revealed macrophage M1 to M2 switch and an increased proportion of activated dendritic cells with *Pbrm1* and *Kdm5c* loss, supporting a unique immune environment.

**Conclusions**. Mutations in *PBRM1* and *KDM5C* shape specific transcriptional profiles in the cancer cells, which modulate the tumor microenvironment and potentially immunotherapy response.

**Conflict of Interest:** None declared

### C27.5 Reactivation of human fetal microRNAs in liver cancer

***Nikita Telkar***[1;2;3], *Greg Stewart*[1], *Michelle Pewarchuk*[1], *Katya Benard*[1], *David Cohn*[1], *Wendy Robinson*[2;3], *Wan Lam*[1]

[1]*British Columbia Cancer Research Centre, Vancouver, Canada;* [2]*British Columbia Children's Hospital Research Institute, Vancouver, Canada;* [3]*University of British Columbia, Department of Medical Genetics, Vancouver, Canada*

Rapid cell proliferation and differentiation are hallmarks shared by fetal development and cancer. While these highly regulated processes cease functioning after fetal growth, they show aberrant re-activation in cancer (oncofetal). MicroRNAs regulate gene expression and have known clinical roles in normal adult and tumour tissue. However, due to the scarcity of fetal samples, the processes causing this re-activation of fetal microRNAs and the parallels between tumour and fetal development remain to be explored.

We profiled the microRNA transcriptome of 10 fetal liver samples from second trimester elective terminations and compared them with 345 hepatocellular carcinoma and 50 non-malignant (NM) matched samples from The Cancer Genome Atlas (TCGA). MicroRNAs which showed significant differential expression after multiple-testing correction between tumour-NM and fetal-NM but not between tumour-fetal were termed oncofetal microRNAs ($n = 113$). The Kaplan-Meier survival curve using this 113 miRNA-signature was highly significant ($p = 0.0019$), and six displayed standalone significant difference ($p <= 0.05$) in patient survival based on expression. Given the higher incidence of liver cancer in males than females, sex-stratified analysis revealed 121 hepatic oncofetal microRNAs in males and 166 in females – 62 of the 121 and 49 of 166 had significant patient survival curves. Signalling pathways (mTOR, MAPK, HIPPO), immune pathways, and cancer pathways were the top hits output by multiple pathway analysis databases.

To our knowledge, this is the first report of human fetal liver miRnome profiling and comparison with adult liver and tumour tissue. Our results emphasize the clinical importance of oncofetal microRNAs.

**CIHR:**FRN106430

**Conflict of Interest:** None declared

### C27.6 CTNNA1 germline variants with a premature termination codon are a risk factor for development of early-onset Diffuse Gastric Cancer

***Silvana Lobo***[1;2], *Alexandre Dias*[1;3], *Chrystelle Colas*[4;5], *Robert Hüneburg*[6;7;8], *Lise Boussemart*[9;10], *Liselotte P van Hest*[11;12], *Leticia Moreira*[13;14;15], *Carolyn Horton*[16], *Jennifer Herrera-Mullar*[16], *Dana Farengo Clark*[17], *Sigrid Tinschert*[18;19], *Golmard Lisa*[4;5], *Isabel Spier*[6;8;20], *Adrià López-Fernández*[21;22], *Daniela Oliveira*[23;24], *Marta Ferreira*[1;3], *Magali Svrcek*[25;26], *Pierre Bourgoin*[26], *Helene Delhomelle*[4;5], *Paul Mansfield*[27], *Verena Steinke-Lange*[28;29;30], *Sérgio Sousa*[23;24], *Manuela Balsinha*[31], *Stefan Aretz*[6;8;20], *JUDITH BALMAÑA*[21;22], *Melyssa Aronson*[32], *Augusto Perazzolo Antoniazzi*[33], *Edenir Palmero*[34;35], *Lizet Van der Kolk*[36], *Annemieke Cats*[37], *Jolanda M van Dieren*[37], *Sergi Castellvi-Bel*[13;14;15], *Bryson Katona*[17], *Rachid Karam*[16], *Florence Coulet*[38], *Paulo Pereira*[39;40], *Patrick Benusiglio*[41;42], *Carla Oliveira*[1;43;44]

[1]*i3S/IPATIMUP – Instituto de Investigação e Inovação em Saúde/ Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal;* [2]*Institute of Biomedical Sciences Abel Salazar of the University of Porto, Porto, Portugal;* [3]*Faculty of Science of the University of Porto, Porto, Portugal;* [4]*Institut Curie, University Paris Sciences Lettres, Department of Genetics, Paris, France;* [5]*Full Member of the European Reference Network on Genetic Tumor Risk Syndromes (ERN GENTURIS) – Project ID No 739547, Paris, France;* [6]*National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany;* [7]*University Hospital Bonn, Department of Internal Medicine I, Bonn, Germany;* [8]*Full Member of the European Reference Network on Genetic Tumor Risk Syndromes (ERN GENTURIS) – Project ID No 739547, Bonn, Germany;* [9]*Nantes Université, Univ Angers, CHU Nantes, INSERM, Immunology and New Concepts in ImmunoTherapy (INCIT), Nantes, France;* [10]*Nantes University Hospital, Department of Dermatology, Nantes, France;* [11]*Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, Netherlands;* [12]*University of Amsterdam, Department of Clinical Genetics, Amsterdam, Netherlands;* [13]*Hospital Clínic Barcelona, Department of Gastroenterology, Barcelona, Spain;* [14]*Centro de Investigación Biomédica en Red en Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain;* [15]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain;* [16]*Ambry Genetics, Aliso Viejo, California, United States;* [17]*Division of Gastroenterology and Hepatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Philadelphia, Pennsylvania, United States;* [18]*Division of Human Genetics, Medical University Innsbruck, Innsbruck, Austria;* [19]*IFLb Laboratoriumsmedizin Berlin GmbH, Windscheidstraße, Berlin-Charlottenburg, Berlin, Germany;* [20]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany;* [21]*Hereditary Cancer Group, Medical Oncology Department Hospital Vall d'Hebron, and Vall d'Hebron Institute of Oncology, Barcelona, Spain;* [22]*Full Member of the European Reference Network on Genetic Tumor Risk Syndromes (ERN GENTURIS) – Project ID No 739547, Barcelona, Spain;* [23]*Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal;* [24]*University Clinic of Genetics, Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal;* [25]*Sorbonne Université, UPMC Univ Paris 06, INSERM, UMRS 938, SIRIC CURAMUS, Equipe Instabilité Des Microsatellites Et Cancer, Equipe Labellisée Par La Ligue Contre Le Cancer, Centre de Recherche Saint Antoine, Paris, France;* [26]*Sorbonne Université, Laboratoire D'anatomie Et Cytologie Pathologiques, Hôpital Saint-Antoine, AP-HP, Paris, France;* [27]*The University of Texas MD Anderson Cancer Center, Houston, United States;* [28]*Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany;* [29]*Medizinisch Genetisches Zentrum, Munich, Germany;* [30]*Full Member of the European Reference Network on Genetic Tumor Risk Syndromes (ERN GENTURIS) – Project ID No 739547, Munich, Germany;* [31]*Centro Hospitalar Universitário São João, Serviço de Cirurgia, Porto, Portugal;* [32]*Zane Cohen Centre, Sinai Health System, Toronto, Canada;* [33]*Barretos Cancer Hospital, Cancer Genetics Departament, Barretos, Brazil;* [34]*Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, Brazil;* [35]*National Cancer Institute, Department of Genetics, Rio de Janeiro, Brazil;* [36]*Family Cancer Clinic, Netherlands Cancer Institute, Amsterdam, Netherlands;* [37]*Netherlands Cancer Institute, Department of Gastrointestinal Oncology, Amsterdam, Netherlands;* [38]*Unité fonctionnelle d'Oncoangiogénétique et génomique des tumeurs solides, Département de Génétique médicale, Hôpital Pitié-Salpêtrière, AP-HP, Sorbonne Université, Paris, France;* [39]*i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal;* [40]*IBMC – Instituto de Biologia Molecular e Celular, Porto, Portugal;* [41]*Unité fonctionnelle d'Oncogénétique clinique, Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, Sorbonne Université, Paris, France;* [42]*Chirurgie générale et digestive, Hôpital Saint-Antoine, AP-HP, Sorbonne Université, Paris, France;* [43]*Faculty of Medicine of the University of Porto, Porto, Portugal;* [44]*Full Member of the European Reference*

*Network on Genetic Tumor Risk Syndromes (ERN GENTURIS) – Project ID No 739547, Porto, Portugal*

**Introduction:** Very rare *CTNNA1*-variants have been found in patients with Hereditary Diffuse Gastric Cancer and Macular Dystrophy Patterned-2. To understand whether *CTNNA1*-variants cause specific phenotypes depending on variant gene location and/or molecular type, we analyzed genotype-phenotype associations in a cohort of individuals/families carrying very rare *CTNNA1*-variants.

**Methods:** Sixteen institutions contributed to a *CTNNA1*-variant clinical database, curated for population frequency and molecular impact. Variants were categorized into PTC-variants (generating premature termination codons) or non-PTC-variants through in silico prediction tools. Genotype–phenotype associations were analyzed using a multivariable logistic regression model. Variant functional/clinical impact was assessed using a humanized *Drosophila* model and clinical data.

**Results:** 110 families contributed with 474 phenotypes from 442 individuals carrying 80 very rare *CTNNA1*-variants (FREQ < 0.1%), 75% predicted as PTC-variants. Diffuse Gastric Cancer (DGC) presented an average age-of-onset of 45.2 ± 15.9yo, was significantly more frequent (14.4% vs. 3.90%, $p = 0.004$) and more likely to occur (OR = 3.33; 95%CI [1.15–9.69]; $p = 0.027$) in PTC-variant than non-PTC-variant families, after logistic regression. No significant differences were observed between PTC-variant and non-PTC-variant families for other cancer types. Eye-Disorders were significantly associated with non-PTC-variant families (OR = 59.4; 95%CI [13.8–256]; $p < 0.001$). In αE-catenin knock-out *Drosophila*, organ development/lethality was rescued by a human αE-catenin protein carrying a non-PTC-variant but not by αE-catenin with a PTC-variant.

**Conclusions:** Early-onset DGC was independently associated with *CTNNA1* PTC-variants, and Eye-Disorders with non-PTC-variants. Our *Drosophila* model is able to inform on functional impacts of human germline *CTNNA1*-variants, and *CTNNA1*-tumors somatic analysis (ongoing) is expected to highlight dysregulated mechanisms by specific variant types.

**ACKNOWLEDGEMENTS:** FCT-PhD-fellowship(Ref:2020.05773.BD);ERN-GENTURIS (Project-ID No.739547);LEGOH-Project(Ref:PTDC/BTM-TEC/6706/2020).

**Conflict of Interest:** Silvana Lobo: None declared, Alexandre Dias: None declared, Chrystelle Colas: None declared, Robert Hüneburg: None declared, Lise Boussemart: None declared, Liselotte P van Hest: None declared, Leticia Moreira: None declared, Carolyn Horton Full Time Paid Employee at Ambry Genetics, Jennifer Herrera-Mullar Full Time Paid Employee at Ambry Genetics, Dana Farengo Clark: None declared, Sigrid Tinschert: None declared, Golmard Lisa: None declared, Isabel Spier: None declared, Adrià López-Fernández: None declared, Daniela Oliveira: None declared, Marta Ferreira: None declared, Magali Svrcek: None declared, Pierre Bourgoin: None declared, Helene Delhomelle: None declared, Paul Mansfield Has common stock in Amgen and Stryker, Verena Steinke-Lange: None declared, Sérgio Sousa: None declared, Manuela Balsinha: None declared, Stefan Aretz: None declared, JUDITH BALMAÑA: None declared, Melyssa Aronson: None declared, Augusto Perazzolo Antoniazzi: None declared, Edenir Palmero: None declared, Lizet Van der Kolk: None declared, Annemieke Cats: None declared, Jolanda M van Dieren: None declared, Sergi Castellvi-Bel: None declared, Bryson Katona: None declared, Rachid Karam Full Time Paid Employee at Ambry Genetics, Florence Coulet: None declared, Paulo Pereira: None declared, Patrick Benusiglio Received honoraria from AstraZeneca, BMS and MSD (Merck pharmaceuticals), Carla Oliveira: None declared

## C28 INNOVATIVE GENOMIC SERVICES

### C28.1 Optimising dynamic consent platforms: learnings from evaluation of 'CTRL' in a cardiovascular genetic disorders cohort

***Matilda Haas***[1;2], *Evanthia Madelli*[1;2], *Rosie Brown*[1;2], *Megan Prictor*[3], *Tiffany Boughtwood*[1;2]

[1]*Australian Genomics, Melbourne, Australia;* [2]*Murdoch Children's Research Institute, Melbourne, Australia;* [3]*University of Melbourne, Melbourne Law School, Melbourne, Australia*

**Background/Objectives:** There has been great interest in whether dynamic consent should be used in health research, but few studies have evaluated its uptake and acceptability. Australian Genomics piloted and evaluated CTRL ('control'), a dynamic consent tool incorporating granular, dynamic decision-making and communication for genomic research.

**Methods:** Individuals from a Cardiovascular Genetic Disorders Flagship were invited in person or by email to register for CTRL. Participant demographics, consent choices, experience measures and website analytics were analysed using descriptive statistics.

**Results:** Ninety-one individuals registered to CTRL during the study period. More males than females registered when invited retrospectively ($p = 0.0276$), but there was no difference in age, gender, or education level between those who did and did not use CTRL. Most people permitted secondary use of their data for general research (80%) and wanted to be notified every time their data were shared (64%). Most people wanted all incidental findings returned (69%). No differences emerged between CTRL and non-CTRL groups on outcome measures including satisfaction, trust, decision regret and understanding of genomic testing. Most people logged in once and nearly all (92%) made choices for all 17 consent questions. Other pages (news, contact us) were not frequently accessed.

**Conclusion:** Variation in individual consent choices supports the desirability of providing granular consent options. CTRL was acceptable to those who used it, but it was underutilised. This is one of the first studies globally evaluating dynamic consent, and will inform refinement of future platform design.

**Grant References:** NHMRC (1113531, 2000001); MRFF (EPCD000028).

**Conflict of Interest:** None declared

### C28.2 Long-term clinical follow-up for DECIDE: a patient decision-aid for genomic sequencing

***SHELIN ADAM***[1;2], *Patricia Birch*[1;2], *Michelle Demos*[2;3], *Sylvia Stockler*[2;4], *Rhea Beauchesne*[1], *Simone Race*[4], *Alison Elliott*[1;2;5]

[1]*University of British Columbia, Medical Genetics, Vancouver, Canada;* [2]*BC Children's Hospital Research Institute, Vancouver, Canada;* [3]*University of British Columbia, Neurology, Vancouver, Canada;* [4]*University of British Columbia, Biochemical Diseases, Vancouver, Canada;* [5]*Women's Health Research Institute, Vancouver, Canada*

**Background/Objectives:** DECIDE is an interactive online tool that provides information and decision-support to people considering genomic sequencing. In a non-inferiority trial, DECIDE was equivalent to conventional genetic counselling in increasing users' knowledge about sequencing, and was highly acceptable to most individuals: https://doi.org/10.1007/s10897-018-0281-1.

We emulated clinical usage and evaluated DECIDE in two non-genetics specialty clinics with no clinical genetic counsellors (GC). We assessed short and long-term impact of DECIDE use.

**Methods:** We assessed users' knowledge and decisional conflict using two validated instruments. Long-term follow up, 11-17 months after using DECIDE, included semi- structured interviews, decisional regret, and decisional conflict scales.

**Results:** 57 of 68 eligible families completed DECIDE. 20% failed the knowledge quiz or demonstrated decisional conflict and were therefore referred to the research GC. In addition, 12% chose to see a GC to clarify information. 63% made their sequencing decision based on DECIDE alone. 84% said DECIDE helped with their sequencing decisions. In long-term follow-up with 27 families, DECIDE users stated they were comfortable with their decision, had enough information to make an informed choice and felt the decision was generally easy, yet stressful. Users found DECIDE clear and helpful but some wanted information specific to their family's situation. Information on anti-discrimination legislation and incidental findings were among the most helpful parts of DECIDE.

**Conclusions**: In long-term follow-up, families appreciated DECIDE including the convenience of remote access, and additional information and explanations, even when they felt the choice for clinical sequencing was self-evident.

**Funding:** GenomeCanada LSARP to AE.

**Conflict of Interest:** SHELIN ADAM University of British Columbia, Department of Medical Genetics, Collaborator, Patricia Birch University of British Columbia, Department of Medical Genetics, Collaborator, Michelle Demos University of British Columbia, Department of Neurology, Sylvia Stockler University of British Columbia, Department of Biochemical Diseases, Rhea Beauchesne University of British Columbia, Department of Medical Genetics, Simone Race University of British Columbia, Department of Biomedical Diseases, Alison Elliott University of British Columbia, Department of Medical Genetics, Principal Investigator

### C28.3 Development and Implementation of Novel Chatbot-based Genomic Research Consent

*Erica Smith[1], Sarah Savage[1], Hallie Andrew[2], Gloria Mas Martin[1], Amanda Kahn-Kirby[1], Jonathan LoTempio[3], Emmanuèle Délot[2], Andrea Cohen[2], Georgia Pitsava[2], Seth Berger[2], Vincent Fusaro[1],* ***Eric Vilain***[3]

*[1]Invitae Corp, San Francisco, United States; [2]Children's National Hospital, Center for Genetic Medicine Research, Washington, United States; [3]University of California Irvine, Institute for Clinical and Translational Science, Irvine, United States*

**Background:** The informed consent process is known to be time intense. We sought to use a chatbot to alleviate this bottleneck.

**Objective:** To conduct a retrospective analysis comparing traditional human-based consenting to an automated chat-based consenting process.

**Methods:** We developed a new chat-based consent using our IRB-approved consent forms. We leveraged a previously developed platform (Gia®, or "Genetic Information Assistant") to deliver the chat content to candidate participants. The content included information about the study, educational information, and a quiz to assess understanding. We analyzed 144 families referred to our study during a 6-month time period. A total of 37 families completed consent using the traditional process, while 35 families completed consent using Gia.

**Results:** The median length of the consent conversation was shorter for Gia users compared to traditional (44 vs. 76 min). Additionally, the total time from referral to consent completion was faster with Gia (5 vs. 16 days). Within Gia, understanding was assessed with a 10-question quiz that most participants (96%) passed. Feedback about the chat consent indicated that 86% of participants had a positive experience.

**Discussion:** Using Gia resulted in time savings for both the participant and study staff. The chatbot enables studies to reach more potential candidates. We identified five key features related to human-centered design for developing a consent chat.

**Conclusion:** This analysis suggests that it is feasible to use an automated chatbot to scale obtaining informed consent for a genomics research study. We further identify a number of advantages when using a chatbot.

**Conflict of Interest:** Erica Smith Invitae Corp, Invitae Corp, Sarah Savage Invitae Corp, Invitae Corp, Hallie Andrew: None declared, Gloria Mas Martin Invitae Corp, Invitae Corp, Amanda Kahn-Kirby Invitae Corp, Invitae Corp, Jonathan LoTempio: None declared, Emmanuèle Délot: None declared, Andrea Cohen: None declared, Georgia Pitsava: None declared, Seth Berger: None declared, Vincent Fusaro Invitae Corp, Invitae Corp, Eric Vilain: None declared

### C28.4 Digitalising genetic cascade screening in cardiogenetics: introducing a new digital family clinic

***Marlies van Lingen***[1], *Lieke van den Heuvel[1], Noor Giesbertz[2], Marten Siemelink[1], Peter van Tintelen[1]*

*[1]UMC Utrecht, Genetics, Utrecht, Netherlands; [2]The Netherlands Cancer Institute (NKI), Genetics, Amsterdam, Netherlands*

**Background** In genetic cascade screening for cardiac diseases, only 40% of at-risk family members attend genetic counselling. Moreover, although the demand for genetic counselling increases, financial and human resources are limited, causing growing waiting lists. To lower the threshold for cardiogenetic counselling and to increase efficiency, we designed a digital platform for presymptomatic genetic counselling of inherited cardiomyopathies with tailored information provision and virtual assistance based on Artificial Intelligence.

**Methods** The online platform was developed and designed based on an iterative design thinking method. We used co-creation to optimize this digital intervention: stakeholder input was collected through 7 focus groups with 10 probands, 11 family members, and 16 healthcare professionals. Subsequently, multiple online stakeholder assessments were performed and analysed with thematic analysis.

**Results** Iterative co-creation resulted in a technical and design concept of the digital clinic and virtual assistant, which includes stakeholder journeys, front-end design, and integration maps. Stakeholder analysis showed positive attitudes towards the proposed concept, alongside several challenges for future development. The main concerns involved themes of in-person contact and psychosocial support, quality of care and decision-making, care for vulnerable groups, virtual assistance, and digital safety.

**Conclusion** We propose a digital platform for the full process of presymptomatic counselling for families with inherited cardiomyopathies. Although stakeholders expressed positive attitudes towards digital counselling, they also raised concerns. In near future, we will pilot this intervention and compare it to current clinical care.

**Grant references** This project is financially supported by ZonMW/IMDI (104021006) and the Dutch Heart Foundation (2019B012).

**Conflict of Interest:** Marlies van Lingen Full time UMC Utrecht Genetics department, Lieke van den Heuvel Part-time UMCU Utrecht Genetics department, Netherlands Heart Foundation and ZonMw/NWO, Noor Giesbertz: None declared, Marten Siemelink

Part-time UMCU Genetics department, Netherlands Heart Foundation and ZonMw/NWO, Peter van Tintelen Fulltime UMC Utrecht, Netherlands Heart Foundation/ PLN Foundation/ Leducq Foundation/ZonMw, Cardiac Tissue Bank, Netherlands Heart Institute, Utrecht, the Netherlands (unpaid)

### C28.5 The use of AI in variant interpretation in newborn screening: Professionals' perspectives

***Mirjam Plantinga***[1], *Sara Longaron*[2], *Marielle E. van Gijn*[1], *Lennart Johansson*[1], *Irene van Langen*[1], *Adelita Ranchor*[2]

[1]*UMCG, genetics, Groningen, Netherlands;* [2]*UMCG, Health Psychology, Groningen, Netherlands*

**Background/objectives:** Currently, the Dutch newborn screening (NBS) program detects 26 rare diseases by biochemical techniques in bloodspots. With the use of next generation sequencing (NGS) it would be possible to include more diseases, but it warrants the use of artificial intelligence (AI) for variant interpretation because of the enormous amounts of data produced by NGS. The development and implementation of AI, however, raises ethical, legal, and psychosocial questions. This research explores the opinions of professionals that would be involved in NGS based NBS, using a Q-sort methodology.

**Methods:** First, an online focus group study was held with a heterogenous sample of Dutch professionals ($n = 10$) to collect ideas, concepts, or statements related to the use of AI for variant interpretation in NBS. Second, the q sorting exercise was done with a larger group of Dutch professionals ($n = 35$). The data was analysed with factor analysis, factor extraction, factor rotation and factor interpretation.

**Results:** The focus group brought forward different factors related to responsible implementation of AI, such as efficiency, trust, acceptability, transparency, usability, validity, privacy and human agency. These factors were combined with literature and developed into 40 conditions for responsible implementation of AI for variant interpretation for NGS based NBS. The Q sort provided an overview of the conditions that involved professionals deemed most important.

**Conclusion:** The findings will be useful to make informed decisions regarding the integration of a next-generation sequencing-first approach in the Dutch NBS program.

**Grant references:** NWA.1332.20.006

**Conflict of Interest:** Mirjam Plantinga https://www.nwo.nl/en/news/more-10-million-euros-human-centred-ai-research-elsa-labs, Sara Longaron: None declared, Marielle E. van Gijn: None declared, Lennart Johansson: None declared, Irene van Langen: None declared, Adelita Ranchor: None declared

### C28.6 Data counselling: providing support for decision-making about genomic and health data sharing for research

***Cláudia de Freitas***[1;2;3], *Ana Pinho*[2;3], *Ana Henriques*[1;2;3]

[1]*EPIUnit - Institute of Public Health of University of Porto, Porto, Portugal;* [2]*Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional, Porto, Portugal;* [3]*Faculdade de Medicina da Universidade do Porto, Departamento de Ciências da Saúde Pública e Forenses e Educação Médica, Porto, Portugal*

**Background:** Advancements in genomic research are rendering the ethical, legal and psychosocial implications of data sharing ever more complex. Data counsellors can support lay people in navigating through this complexity and in anticipating the consequences of sharing their data. This study explores participants' preferences concerning decision-making about genomic and health data sharing for research.

**Methods:** Multi-methods study drawing on 638 structured questionnaires and 41 semi-structured interviews with rare disease patients and informal carers recruited in a Portuguese hospital. Descriptive statistics were used to analyse preferred modes of decision-making: deciding alone, delegating decision-making or deciding with support (e.g. from family/friends, healthcare professionals, data counsellors). The reasons underlying participants' preferences were explored through interpretative analysis.

**Results:** Most participants preferred to decide with support (62%), 37% opted to decide alone and 1% chose to delegate decisions about data sharing. Almost 60% of those who opted for support selected data counsellors as their preferred source of support. Reasons underpinning participants' preference for support in decision-making include the need for assistance to unpack intricate issues, make informed decisions, keep their futures safe and open, and devise spaces for dialogue and participation in research and data governance.

**Conclusion:** Participants' significantly high preference for data counsellors as supporters in decision-making about data sharing for research uncovers several unattended needs whose disregard can undermine the ethical governance of data and, subsequently, the inclusiveness and diversity of genomic research. It also lends strength to claims for the development of a new professional specialism in data counselling.

**Funding:** PTDC/SOC-SOC/32194/2017

**Conflict of Interest:** None declared

## C29 SKIN AND SKELETAL DISORDERS: NEW GENES AND PATHWAYS

### C29.1 Provision of a diagnostic service for mosaic skin disorders; experience from the first 500 cases including novel variants and mutational hot-spots

***Thomas Cullup***[1], *Mariana Grobler*[1], *Maria Lock*[1], *Patrick Lombard*[1], *Satyamaanasa Polubothu*[2;3], *Clinda Puvirajasinghe*[1], *Joe Shaw*[1], *Lucy Jenkins*[1], *Veronica Kinsler*[2;3]

[1]*Rare and Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom;* [2]*Great Ormond Street Hospital for Children NHS Foundation Trust and UCL GOS Institute of Child Health, Paediatric Dermatology, London, United Kingdom;* [3]*The Francis Crick Institute, Mosaicism and Precision Medicine Laboratory, London, United Kingdom*

**Background/Objectives:** Mosaic disorders are a rapidly expanding area of genetics, diagnosable generally only with high depth sequencing of affected tissue. Diagnostic grade testing for most mosaic disorders was previously unavailable in the United Kingdom. With the development of genotype-specific targeted therapies and the increased appreciation of potential germline transmission to offspring of probands, it has become highly clinically relevant to make a genetic diagnosis in mosaic disorders.

**Methods:** A bespoke next generation sequencing panel was developed covering 30 genes proven as causal in mosaic disorders, with sequencing conducted at high read depth. A two-tier analysis strategy was designed, comprising both a variant calling pipeline designed for low-level variant detection and a base-counting pipeline to interrogate variant hot-spots.

**Results:** Validation studies and ongoing testing have demonstrated that the testing strategy is able to detect variants at 1% variant allele frequency (VAF) with the variant calling pipeline and

0.4% VAF with the base-counting pipeline. The diagnostic yield of the service is 50% when testing is conducted on affected tissue, with pathogenic variants detected in 20 of 30 targeted genes. A novel hot-spot has been identified in *ACTB* and novel variants outside of known canonical hot-spots have also been detected.

**Conclusion:** We have demonstrated the development of a highly sensitive testing strategy for mosaic disorders within the context of a nationalised healthcare system. The benefits of a dual analysis strategy are highlighted; allowing detection of both novel variants and very low level variants at existing hot-spots.

**Conflict of Interest:** Thomas Cullup Rare and Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, Mariana Grobler Rare and Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, Maria Lock Rare and Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, Patrick Lombard Rare and Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, Satyamaanasa Polubothu: None declared, Clinda Puvirajasinghe Rare and Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, Joe Shaw Rare and Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, Lucy Jenkins Rare and Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, Veronica Kinsler: None declared

### C29.2 ZIC1 variants in neurodevelopmental disorder with and without craniosynostosis

***Laura Watts***[1], *Elizabeth Lewis-Orr*[2], *Isaac Walton*[2], *Rebecca Tooze*[2], *YANG PEI*[2], *Dora Steel*[3;4], *Manju Kurian*[3;4], *Shekeeb Mohammad*[5], *Moira Blyth*[6], *Alison Kraus*[7], *Fuad Al Mutairi*[8;9], *Susan Holder*[10], *Virginia Clowes*[10], *Jan Cobben*[10], *Helen Stewart*[1], *Diana Johnson*[11], *Julie Cohen*[12;13], *Sophia Ceulemans*[14], *Rita Ortega Rico*[15], *Marte Haug*[16], *Siren Berland*[17], *Hannah M Bombei*[18], *Alpa Sidhu*[19], *Catherine Gooch*[20], *Maria Rita Passos-Bueno*[21], *Tiong Yang Tan*[22;23], *Emma McCann*[24], *Helen Lord*[25], *Hui-Lin Chin*[26;27], *Denise Li-Meng Goh*[26;27], *Jeremy Lin*[26;27], *Boris Keren*[28], *Perrine Charles*[28], *Trayan Delchev*[29], *Daniela Avdjieva-Tzavella*[29], *Salem Alawbathani*[30], *Ligia Almeida*[30], *Ameni Kdissa*[30], *Ruslan Al-Ali*[30], *Aida Bertoli-Avella*[30], *Andrew Wilkie*[2;31], *Deborah Shears*[1], *Stephen Twigg*[2]

[1]*Oxford Centre for Genomic Medicine, Oxford University NHS Foundation Trust, Oxford, United Kingdom;* [2]*Clinical Genetics Group, MRC Weatherall Institute of Molecular Medicine, Oxford, United Kingdom;* [3]*Molecular Neurosciences, Developmental Neurosciences, Zayed Centre for Research into Rare Disease in Children, UCL Great Ormond Street Institute of Child Health, London, United Kingdom;* [4]*Department of Neurology, Great Ormond Street Hospital for Children, London, United Kingdom;* [5]*Kids Neuroscience Centre, The Children's Hospital at Westmead, Faculty of Medicine and Health, University of Sydney, Sydney, Australia;* [6]*North Scotland Clinical Genetics Service, Department of Medical Genetics, Aberdeen, United Kingdom;* [7]*Yorkshire Regional Clinical Genetics Service, Chapel Allerton Hospital, Leeds, United Kingdom;* [8]*Division of Genetics, Department of Pediatrics, King Abdullah Specialized Children Hospital, King Abdulaziz Medical City, MNGHA, Riyadh, Saudi Arabia;* [9]*King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences, MNGHA, Riyadh, Saudi Arabia;* [10]*North West Thames Regional Clinical Genetics Service, London North West University Healthcare NHS Trust, London, United Kingdom;* [11]*Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, United Kingdom;* [12]*Department of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, United States;* [13]*Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, United States;* [14]*Department of Genetics, Department of Neurology, Rady Children's Hospital, San Diego, United States;* [15]*Fundación Centro Colombiano de Epilepsia y Enfermedades Neurológicas, FIRE, Cartagena de Indias, Colombia;* [16]*Department of Medical Genetics, St. Olav's University Hospital, Trondheim, Norway;* [17]*Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway;* [18]*Division of Medical Genetics and Genomics, The Stead Family Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, United States;* [19]*Division of Medical Genetics and Genomics, The Stead Family Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States;* [20]*Department of Genetics, University of Alabama at Birmingham, Birmingham, Alabama, United States;* [21]*Human Genome and Stem Cell Research Center, University of São Paulo, Institute of Bioscience, Department of Genetics and Evolutionary Biology., Sao Paulo, Brazil;* [22]*Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia;* [23]*Department of Paediatrics, University of Melbourne, Melbourne, Australia;* [24]*Department of Clinical Genetics, Liverpool Women's NHS Foundation Trust, Liverpool, United Kingdom;* [25]*Oxford Genetics Laboratories, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom;* [26]*Division of Genetics and Metabolism, Khoo Teck Puat-National University Children's Medical Institute, National University Hospital, Singapore, Singapore;* [27]*Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore;* [28]*Département de Génétique, Assistance Publique-Hôpitaux de Paris, Hôpital Pitié-Salpêtrière, Paris, France;* [29]*Department of Clinical Genetics, University Pediatric Hospital, Sofia, Bulgaria;* [30]*CENTOGENE GmbH, Rostock, Germany;* [31]*Craniofacial Unit, Oxford University Hospitals NHS Trust, John Radcliffe Hospital, Oxford, United Kingdom*

*ZIC1* encodes a member of the ZIC (zinc finger of cerebellum) transcription factor family with critical roles in vertebrate neural and skeletal development. Heterozygous deletions encompassing *ZIC1* and *ZIC4* can cause Dandy-Walker malformation, whilst heterozygous intragenic *ZIC1* alterations within the final exon result in a distinct phenotype of craniosynostosis with variable intellectual disability through a gain-of-function mechanism. Through international collaboration we have identified the largest group of individuals with *ZIC1* variants to date, significantly expanding the phenotypic spectrum and allowing genotype-phenotype correlation. We present detailed phenotypic descriptions of nineteen families comprising 22 individuals with seventeen different heterozygous *ZIC1* variants. Ten individuals had a phenotype that included craniosynostosis with facial dysmorphism, structural brain abnormalities and developmental delay. Eleven individuals had a neurodevelopmental disorder (NDD) alone without craniosynostosis, which has not previously been described with *ZIC1* SNVs. Variants associated with craniosynostosis were clustered at the C-terminal end of the protein, predominantly nonsense variants predicted to escape nonsense mediated decay. Variants associated with NDD alone include missense alterations within exons 1 and 2 predicted to disrupt the normal function of the DNA binding zinc fingers, likely leading to loss of normal ZIC1 function. Together this suggests that a gain-of-function mechanism involving the C-terminus of ZIC1 is responsible for the craniosynostosis phenotype, whilst more proximal and loss-of-function alterations cause a novel NDD phenotype without craniosynostosis. We wish to acknowledge

funding from the NIHR Oxford Biomedical Research Centre for this work.

**Conflict of Interest:** None declared

### C29.3 Skeletal phenotype of a novel Smad4 mouse model for Myhre syndrome

***Alice Costantini***[1;2], *François Bouvard*[1], *Florine Cattin*[1], *Neha Mohanpuria*[1], *Johanne Dubail*[1], *Valérie Cormier-Daire*[1;3]

[1]*Paris Cité University, INSERM UMR1163, Institut Imagine, Paris, France;* [2]*Karolinska Institutet, Department of Molecular Medicine and Surgery and Center for Molecular Medicine, Stockholm, Sweden;* [3]*Reference Center for Skeletal Dysplasia, Hôpital Necker Enfants Malades, AP-HP, Paris, France*

**Background/Objectives**: Myhre syndrome (MS) is characterized by short stature, short extremities, muscular build, deafness, and cognitive delay. MS is caused by monoallelic missense variants in the MH2 domain of SMAD Family Member 4 (*SMAD4*), encoding a critical mediator of TGF-β/BMP signaling. To better understand the physiopathological mechanisms occurring in MS, we established and phenotyped the first knock-in mouse model of MS, which harbors the missense variant identified in most of MS patients.

**Methods**: We examined mouse neonates and performed whole-mount skeletal staining, (immuno)histochemistry, and micro-CT to evaluate the skeletal phenotype. To identify the pathomolecular mechanisms underlying MS, we carried out RNA-sequencing on mRNA extracted from cultures of primary costal chondrocytes of neonatal mice.

**Results**: $Smad4^{I499V/+}$ mouse survival rate, normal at birth, was strongly reduced from postnatal day 4. At birth, $Smad4^{I499V/+}$ mice were significantly smaller than their wild-type littermates and remained smaller at 8 weeks of age. Skeletal analysis at birth showed reduced long bone length, delayed bone ossification, and more rounded head. Immunohistochemistry analysis suggested reduced type X collagen expression in the growth plate. At 8 weeks, micro-CT $Smad4^{I499V/+}$ mice featured head shape alteration and smaller eyelid opening, resembling prognathism and short palpebral fissures observed in MS patients. Preliminary RNA-sequencing results highlighted aberrant expression of genes partaking in TGF-β/BMP signaling.

**Conclusion**: Our MS mouse model mimics the human phenotype, thus constituting a good model for understanding the disease pathogenesis and identifying a treatment for MS.

**Grant References**: AC is financed by a grant from the Swedish Research Council.

**Conflict of Interest:** None declared

### C29.4 Bi-allelic TUFT1 variants cause woolly hair, superficial skin fragility and desmosomal defects

***Adam Jackson***[1], *Celia Moss*[2], *Kate Chandler*[3], *Pablo Lopez Balboa*[4], *Maria L Bageta*[4], *Gabriela Petrof*[4], *Anne Martinez*[4], *Liu Lu*[5], *Alyson Guy*[5], *Jemima Mellerio*[6], *John Lee*[6], *Malobi Ogboli*[2], *Gavin Ryan*[7], *Genomics England Research Consortium*[8], *John A McGrath*[6], *Siddharth Banka*[1]

[1]*Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK., Manchester, United Kingdom;* [2]*Department of Dermatology, Birmingham Children's Hospital, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK, Birmingham, United Kingdom;* [3]*Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Health Innovation Manchester, Manchester, UK., Manchester, United Kingdom;* [4]*Department of Dermatology, Great Ormond Street Hospital NHS Foundation Trust, London, London, United Kingdom;* [5]*Viapath, National Diagnostic Epidermolysis Bullosa Laboratory, Guy's Hospital, London, London, United Kingdom;* [6]*St John's Institute of Dermatology, King's College London (Guy's campus), London, London, United Kingdom;* [7]*West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK, Birmingham, United Kingdom;* [8]*Genomics England, London, United Kingdom*

**Background:** Desmosomes are complex cell junction structures that connect intermediate filaments providing strong cell-to-cell adhesion in tissues exposed to mechanical stress.

**Objectives:** To identify causal variants in individuals with woolly hair, skin fragility and unknown genetic cause.

**Methods:** Whole genome sequencing, whole exome sequencing, clinical phenotyping, haplotype analysis, single-cell RNA sequencing data analysis, immunofluorescence microscopy, and transmission electron microscopy.

**Results:** We identified homozygous predicted loss-of-function (pLoF) TUFT1 variants in nine individuals, from three families, with woolly hair and skin fragility. One donor splice site variant, c.60+1G > A, was present in two families, while a frameshift variant, p.Gln189Asnfs*49, was found in the third family. Haplotype analysis showed the c.60+1G > A substitution to be a founder variant in the Irish population that likely arose ~20 generations ago. Human and mouse single-cell RNA sequencing data showed TUFT1 expression to be enriched in hair dermal sheath and keratinocytes. TUFT1 expression was highly correlated with genes encoding desmosomal components implicated in diseases with phenotypes overlapping with the cohort presented here. Immunofluorescence showed tuftelin-1 to be mainly localized to the peripheral cell membranes of keratinocytes in normal skin. Skin samples from individuals with TUFT1 variants showed markedly reduced immunoreactivity for tufetlin-1, with a loss of the keratinocyte cell membrane labeling. Light microscopy revealed keratinocyte adhesion, mild hyperkeratosis and areas of superficial peeling.

**Conclusions:** Bi-allelic loss of function TUFT1 variants cause a new autosomal recessive skin/hair disorder characterized by woolly hair texture and early-onset skin fragility. Tuftelin-1 has a role in desmosomal integrity and function.

**Conflict of Interest:** None declared

### C29.5 Disease-specific biomarkers of pathogenic HRAS variants in skin

***Theresa Nauth***[1], *Hannah Voß*[2], *Thomas Mair*[2], *Verena Rickassel*[1], *Sophia Deden*[1], *Hartmut Schlüter*[2], *Georg Rosenberger*[1]

[1]*Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [2]*Section for Mass spectrometry and proteomics, University Medical Center Hamburg Eppendorf, Hamburg, Germany*

The small GTPase HRAS is a crucial modulator in epidermal homeostasis as HRAS effector proteins control keratinocyte proliferation and differentiation. Pathogenic germline variants in *HRAS* result in the non-mosaic RASopathy Costello syndrome (CS), a rare developmental disorder with >80% of patients showing the HRAS p.G12S variant. Individuals with CS present specific dermatological features including loose, redundant and soft skin, hyperpigmentation, prematurely aged skin and others. Postzygotic *HRAS* variants have been identified in disorders of the skin, so-called mosaic RASopathies (e.g., Nevus sebaceous/Schimmelpenning syndrome) and >90% of patients show the HRAS p.G13R variant. Finally, oncogenic *HRAS* variants are common in dermatological and other malignancies, and the p.G12V variant

pre-dominantes. Although all a. m. amino acid substitutions result in a constitutively active HRAS, unique biological consequences of different *HRAS* variants may underlie the phenotypical pleiotropy of *HRAS*-associated disorders. To systematically study *HRAS* variant-specific skin phenotypes, we stably expressed HRAS mutants in HaCaT keratinocytes and determined proteome-wide differences by applying immunoblotting and mass spectrometry. We identified significant differences between HRAS mutants (i) in the expression of differentiation and proliferation markers KRT10 and KRT14, respectively, (ii) in the activation of canonical AKT and MAPK signaling, and (iii) in the activation/expression levels of several other proteins, e.g., CAV1. Our preliminary results suggest that a specific *HRAS* variant can be determined based on its functional consequences. This underscores the existence of *HRAS* variant-specific qualities of dysregulation. Therefore, we aim to characterize proteome-wide variant-specific consequences to delineate the pathophysiological architecture of *HRAS*-associated phenotypes.

**Conflict of Interest:** None declared

### C29.6 Biallelic truncating variants in VGLL2 cause syngnathia in humans

*Christopher Gordon[1], valeria agostini[1], Aude Tessier[1], Roman Khonsari[2], Eva Galliani[2], Yukiko Kurihara[3], Hiroki Kurihara[3], Arnaud Picard[2], Ersoy konas[4],* ***Jeanne Amiel***[1;5]

*[1]Institute Imagine, Embryology and Genetics of malformations, Paris, France; [2]Necker Hospital, Maxillofacial surgery, Paris, France; [3]The University of Tokyo, Physiological Chemistry and Metabolism, Bunkyo City, Japan; [4]Hacettepe University Faculty of Dentistry, ankara, Turkey; [5]Service de Médecine Génomique des Maladies Rares, Necker Hospital, Paris, France*

Syngnathia is an ultra-rare craniofacial malformation characterized by an inability to open the mouth due to congenital fusion of the upper and lower jaws. We report four patients from three families with isolated bony syngnathia and homozygous truncating variants in vestigial-like family member 2 (VGLL2), identified through whole exome and direct sequencing. Two of the patients, of common geographic origin but not known to be related, shared the same variant on a common haplotype, indicating a founder effect. The VGLL gene family encodes cofactors of TEAD transcriptional regulators, which play roles in patterning and cell fate specification during development. Vgll2 is regionally expressed in the pharyngeal arches of model vertebrate embryos, and morpholino-based knockdown of vgll2a in zebrafish has been reported to cause defects in development of pharyngeal arch cartilages. However, we generated vgll2a germline mutant zebrafish by CRISPR/Cas9 and did not observe craniofacial anomalies. Knockout of vgll4l, a related gene previously implicated in zebrafish craniofacial development, or knockout of vgll2a and vgll4l simultaneously, also had no discernible effect on jaw development. Vgll2-/- mice present a skeletal muscle phenotype but normal craniofacial development. Our results identify loss of VGLL2 as the first known genetic cause of isolated bony syngnathia in humans and suggest that other vertebrates may have the capacity to compensate for its absence during craniofacial development. Future work will require further combinatorial knockouts in animal models in order to understand the nature of compensation amongst VGLL gene family members.

**Conflict of Interest:** Christopher Gordon: None declared, valeria agostini: None declared, Aude Tessier: None declared, Roman Khonsari: None declared, Eva Galliani: None declared, Yukiko Kurihara: None declared, Hiroki Kurihara: None declared, Arnaud Picard: None declared, Ersoy konas: None declared, Jeanne Amiel MarMaRa

## CONCURRENT SYMPOSIA

## S01 TRANSLATIONAL METABOLISM: FROM INBORN ERRORS OF METABOLISM TO MULTIFACTORIAL DISEASES

### S01.1 Development of novel therapies for inherited metabolic diseases based on a multi-omics approach

***Sean Froese***

Methylmalonic aciduria (MMA) is an inborn error of metabolism with multiple monogenic causes and a poorly understood pathogenesis, leading to the absence of effective causal treatments. Here we employ multi-layered omics profiling combined with biochemical and clinical features of individuals with MMA to reveal a molecular diagnosis for 177 out of 210 (84%) cases, the majority (148) of whom display pathogenic variants in methylmalonyl-CoA mutase (MMUT). Stratification of these data layers by disease severity shows dysregulation of the tricarboxylic acid cycle and its replenishment (anaplerosis) by glutamine. The relevance of these disturbances is evidenced by multi-organ metabolomics of a hemizygous Mmut mouse model as well as through identification of physical interactions between MMUT and glutamine anaplerotic enzymes. Using stable-isotope tracing, we find that treatment with dimethyl-oxoglutarate restores deficient tricarboxylic acid cycling. Our work highlights glutamine anaplerosis as a potential therapeutic intervention point in MMA.

**Conflict of Interest:** None declared

### S01.3 Cell and tissue-specific roles of urea cycle enzymes in cancer

***Ayelet Erez***

Efforts to understand genetic syndromes such as urea-cycle disorders (UCD) have been invested for many years and provide valuable means for understanding the mechanism behind the consequent phenotype. We can further utilize this knowledge to gain insights into the role of UC enzymes in complex diseases such as cancer and dissect the direct downstream domino effects of their dysfunction. In addition, congenital mutations in an isolated gene causing UCD enable us to predict the systemic consequences of targeting it as a potential therapy.

Interestingly, multiple cancers have been found to have altered levels of UC enzyme expression, the most studied of which is ASS1. We have found that changes in the expression of UC enzymes, specifically ASS1, can maximize nitrogen incorporation into biomasses such as amino and nucleic acids, thus supporting tumor growth and aggressiveness. The rewired flux of UC substrates and intermediates into biosynthetic routes results in distinctive signatures manifested by synthesizing unique peptides and metabolites that can affect the immune response. The shift in nitrogenous metabolites to the tumor systemically alters the host metabolism. Thus, changes in the expression of UC enzymes have diagnostic, prognostic, and therapeutic implications for cancer patients.

**Conflict of Interest:** None declared

## S02 SEX DIFFERENCES IN NEURODEVELOPMENTAL DISORDERS

### S02.1 Reduced reproductive success is associated with selective constraint on human genes

*Eugene Gardner*

Genome-wide sequencing of human populations has revealed substantial variation among genes in the intensity of purifying selection acting on damaging genetic variants. Although genes under the strongest selective constraint are highly enriched for associations with Mendelian disorders, most of these genes are not associated with disease and therefore the nature of the selection acting on them is not known. Here we show that genetic variants that damage these genes are associated with markedly reduced reproductive success, primarily owing to increased childlessness, with a stronger effect in males than in females. We present evidence that increased childlessness is probably mediated by genetically associated cognitive and behavioural traits, which may mean that male carriers are less likely to find reproductive partners. This reduction in reproductive success may account for 20% of purifying selection against heterozygous variants that ablate protein-coding genes. Although this genetic association may only account for a very minor fraction of the overall likelihood of being childless (less than 1%), especially when compared to more influential sociodemographic factors, it may influence how genes evolve over time.

**Conflict of Interest:** None declared

### S02.3 Sex-Based Analysis of De Novo Variants in Neurodevelopmental Disorders

*Tychele Turner*

Autism is a neurodevelopmental disorder that affects 1 in 36 individuals and eighty percent of individuals with autism are male. Sex bias in phenotypes is prevalent and reasons for this bias include sex chromosomes, hormones, sex-specific imprinting, and a protective effect. Considering autism to be a 'complex' or 'multifactorial' disorder with a contribution of both genetic and environmental factors, individuals of the rarer class (females) have a higher biological threshold for being affected with autism. In terms of the genetics of autism, this means that females require a larger genetic burden than males to reach the biological threshold. Previous work has shown that there is an excess of de novo variants (DNVs) in females with autism consistent with a female-protective effect. Through assessment of published data in denovo-db, I previously identified specific genes with excess DNVs in females. Since establishing my independent Turner laboratory in September of 2019, I have been developing several approaches to study genomic variation in autism with direct implication in the study of sex bias. For this talk, I will review and share some of our developed computational tools to detect DNVs (HAT), QC DNVs (acorn), assess spatial clustering of missense variation (3D-CLUMP), statistically evaluate noncoding DNVs (fitDNM), and to examine conservation of genomic regions (ACES). We have applied these methods to 44,282 families with whole-exome sequencing data, 3070 families with whole-genome sequencing data, and one family with multiple females with autism that we solved using highly accurate long-read whole-genome sequencing. Moving forward with colleagues and collaborators across the medical and scientific fields, I plan to focus efforts and research specific to this topic on further developing and applying our current and new computational methods and functional approaches for studying genomic aspects of the sex bias in autism.

**Conflict of Interest:** None declared

## S03 APPLICATION OF GWAS IN SOCIAL SCIENCES

### S03.1 Applications of genetics in reproductive behaviour and related phenotypes

*Melinda Mills*

Reproductive behaviour—age at first sexual intercourse (AFS), age at first birth (AFB) and number of children ever born (NEB) – has implications for health and evolutionary fitness. This talk provides an overview of reproductive behaviour GWASs and the relationship of these phenotypes to reproductive biology (e.g., FSHB, ESR1, PCOS), externalizing behaviour (e.g., ADHD), education and health. Late AFB is associated with a reduced incidence of Type 2 Diabetes and Cardiovascular Disease and higher childhood socioeconomic circumstances. AFB and NEB are related to longevity, suggesting a trade-off between reproductive ageing and intensity. Findings are also linked to historical selection scans of ancient genome data, highlighting an allele in the FADS1/2 gene locus that has been under selection for thousands of years and remains so today. The talk concludes with a reflection on the highly polygenic nature of these traits, their interaction with the environment, variation in estimates of within- and between-family models and distance from use in a clinical setting.

**Conflict of Interest:** None declared

### S03.2 The genetics of educational attainment

*Aysu Okbay*

Educational attainment (EA) is an important dimension of socioeconomic status that features prominently in research by social scientists, epidemiologists, and other medical researchers. EA is strongly related to a range of health behaviors and outcomes, including mortality. For this reason, and because EA can be measured accurately at low cost, cohort studies used in genetic epidemiology and medical research routinely measure participants' EA.

There have been four genome-wide association study (GWAS) meta-analyses of EA so far [1–4], each expanding the sample size and identifying new genetic variants associated with EA, as well as shedding light on the possible pathways and mechanisms through which these variants operate. In this talk, I will provide an overview of findings from the first three EA GWAS and present results from the most recent one conducted in a sample of ~3 million individuals. In this fourth GWAS of EA[4], we identify 3952 approximately uncorrelated genome-wide-significant SNPs. A genome-wide polygenic predictor, or polygenic index (PGI), explains 12–16% of EA variance and contributes to risk prediction for ten diseases. Direct effects (i.e., controlling for parental PGIs) explain roughly half the PGI's magnitude of association with EA and other phenotypes. The correlation between mate pairs' PGIs is far too large to be consistent with phenotypic assortment alone, implying additional assortment on PGI-associated factors. In an additional GWAS of dominance deviations from the additive model, we identify no genome-wide-significant SNPs, and a separate X-chromosome additive GWAS identifies 57.

**References**

1. Rietveld, C. A. et al. GWAS of 126,559 individuals identifies genetic variants associated with educational attainment. *Science (80-.)*. **340**, 1467–1471 (2013).

2. Okbay, A. et al. Genome-wide association study identifies 74 loci associated with educational attainment. *Nature* **533**, 539–542 (2016).

3. Lee, J. J. et al. Gene discovery and polygenic prediction from a 1.1-million-person GWAS of educational attainment. *Nat. Genet.* **50**, 1112–1121 (2018)

4. Okbay, A. et al. Polygenic prediction of educational attainment within and between families from genome-wide association analyses in 3 million individuals. *Nat. Genet*. 2022 544 54, 437–449 (2022).

**Conflict of Interest:** None declared

### S03.3 Social implications of Behavioral DNA testing and GWAS specifically

*Paul Appelbaum*

Polygenic scores have been generated for a broad array of sociobehavioral outcomes. A sampling includes educational attainment, obesity, addiction, income, religious affiliation, same-sex behavior, and likelihood of voting. But linking these outcomes to genetics has generated a host of concerns. In this presentation, I will focus on two of these worries: the tendency of polygenic scores to reinforce genetic determinism, and their potential to produce stigma and lead to discrimination. At the individual level, genetic determinism can lead to fatalism or a sense of genetic invulnerability, both of which can interfere with behaviors that reduce risks of poor outcomes and increase the likelihood of good ones. This effect may be enhanced by the inherent uncertainty in these probabilistic results, as the quest for certainty leads to poor decision making. Stigma and consequent discrimination often reflect genetic essentialist attitudes, i.e., that genes establish "who we are," which in the case of scores that predict poor outcomes may lead to negative perceptions of others. Whether at the individual or group level, polygenic scores can be (and have been) used to suggest the futility of efforts to achieve better outcomes. Concerns about use of PGS are not a reason to preclude their use in all circumstances. But any such use should prospectively consider and balance the likely benefits and the potential negative consequences, with the burden of anticipating and addressing negative outcomes borne by those who are proposing to generate and use them.

**Conflict of Interest:** None declared

## S04 HONORING DIVERSITY: TOWARD CULTURALLY COMPETENT GENETIC COUNSELING

### S04.1 Psycho-social and Genetic Counselling Issues in Prenatal Setting in Southeast Asia

*Juliana MH Lee*

Clinical genetic services were established in Southeast Asia (SEA) by clinical geneticists in mid 1990s and genetic counsellors only began practice in 2004. Due to limited resources, only 6 out of 11 countries in SEA have established genetic services. In the 2000s, prenatal karyotyping and testing for known pathogenic variant were available upon request, when there was an increased risk for trisomy 21,13 or 18 detected in maternal serum screening/first trimester screening, abnormal ultrasound observed or a family history with a known pathogenic variant. Since 2014, the introduction of prenatal cell-free DNA screening has made an impact in the uptake of prenatal testing among pregnant women especially in the private services. An increased risk of a chromosomal abnormality detected in the prenatal cell-free DNA screening is recommended to be confirmed by karyotyping or chromosomal microarray. This has increased detection of chromosomal abnormalities in pregnancy usually among couples without any family history, which would have caused anxiety if they were not provided genetic counselling and support. The psycho-social issues experienced by most couples in the prenatal setting ranged from difficulty in understanding complex genetic information, managing uncertainty of a genetic condition diagnosed in pregnancy, feeling guilty for considering termination a pregnancy and paying high cost of testing. Religion, family values and perception towards disability were among influencing factors for couples when deciding on termination of pregnancy. There is an urgent need to train more local genetic counsellors in the prenatal setting to ensure patients have the opportunity to make informed decision making through genetic counselling.

**Keywords:** genetic counselling, non-invasive prenatal screening, prenatal testing, psycho-social issues

**Conflict of Interest:** None declared

### S04.2 Exploring models of service delivery: the case of religious lay counselling practices in Ghana

*Annabella Osei-Tutu*

Religious lay counselling is a common informal psychosocial support service provided by Imams, pastors, elders, and some congregants in Christian and Muslim communities in Ghana. Religious lay counsellors are often sought to offer advice and support around a wide range of issues, including marriage, parenting, and well-being. This presentation draws on previous work with Ghanaian Christian and Muslim lay counsellors to examine common practices, some of which have implications for genetic counselling practice. Specifically, the presentation will explore conceptualizations, common presenting concerns, and counselling practices. It is hoped that the presentation would contribute to discussions around honoring diversity in genetic counselling practice.

**Conflict of Interest:** None declared

### S04.3 Genetic counselling in the Arab world: Challenges and implications

*Khalsa Al Kharusi*

Genetic counseling is relatively a new and promisingly growing health profession around the world. Nevertheless that the profession is barely known or hardly recognized in some areas of the Arab world, it has a developmental leap in others. The profession is confronted by several challenges that can be stemmed from two main domains: Resources and awareness about the profession. Considering the unique features of the Arab culture, how far the principles of genetic counselling and Western frameworks can be accommodated?

**Conflict of Interest:** None declared

## S05 OLIGOGENICS IN RARE DISEASE

### S05.2 Oligogenic mechanisms - Implications of the digenic TIA1/SQSTM1 disease

*Bjarne Udd*

Most neuromuscular diseases (NMD) are not aquired conditions, but despite extensive exome and genome sequencing a large proportion of NMD patients remain without final causative diagnosis. The reason may be due to complex genetic changes, epigenetic mechanisms or more than one gene being involved in the genetic background. Such digenic or oligenic mechanisms have been observed and largely accepted as the background in ALS disease for which more than 40 genes have been associated, but with just small proportions of each gene in monogenic ALS conditions.

We have identified the first digenic cause of a muscle disease, late onset distal myopathy, in which the major gene variants in the SQSTM1 gene are known to cause dominant Paget bone disease with incomplete penetrance. But when combined with a frequent TIA1 SNP the target tissue is changed from bone to the muscle and cause a rimmed vacuolar myopathy.

Since primary dominant variants in TIA1 are known to cause Welander distal myopathy, or ALS with or without frontotemporal dementia, the digenic mechanism opens a window also to the field of multisystem proteinopathies (MSP). These are characterized by protein aggregates in postsynaptic tissues such and neurons, muscle and bone and mediated by defects in stress granule RNA-metabolism or clearance of misfolded proteins. Again, the same major gene defect may target different tissues depending on secondary hits.

**Conflict of Interest:** None declared

### S05.3 A digenic muscle disease caused by TTN and SRPK3

***Volker Straub***

***Digenic inheritance involving a muscle specific protein kinase and the giant titin protein causes a skeletal muscle myopathy***

Volker Straub and Ana Topf on behalf of the *SRPK3* Study Group

John Walton Muscular Dystrophy Research Centre, Institute of Translational and Clinical Research, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK.

In true digenic inheritance (DI), pathogenic variants at two independent loci must be inherited together to result in disease manifestation. While thousands of monogenic diseases have been identified, only a very small number of DI diseases are known. We had originally proposed *SRPK3*, an X-linked serine/arginine protein kinase, as a candidate gene for centronuclear myopathy with cores. However, further interrogation of the *SRPK3* pedigrees suggested that variants in this gene were not sufficient to cause disease. Through whole exome sequencing analysis, we identified heterozygous, predominantly truncating, variants in a second locus, the *TTN* gene, in all patients of the initial cohort. Thanks to an extensive international collaboration, we have now gathered a cohort of 36 families where pathogenic variants in both genes must be present for the myopathy to manifest. The double heterozygosity was not seen amongst 125,000 control individuals interrogated, nor is it due to an overall high frequency of *TTN* truncating variants, as these were significantly more common in the *SRPK3* patients than in other genetically diagnosed recessive LGMD cohorts, strongly suggesting our findings are not due to chance. Furthermore, double mutant zebrafish reproduce our findings, where the srpk3-/-;ttn1 + /- embryos show a severe muscle phenotype not observed in the srpk3-/- or ttn1 + /- embryos alone. We therefore propose that this novel congenital myopathy is caused by digenic inheritance of pathogenic variants in *SRPK3* and *TTN*.

**Conflict of Interest:** None declared

## S06 TREATMENT FOR INTERFERONOPATHIES

### S06.1 Treating Aicardi-Goutieres Syndrome

***Yanick Crow***

As brutally demonstrated by the COVID-19 pandemic, an effective immune system is essential for survival. Developed over evolutionary time, viral nucleic acid detection is a central pillar in the defensive armamentarium used to combat foreign microbial invasion. To ensure cellular homeostasis, such a strategy necessitates the efficient discrimination of pathogen-derived DNA and RNA from that of the host. In 2011, it was suggested that an upregulation of type I interferon signalling might serve as a defining feature of a novel set of Mendelian inborn errors of immunity, where antiviral sensors are triggered by host nucleic acids due to a failure of self versus non-self discrimination. These so-called type I interferonopathies, including Aicardi-Goutières syndrome, constitute an expanding group of inborn errors of innate immunity where enhanced type I interferon signalling is considered directly relevant to disease pathogenesis. Neurological involvement represents a major feature of these disorders, encompassing a diverse set of clinical phenotypes ranging from mimics of congenital infection, through acute bilateral striatal necrosis, progressive non-syndromic dystonia, progressive non-syndromic spastic paraparesis, neuromyelitis optica, moyamoya and cerebrovascular disease.

With the continued identification of genotypes associated with upregulated type I interferon signalling, the importance of strategies to block elements in this pathway has become apparent. Furthermore, genetic and clinical advances have been coupled with better definition of innate immune pathways and the interest of pharmaceutical companies in developing relevant therapeutic molecules. Examples of such novel approaches include JAK1 inhibition and the use of reverse transcriptase inhibitors - premised on a reduction of immunostimulatory DNA derived through a reverse transcription step in the lifecycle of LINE-1 endogenous retroelements. Other modalities are very likely to become available with time, including antibodies against the type I interferon receptor and STING blockade.

**Conflict of Interest:** None declared

### S06.2 Trisomy 21 - a treatable interferonopathy

***Joaquin Espinosa***

**Trisomy 21 – a treatable interferonopathy**

*Joaquin M. Espinosa*

*Linda Crnic Institute for Down Syndrome, Department of Pharmacology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA.*

It is well established that individuals with Down syndrome display chronic hyperactivation of interferon signaling associated with overexpression of four interferon receptors encoded on human chromosome 21 (IFNAR1, IFNAR2, IFNGR2 and IL10RB). However, the clinical impacts of interferon hyperactivity in Down syndrome are ill-defined. We will present the results of a multiomics investigation of interferon signaling in hundreds of individuals with Down syndrome. Using interferon scores derived from the whole blood transcriptome, we defined the proteomic, immune, metabolic, and clinical features associated with interferon hyperactivity in Down syndrome. Interferon hyperactivity associates with a distinct pro-inflammatory phenotype and dysregulation of major growth signaling and morphogenic pathways. Individuals with the highest interferon activity display the strongest remodeling of the peripheral immune system, including increased cytotoxic T cells, B cell depletion, and monocyte activation. Interferon hyperactivity accompanies key metabolic changes, most prominently dysregulated tryptophan catabolism. Furthermore, high interferon signaling stratifies a subpopulation with elevated rates of congenital heart disease and autoimmunity.

To define the contribution of the IFNR gene clusters to Down syndrome phenotypes, we used genome editing to correct its copy number in a mouse model of Down syndrome, which normalized antiviral responses, prevented heart malformations, ameliorated developmental delays, improved cognition, and attenuated craniofacial anomalies. Therefore, triplication of the *IFNR* locus modulates hallmarks of Down syndrome in mice, suggesting that trisomy 21 elicits an interferonopathy amenable to therapeutic intervention.

Lastly, we will present results from an ongoing clinical trial testing the safety and efficacy of the JAK inhibitor tofacitinib for multiple therapeutic endpoints in Down syndrome. Tofacitinib treatment normalizes interferon scores and reduces elevation of pathogenic cytokines in Down syndrome, while resolving multiple autoimmune skin conditions more prevalent in this population, also decreasing levels of autoantibodies causative of autoimmune thyroid disease.

Altogether, these results demonstrate that interferon hyperactivity plays multiple pathogenic roles in Down syndrome, indicating that trisomy 21 elicits an interferonopathy amenable to therapeutic intervention.

**Conflict of Interest:** None declared

## S07 UNDERSTANDING PRE-CANCER: MOLECULAR ANALYSIS TO CLINICAL CANCER PREVENTION

### S07.2 Barrett's

***Sarah Killcoyne***

**Understanding genomic instability will improve early cancer diagnosis in Barrett's oesophagus**

Oesophageal cancer is the 8th most common cancer worldwide and the 6th leading cause of cancer-related deaths. Less than 20% of patients survive 5 years after diagnosis, mostly due to late diagnosis. However, oesophageal adenocarcinoma (OAC) has a well-characterized premalignant condition known as Barrett's oesophagus. In recent years techniques to better analyze genomic changes have identified high rates of structural variation include widespread copy number gains and losses and extrachromosomal DNA content related to malignant changes in the Barrett's prior to a cancer diagnosis. This instability, affecting the entire genome in most cases, can help to risk stratify Barrett's patients as well as diagnosing early stage disease improving patient outcomes.

**Conflict of Interest:** None declared

## S08 EXPLOITING NEW APPROACHES IN GWAS

### S08.1 Using haplotype information to empower GWAS

***Po-Ru Loh***

Genetic association studies have discovered hundreds of thousands of common single-nucleotide polymorphisms (SNPs) associated with human phenotypes. However, such associations have generally been difficult to interpret, often only providing hints of other nearby genetic variants that causally modify traits. In this talk, I will describe progress on ascertaining and evaluating the effects of understudied forms of genetic variation, including variable number tandem repeats (VNTRs) and copy-number variants (CNVs). This work has been powered by statistical methods that leverage haplotype-sharing among distantly related individuals in large biobank cohorts.

**Conflict of Interest:** None declared

### S08.3 Joining forces across disciplines: accelerating the progress of GWAS variant-to-function in the microbiome

***Alexandra Zhernakova***

The human gut microbiome is a complex organ that plays a vital role in host metabolism, immunity, and overall health. While environmental factors are known to be the primary drivers of inter-individual variations in gut microbial composition, recent research has revealed that host genetics also contribute to these variations, with over 10% of gut microorganisms being heritable. Over the past 5 years,12 microbial genome-wide association studies (mbGWAS) have been published, each involving more than 1,000 participants. Notably, a large-scale Dutch study encompassing 10,000 participants and an international collaboration, the MiBioGen consortium, consisting of more than 18,000 participants from 23 research centers, have contributed to these efforts. However, despite these extensive endeavours, only a limited number of genetic loci have consistently been confirmed across multiple studies. In my presentation, I will delve into the results, challenges, and future directions arising from these studies. Next, I will present the findings from our recent large-scale analysis of common antibodies targeting microbial antigens, shedding light on the intricate interplay between genetics and other factors in shaping immune responses against commensal bacteria.

**Conflict of Interest:** None declared

## S09 DATA ALTRUISM AND FUTURE OF DATA SHARING IN GENOMICS

### S09.1 The governance concerns related to developing diverse genomic databases

***Robert Cook-Deegan***

Many efforts to build global genomic data resources are encountering legal and ethical complexities. Examples are several human pangenome projects, the Earth BioGenome, the Human Cell Atlas. The goals of unfettered open science are not completely compatible with efforts to retain sovereignty over samples and data that are inputs to global datasets and sample repositories. Indigenous authorities and nations have laws and policies governing export and use of genetic data and related samples. The technical capacity for using DNA as a surveillance tool have raised human rights concerns. Economic nationalism and efforts to reverse practices of extractive biocolonialism confront common practices in science that treat inputs from individuals as donations but then convert them to "assets" that can be licensed and monetized. The global resources are generally not directly useful commercially, but they are inputs to many forms of subsequent commercialization. Yet there is no international structure, or even a consensus framework, for benefit-sharing and reciprocity between those contributing samples and data and the institutions building the resources. For nonhuman organisms, the benefit-sharing provisions for the next Conference of Parties under the Nagoya Protocol (for the Convention on Biological Diversity) will address this issue, and present a draft framework. For human samples and data, privacy laws in different jurisdictions are crucial to sharing data, but those laws are inconsistent. Moreover, the arguments for benefit-sharing regarding nonhuman resources are even more compelling when addressing fairness among human beings. The call for fairness is likely to grow ever stronger and spill over from Nagoya to human data and samples. The leaders of efforts to build global public good resources will therefore confront challenges to deal with inconsistent privacy laws and policies, legal constraints on free flow of data and samples, and what to do about deliberate misuse, including human rights abuse. The issues can be addressed by forethought with explicit attention to reciprocity and fairness, by reacting to scandal and crisis, or most likely, some combination.

**Conflict of Interest:** None declared

## S10 SOMATIC MUTATIONS IN IMMUNE DISORDERS

### S10.2 Somatic mutations in 'benign' blood diseases

***Satu Mustjoki***

#### Somatic mutations in immune disorders

*Satu Mustjoki*[1-3]

*[1]Hematology Research Unit Helsinki, Helsinki University Hospital Comprehensive Cancer Center, Helsinki, Finland [2]Translational Immunology Research Program and Department of Clinical Chemistry and Hematology, University of Helsinki, Helsinki, Finland [3]iCAN Digital Precision Cancer Medicine Flagship, Helsinki, Finland*

Recent studies have revealed that somatic mutations are common in normal cells such as skin epithelial cells. Yet, the role of somatic mutations in normal lymphoid cells has not been studied in detail. There are a couple of examples where somatic mutations in immune cells lead to lymphoproliferation and associated autoimmune disorders such as in autoimmune lymphoproliferative syndrome (ALPS) and large granular lymphocyte (LGL) lymphoproliferation. Somatic mutations in the *FAS* gene can cause sporadic cases of ALPS leading to a similar phenotype as germline defects. In LGL lymphoproliferation, somatic *STAT3* mutations are common in CD8+ effector T cells and associate with autoimmune manifestations. In addition to these lymphoproliferative diseases, recent discoveries in rheumatoid arthritis (RA), multiple sclerosis, aplastic anemia (AA, hematologic autoimmune disease), and chronic graft-versus-host disease (cGVHD, a severe complication after allogenic hematopoietic stem cell transplantation) suggest that somatic mutations are common in T cells, and that they affect T-cell phenotype and function. Interestingly, in none of these conditions T-cell mutations produce uncontrolled, leukemia-like proliferation, but they can promote expansion and persistence of lymphocyte clones. Somatic mutations have also been discovered in normal B cells. In Sjögren's syndrome associated cryoglobulinemic vasculitis, lymphoma-associated somatic mutations were found in memory B cells which were producing pathogenic autoantibodies. Mutations may have helped autoantibody producing B cells to evade from normal tolerance checkpoints.

Thus, these findings suggest that somatic mutations in T and B cells can affect immune cell function which may cause aberrant reactivity that can be tolerogenic or immunostimulatory. However, further studies are needed to understand the normal spectrum of somatic mutations in immune cells and what is required that these somatic alterations can cause pathogenic immune cell reactivity and actual immune-mediated disease.

**Conflict of Interest:** None declared

### S10.3 Somatic mutations in primary immunodeficiencies: friends or foes?

***Roger Colobran***

The majority of primary immunodeficiencies (PIDs, also known as inborn errors of immunity, IEI) are caused by germline mutations and follow the three classic Mendelian modes of inheritance (autosomal recessive, autosomal dominant and X-linked). However, in the last two decades, a growing number of PIDs caused by somatic mutations have been discovered. In this conference, I will summarize the role of somatic variants in autoimmune lymphoproliferative syndrome (ALPS). ALPS is a disorder of lymphocyte apoptosis mainly caused by defects in *FAS* and manifesting with non-malignant lymphoproliferation, autoimmune cytopenias and an increased risk of lymphoma. Besides germline variants in FAS (accounting for 70% of ALPS patients), up to 15% of patients carry somatic variants in FAS. These somatic variants are principally detectable in a population of TCR alpha-beta double negative T cells (DNTs), which is typically elevated in ALPS patients (but it represents a minor cell population in peripheral blood). Somatic mutations in *FAS* are probably underdiagnosed for several reasons related with the low variant allele frequency (VAF) of these variants in peripheral blood. I will discuss the different methods to detect these somatic variants and its limitations in genetic diagnosis, and I will show recent data about the identification and evolutionary dynamics of somatic *FAS* variants in ALPS patients pre- and post-treatment.

Beyond the role of somatic mutations as disease-causing, in the second part of this conference I will talk about the phenomenon of reversion mosaicism (also known as somatic reversion or somatic genetic rescue). Reversion mosaicism refers to somatic mosaicism due to a reversion to normal of an inherited pathogenic mutation. In reversion mosaicism, reversion mutations partially or fully restore the effect of the primary disease-causing variant. I will show you a fascinating case of somatic reversion in a recently discovered primary immunodeficiency in which somatic reversion has not been described so far.

**Conflict of Interest:** None declared

## S11 COMPLETE GENOMES

### S11.1 T2T genomes of multiple individuals

***Adam Phillippy***

The Telomere-to-Telomere (T2T) Consortium recently completed the entire sequence of a human genome, introducing over 200 million bases of new sequence and correcting multiple errors in the prior reference. In parallel, the Human Pangenome Reference Consortium (HPRC) released a draft human pangenome reference containing an additional 47 phased, near-complete diploid assemblies from a genetically diverse cohort. This pangenome revealed novel alleles at structurally complex loci and contributed an additional 100 million bases of polymorphic euchromatic sequence. Use of these updated reference sequences was shown to reduce analysis bias and significantly increase the number of variants identified compared to the GRCh38 reference. This added genomic information provides opportunities for new variational and functional studies and is expected to advance our understanding of human genomics and disease.

As one example, the short arms of the human acrocentric chromosomes 13, 14, 15, 21, and 22 have been understudied due to their omission from prior human reference genomes. Our initial analysis of these chromosome arms in the context of the pangenome has revealed the presence of pseudo-homologous regions (PHRs) indicative of recombination between non-homologs. The PHRs include sequences previously shown to lie at the breakpoint of Robertsonian translocations, and their arrangement is compatible with crossover in inverted duplications on chromosomes 13, 14, and 21. The ubiquity of signals of recombination between heterologous chromosomes seen in the HPRC draft pangenome's acrocentric assemblies suggests that these shared sequences form the basis for recurrent Robertsonian translocations, providing sequence and population-based confirmation of hypotheses first developed cytogenetically 50 years ago.

**Conflict of Interest:** None declared

### S11.2 Centromere variation and evolution

*Glennis Logsdon*

Advances in long-read sequencing technologies and associated algorithms have enabled the complete assembly of human centromeres for the first time. This achievement has provided the first high-resolution map of each centromere and reveals remarkable diversity in their sequence, structure, and organization. However, because these centromeres were assembled from a single human genome, they do not adequately represent the natural variation of centromeres, and as a result, our understanding of centromere variation in its natural context is still limited. In this talk, I will debut a second set of complete human centromeres and highlight the genetic and epigenetic variation between these two reference sets and within the broader context of the human population. I will also present the complete assembly of 35 orthologous centromeres from human, chimpanzee, orangutan, and macaque genomes and provide a comparative and phylogenetic analysis showing the rapid and independent evolution of these regions over the last 25 million years. Finally, I will discuss future efforts to investigate centromere variation and evolution at scale in both health and disease.

**Conflict of Interest:** None declared

### S11.3 The reference genome

*Valerie Schneider*

In the 20 years since the completion of the Human Genome Project (HGP), the human reference genome assembly has continued to play a vital role in supporting diverse aspects of biological research. In addition to providing a coordinate system, it is also a baseline for variation analyses, a template for annotation, a point of reference for disease research and comparative genomics, a benchmark for sequencing and assembly technologies, and a gateway to diagnostic testing and personalized medicine. The enduring value of the reference reflects not only the original HGP design, but its deliberate and continued curation by the Genome Reference Consortium (GRC). In addition to correcting errors and closing gaps, this curation has allowed the reference to improve its representation of human population diversity and serve as a more robust substrate for genomic analysis in the years since its initial release. Today, the role of the reference continues to evolve, as it now informs activities such as the development of the human pangenome reference. We will present the current state of the reference genome (GRCh38.p14), highlight recently developed resources, such as the MANE annotation set, that are derived from it, and discuss ways in which it is contributing to the pangenome. Finally, we will share lessons learned from more than a decade of curating an essential public resource and how they might be used to promote the adoption and lasting value of new representation of the human genome.

This work of VAS was supported by the National Center for Biotechnology Information of the National Library of Medicine (NLM), National Institutes of Health.

**Conflict of Interest:** None declared

## S13 LEVERAGING GENETIC STUDIES FOR DRUG DISCOVERY

### S13.3 The long journey from GWAS SNPs to drug target genes

*Brent Richards*

GWAS has identified thousands of loci that are strongly associated with disease. Assuming, that each association reflects a causal biological signal, such loci should provide ample opportunities to identify high-yield drug targets for these diseases. This is because drug targets with evidence from human genetics increase the probability of successful drug development 2-4 fold.

Yet, despite this promise, few medicines have been developed directly from observations arising from GWAS. This is most often because the gene responsible for the signal at the GWAS locus is unclear, and the effect size on disease that can be anticipated via pharamcological perturbation of the causal gene's protein is difficult to predict.

In this talk, I will review progress on methods to map causal genes at GWAS loci and provide an overview of challenges in the field, but also ways that current methods can be used to accelerate drug development. I will then discuss relationships between GWAS effect sizes and anticipated effect sizes of pharmacological perturbation of a gene's protein.

**Conflict of Interest:** None declared

## S14 PRECISION MEDICINE IN RENAL CANCER

### S14.1 Hereditary renal cell carcinoma syndromes

*Eamonn Maher*

Though hereditary causes of renal cell carcinoma (RCC) account for only about 5% of all cases, the opportunities for early detection and treatment of RCC and syndrome-specific associated tumours in affected patients and their relatives means that early diagnosis is important. Hereditary RCC syndromes are inherited in an autosomal dominant manner but a family history may not be present because of non-penetrance in other family members or, less frequently a "de novo mutation". A syndromic cause may be suspected by non-RCC features (e.g. VHL disease, Birt-Hogg-Dube syndrome, HLRCC, Tuberose Sclerosis) but syndromic cases can also present as isolated RCC, particularly if a young onset and/or bilateral RCC. For the most part, the management of hereditary RCC is based around tumour surveillance and nephron-sparing surgery but there are important differences between the management of specific syndromes that makes precise diagnosis important. Though a positive family history, early age of onset, or multifocal RCC should trigger consideration of genetic testing, current genetic testing panels (e.g. in the UK BAP1, FH, FLCN, MET, SDHB, VHL and cytogenetic analysis) and testing eligibility criteria result in incomplete ascertainment of hereditary cases which might be addressed by more extensive testing. However, efforts to identify novel genes to further improve diagnostic yield are still ongoing. The identification of the molecular basis of hereditary RCC syndromes, as exemplified by von Hippel-Lindau disease, has led to the development of novel targeted therapies (e.g. the HIF2 antagonist belzutifan) and hereditary renal cell carcinoma syndromes provide a model for precision medicine in human cancer genetics.

**Conflict of Interest:** None declared

### S14.3 Molecular genetics and precision medicine in renal cancer

*James Brugarolas*

Kidney cancer is one of the top ten most common cancers. Despite recent advances, when metastatic, it is largely incurable. Clear cell renal cell carcinoma (ccRCC), the most common type, is characterized by *VHL* inactivation. However, *VHL* inactivation is insufficient for ccRCC development. In addition to *VHL*, mutations in *BAP1* or *PBRM1* are commonly found in ccRCC tumors. Interestingly, *BAP1* and *PBRM1* mutations anticorrelate and are

associated with differential tumor architectures, tumor grade, treatment response and survival in patients. While the precise molecular mechanism whereby these genes suppress tumorigenesis is unclear, a key effector downstream of *VHL* is the HIF2a transcription factor. Arguably the most important driver of ccRCC, HIF2a was regarded as undruggable. However, structural analyses identified a vulnerability, which was exploited through a chemical screen leading to the identification of lead compounds, and the founding of Peloton Therapeutics in the university (UT Southwestern) BioCenter. Peloton developed 3 highly-related drugs (PT2385, PT2399 and PT2977), referred herein as PT. PT effectively and specifically inhibited HIF2a in human ccRCC transplants in mice and was more effective and better tolerated than sunitinib (standard of care). In a phase I clinical trial, PT inhibited HIF2a in patient tumors, was well tolerated and active (video). Subsequent studies in patients with germline *VHL* mutations, which are predisposed to ccRCC, showed that PT induced regression of the vast majority of ccRCCs, and PT was approved for use by the FDA (story). However, prolonged PT treatment results in resistance mutations, which we previously identified in preclinical models. In collaboration with Arrowhead Pharmaceuticals, we show that a tumor directed HIF2a siRNA drug (siHIF2) inhibits both wild-type and mutant HIF2a, and that siHIF2 effectively inhibits ccRCC in both preclinical models and patients (story). Overall, these studies set a foundation for the first molecular genetic classification of ccRCC, establish HIF2a as a core dependency and therapeutic target, and set a paradigm for tumor-directed RNA-based therapeutics in cancer.

**Conflict of Interest:** None declared

## S15 PLACENTA: THE FORGOTTEN ORGAN

### S15.1 Regulation of gene expression in human placenta and its disturbances

***David Monk***

The placenta is unique among human tissues for its rapid development from the extra-embryonic lineages, its short functional lifespan and its distinctive epigenetic state. It is the interface between the fetus and mother, responsible to nutrient exchange, hormone secretion and has an immunological role in protecting the fetus all of which must function to ensure healthy development in eutherian mammals. Placenta cells result from the first lineage decision in pre-implantation embryos, when the trophectoderm segregates from the inner cell mass responsible for the embryo proper. The developmental queues responsible to determining trophectoderm fate are largely unknown, but recent advances in single-cell and low input technologies have revealed these are largely DNA methylation independent. However, as the placenta develops, trophoblast cells acquire a unique epigenetic profile, highlighted by its hypomethylated state compared to other embryonic tissues, as well as the acquisition of large partially methylated domains (PMDs) and placenta-specific imprinted loci. Such a privileged epigenetic state enables unique transcriptional networks to assist in placenta development, such as the role of hypomethylated LTR-repeats as cryptic promoter or enhancers. Recently work by our group and others has revealed that the different DNA methylation features that are unique to the placenta epigenome can be cell-type specific and be subject to temporal changes during gestation. In my presentation I will talk about the unique nature of the placenta epigenetic landscape, transcriptional factor usage that defines cell-type and highlight the recent advances in placenta-specific imprinting, as well as discuss their implications for placenta-associated pregnancy complications.

**Conflict of Interest:** None declared

### S15.3 The placenta, reproductive and cardiovascular health: the need for population based placental sciences

***Abigail Fraser***

Despite its vital role in human pregnancy, the placenta remains under-studied. This talk will cover current thinking around the aetiology of placental syndromes and the relationship between placental pathology, maternal health in pregnancy and cardiovascular health in later life. I will propose that studying placenta may offer insight into the aetiology of both placental syndromes and cardiovascular disease; and address the potential of placental investigations to provide a non-invasive cardiovascular screening opportunity, as well as associated challenges.

**Conflict of Interest:** None declared

## S16 HIGH-THROUGHPUT FUNCTIONAL ANALYSIS OF VARIANTS

### S16.1 Systematically editing the human genome at scale

***Gregory Findlay***

Our incomplete understanding of how rare variants contribute to disease phenotypes substantially limits the clinical utility of genomic data. To address this, we developed Saturation Genome Editing (SGE), a CRISPR-based method to assay all possible single nucleotide variants across targeted genomic regions. We've used SGE to functionally characterise over 10,000 variants across the tumour suppressor genes *BRCA1* and *VHL*. The resulting variant effect maps reveal loss-of-function variants acting via diverse mechanisms and predict human disease risk with extremely high accuracy. Ongoing work centres on scaling SGE and related technologies to more cell types, phenotypic assays, and genes, towards the ultimate goal of being able to predict the consequences of all variants encountered clinically.

**Conflict of Interest:** None declared

### S16.2 Novel high throughput functional genomics approaches

***Jozef Gecz***

**Pathogenic Evaluation of Recalcitrant Variants through Systematic Transactivation**

***Jozef Gecz***[1], *Emmylou Nicolas*[1], *Mark Corbett*[1], *Tarin Ritchie*[1], *Ingrid Scheffer*[2], *Samuel Berkovic*[2], *Michael Hildebrand*[2], *John Grigg*[3], *Sandra Cooper*[4,5,6], *Christian Pflueger*[7], *Ryan Lister*[7] *and Lachlan Jolly*[1]

[1]*The University of Adelaide and Robinson Research Institute, Adelaide, SA, Australia.* [2]*Epilepsy Research Centre, Department of Medicine, The University of Melbourne, Heidelberg, VIC, Australia* [3]*Speciality of Ophthalmology, Save Sight Institute, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia* [4]*Kids Neuroscience Centre, Kids Research, Children's Hospital at Westmead, Westmead, NSW, Australia,* [5]*Discipline of Child and Adolescent Health, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, Australia,* [6]*Functional Neuromics, Children's Medical Research Institute, Westmead, NSW, Australia* [7]*The University of Western Australia and Harry Perkins Institute of Medical Research, Nedlands, WA, Australia.*

Many individuals with suspected monogenic disorders do not receive a genetic diagnosis. A significant proportion have variants

of uncertain significance (VUS) for which functional studies may help confirm pathogenicity. For VUS predicted to impact splicing, analysis of gene splicing at the RNA level, ideally studied in the context of the patient genetic and cellular background, is often sufficient to reclassify the variant. Patient blood and/or skin derived cell lines are frequently used for RNA analyses, however, >1400 Mendelian disease genes are recalcitrant to RNA studies as they are not sufficiently expressed in these clinically accessible tissues. To overcome this limitation, we adapted a CRISPR-based gene transactivation technology to induce the expression of otherwise non-expressed disease genes in patient cell lines. Thus far, we have activated the expression of 14/15 non-expressed disease genes tested, including those causing epilepsy (e.g., *PCDH19*, *SCN1A*), intellectual disability (e.g., *MYT1L*, *PAK3*), blindness (*USH2A*), Osteomyelitis (*IL1RN*) and neuromuscular disorders (*DMD*). Activation levels range from 10 to >20,000-fold. To exemplify our approach, we combined gene transactivation with long-read RNA sequencing in patient cells to resolve the deleteriousness of VUS suspected to alter splicing in *USH2A* and *PAK3*. Our study highlights the utility of CRISPR based gene transactivation as a diagnostic technology able to provide functional evidence of variant pathogenicity in non-expressed Mendelian genes in patient cells. The approach is adaptable to any gene and can be re-used to study different variants within a gene.

**Conflict of Interest:** None declared

## S17 LONG READS FOR SOLVING OLD PROBLEMS

### S17.1 Diagnostic applications of long-read RNAseq

***Mina Ryten***

Genetic and transcriptomic studies are fundamentally reliant on accurate and complete human gene annotation, being defined as the genetic coordinates of all transcripts of a given gene. Among other analyses, this is required for the quantification of expression or splicing from RNA experiments, interpretation of significant genome-wide association study signals, and variant interpretation from genetic tests. As our understanding of transcriptomic complexity improves, it is apparent that existing gene annotation remains incomplete with perhaps the most incomplete being the human brain transcriptome. By using a combination of untargeted and targeted short- and long-read RNA-sequencing approaches, we find that core assumptions, including that genes have a single major transcript, and that existing annotation is largely correct, do not hold for the important disease-associated genes, *SNCA* and *GBA1*. Focusing on the latter, and its pseudogene pair, *GBAP1*, and using short-read RNA-sequencing data from human brain samples, we found that only 42% of all reads mapping to *GBA1* did so uniquely, with the remaining reads mapping primarily to *GBAP1*. This resulted in a significant misestimation of the relative expression of *GBA1* to *GBAP1*. Furthermore, using targeted long-read RNA-sequencing of 12 human brain regions we identified 18 *GBA1* transcripts that had a novel open reading frame and 7 *GBAP1* transcripts predicted to encode a protein. We showed that a subset of these transcripts generate stable protein that lacked GBA's function as a lysosomal glucocerebrosidase, that such transcripts were common, and that their usage showed cell type selectivity in human brain. Finally, we used annotation-independent analyses of both long- and short-read RNA-sequencing data to show that parent genes are more likely to have incomplete annotation. Given that 734 genes causing Mendelian disease have at least one pseudogene, these findings highlight the need for long-read RNA-sequencing analyses at many disease loci.

**Conflict of Interest:** None declared

### S17.2 Toward improved diagnosis of genetic disease with nanopore long-read sequencing

***Ira Deveson***

Nanopore long-read sequencing offers many opportunities to streamline, improve or completely reimagine the diagnosis of genetic disease. For example, we recently demonstrated how Oxford Nanopore's 'ReadUntil' functionality could be used to accurately genotype all disease-associated short-tandem repeats in a single, simple assay. This capacity for programmable targeted sequencing and accurate resolution of repetitive genes and structural variation (in addition to small variants and DNA methylation) underpins diagnostic opportunities in many different disease areas. However, several key barriers remain to be overcome, including: (a) generation of long-read reference data across large, diverse human cohorts to define the background of 'normal' variation against which pathogenic variants may be interpreted and; (b) development of more sophisticated, robust, efficient and scalable computational methods for nanopore data analysis. While many in the genomics community continue to obsess over the nanopore sequencing error rate, I will discuss our ongoing efforts to tackle these more meaningful challenges, and the many diagnostic opportunities that may follow.

**Conflict of Interest:** None declared

## S18 CO-CREATING RESEARCH WITH PARTICIPANTS

### S18.1 Training researchers to engage with patients: the importance of co-creation

***Emma Dorris***

In the framework of a Horizon 2020 project (EATRIS-Plus), EATRIS (European infrastructure for translational medicine) and EPF (European Patients' Forum) and affiliated EPF member EATG (European Aids Treatment Group) joined forces to improve responsible research practices in the field of patient engagement and address the multiple barriers currently preventing academic researchers from meaningfully engaging with patients in their research.

Partners dedicated the year of 2022 to a co-creation process to better understand how researchers could be further supported and incentivised to implement meaningful patient engagement in their research. This was achieved through the establishment of a pan-European multi-stakeholder taskforce, a public consultation and focus group meetings with patient organisations, researchers and research funders.

This bottom-up approach lead to the co-development of the "Patient Engagement Resource Centre", which was launched in March 2023.

The Patient Engagement Resource Centre is an easy to navigate platform to help researchers get started with patient engagement. It is a repository of publicly available guidance, training and practical tools that support researchers with every stage of their patient engagement activity: from planning, to conducting and evaluating. The "Fundamentals" section offers a curated list of materials, which are deemed essential to understand the basics of good patient engagement practices. The Resource Centre also features "Stories" from researchers, patients and caregivers, highlighting in short videos how they have collaborated and their recommendations to others who would like to start engaging with patients.

**Conflict of Interest:** None declared

### S18.2 Engaging patients in clinical trials endpoints: A Gaucher Disease Registry perspective

*Tanya Collin-Histed*

**Background:** Type 2 and Type 3 Gaucher Disease (GD), a rare inherited metabolic disorder, are neuronopathic GD (nGD) and result in infant death or progressive neurological deterioration. Current drug therapies do not cross the blood brain barrier and thus do not treat nGD.

**Objectives:** The development of a patient registry specific to nGD through collaboration between patients, caregivers, clinical experts, researchers, and industry.

**Methodology:** Led by the International Gaucher Alliance (IGA), multiple stakeholders, including patients, caregivers, clinicians, and researchers, partnered to develop a web-based platform for patients with nGD and their caregivers. Questionnaires (baseline and follow-up) were designed to capture data relevant to patients, including neuronopathic Gaucher-specific Patient Reported Outcomes (nGD-PRO) and Observer Reported Outcomes (nGD-ObsRO). Qualitative interviews with patients and caregivers ensured the use of relevant terminology. Clinicians informed the process of diagnosis confirmation.

**Results:** The Gaucher Registry for Development Innovation and Analysis of Neuronopathic Disease (GARDIAN) is a global, longitudinal, prospective patient registry with no age restrictions. GARDIAN will capture data at baseline and every 6 months for 3 years. Data collected will include enzyme/genetic results, patient characteristics, symptoms (neurological/non-neurological), medical history, treatment, and comorbidities. Patient- and caregiver-reported outcomes include the PedsQL, PGI-S, GAD-7, PHQ-9 and an nGD-PRO and nGD-ObsRO to be validated within the registry.

**Conclusions:** Patient engagement in the development of GARDIAN optimizes its value as a real-world data source to assist clinicians in decision making and addresses the unmet needs of patients. The systematic and standardized approach of real-world data collection will improve our understanding of nGD patients' experiences, perspectives, needs, and priorities.

**Conflict of Interest:** None declared

### S18.3 Inclusive genetics research with people with intellectual disability

*Elizabeth Palmer*

1% of the population have intellectual disability, with an underlying genetic cause increasingly being able to be identified. The recent Australian National Roadmap for Improving the Health of People with Intellectual Disability highlighted significant health inequities: people with intellectual disability have more than twice the rate of avoidable deaths and lower rates of access to preventative healthcare. Exclusionary and discriminatory healthcare practices contribute to this inequity: the opinions and preferences of people with intellectual disability have not previously been considered in the planning of an appropriate and accessible genetic healthcare model (Strnadová et al., 2022).

GeneEQUAL is an inclusive research program aiming to improve the empowerment and genetic healthcare of people with intellectual disability. Our foundational qualitative research revealed that people with intellectual disability recommended accessible, multimodal health-literacy resources, and clinician education on trauma-informed, respectful, and person-centred clinical approaches (Strnadová et al., 2023). This research informed the co-production of an Educational Toolkit including Easy Read genetic booklets and a video series demonstrating inclusive and respectful clinical practice. The Toolkit aims to empower people with intellectual disability to make informed decisions about clinical genetic services and genetic testing, enhance their support before and after a genetic diagnosis, and improve current clinical practice. Focus groups and multi-stakeholder advisory workshops with over 50 individuals have guided the co-production process. Mixed-methods evaluations with clinicians and people with intellectual disability are in progress. We propose that this inclusive research model has scope to improve the equity and accessibility of genetic healthcare for other vulnerable populations, and highlight reflections on the value of the co-production process from the participants with intellectual disability themselves.

**Conflict of Interest:** None declared

## S19 ADDRESSING DIVERSITY IN THE GENOMIC WORLD

### S19.1 Mexican Biobank advances population and medical genomics of diverse ancestries

*Mashaal Sohail*

Latin America continues to be severely underrepresented in genomics research, and fine-scale genetic histories and complex trait architectures remain hidden due to a lack of sufficient data. To fill this gap, the Mexican Biobank project genotyped 6,057 individuals from 898 rural and urban localities across all 32 states in Mexico at 1.8 million genome-wide markers with linked complex trait and disease information creating a valuable nationwide genotype-phenotype database. Using ancestry deconvolution and inference of identity-by-descent (IBD) segments, we inferred detailed ancestral histories across Mesoamerican regions, unraveling indigenous, colonial, and post-colonial demographic dynamics. We observed variation in runs of homozygosity (ROH) among genomic regions with different ancestries reflecting distinct demographic histories and in turn, different distributions of rare deleterious variants. We conducted genome-wide association studies (GWAS) for 22 complex traits and found that several traits are better predicted using the MXB GWAS compared to the UK Biobank GWAS. We identified genetic and environmental factors associating with trait variation, such as the length of genome in ROH as a predictor for body mass index, triglycerides, glucose, and height. This study provides new insights into the genetic histories of individuals in Mexico and dissects their complex trait architectures, both crucial for making precision and preventive medicine initiatives accessible worldwide.

**Conflict of Interest:** None declared

### S19.3 BioBank Japan and what we have learnt so far

*Yukinori Okada*

We here introduce Biobank Japan Project (BBJ), one of the largest non-European biobanks with deep phenotype information. BBJ is a nation-wide hospital-based cohort of Japan launched in 2003. BBJ consists of ~260K Japanese affected with 51 diseases (immune, metabolic, musculocutaneous, neuronal diseases and cancers), which collected DNA, sera, and deep clinical information (clinical measurements, dietary and lifestyles) of participants. GWAS data (~260K) and deep WGS data (~10K) have been developed. We have massively expanded international collaboration network with global biobanks and consortia, such as Global Biobank Meta-analysis Initiative (GBMI). Cross-population GWAS meta-analysis of BBJ, UK biobank, and FinnGen (>650K individuals) on 220 human complex phenotypes and biomarkers has identified global atlas of genotype-phenotype associations (Sakaue S et al. ***Nat Genet*** 2021). GWAS summary statistics are publicly available

at Pheweb.jp (https://pheweb.jp/). In this session, we would like to introduce our achievements and future directions.

**Conflict of Interest:** None declared

## S21 GENE-DISEASE RELATIONSHIPS

### S21.1 Lumping vs. splitting – how to approach defining a disease for accurate curation - virtual

*Courtney Thaxton*

The dilemma of how to categorize and classify diseases has been debated for centuries. The field of medical genetics has historically approached the classification of disease, or nosology, based on the set of clinical phenotypes observed in patients and families, either lumping phenotypes together as part of a syndrome, or splitting clinical features into separate disease entities. However, advances in genomic sequencing technology have turned nosology on end. We are now able to identify the underlying genetic etiologies of diseases that may have previously been "lumped" or "split" based on phenotypic characteristics; when these entities are found to have a more distinct basis in genetic variation, systematic reclassification and re-categorization of disease entities may be necessary. The Clinical Genome Resource (ClinGen) aims to define the clinical relevance of genes and variants for use in precision medicine and has developed frameworks to classify the strength of evidence underlying monogenic gene-disease relationships, variant pathogenicity, and clinical actionability. In each of these frameworks it is first necessary to define, in nosological terms, the entity being evaluated. While this task is straightforward when a single gene has been reported in association with a single distinct disorder, it can be challenging to decide how to curate genes associated with multiple disease entities and/or a broad phenotypic spectrum. We therefore developed a set of criteria to guide "lumping and splitting" decisions and improve consistency across the consortium. These criteria may also be useful to other groups who are involved in defining monogenic gene-disease relationships. We also formulated a pre-curation process that facilitates initial assessment of disease entities associated with a gene. As part of this process, biocurators and disease experts categorize disease associations as having an isolated phenotype, complex organ-specific phenotype, or syndromic phenotype, to aid in defining the disease entity for the curation. Here, we outline the precuration process, the lumping and splitting guidelines with examples, and describe the implications for clinical diagnosis, informatics, and care management.

**Conflict of Interest:** None declared

### S21.2 International efforts for gene curation and the interpretation of genome variation

*Julia Foreman*

**International efforts for gene curation and the interpretation of genome variation**

Julia Foreman, on behalf of Genome Assembly and Annotation, EMBL-EBI

High quality gene-disease information, international standards and robust tools are essential to enable robust variant interpretation and accurate diagnosis. At EMBL-EBI, we create tools and reference datasets to enable interpretation of genome variation, developing systems to support experts in the filtering, interpretation and sharing of candidate diagnostic variants. The breadth of the tools developed at EMBL-EBI, along with collaborative efforts, will be presented.

The Ensembl Variant Effect Predictor (VEP), is a powerful toolset for the large-scale annotation and prioritisation of genomic variants, which applies novel algorithms and integrates comprehensive reference datasets and tools to create detailed variant annotations. For the analysis and sharing of rare disease patient phenotype-linked candidate diagnostic variants, DECIPHER is available. It provides interfaces which summarise and contextualise genotypic and phenotypic data, enabling variant interpretation according to international standards.

Accurate curation of gene-phenotype associations is essential to support genomic interpretation. Genotype2Phenotype (G2P) enables the curation of detailed, evidence supported gene-phenotype associations. An Ensembl VEP extension has also been developed that uses G2P data to support diagnostic variant filtering. In addition, we collaborate with the Gene Curation Coalition to agree standards for the reporting of gene disease evidence.

The use of standard, common transcripts is key to robust variant reporting. We collaborate with NCBI to create the Matched Annotation from NCBI and EMBL-EBI (MANE) transcript set. We have defined a default representative 'MANE Select' transcript for each human protein coding gene and further 'MANE Plus Clinical' transcript where needed to cover all clinically reported variants.

We have recently established the PARADIGM initiative to create **P**rimary **A**nnotated **R**esources to **A**dvance **D**iscovery **I**n **G**enomic **M**edicine and thus find novel genetic causes of disease and improve diagnosis rates.

**Funding:** DECIPHER receives funding from Wellcome [WT223718/Z/21/Z]. Ensembl receives majority funding from Wellcome [WT222155/Z/20/Z] with additional funding for specific project components. Research reported in this publication was supported by National Human Genome Research Institute of the National Institutes of Health under award number 2U24HG007234-09. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. G2P receives funding from Wellcome [WT226083/Z/22/Z]. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825575 (EJP RD) and the European Molecular Biology Laboratory.

**Conflict of Interest:** None declared

### S21.3 A framework for an evidence-based gene list relevant to autism spectrum disorder

*Jacob Vorstman*

Until recently, there was no standardized approach for evaluating a gene's relevance to autism spectrum disorder (ASD). Consequently, substantial variability exists between various proposed lists of "ASD risk" genes. Given the increasing use of genetic testing in clinical settings for patients with ASD and other neurodevelopmental disorders, this limitation hampers a uniform interpretation of genetic results and has important consequences: individuals with ASD are not tested for the same genes in different health care settings, and research groups are working under different assumptions regarding the genes underpinning ASD.

To address this issue, we developed, with an international multidisciplinary group of ASD experts, a systematic framework to curate genes with potential relevance to ASD (Schaaf *et al*. Nat Rev Genet, 2020, PMID: 32317787). This method, coined EAGLE (Evaluation of Autism Gene Link Evidence), aims at developing a comprehensive, transparent, and evidence-based gene list for ASD.

Briefly, EAGLE reviews and curates published genetic, phenotype, and experimental ASD data. Using the Clinical Genome (ClinGen) Gene-Disease Validity framework as a starting place,

points are awarded to each clinical/experimental case. These points are summed across all studies for a given gene, with the total dictating the classification a gene receives (limited, moderate, or definitive relationship to ASD). An additional feature of EAGLE curation is that it includes an evaluation of the quality of the phenotype data relevant to ASD.

Here, I will discuss rationale, methods, and utility of the growing list of genes curated through EAGLE and how it is made available to the clinical and research community through the SFARI Gene database.

**Conflict of Interest:** None declared

## S22 DIAGNOSTICS IN PRENATAL SETTING - THE PRESENT AND THE FUTURE

### S22.1 Prenatal exome sequencing - facing uncertainty

***Antoni Borrell***

### Clinical Application of Prenatal Exome Sequencing—Facing Uncertainty

*Antoni Borrell, BCNatal-Hospital Clínic, Barcelona*

Any prenatally applied genetic test carries a certain degree of uncertainty, from the classic karyotype to the most recently introduced tests based on next-generation sequencing. Roughly, uncertainties raised in the clinical application of exome sequencing can be related to: a) clinical utility and effectiveness; b) incomplete knowledge of the prenatal phenotype and variant classification; and c) unexpected findings.

The eligibility criteria for testing are uncertain, however, in most centers, a 20% yield has been set as a cut-off when structural anomalies are detected at ultrasound examination. This cut-off has not been recommended by any guideline, rather it has been widely adopted based on common sense.

Uncertainties can be raised by incomplete knowledge on prenatal phenotypes since only postnatal phenotypes are clearly defined. In addition, variant classification is an inexact science as it is subject to interpretation. To address both issues, a multidisciplinary team is required to review any single diagnosis.

A final source of uncertainty are incidental findings, which increase with a wider exome sequencing analysis. It is uncertain whether a targeted analysis with a multigene panel is preferred to whole or clinical exome sequencing. In addition, recommendations on reporting incidental and secondary findings are totally discrepant according to the different guidelines.

**Conflict of Interest:** None declared

## S23 BEYOND CODING POINT MUTATIONS: NEW MUTATION TYPES IN CANCER

### S23.1 Hidden germline variants in Lynch syndrome

***Richarda de Voer***

Individuals with Lynch syndrome (LS) are prone to develop early-onset mismatch repair deficient (MMRd) colorectal- and endometrial cancers due to germline pathogenic variants (PVs) in one of the mismatch repair (MMR) genes *MLH1, MSH2, MSH6, PMS2*, or deletions affecting the 3′ region of *EPCAM*. Current germline diagnostics for LS, after the exclusion of somatic *MLH1*-promoter hypermethylation, include targeted short-read sequencing and multiplex ligation-dependent probe amplification (MLPA) of the coding regions of the MMR genes. In the absence of a germline PV in an MMR gene, the presence of somatic MMRd is investigated. Individuals who remain genetically unresolved after germline and somatic analysis are considered to have unexplained MMRd (UMMRd). For individuals with UMMRd and their relatives treatment options and surveillance advices are unclear. The application of long-read sequencing, both targeted- and whole-genome sequencing, has opened up new possibilities to screen for hidden germline variants in UMMRd. I will present our work on the application of targeted long-read sequencing to identify non-coding variants in individuals with UMMRd and their functional follow-up to determine pathogenicity. Our work suggests that in at least 18% of individuals with UMMRd a germline non-coding PV in an MMR gene is missed by current routine diagnostic approaches. Our findings suggest that in order to improve LS diagnostics, treatment and cancer surveillance in patients and their relatives, germline sequencing of the full MMR gene loci should be performed.

**Conflict of Interest:** None declared

### S23.3 Genome-wide analysis of somatic noncoding mutation patterns in cancer

***Felix Dietlein***

In protein-coding regions of the genome, hundreds of "driver" mutations have been characterized that contribute to the uncontrolled growth of tumors. In the remaining 98% of the genome, our knowledge of drivers is limited to a handful. A systematic understanding of noncoding events in tumor development is lacking.

Here, we developed a new statistical framework to identify significant mutation events in the noncoding genome and used it to analyze somatic noncoding mutations in >3500 whole cancer genomes. On average, we identified ~5 novel noncoding events per cancer type, including many of the noncoding findings from previous studies (e.g., PCAWG consortium) along with novel observations, including highly localized mutagenic processes not observed in the rest of the genome and significantly mutated promoter and enhancer regions of genes associated with tumor signaling. We validated a subset of these findings by performing CRISPR-interference and luciferase reporter experiments in cancer cell lines. Broadly, our work provides a catalog of noncoding events across 19 major cancer types and reveals that interpreting whole cancer genomes involves challenges specific to noncoding regions.

**Conflict of Interest:** None declared

## S24 HEALTH ECONOMICS IN GENOMIC MEDICINE

### S24.1 Economics of Precision Medicine and Genomic Technologies

***Ilias Goranitis***

The presentation focuses on the 5-year journey (2018-23) of the health economics program of the Australian Genomics. It will provide insights into how the program was set up and how it addressed key evaluation challenges to inform the translation of Genomic Medicine in Australia. Using empirical evidence, the talk will demonstrate how health economics can contribute to the pursuit of efficient, sustainable and equitable implementation of Genomic Medicine and the design of high-value services and systems.

**Conflict of Interest:** None declared

### S24.2 The economics of genomics within resource-limited environments

*George P. Patrinos*

Nowadays, many relevant drug-gene associations have been discovered, but pharmacogenomics-guided treatment needs to be cost-effective as well as clinically beneficial to be incorporated into standard healthcare. There are several cost-effectiveness analyses of genome-guided drug treatment interventions in various medical specialties, mostly in cardiology and oncology but also in psychiatry. Health economic evaluation in genomic and personalized medicine are based mostly on simulated data (~90%) and only ~10% of the studies are based on real-life clinical data. Amongst the limitations of the field are the unclear explanation of study perspective and cost inputs, as well as the underreporting of study design elements, which can influence though the economic evaluations. We will focus on the basics of health economic evaluation in genome-guided treatment modalities, and discuss case examples of economic evaluations in developing countries in Europe, demonstrating that although there is growing evidence on the cost-effectiveness of genome-guided interventions, there is still a need for performing additional research on economic evaluations towards implementation of pharmacogenomics in the clinical settings.

**Conflict of Interest:** None declared

## EDUCATIONAL SESSIONS

## E01 NEW TECHNOLOGIES: RECENT ADVANCES IN SEQUENCING TECHNOLOGIES

### E01.2 Sequencing by Binding (SBB): increase accuracy of short-read genomes

*Jonas Korlach*

Sequencing by Binding (SBB) is a new short-read sequencing method which separates the labeled nucleotide binding step from the nucleotide incorporation step, allowing for optimization of each step separately and thereby resulting in higher sequencing accuracy. I will describe the performance and benchmarking of SBB sequencing, following by examples where higher short-read sequencing accuracy can be a benefit, including higher accuracy in sequencing long homonucleotide stretches, and higher sensitivity and specificity for rare variant detection for cell-free DNA and infectious disease applications.

**Conflict of Interest:** None declared

## E02 NOT ONLY DNA EDITING: GENTLER WAYS TO TWEAK GENES

### E02.2 SINEUP: A New Modular Tool to Increase Protein Translation

*Marta Biagioli*

Initially discovered in 2012, this class of functional natural non-coding RNAs (ncRNAs) have a modular structure with the Binding Domain (BD) providing specificity to the target transcript and an effector domain (ED) - containing an inverted, repetitive SINEB2 element - able to UP-regulate protein translation of the target mRNA. Because of this, they have been named SINEUP. These molecules are able to augment, in a specific and controlled way, the expression of the target protein, with no alteration of target mRNA levels.

Since the understanding of its modular structure, synthetic SINEUP molecules can be engineered by creating a specific BD for the gene of interest, located upstream the invSINEB2 ED, with broad applications in protein manufacturing as well as in therapy of haploinsufficiencies.

Here we propose an overview of the SINEUP structure, functional mechanisms and the current status of therapeutic development for Autism Spectrum Disorders and other neurodevelopmental conditions associated to dominant haploinsufficiencies.

**Conflict of Interest:** None declared

## E03 LET'S GET UP TO DATE ON BLOOD CANCER GENETICS AND GENOMICS

### E03.2 Blood Cancer Susceptibility genes, new kids on the block

*Ana Rio-Machin*

While haematological malignancies have historically been considered sporadic disorders, there are occurrences of familial cases where two or more individuals within the same family are affected. The inclusion of familial myeloid malignancies as a separate disease entity in the revised WHO classification has renewed efforts to improve both the recognition and the management of this group of at-risk individuals.

Myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) are related and aggressive myeloid neoplasms characterised by the accumulation of immature blast cells within the bone marrow. Familial MDS/AML cases represent a high-risk group of patients, accounting for ~10–15% of all cases, who require a unique management and whose only curative treatment is stem cell transplantation. Early detection of familial patients is key, as any relatives harbouring the same inherited variant should be discounted as potential bone marrow donors and given access to surveillance programmes.

The RNA-helicase *DDX41* was identified as predisposing gene of familial MDS/AML in 2015, and germline *DDX41* variants, accounting for ~5% of all AMLs, are considered now the most frequent cause of these inherited forms of the disease. However, one third of the *DDX41* germline variants identified in diagnostic screenings are classified as variants of unknown significance (VUS) and there are no definitive guidelines about how to proceed with the VUS. Although, in addition to *DDX41*, germline variants in more than 20 genes have been associated to MDS/AML predisposition, the aetiology of 40% of familial cases remains unknown. In addition, each predisposing gene and the type/location of the variants display marked variability in disease phenotype, latency and penetrance, altogether making the diagnosis and management of these patients/relatives extremely challenging.

This clinical and molecular heterogeneity of inherited MDS/AML was reflected in our series of families, where variants in 16 previously defined loci were detected in 49 (57%) families, and whole exome sequencing in a further 37 'uncharacterized' families (43%) revealed 56 new potential candidate genes. Several of these genes are linked to rare inherited myeloid disorders, but we have also identified novel genes not previously related to haematological diseases. One example is DHX34, another RNA-helicase, likewise DDX41, with functions in both Nonsense mediated decay (NMD) and RNA splicing. Although *DXH34* is not mutated in sporadic AML, it has been recently shown that it exhibits alternative splicing in one third of sporadic cases, resulting in a premature stop codon that phenocopies our

loss-of-function germline mutations observed in our familial patients.

Further functional and multi-omic approaches will offer new insights into the aetiology of myeloid malignancies and will provide a framework to identify novel predisposing genes for inclusion into routine diagnostics, ultimately improving the diagnosis, early detection and management of familial cases.

**Conflict of Interest:** None declared

## E04 HARNESSING PLEIOTROPY

### E04.1 Pleiotropic associations: methods and insights

***Luke O'Connor***

Pleiotropy is the phenomenon that one gene or allele affects multiple phenotypes. This talk will be an overview of pleiotropy in common diseases and traits: what it is; how it is estimated; what it can teach us about disease biology. It will include a discussion of Mendelian Randomization, which is a commonly used - but sometimes abused - method for causal inference using genetic association data. It will also touch on question of whether common and rare genetic variants have similar or different pleiotropic effects.

**Conflict of Interest:** None declared

## E05 THERAPEUTIC GENE EDITING IN EUROPE: WHAT WILL IT TAKE TO MAKE IT HAPPEN?

### E05.1 Laws in the EU: How do they apply to gene editing?

***Vera Lúcia Raposo***

Gene therapy has the potential to treat a wide range of genetic disorders. However, it is still a relatively new field and there are many ethical and regulatory issues that need to be considered. Most limitations are imposed under the assumption that gene editing – even when targeting therapeutic purposes – can threaten human dignity, broadly speaking. Therefore, I will start by considering the ethical implications of gene editing and the need to ensure that the technology is used in ways that uphold human dignity, having as legal basis the norms on fundamental rights, in particular, Article 3 of the Charter of Fundamental Rights of the European Union and Article 13 of the Convention on Human Rights and Biomedicine. After the analysis of the general ethical/legal framework, I will dive into more practical issues involving therapeutic gene editing. I will address the technical challenges imposed by existing pharmaceutical norms that rule the approval and marketing of drugs used to deliver the pharmaceutical products through which most gene therapies are delivered to patients, known in European pharmaceutical law as Advanced Therapy Medicinal Products (ATMPs), and highlight the main difficulties they raise.

**Conflict of Interest:** None declared

## E06 SPATIAL TRANSCRIPTOMICS/CELL LINEAGES

### E06.1 Single cell perturbation screen

***Malte Spielmann***

Single-cell sequencing is a powerful approach that can detect genetic alterations and their phenotypic consequences in the context of human development, with cellular resolution. Whether germline or somatic in nature, some mutations may have significant genotypic impact and lead to diseased cellular phenotypes, either systemically or confined to a tissue. Single-cell sequencing enables the detection and monitoring of the genotype and the consequent molecular phenotypes at a cellular resolution. It offers powerful tools to compare the cellular lineage between 'normal' and 'diseased' conditions and to establish genotype-phenotype relationships. While single cell approaches are currently used mainly in basic research, it can be expected that applications of these technologies in the clinic may aid the detection, diagnosis and eventually the treatment of rare genetic diseases as well as cancer.

In my talk I will highlight our recent work on establishing single cell RNA sequencing (sc-RNA-seq) of the wholeembryo as a scalable platform for the systematic phenotyping of mouse genetic models. We applied combinatorial indexing-based sc-RNA-seq to profile 101 embryos of 22 mutant and 4 wildtype genotypes at embryonic stage E13.5, altogether profiling over 1.6M nuclei. We developed and applied several analytical frameworks for detecting differences in composition and/or gene expression across 52 cell types or trajectories. Some mutants exhibit changes in dozens of trajectories while others only in a few cell types. We also identify differences between widely used wildtype strains, compare phenotyping of gain vs. loss of function mutants, and characterise deletions of topological associating domain (TAD) boundaries. Our findings show how single cell profiling of whole embryos can enable the systematic molecular and cellular phenotypic characterization of mouse mutants with unprecedented breadth and resolution.

**Conflict of Interest:** None declared

## E08 SHOULD WE BE ROUTINELY RETURNING RESULTS FROM GENOMIC RESEARCH?

### E08.2 A cutting edge approach to returning genomic results

***Mary-Anne Young***

**Background:** Increased use of genomic sequencing in research is resulting in large volumes of data including clinically actionable genomic information. Guidelines are emerging with regards to returning genomic research results although provide a high-level view and do not explicitly outline a return of results pathway. Most research participants want to be notified of this information, not only for themselves but their family members. Whilst researchers support return of results, most do not have the communication skills or resources to facilitate return of results. There is ongoing debate regarding types of results to be returned and the best methods to return.

This presentation will examine the history and evidence regarding return of research results in Australia and present a novel program returning results known as My Research Results.

**My Research Results:** My Research Results (MyRR) is an evidence based genetic counsellor led Australian program facilitating the return of primary and secondary research results to adult research participants or their next of kin. MyRR connects researchers, research participants and clinical genetic services. The return of results process designed by MyRR is collaborative, client centred and supports research participants and their family to make an informed choice about receiving/not receiving results.

**Implications:** Returning genomic research results will impact the profession and practice of Genetic Counsellors.The skillsets of

genetic counsellors are well aligned to the skills required for returning research results i.e., providing information and support to facilitate decision making, exploring the familial nature of the information in a client centred way. In addition, changes to models of genetic counselling practice will be required from those that are traditionally based on personal or family history to models where results from genomic sequencing are the entry point.

**Conclusion:** MyRR has been developed in response to an identified need of an ethical, practical process to return research results. It is a flexible, adaptive, genetic counsellor led program facilitating the return of primary and secondary research results to adult research participants.

**Conflict of Interest:** None declared

## E09 EPILEPSY: GENETICS & TREATMENT

### E09.2 A self-regulating gene therapy for Rett syndrome

***Stuart Cobb***

A self-regulating gene therapy for Rett syndrome

*Stuart Cobb*[1,2]

[1]*University of Edinburgh, Edinburgh, UK and* [2]*Neurogene Inc., New York, NY, USA*

Rett syndrome is a severe neurological disorder characterized by developmental regression and a constellation of clinical phenotypes including seizures. The disorder is caused by loss-of-function variants in the X-linked *MECP2* gene. Females with Rett syndrome exhibit a mosaic pattern of expression whereby approximately half of cells exhibit substantial deficiency in functional MeCP2. Conversely, individuals with duplication at the *MECP2* locus also experience severe neurological disease, indicating that the gene is highly dosage sensitive and creating challenges for conventional gene therapy. NGN-401 is an investigational AAV9 gene therapy for Rett syndrome designed to provide full length and fully functional MeCP2 protein within a narrow expression range via a self-regulating miRNA circuit, termed EXACT. Studies in *Mecp2* knockout mice, a model that shares phenotypic features of the human disease, have shown that delivery of NGN-401 extends survival and ameliorates disease phenotypes. The EXACT miRNA circuit also affords safety features over conventional gene therapy. Overall, the totality of data generated in multiple preclinical models demonstrated that NGN-401 allowed for MeCP2 expression that provided therapeutic benefit while mitigating the risk of overexpression toxicity, supporting FDA clearance to initiate a first-in-human paediatric clinical study.

**Conflict of Interest:** None declared

## E10 SPINAL MUSCULAR ATROPHY TREATMENTS

### E10.2 AAV therapy for SMA

***Francesco Muntoni***

Spinal muscular atrophy is an ideal condition in terms of AAV gene therapy. All patients with SMA, irrespective of their severity, have homozygous deletions (or other mutations) of the SMN1 gene, but carry at least one intact copy of the SMN2 gene.

These two genes are virtually identical, with the exception of the splicing regulation of exon 7, excluded from the majority of the transcript in SMN2. Nevertheless a small amount of SMN protein is produced by all individuals affected by SMA, hence providing an important protection from the possibility of developing transgene related immune reactions.

Furthermore the SMN gene cDNA can be effectively packed within an AAV, together with relevant regulatory elements and the promoter.

Finally motorneurons, the primary target of the disease, are non-dividing cells, hence long term persistency is expected and indeed so far demonstrated up to 7.5 years after receiving the gene therapy in the early treated patients.

This led to the approval of zolgensma, an AAV9 gene therapy product for the severe form of SMA1 in 2019, and at the time of writing this abstract more than 3000 children worldwide have been treated.

With the recruitment of many more children in the real world compared to the clinical trials, novel, rare adverse events have transpired. In a few cases in which the treated children have succumbed due to the severity of their disease, postmortem examination have provided an unique insight of the biodistribution and SMN protein production in the entire body.

In my presentation I will summarise results from clinical trials, real world, and with special consideration for safety profile

**Conflict of Interest:** None declared

## E11 GENETICS CARE FOR TRANS AND GENDER DIVERSE PATIENTS: FROM MEDICALIZATION TO EMPOWERMENT

### E11.1 Sex, gender, and pedigrees in clinical genetics: What to record, how to record it, and why

***Jehannine Austin***

Pedigrees are a fundamental tool that are used ubiquitously in genetics practice. But some of the core assumptions underlying how we construct these important pieces of the medical record are often unclear. In this presentation, we will examine the assumptions underlying the symbols we draw, consider the ways in which information about sex and gender are important to the genetics interactions, and discuss the ways in which we can gather information about these variables in clinical settings.

**Conflict of Interest:** None declared

### E11.2 Gender Affirming Genetics Practices

***Kimberly Zayhowski***

Binary frameworks for classifying gender are exclusionary, disrespectful, and harmful to transgender and gender diverse people seeking genomic healthcare or enrolled in genomic research. In this session we will review how disparities in access, care, and health outcomes for gender diverse patients manifest in a variety of clinical genomic practice settings, including oncology and prenatal clinics. We will discuss data from qualitative research conducted with gender diverse patients, shining a light on patients' experiences of hereditary cancer risk assessment and prenatal risk assessment. Moreover, we will explore experiences of gender diverse individuals in genomics research. Throughout the presentation, we will provide practical suggestions on how to provide respectful, empathic, and sensitive care to gender diverse patients. The overarching goal of the session is to demonstrate the cascading effects of inaccurate uses of genetics for gender diverse people, and show the audience how to shift their own practices to be more inclusive and just for all.

**Conflict of Interest:** None declared

## E13 NEW MUTATIONS AND EVOLUTION

### E13.1 Naked-mole rat and aging - virtual

***Vera Gorbunova***

Abundant high molecular weight hyaluronic acid (HMW-HA) contributes to cancer resistance and possibly longevity of the longest-lived rodent, the naked mole-rat. To study whether the benefits of HMW-HA could be transferred to other animal species, we generated a transgenic mouse overexpressing naked mole-rat hyaluronic acid synthase 2 gene (nmrHAS2). nmrHAS2 mice showed increase in hyaluronan levels in several tissues, and lower incidence of spontaneous and induced cancer, extended lifespan and improved healthspan. The transcriptome signature of nmrHAS2 mice shifted towards that of longer-lived species. The most striking change observed in nmrHAS2 mice was attenuated inflammation across multiple tissues. HMW-HA reduced inflammation via several pathways including direct immunoregulatory effect on immune cells, protection from oxidative stress, and improved gut barrier function during aging. These findings demonstrate that the longevity mechanism that evolved in the naked mole-rat can be exported to other species, and open new avenues for using HMW-HA to improve lifespan and healthspan.

**Conflict of Interest:** None declared

### E13.2 Evolution of somatic mutation rates

***Alex Cagan***

Somatic mutations accumulate in the DNA of healthy cells throughout life. They underpin the development of cancer and may also play a role in ageing and age-associated diseases. Directly studying these mutations in normal tissues has been challenging due to the difficulty of detecting mutations present in single cells or small clones in a tissue. Recent technical advances are enabling the study of somatic mutation in normal tissues, revealing how our cells accumulate mutations at different rates and how clonal expansions of mutant cells can colonise tissues. Yet relatively little is known about how these processes operate in non-human species. We performed whole-genome sequencing of 208 intestinal crypts from 56 individuals to study the landscape of somatic mutation across 16 mammalian species. This comparative analyses of somatic mutagenesis sheds light on the diversity of mutagenic processes across species, and on long-standing questions regarding the evolution of somatic mutation rates and their role in cancer and ageing.

**Conflict of Interest:** None declared

## E14 3D GENOMES EXPLAINED

### E14.2 Resolution of regulatory conflicts through genome 3D-restructuring

***Michael Robson***

3D chromatin folding physically isolates enhancer elements with their target genes in topologically-associated domains (TADs) which drive gene mis-expression and disease when disrupted. Yet, many apparently TAD-disrupting mutations are benign in patients indicating additional mechanisms must restrict enhancers to their correct targets. Here, we identify these mechanisms by examining how similar regulatory conflicts were resolved by evolution. Specifically, we dissected how a new gene, *Zfp42*, inserted in an existing TAD in placental mammals without adopting or disrupting the conserved expression of its gene, *Fat1*. In ESCs, TAD partitioning physically separates *Zfp42* and *Fat1* with distinct enhancers that drive their independent activity. By contrast, in embryonic limbs, inactive *Zfp42* shares *Fat1*'s intact TAD but does not respond to active *Fat1* enhancers. Rather, *Zfp42*'s promoter is rendered inert to enhancers by context-dependent DNA methylation. Diverse mechanisms thus enabled the incorporation of independent *Zfp42* regulation in the *Fat1* locus. Moreover, genome-wide, we find that most TADs similar contain multiple independently expressed genes. We therefore propose the capacity to resolve regulatory conflicts is a generalisable feature of genomes and must be accounted for when interpreting genomic variants in patients.

**Conflict of Interest:** None declared

## E16 PGS: HOW CLOSE IS IT TO CLINICAL APPLICABILITY

### E16.1 Realising the benefit to healthcare through population-wide application of polygenic risk scores

***Gil McVean***

Realising the benefit to healthcare through population-wide application of polygenic risk scores

Polygenic risk scores, which combine the effects of variants from across the genome to characterise an individual's inherited risk for disease, have the potential to identify people who can benefit from enhanced screening, preventative programs and early intervention but who are currently invisible to healthcare practice. However, delivering the benefit of such technologies in real-world settings requires solving many challenges, including: establishing analytical stability of the technology; ensuring validity of the risk predictions within the population to which it will be applied and across the breadth of context (including age, sex, ethnicity and geography); providing an evidence base for the clinical benefit that can be achieved within the pathways where such information is deployed; and demonstrating implementation feasibility. Using examples from our own studies, I will assess where we are on the path to successful realisation of the promise of precision prevention.

**Conflict of Interest:** None declared