European Journal of Human Genetics (2019) 27:748–869
https://doi.org/10.1038/s41431-019-0407-4

MEETING ABSTRACTS

Abstracts from the 51st European Society of Human Genetics Conference: Oral Presentations

June 16–19, 2018, Fiera Milano Congressi, Milan Italy

Sponsorship: Publication of this supplement is 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".

Plenary Sessions

PL1 Opening plenary lecture

PL1.1
Leena Peltonen Lecturer - Complex Genetics

T. Lappalainen[1,2]

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Detailed characterization of cellular effects of genetic variants is essential for understanding biological processes that underlie genetic associations to disease, to improve the interpretation of the personal genome, and to characterize the genetic architecture of molecular variation. This has inspired large consortium projects to create and integrate population-scale genome data with transcriptome data – as well as other molecular phenotype data – in human populations. The catalogs of genetic effects on the transcriptome across multiple human tissues and conditions now allows downstream discovery in diverse questions in genetics, including joint effects of regulatory and coding variants underlying modified penetrance of disease-causing variants, and novel methods to understand variation in gene dosage in human populations and patients.

T. Lappalainen: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Merck.

PL1.2
Recent advances in mutational signatures of human cells

S. Nik-Zainal

Department of Medical Genetics, Cambridge, United Kingdom

A cancer genome contains the historic mutagenic activity that has occurred throughout the development of a tumour. While driver mutations were the main focus of cancer research for a long time, passenger mutational signatures - the imprints of DNA damage and DNA repair processes that have been operative during tumorigenesis - are also biologically informative. In this lecture, I provide a synopsis of this concept, describe the insights that we have gained through combinations of computational analysis and experiments in cell-based systems, and showcase how we have developed the concept into applications that we hope to translate into clinical utility in the near future.

S. Nik-Zainal: None.

PL2 What's New and Late Breaking Session

PL2.1
Genomic sequencing 15,000 healthy elderly individuals - Implications for clinical genetics

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Introduction: The variable penetrance of pathogenic variants remains a major challenge to clinical genetics practice, particularly in predictive testing of hereditary cancer risk genes. Ascertainment bias towards affected individuals and their families has in part shaped our understanding of gene penetrance. Here we report, for the first time, rates of pathogenic variation in hereditary cancer genes across thousands of healthy elderly individuals, ascertained without reference to clinical phenotype or family history, enrolled in an aspirin primary prevention trial.

Materials and Methods: Individuals enrolled in the ASPirin in Reducing Events in the Elderly (ASPREE) Healthy Ageing Biobank were screened using a 760 gene 'super-panel' including pan-cancer gene coverage. The average age was 75 years (min 65 years, max. 98 years), and all participants were confirmed to be free of life-threatening cancer diagnoses, cardiovascular disease or cognitive decline at time of enrolment. Medical records, family history and clinical data were mined for evidence of cancer phenotypes in mutation carriers.

Results: We detected hundreds of pathogenic and high-confidence predicted-deleterious mutations in hereditary cancer genes in elderly individuals (>10,000 samples sequenced Feb 2018). Mutations were often detected in the absence of any reported disease phenotype or family history. Overall, pathogenic variants were found at a comparable rate to population-based cohorts, suggesting over-representation of non-penetrant individuals in this study.

Conclusions: Pathogenic germline mutations in cancer predisposition genes may exist more commonly in the population than expected, raising questions about clinical actionability, risk and penetrance.

P. Lacaze: F. Consultant/Advisory Board; Modest; BC Platforms. **R. Sebra:** A. Employment (full or part-time); Significant; Sema4. **M. Riaz:** None. **R. Woods:** None. **I. Winship:** None. **E. Schadt:** A. Employment (full or part-time); Significant; Sema4. **J. McNeil:** None.

PL2.2
CRISPR-QTL mapping as a genome-wide association framework for cellular genetic screens of the noncoding genome

M. Gasperini[1], A. Hill[1], J. L. McFaline Figueroa[1], B. Martin[1], C. Trapnell[1], N. Ahituv[2], J. Shendure[1,3,4]

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Almost all noncoding variants returned from whole genome sequencing (WGS) cannot be confidently interpreted. Although most are thought to disrupt gene function by disrupting regulatory-elements such as enhancers, it is still largely unknown which noncoding regions constitute true enhancers, nor which genes each enhancer influences. To empirically address this, we have developed CRISPR-QTL mapping, a framework in which large numbers of noncoding perturbations are introduced to each cell on an isogenic background, followed by single-cell RNA-sequencing (scRNA-seq). This framework is borrowed from conventional human expression quantitative loci (eQTL) studies, but with individual humans replaced by individual cells; variants replaced by 'unlinked' combinations of guide RNA-programmed perturbations per cell; and tissue-level RNA-seq of many individuals replaced by scRNA-seq of many cells. We applied CRISPR-QTL mapping to evaluate 1,119 candidate enhancers with no strong *a priori* hypothesis as to their target gene(s). Perturbations were made by a nuclease-dead Cas9 tethered to KRAB, and introduced at a mean 'allele frequency' of 1.1% into a population of 47,650 profiled human K562 cells (median of 15 gRNAs identified per cell). We tested for differential expression of all genes within 1 megabase of each candidate enhancer, evaluating 17,584 potential enhancer-target gene relationships in one experiment. We identify 128 *cis* CRISPR-QTLs whose targeting resulted in downregulation of 105 nearby genes (of which 49 genes are thought to underlie disease). We anticipate that the power of CRISPR-QTL mapping will facilitate the comprehensive characterization of enhancer-gene relationships, thus advancing interpretation of noncoding variants discovered through WGS.

M. Gasperini: None. **A. Hill:** None. **J.L. McFaline Figueroa:** None. **B. Martin:** None. **C. Trapnell:** None. **N. Ahituv:** None. **J. Shendure:** None.

PL2.3

Elimination of aneuploid cells in the early mammalian embryo

S. Singla, M. Zernicka-Goetz

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Whole chromosomal aneuploidies - loss and/or gain of chromosomes from a euploid complement - are responsible for the low fecundity in humans. Here we use mouse embryo as a model system to understand how mosaic aneuploidy affects embryonic development. To experimentally induce chromosome missegregation in the cleavage stage embryos, we inhibit the master spindle assembly checkpoint kinase Mps1, which results in a high percentage of aneuploid cells in the embryos. We introduce mosaicism in the system by making chimeras using euploid and aneuploid cells. Detailed analysis of such chimeras revealed that during blastocyst maturation abnormal cells contributing to the inner cell mass undergo preferential elimination up to 40%. Using an in vitro culture system capable of recapitulating in vivo mosaic embryos during peri-implantation development and time-lapse imaging, we have found aneuploid cells are further preferentially eliminated from the epiblast via apoptosis. Moreover, we demonstrate that autophagy genes are significantly upregulated in aneuploid cells. Using pharmacological treatments to knockdown genes involved in autophagy apoptosis, we have found the onset of autophagy-mediated apoptosis during embryogenesis. Overall, our findings suggest that p53-mediated autophagy promotes apoptosis of aneuploid cells in mouse preimplantation embryos. This work gives an insight into the mechanisms behind subfertility, developmental defects, and miscarriages during pregnancy. Funding Wellcome Trust; Cambridge Commonwealth, European and International Trust.

S. Singla: None. **M. Zernicka-Goetz:** None.

PL2.4

***SLC10A7* mutations in human and mouse cause a skeletal dysplasia with amelogenesis imperfecta mediated by GAG biosynthesis defects**

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Skeletal dysplasias with multiple dislocations are a group of severe disorders characterized by dislocations of large joints, scoliosis, short stature and a variable combination of cleft palate, heart defects, intellectual disability and obesity. With the help of massively parallel sequencing technologies, the majority of these rare disorders have been linked to pathogenic variants in genes encoding glycosyltransferases ("linkeropathies"), sulfotransferases, epimerases or transporters, required for glycosaminoglycan (GAG) biosynthesis. These findings support the existence of a new group of inborn errors of development defined by impaired GAG biosynthesis. However, several findings suggest that GAG synthesis is more complex than previously described and that there are a number of partners of unknown function still to be identified.

Using exome sequencing, we identified homozygous mutations in *SLC10A7* in five individuals with a skeletal dysplasia with dislocations and amelogenesis imperfecta. Common features were severe growth retardation< -3SD, cleft palate, yellow/brown teeth, knee dislocations, spine anomalies and advanced carpal ossification. *SLC10A7* encodes a 10-transmembrane-domain transporter located at the plasma membrane, with a yet unidentified substrate. Functional studies in vitro demonstrated that *SLC10A7* mutations were loss-of-functions mutations reducing SLC10A7 protein expression. We generated a *Slc10a7*$^{-/-}$ mouse model which displayed short long bones, growth plate disorganization and tooth enamel anomalies, recapitulating the human phenotype. Furthermore, we identified decreased heparan sulfate levels in *Slc10a7*$^{-/-}$ mouse cartilage and patient fibroblasts. Finally, we found an abnormal N-glycoprotein electrophoretic profile in patient blood samples. Together, our findings support the involvement of SLC10A7 in glycosaminoglycan synthesis and specifically in skeletal and tooth development.

J. Dubail: None. **C. Huber:** None. **S. Chantepie:** None. **S. Sonntag:** None. **B. Tüysüz:** None. **E. Mihci:** None. **C. Gordon:** None. **E. Steichen-Gersdorf:** None. **J. Amiel:** None. **B. Nur:** None. **I. Stolte-Dijkstra:** None. **A. van Eerde:** None. **K. van Gassen:** None. **C. Breugem:** None.

A. Stegmann: None. **A. Bruneel:** None. **N. Seta:** None. **A. Munnich:** None. **D. Papy-Garcia:** None. **M. De La Dure-Molla:** None. **V. Cormier-Daire:** None.

PL2.5

Local and global chromatin interactions are altered by large genomic deletions associated with human brain development

X. Zhang[1], Y. Zhang[2], X. Zhou[1], C. Purmann[1], M. S. Haney[1], T. R. Ward[1], J. Yao[3], S. M. Weissman[3], A. E. Urban[1]

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Large copy number variants (CNVs) in the human genome are strongly associated with common neurodevelopmental, neuropsychiatric disorders such as schizophrenia and autism. Using Hi-C analysis of long-range chromosome interactions, including a novel approach for haplotype-specific Hi-C analysis, and ChIP-Seq analysis of regulatory histone marks, we studied the epigenomic effects of the prominent heterozygous large deletion CNV on chromosome 22q11.2, with replication analyses for the CNV on 1q21.1 [BioRxiv 182451].

There are local and global gene expression changes as well as pronounced and multilayered effects on chromatin states, chromosome folding and topological domains of the chromatin, that emanate from the large CNV locus. Regulatory histone marks are altered in the deletion flanking regions, in opposing directions for activating and repressing marks. Histone marks are changed along chromosome 22q and genome wide. Chromosome interaction patterns are weakened within the deletion boundaries and strengthened between the deletion flanking regions. The long-range folding contacts between the telomeric end of chromosome 22q and the distal deletion-flanking region are increased. Using haplotype specific Hi-C analysis we determined that on the chromosome 22q with deletion the topological domain spanning the CNV boundaries is deleted in its entirety while neighboring domains interact more intensely with each other. Finally, there is a widespread and complex effect on chromosome interactions genome-wide, i.e. involving all other autosomes, with some of this effect tied to the deletion region on 22q11.2.

These findings suggest novel principles of how such large genomic deletions can alter nuclear organization and affect genomic molecular activity.

NIH grants

X. Zhang: None. **Y. Zhang:** None. **X. Zhou:** None. **C. Purmann:** None. **M.S. Haney:** None. **T.R. Ward:** None. **J. Yao:** None. **S.M. Weissman:** None. **A.E. Urban:** None.

PL2.6

miR-204 overexpression exerts a protective role in inherited retinal diseases

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Introduction: Inherited retinal diseases (IRDs) are progressive neurodegenerative conditions of the retina that represent a main cause of genetic blindness in the Western world. Their high genetic heterogeneity hinders the development of effective gene-based therapies. We have recently demonstrated that the microRNA miR-204 is essential for retinal function and plays a pathogenic role in IRD in humans. Therefore, we investigated the potential therapeutic action of this microRNA in IRDs.

Materials and Methods: We delivered by subretinal injection an adeno-associated viral vector carrying the miR-204 precursor to the *Aipl1* knockout and the P347S-*RHO* transgenic mouse lines, models of autosomal recessive and dominant forms of IRDs, respectively. The impact on retinal function and degeneration was assessed by electroretinographic and immunohistological analyses.

Results: We detected a notable improvement of the ERG response in the miR-204-injected eyes of P347S-*RHO* mice. This effect persisted for two months post-injection and was prevalent under photopic conditions that reflect cone activity. We also observed a preservation of cone photoreceptors and a significant decrease in apoptotic photoreceptor cells. Photoreceptors were better preserved also in the miR204-injected eyes of *Aipl1* knockout mice. Transcriptome analysis suggested that dampening of microglia activation represents one of the main mechanisms underlying the neuroprotective effect of miR-204.

Conclusions: Our findings indicate that the subretinal delivery of miR-204 attenuates retinal degeneration in IRD mouse models and preserves retinal function, supporting the gene-independent therapeutic potential of this microRNA.

Supported by the Foundation Fighting Blindness and the Italian Telethon Foundation.

M. Karali: None. **E. Marrocco:** None. **M. Pizzo:** None. **I. Guadagnino:** None. **A. Carissimo:** None. **I. Conte:** None. **E.M. Surace:** None. **S. Banfi:** None.

LB1

***NAXD*mutations cause a novel neurodegenerative disorder exacerbated by febrile illnesses**

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Introduction: Niacin (vitamin B3) is the precursor for the nicotinamide nucleotide cofactors NAD^+ and $NADP^+$, which are critical coenzymes for a number of key metabolic processes involving oxidation/reduction, including glycolysis, the citric acid cycle and the mitochondrial respiratory chain. Hydration of their reduced forms (either spontaneously if the temperature is elevated or following specific enzymatic processes) leads to the accumulation of NADHX and NADPHX, which are toxic and can inhibit a number of dehydrogenases. Highly conserved dehydratase NAD(P)HX Dehydratase (NAXD) is one of the key intracellular NADHX/NADPHX repair enzymes.

Materials and Methods: We describe 6 unrelated children with generally fever-induced recurrent episodic neurological impairment and/or cardiac failure with early demise, and its genetic basis.

Results: Genomic sequencing revealed biallelic *NAXD* variants in all cases, and *in silico* analyses predicting potential pathogenicity for each of them. Pathogenicity was functionally validated using patient fibroblasts through a combination of transcript analysis (splicing abnormality in one case), demonstration of markedly elevated S-NADHX, R-NADHX and cyclic-NADHX, impaired mitochondrial function, and reduced viability in culture media containing galactose and azide but not glucose. Transduction with wildtype *NAXD* restored damaged metabolite levels back to normal. Finally, in vitro studies revealed that the two missense variants [(p.(Gly63Ser) and p.(Arg608Cys)] were thermolabile, and specific ATP-dependent NADHX dehydratase activity showed abnormal enzyme kinetics.

Conclusion: This is the first report of a human disorder caused by NAXD deficiency, showing remarkable clinical overlap with the other known defect of the NADH/NADPH repair pathway, NAXE deficiency.

N.J. Van Bergen: None. **Y. Guo:** None. **J. Rankin:** None. **N. Paczia:** None. **J. Becker-Kettern:** None. **L.S. Kremer:** None. **A. Pyle:** None. **J. Conrotte:** None. **C.J. Ellaway:** None. **P. Procopis:** None. **K. Prelog:** None. **T. Homfray:** None. **J. Baptista:** None. **E. Baple:** None. **M. Wakeling:** None. **S. Massey:** None. **D.P. Kay:** None. **A. Shukla:** None. **K.M. Girisha:** None. **L.E.S. Lewis:** None. **S.D. Santra:** None. **R. Power:** None. **P. Daubeney:** None. **J. Montoya:** None. **E. Ruiz-Pesini:** None. **R. Kovacs-Nagy:** None. **M. Pritsch:** None. **U. Ahting:** None. **D.R. Thorburn:** None. **H. Prokisch:** None. **R. Taylor:** None. **J. Christodoulou:** None. **C. Linster:** None. **S. Ellard:** None. **H. Hakonarson:** None.

LB2

GTF2I regulates social behavior by promoting neuronal maturation: implications for Williams-Beuren and 7q11.23 microduplication syndromes

A. Lopez Tobon[1,2], R. Shyti[1,2], E. Villa[1], N. Caporale[1], S. Trattaro[1,2], C. Cheroni[1], F. Troglio[1], B. Marcó de la Cruz[1], M. Gabriele[1], N. Malerba[1], F. Kooy[3], G. Merla[4], G. Testa[1,2]

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Copy number variations at the 7q11.23 locus comprising 26-28 genes cause neurodevelopmental disorders with shared and opposite phenotypes. Deletion cause Williams-Beuren syndrome (WBS) characterized by hypersociability, anxiety and intellectual disability, while duplication give rise to 7q duplication syndrome (7DupASD) with severe impairments in language, anxiety and autism spectrum disorder (ASD). Despite the well-established genetic underpinnings of these disorders effective treatments are unavailable. Converging evidence points to GTF2I as a major player mediating the cognitive-behavioral phenotype associated with WBS and 7DupASD. We showed previously in patient-derived iPSC and neural progenitors that GTF2I associates with LSD1 to suppress expression of genes involved in neuronal function, whereas inhibition of LSD1 restored gene expression balance. Here we used mouse models together with patient-derived 3D cortical organoids and single-cell transcriptomics to dissect the molecular foundations of WBS and 7DupASD. Mice with duplication of GTF2I faithfully recapitulated the ASD-like phenotype in the three-chambered sociability apparatus. Remarkably, inhibition of LSD1 with an irreversible inhibitor, rescued the ASD-like phenotype. Bulk and single-cell RNAseq from cortical organoids revealed divergent dynamics of neuronal maturation with accelerated maturation in 7DupASD and decelerated in WBS organoids resulting in a divergent neural fate, which was confirmed by relative abundance of basal progenitor populations. Strikingly, knockdown of GTF2I in 7DupASD organoids rescued neuronal maturation rate to control levels. These findings suggest that GTF2I-LSD1 axis plays a critical role in social behavior and cognition by controlling the timing of cortical maturation.

A. Lopez Tobon: None. **R. Shyti:** None. **E. Villa:** None. **N. Caporale:** None. **S. Trattaro:** None. **C. Cheroni:** None. **F. Troglio:** None. **B. Marcó de la Cruz:** None. **M. Gabriele:** None. **N. Malerba:** None. **F. Kooy:** None. **G. Merla:** None. **G. Testa:** None.

LB3

Biallelic mutations of the dihydroceramide desaturase *DEGS1* gene cause a novel hypomyelinating leukodystrophy with a therapeutic hope

A. Pujol[1], D. Pant[1], A. Schluter[1], N. Launay[1], S. Aguilera[2], M. Willis[3], L. Colleaux[4], I. Dorboz[5], M. Patterson[6], J. Gleeson[7], O. Boespflug-Tanguy[8], A. Fatemi[9], J. Terriente[10], C. Pujades[11]

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Sphingolipid imbalance is the culprit in a variety of neurological diseases, some affecting the myelin sheath as the lysosomal storage disorders Krabbe and Metachromatic leukodystrophy, among others. Here we have used whole exome sequencing to uncover the endoplasmic reticulum lipid desaturase *DEGS1* as the causative gene of a novel leukodystrophy. This enzyme catalizes the final step in the *de novo* biosynthesis of ceramides controlling the conversion of dihydroceramide (DhCer) to ceramide (Cer), and may play intriguing roles in antiapoptotic, and anabolic and insulin signalling. Through international collaborative networks, we have identified eighteen patients from twelve unrelated families carrying biallelic deleterious variants in the *DEGS1* gene. Common features among the cases include severe hypomyelination, cerebellar atrophy, and thinning of the corpus callosum on MRI, accompanied by speech abnormalities, developmental delay and intellectual disability, suggesting a critical role of DEGS1 and ceramide metabolism in myelin development and maintenance. Patients' fibroblasts exhibited accumulation of DhCer, which increased ROS production. Knockdown of the *DEGS1* ortholog in *Danio rerio* recapitulated the biochemical imbalance of ceramides, showed diminished numbers of mature oligodendrocytes and impaired locomotor abilities. The enzymatic inhibition of ceramide synthase, one step prior to DEGS1 in the pathway, by the multiple sclerosis treatment Fingolimod (FTY720), restored the critical DhCer/Cer balance, and ameliorated the cellular and locomotor phenotypes in the zebrafish model. These results pave the way to clinical translation, illustrating the transformative, timely impact of clinical genomics in patient care. Funded by Marató TV3; Fondo de Investigación Sanitario ISCiii, Hesperia Foundation.

A. Pujol: None. **D. Pant:** None. **A. Schluter:** None. **N. Launay:** None. **S. Aguilera:** None. **M. Willis:** None. **L. Colleaux:** None. **I. Dorboz:** None. **M. Patterson:** None. **J. Gleeson:** None. **O. Boespflug-Tanguy:** None. **A. Fatemi:** None. **J. Terriente:** None. **C. Pujades:** None.

PL3 Mendel Lecture

PL3.1
CRISPR-Cas9: How bacteria revolutionize genome engineering

E. Charpentier[1,2]

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The CRISPR-Cas9 system has recently emerged as a transformative technology in biological sciences, allowing rapid and efficient targeted genome editing, chromosomal marking and gene regulation in a large variety of cells and organisms. The system consists of Cas9, an enzyme that can be programmed with RNA guides to target site-specifically any DNA sequence of interest. The system is efficient, versatile and easily programmable. CRISPR-Cas9 research has developed into one of the most dynamic and fastest-moving fields in life sciences and holds great promise for future biotechnical and biomedical applications. The CRISPR-Cas9 system is remarkably simple in its design, close to a plug-and-play method, which can therefore be easily leveraged for a large variety of gene targeting. Application of the system has been extraordinarily broad, including among many others the generation of transgenic animals, genetic modification of various eukaryotic cell types, and genetic modification of plants and crops. Tool and kit service companies offer CRISPR-Cas9-related products and at least three biotechnology companies have been founded during the past years to develop the technology for the treatment of serious human genetic disorders, with probably many more to come. Already, the CRISPR-Cas9 system is an integral and critical part of the toolbox for any researcher who intends to modify genetic information by means of targeted introduction or correction of mutations, replacement of genes, modification of DNA or modulation of transcription in any cell or organism - and the applications of this breakthrough technology are continuing to increase at a rapid pace.

E. Charpentier: None.

Concurrent Symposia

S01 Prenatal Genetics - joint with EMPAG

S01.1
The utility of sizing up cell-free fetal DNA

R. Chiu

Hong Kong, Hong Kong

Cell-free DNA molecules circulate in human plasma in the form of short fragments. Cell-free fetal DNA demonstrate an even shorter size profile than the circulating maternal DNA in maternal plasma. This observation could be exploited as a means to differentiate the fetal DNA population from the maternal DNA in maternal circulation. For example, aneuploidies of fetal origin may result in aberrant genomic representation among the shorter DNA molecules in the maternal plasma sample. While noninvasive prenatal testing by maternal plasma DNA sequencing generally provides high sensitivities and specificities for the detection of common fetal chromosomal aneuploidies, false-positive results may occur. We reasoned that the combined used of cell-free DNA counting and size measurements may allow one to identify the false-positive cases. We applied the combined protocol to test for fetal chromosomal aneuploidies among 20,000 cases seeking NIPT screening. The approach was able to identify sex chromosome aneuploidies of maternal origin, copy number variations of maternal origin, artefacts caused by sequencing biases or conditions that perturbed the maternal plasma DNA profile, such as systemic lupus erythematosus. The adoption of the approach led to a substantial reduction in the number of reports that would otherwise become a false-positive result with respect to the fetal chromosome profile. Next, we reasoned that if cell-free fetal DNA molecules were shorter than the cell-free maternal DNA molecules, the fragmentation sites of the two different population of DNA molecules might be different. Interestingly, we demonstrated that the ending sites between the cell-free fetal and maternal DNA molecules indeed showed consistent differences. Some of the ending sites were reproducibly detectable from maternal plasma collected from different pregnancies and served as a means to assess the fetal DNA fraction in the sample. (supported by the Research Grants Council of the Hong Kong SAR Government under the Theme-based research scheme (T12-403/15-N).)

S01.2
Mommy and me sequencing: incidental detection of maternal abnormalities via non-invasive prenatal testing

D. W. Bianchi

National Institute of Child Health and Human Development, Bethesda, MD, United States

Noninvasive prenatal testing (NIPT) using circulating cell-free (cf) DNA in the plasma is currently the most mature example of implementation of genomics into clinical medicine. Since 2011, the testing has been widely incorporated

into prenatal care, resulting in a significant reduction in the number of invasive diagnostic procedures and cytogenetic analyses performed globally. Most women who undergo prenatal cf DNA screening do not realize that the testing analyzes placental as well as maternal DNA, and furthermore, that maternal DNA abnormalities are sometimes the underlying biological explanation for false positive results. When the NIPT result is abnormal and the fetal karyotype or chromosome microarray results are normal, the differential diagnosis includes confined placental mosaicism, twin demise and maternal findings. In this presentation I will review the increasing number of maternal abnormalities that have been detected via NIPT, including autoimmune and metabolic conditions, benign and malignant tumors, autosomal and sex chromosome aneuploidies, and copy number variants with clinical significance. The ethical and medical management implications of the release of these secondary maternal findings will also be discussed.

D.W. Bianchi: None.

S01.3
Supporting informed choice for non-invasive prenatal testing in clinical practice: How well are we doing?

C. Lewis

North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom

Non-invasive prenatal testing (NIPT) using cell-free DNA in maternal blood is an advanced screening test which has been shown to be highly accurate for Down syndrome as well as trisomies 13 and 18. The clinical advantages of NIPT are that the test is safe, easy to conduct and can be administered within the first trimester of pregnancy. Nevertheless, there are concerns that NIPT may compromise informed decision-making as the risk-free nature of the test may lead parents to agree to testing without adequate consideration of the potential outcomes including a positive NIPT result (thus requiring consideration about invasive testing) or an inconclusive result. Moreover, because NIPT is a blood test, consent procedures may potentially become less rigorous than those used for invasive testing and the ease with which the test can be conducted may promote routinisation of testing. A number of studies have been conducted in recent years to establish whether NIPT is undermining informed decision-making. In this talk I will present key findings from this body of literature as well as presenting the findings from my own research from the RAPID study where we adapted the Multidimensional Measure of Informed Choice (MMIC) developed by Marteau et al. (2001) for NIPT. The adapted measure was used in both a research and a routine clinical setting and rates of informed choice were measured and compared. Recommendations for practice relating to how best to facilitate informed decision-making in the prenatal clinic will also be discussed.

C. Lewis: None.

S02 DNA damage and repair in cancer

S02.1
DNA damage and non coding RNA in cancer and ageing

F. d'Adda di Fagagna

IFOM Foundation, Milan, Italy

Cellular senescence in the context of ageing and cancer is often associated with DNA damage generation and DNA damage response (DDR) activation. I will discuss the evidence in support of the presence of DNA damage in senescence cells and the emerging role of non coding RNA in DDR signaling and the potential of their inhibition by new therapeutic approaches.

F. d'Adda di Fagagna: None.

S02.2
Differential DNA repair across human chromosomes shapes somatic mutation landscapes

F. Supek[1], B. Lehner[2,3,4]

[1]Institut de Recerca Biomedica (IRB Barcelona), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, [2]Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, [3]Universitat Pompeu Fabra (UPF), Barcelona, Spain, [4]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Cancer genome sequencing has revealed considerable variation in somatic mutation rates across the human genome, with mutations accumulating faster in heterochromatic late replicating regions and slower in early replicating euchromatin. We identify variable DNA mismatch repair (MMR) as the basis of this domain-scale variation. While regional mutation rates are broadly stable between tumors, there are systematic differences that accurately reflect the cancer cell-of-origin, related to changes in replication timing and gene expression. However, mutations arising after the inactivation of MMR are no longer enriched in late replicating heterochromatin relative to early replicating euchromatin. This implicates differential DNA repair as the primary cause

of the large-scale regional mutation rate variation across the human genome (Supek and Lehner, 2015 Nature).

At the smaller, sub-megabase scale, we find that differential MMR also shapes gradients in mutation rates across gene bodies. In particular, we examined clustered mutation patterns across >1,000 tumor genomes and identified a novel and prevalent mutational signature matching the error-prone DNA polymerase eta (POLH). Such clustered mutations occur in tumors associated with carcinogen exposure and they target H3K36me3-marked chromatin, commonly found at 3' ends of transcribed genes. In the absence of carcinogens however, the H3K36me3 regions have a considerably lower mutation rate. This is because the canonical, error-free MMR is normally targeted towards the H3K36me3 histone mark, protecting active genes from alterations. Carcinogens therefore not only increase mutation burden, but can also redistribute mutations to the more important regions of the genome by activating error-prone DNA repair. We predict this can contribute a substantial mutation load in many tumors such as melanoma, gastrointestinal tract and lung cancers, with high potential to yield driver mutations (Supek and Lehner, 2017 Cell).

F. Supek: None. **B. Lehner:** None.

S03 Genome Organization and Function

S03.2
The Dynamics of 3D Chromatin Structures Influence Transcription and Morphogenesis

G. Andrey

Max Planck Institut for Molecular Genetics, Berlin, Germany

The complex gene expression of developmental genes often relies on the communication between promoters and long-range regulatory elements called enhancers. Enhancers and promoters can interact in the nuclear space in a tissue-specific or tissue-unspecific manner. Yet little is known about the functional role of these different modes of interactions. Here we present two in-depth functional studies illustrating how these modes of interaction contribute to achieve specific expression patterns and transcriptional robustness in vivo. In the first case, the disruption of a pre-established, tissue-unspecific interaction between the *Shh* promoter and its limb enhancer strongly reduces *Shh* transcription. In the second case, a tissue-specific enhancer-promoter interaction modulates the fore- and hindlimb activity of an enhancer to drive *Pitx1* transcription in hindlimb only. The disturbance of this particular communication in human patients and in mice with structural variations leads to gene *endo-activation* and a partial arm-to-leg transformation. In conclusion, our data not only suggest an interplay between enhancer specificity, transcriptional robustness and 3D chromatin dynamics but also provide a framework to understand the pathomechanism of many non-coding mutations.

G. Andrey: None.

S03.3
Transgenerational inheritance: The role of CTCF and 3D genome organization

V. Corces

Emory University, Atlanta, GA, United States

Mechanisms by which epiphenotypes are transmitted between generations through the paternal germline remain poorly understood. The nuclei of mammalian sperm are thought to be highly condensed and the DNA mostly covered by protamines with only a few retained nucleosomes. Epigenetic information stored in the form of DNA methylation is erased from paternal chromosomes after fertilization. Results from our lab suggest a more complex picture of the mouse sperm epigenome, indicating the presence of multiple histone modifications at retained nucleosomes. Most promoters in mouse sperm contain the elongating form of RNA polymerase II, and are flanked by several positioned nucleosomes marked by a variety of active histone modifications. The sperm genome is bound by several transcription factors, including Mediator, the pioneer factor FoxA1, and estrogen receptor alpha. These proteins are found at promoters, enhancers, and super-enhancers, many of which are active in mESCs or adult tissues. CTCF and cohesin are also present in sperm DNA, where they organize the sperm genome into domains that overlap extensively with those found in mESCs. This information suggests that mammalian sperm contain a rich and complex palette of epigenetic information that could be altered by environmental factors to paint novel phenotypic outcomes in the next generation. This is supported by experiments in which pregnant females exposed to endocrine disruptor chemicals give rise to progeny showing a variety of phenotypes, including obesity. The obese phenotype is transmitted between generations in the absence of further exposure. Experiments indicate that approximately 110 new protein binding sites are present in the sperm and fat tissue of obese mice from the F1 through the F4 generations. These new binding sites correspond to CTCF and ERalpha, suggesting that effects of these proteins on 3D chromatin organization and transcription of specific genes are responsible for the establishment and transmission of epiphenotypes.

V. Corces: None.

S04 Genetics of dizziness

S04.2
Genetic basis of Meniere's disease

J. Lopez-Escamez[1,2,3]

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Meniere Disease (MD) represents a heterogeneous group of relatively rare disorders with three core symptoms: episodic vertigo, tinnitus and sensorineural hearing loss involving low and medium frequencies. Although the majority of cases are considered sporadic, familial aggregation has been recognized in European and Korean populations, but the finding of familial MD genes has been elusive until the last few years. Detailed phenotyping and hierarchical cluster analyses have found several clinical predictors for different subgroups of patients, which may indicate different mechanisms of disease, including genetic and immune factors. The familial cluster include all cases with unilateral or bilateral MD, and most families show an autosomal dominant pattern of inheritance with incomplete penetrance and variable expressivity in European population. Whole-exome sequencing have found rare and novel variants in 6 genes associated with familial MD including *COCH, FAM136A, DTNA, PRKCB, SEMA3D* and *DPT genes;* however, no consistent gene has been described in multiple unrelated families. Although the significance of these variants remain to be established in animal models, the candidate genes are involved in very different functions such as protein phosphorylation, axonal guiding signalling or structural proteins in the cytoskeleton or extracellular matrix. So, familial MD shows genetic heterogeneity as it has been also demonstrated for syndromic and non-syndromic sensorineural hearing loss. To investigate the immunogenetic contribution in MD, we have also conducted a genome association study using a high density genotyping array containing covering loci associated with autoimmunity (Immunochip). Our group have identified a locus in the classical class I subregion of the MHC ~9 kb at 6p21.33 (31,081,878-31,090,401), being the leading signal rs4947296 (metanalysis,OR = 2.089 (1.661-2.627); $p = 1.39\text{x } 10^{-09}$). The variant is associated with bilateral MD in the Spanish population and it is enriched in patients with a comorbid autoimmune disorder. Moreover, rs4947296 is a trans-expression quantitatve trait locus (eQTL) which regulates gene expression of multiple genes in the tumor necrosis factor-like weak induced of apoptosis (TWEAK)/Fn14 pathway in peripheral mononuclear cells (PBMCs). By using genotype-conditioned PBMCs, we found an increase of NF-κB-mediated inflammatory response in the carriers of the risk genotype. Meniere disease shows clinical and genetic heterogeneity and common and rare variants may contribute to shape the phenotype in a given patient. Ongoing projects include exome sequencing in >100 familial MD patients or early onset sporadic cases and the analysis of two custom sequencing panels designed by our group using data-mining, phenotyping information and gene expression profiles in cochlear and vestibular supporting cells.

Acknowledgments JALE is supported by Instituto de Salud Carlos III (PI17/01644 Grant) by FEDER Funds and H2020-MSCA-ITN-2016-722046 from EU, Luxembourg National Research Fund (INTER/Mobility/17/11772209 Grant), Meniere's Society-UK and Asociación Sindorme de Meniere España (ASMES).

J. Lopez-Escamez: None.

S04.3
An approach to restoring vestibular function?

A. Forge[1]*, R. R. Taylor*[2]

[1]*London, United Kingdom,* [2]*UCL Ear Institute, London, United Kingdom*

The sensory epithelia of the cochlea and vestibular system are composed of sensory "hair" cells and non-sensory supporting cells. Supporting cells surround each hair cell and separated it from its neighbours. Hair cells derive their name from the organised bundle of projections from their apical surface. During development expression of the transcription factor Atonal Homologue 1(Atoh1) in precursor cells directs differentiation towards a hair cell fate. The first cells to do so activate Notch-Delta signalling in their immediate neighbours to inhibit Atoh1 expression. The laterally inhibited cells differentiate as supporting cells. Death of hair cells is a major cause of hearing loss and vestibular dysfunction, which manifests as dizziness, vertigo and imbalance. Ageing and certain drugs with ototoxic side-effects, most notably aminoglycoside antibiotics, are amongst the primary causes of hair cell death. When a hair cell dies the surrounding supporting cells close the lesions. In non-mammalian vertebrates, new hair cells are generated from the supporting cells, some following supporting cell division, others by direct conversion of supporting cells.

There is no hair cell regeneration in the mammalian cochlea. However, there is a limited capacity to regenerate hair cells in the mammalian vestibular system through direct phenotypic conversion of supporting cells. We have some evidence that spontaneous regeneration of hair cells may occur throughout life in the human vestibular system. Using human vestibular sensory epithelia in explant culture, hair cells were ablated by incubation in an aminoglycoside (gentamicin). Subsequent transduction of supporting cells with *ATOH1* using an Ad-2 viral vector resulted in generation of highly significant numbers of cells expressing the hair cell maker protein myosin VIIa. Cells expressing myosin VIIa were also generated after blocking the Notch signalling pathway with TAPI-1 but much less efficiently. Transcriptomic analysis following *ATOH1* transduction confirmed up-regulation of 335 putative hair cell marker genes, including several postulated downstream targets of *ATOH1*, as well as downregulation of 5 chromatin modifiers. Morphological analysis revealed numerous cells bearing dense clusters of microvilli at the apical surfaces. However, no cells bore organised hair bundles and several expected hair cell markers genes were not expressed. The results show a potential to induce conversion of supporting cells in the vestibular sensory tissues of humans, but that differentiation of fully functional hair cells is incomplete under the conditions used. This suggests the likely necessity of additional factors to achieve this goal.

R.R. Taylor: None.

S05 Large-scale genetic studies in complex diseases

S05.2
Large-scale sequencing studies in coronary artery disease

H. Schunkert

Munich, Germany

The primary manifestation of coronary disease occurs often suddenly and unexpectedly in form of myocardial infarction. Thus, the prediction of silent atherosclerotic alterations in coronary arteries is a highly relevant medical need. Recent genomic research identified numerous genetic variants that associate with a higher prevalence of coronary disease. At present, association with coronary artery disease has been demonstrated at more than 160 chromosomal locations with risk alleles increasing relative risk by 5-25% per allele. Moreover, genetic variants primarily affecting cardiovascular risk factors such as hypertension or LDL cholesterol were shown to affect the risk of coronary disease as well.

This enormous progress has been facilitated by genome-wide association studies. By nature, these studies focus on frequent alleles. Thus, the alleles that have been identified to increase the risk of coronary disease are also relatively frequent in our population. As a consequence, virtually all individuals of our population carry a variable degree of genetic predisposition for coronary artery disease. More recently, the focus turned to rare variants with more profound effects. In this regard, the domain of human genetics, i.e. family based research and counseling, received more attention – once again. The presentation will address how this information can be utilized for a better understanding of disease mechanisms as well as for genomic prediction of coronary artery disease.

S05.3
Harnessing large-scale genetics and genomics to derive biological insights in type 2 diabetes

M. McCarthy

Oxford, United Kingdom

Individual risk of common complex diseases such as diabetes (and the complications thereof) is influenced by variation at many hundreds (even thousands) of genetic risk loci, together with a range of exposures experienced over the life course. A deeper understanding of these factors, and the mechanisms through which they influence disease risk and development, is a prerequisite if we are to develop more effective strategies for prevention and treatment. Over the past decade there has been substantial progress towards these goals. The most recent genome-wide association study (involving nearly a million subjects) has extended the number of independent genetic signals for type 2 diabetes beyond 400. For a growing number of these loci, we now know which specific genetic variant is responsible, which regulatory element active in which tissue is disturbed, which downstream effector gene mediates the causal effect, and which pathophysiological process is consequently perturbed. By combining genome-wide association and sequence data, we have a clear description of the genetic architecture of this disease. These and other advances provide exciting translational opportunities. We use genetic associations to highlight novel human validated therapeutic targets and to evaluate those targets for both efficacy and safety. We use individual genetic profiles to explore the potential for stratification of disease risk. We use the combination of human genetics and large-scale omics data sets to identify and characterise biomarkers that may be of clinical value. We use human genetics to highlight which of many potentially modifiable environmental exposures are truly causal for type 2 diabetes, and represent promising

avenues for preventative intervention. And we use all of these data types to inform our understanding of what type 2 diabetes actually is, and how we can account for the evident heterogeneity with respect to both presentation and prognosis.

S06 Liquid biopsies in cancer

S06.3
Liquid Biopsies for Monitoring Temporal Genomic Heterogeneity

G. Siravegna

Torino, Italy

Liquid biopsy -tumor genotyping using circulating cell-free tumor DNA (ctDNA) in the blood- has been recently applied to molecularly profile cancer patients, monitoring tumor burden during therapy and uncovering novel mechanisms of resistance to targeted therapies. We used metastatic colorectal cancer (mCRC) as a model system to assess whether liquid biopsies could in principle uncover molecular mechanisms responsible for the emergence of resistance to targeted therapies, in particular to the monoclonal antibodies (moAbs) cetuximab and panitumumab. Emerging evidence indicates that escape from EGFR targeted therapy might be molecularly heterogeneous and involve several genes. We exploited plasma ctDNA analysis by Next Generation Sequencing (NGS) and Digital PCR (ddPCR) in mCRC patients to define gene alterations responsible for primary or acquired resistance to anti-EGFR therapies.

While most mCRC patients who receive anti EGFR antibodies develop KRAS mutations during the course of therapy we found that additional mutations affecting key nodes of the EGFR signaling pathway can also confer resistance. An interesting mCRC case harbored a novel MAP2K1 mutation (p.K57T) in tissue and liquid biopsies post EGFR blockade. To validate the biological and molecular relevance of this mutation in resistance to anti-EGFR therapy, we used a CRC cell line which harbored a MAP2K1 mutation when made resistant to CMAB. Biochemical analysis showed that MAP2K1 variants affecting position p.K57 drive constitutive activation of MEK and ERK and conferred resistance to CMAB and PMAB. However, the combination of the MEK inhibitor trametinib with either CMAB or PMAB restored sensitivity, suggesting a potential therapeutic strategy to overcome resistance to EGFR blockade caused by this mutation. Accordingly, the patient was treated with the combination of PMAB and trametinib. After 3 months, imaging demonstrated a reduction in size of the biopsied liver metastasis harboring the MAP2K1 mutation, but revealed that some other lesions had progressed. Pre-treatment plasma ctDNA was analyzed using NGS, confirming the presence of MAP2K1 variant, but unveiling an additional KRAS mutation. Longitudinal analysis of plasma samples unveiled that MAP2K1 mutant levels declined, while KRAS mutant increased markedly during therapy, indicating outgrowth of a resistant KRAS-mutant clone. Biopsy of a different liver metastasis' segment that progressed despite PMAB and trametinib revealed the same KRAS mutation identified in ctDNA.

In a separate study, we focused on Her2 amplified CRC. Targeting HER2 is effective in 30% of *ERBB2*-amplified metastatic colorectal cancer, and most sensitive cases eventually relapse. We studied the molecular evolution of individual metastases during treatment to discover spatially resolved determinants of resistance. ctDNA analysis identified alterations associated with lack of response in the majority of refractory patients. CtDNA profiles and lesion-specific radiographic reports revealed organ- and metastasis-private evolutionary patterns in patients who had initially responded. When radiologic assessments documented progressive disease in target lesions, response to HER2 blockade was often retained in other metastases. Genomic and functional analyses on samples and cell models from eight metastases of a patient co-recruited to a post-mortem study unveiled lesion-specific evolutionary trees and pharmacologic vulnerabilities. A correlation between lesions'size and contribution of distinct metastases to plasma ctDNA was observed. These data highlight the significance of coupling radiologic analysis, liquid biopsies and avatar models in precision oncology.

S07 Drug repurposing for treating genetic disorders

S07.1
Drug repurposing to improve cognitive defects in Down syndrome

L. Cancedda[1,2,3]

[1]*Genova, Italy,* [2]*Istituto Italiano di Tecnologia, Genova, Italy,* [3]*Istituto Telethon Dulbecco, Rome, Italy*

Down syndrome (DS) represents the leading cause of genetically-defined intellectual disability. Interestingly, a number of mouse models of DS are currently available and basic research has provided insights on possible molecular targets for pharmacological therapy aimed at treating cognitive impairment in these mice. Nevertheless, developing

new drugs able to act on newly discovered molecular targets is an extremely long, risky and expensive process that proves unsuccessful in most cases, especially for brain disorders. In this context, identifying new therapeutic indications for already existing and clinically used drugs (drug repurposing) may strongly reduce the time, risks and costs associated to classical drug discovery, leading to a faster track from the laboratory benches to the bedsides in the clinics. Here, we summarize recent finding from our laboratory on DS mouse models and we discuss the possibility of drug repurposing to recover cognitive deficits in DS mice in the view of potential translational applications in the near future.

S07.2
Drug repurposing for breast cancer prevention in BRCA1-mutation carriers

E. Nolan[1], J. E. Visvader[2], G. J. Lindeman[2,3]

[1]The Francis Crick Institute, London, United Kingdom, [2]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [3]The Royal Melbourne Hospital, Melbourne, Australia

Women who carry a germline mutation in *BRCA1* are at heightened risk of developing breast (and ovarian) cancer. Tumors often arise at an early age, with the peak relative risk occurring before 40 years of age. Although breast MRI has substantially improved screening for mutation carriers, this is not preventive and its impact on mortality is uncertain. Interval cancers (arising between screening) are not uncommon and screen-detected tumors can be relatively advanced. Furthermore, tumors are usually triple-negative with a basal-like phenotype, necessitating chemotherapy even if identified early. Asymptomatic *BRCA1* mutation carriers have few effective breast cancer prevention options beyond bilateral prophylactic mastectomy (BPM). Although BPM reduces the risk of breast cancer by up to 90%, only a subset of mutation carriers pursue this option. An effective prevention therapy therefore remains an important area of need.

RANKL is an important paracrine mediator of progesterone signaling in breast tissue. Activation of the RANKL/RANK signaling has been shown to accelerate tumor formation and to be associated with aggressive tumor behavior in vivo. Conversely, ablation of RANK signaling in mammary epithelium delays the onset of medroxyprogesterone/DMBA-driven mammary tumors. Accumulating evidence points to a role for progesterone/RANKL/RANK-mediated signaling in breast tumorigenesis in *BRCA1* mutation carriers. *BRCA1* mutation carriers may have elevated levels of serum progesterone during the luteal phase compared to non-carriers and possibly augmented RANKL activity due to reduced levels of the endogenous soluble decoy receptor osteoprotegerin (OPG).

Our recent findings suggest that the RANK and downstream NF-κB pathways are aberrantly activated in luminal progenitor cells in preneoplastic (ostensibly normal) *BRCA1*$^{mut/+}$ breast tissue. The augmented proliferation of these cells and their predilection for DNA damage suggest that they are prime cellular targets for basal-like breast cancers arising in *BRCA1*-mutation carriers. Targeting of RANK^{+} progenitors using a RANKL inhibitor in preclinical models has revealed a promising new prevention strategy for these high-risk women. These findings have raised the possibility that the RANKL inhibitor denosumab (currently only approved for the treatment of osteoporosis and bone metastases) could be 'repurposed' as a breast cancer prevention drug. Plans are underway to formally test this hypothesis in an international, randomized, double-blind, placebo-controlled multi-center phase 3 prevention study, 'BRCA-P', led by the Austrian Breast & Colorectal Study Group.

E. Nolan: None. **J.E. Visvader:** None. **G.J. Lindeman:** None.

S08 Microbiome and Virome

S08.1
Natural selection in humans and pathogens: sequencing the next deadly virus

K. Andersen

La Jolla, CA, United States

Our group is using viral genomics, computational biology, and traditional molecular biology, to gain insights into how viruses emerge and spread in human populations. By generating large-scale genomic datasets of Ebola virus and Lassa virus sequences from hundreds of infected patients, we dissected the trajectory of how these viruses evolved and spread across West Africa.

More recently, our group led efforts to sequence and analyze Zika virus dataset from local human transmissions and mosquitoes in across the Americas. Based on this data, we have been able to demonstrate that the Florida outbreak is much more complex than previously accepted. We show that multiple introductions happened into Florida in the spring of 2016 leading to sustained transmission chains. We show that these Zika virus lineages originated in the Caribbean and were likely brought to the United States via frequent cruise ship traffic. By modeling genomic data and mosquito abundance, we also show that Miami and Southern Florida is at particular risk for future Zika outbreaks.

S09 New Genomic Technologies

S09.1
Applications and analysis methods for nanopore sequencing data

J. Simpson

Ontario Institute for Cancer Research, Toronto, ON, Canada

Nanopore-based sequencing instruments have become commercially available in the past few years. In my talk I will review how these devices sequence DNA and discuss the analysis challenges they present. In particular I will focus on our work on developing signal-level algorithms that operate directly on the measurements made by the instrument. I will highlight how these algorithms can improve the accuracy of genome assemblies and detect base modifications by their subtle perturbations of the electrical current signal. Finally I will discuss how this technology can be used to assemble highly contiguous human genomes, detect methylation and find structural variation in cancer.

J. Simpson: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Oxford Nanopore Technologies.

S09.2
Whole organism lineage tracing

A. Schier

Cambridge, MA, United States

The development of systems ranging from embryos to metastases is governed by transcription factors that regulate specification trajectories and by cell division patterns that determine the lineage relationships of cells. I will illustrate how single-cell RNA sequencing and CRISPR-Cas9 genome editing provide new tools to reconstruct developmental trajectories and lineage trees at unprecedented scales (also see Farrell et al. Science 2018; Raj et al. Nature Biotechnology 2018).

S09.3
Genome-wide identification of human non-coding variants that affect regulatory elements

J. van Arensbergen[1], L. Pagie[1], M. de Haas[1], V. D. FitzPatrick[2], H. J. Bussemaker[2], B. van Steensel[1]

[1]Netherlands Cancer Institute, Amsterdam, Netherlands, [2]Columbia University, New York, NY, United States

We have recently developed 'survey of regulatory elements' (SuRE), a method that assays >150 million DNA fragments (each ~500bp) for their ability to drive transcription in a reporter plasmid. The throughput of the assay enables us to cover entire human genomes at approximately 30 fold, providing high-resolution genome-wide maps of autonomous enhancer and promoter activity. We now report the application of SuRE to systematically determine the impact of non-coding SNPs on promoter and enhancer activity. By surveying four entire human genomes that collectively harbor ~7 million SNPs, we identified thousands of SNPs that alter promoter or enhancer activity in K562 and HepG2 cells. We illustrate how these data help to identify candidate causal variants in GWAS and eQTL data.

J. van Arensbergen: A. Employment (full or part-time); Significant; GenX. **L. Pagie:** None. **M. de Haas:** None. **V.D. FitzPatrick:** None. **H.J. Bussemaker:** None. **B. van Steensel:** None.

S11 Epigenetics of the brain

S11.1
Epigenetic mechanisms regulating energy balance

R. Waterland

Baylor College of Medicine, Houston, TX, United States

Environmental influences during critical periods of prenatal and early postnatal development can cause persistent alterations in regulation of energy balance. Although a potentially important factor in the worldwide obesity epidemic, the fundamental mechanisms underlying such 'developmental programming' of energy balance are poorly understood, limiting our ability to intervene. Most studies of developmental programming of energy balance have focused on persistent alterations in food intake; energy expenditure has been relatively underemphasized. In particular, very few studies have evaluated developmental programming of physical activity. In the past several years we have studied three different mouse models of developmental programming of energy balance. In all three, we observed alterations in body weight regulation that persisted to adulthood, but no group differences in food intake. Rather, programming of energy balance appeared to be due to persistent alterations in energy expenditure and spontaneous physical activity. We are now taking a transgenic approach to assess the role of hypothalamic epigenetics in energy balance. Specifically, mice lacking the *de novo* DNA methyltransferase Dnmt3a in a specific class of hypothalamic neurons exhibit dramatic alterations in epigenetic regulation and, remarkably, voluntary physical activity,

with no change in food intake. Together, these observations indicate that epigenetic mechanisms in the central nervous system play a key role in regulating physical activity behavior and, consequently, energy balance.

R. Waterland: None.

S11.3
Epigenetics of major psychiatric disease: the circadian perspective

A. Petronis

Centre for Addiction and Mental Health, Toronto, ON, Canada

Putative epigenetic misregulation of genes sheds a new light on numerous epidemiological, clinical, and molecular complexities of psychiatric and other complex diseases. Epigenetic modifications of DNA can explain a series of non-Mendelian disease features such as discordance of identical twins, relatively late age of onset, fluctuating course and sometimes partial or even full recovery. Principles for experimental epigenomic studies in disease are still being developed. We recently discovered that DNA modifications in somatic differentiated cells of humans and mice exhibit circadian oscillations. Such oscillating cytosines (osc-modCs) were linked to the circadian transcriptome and explained a fraction of epigenetic variation, which has traditionally been treated as stochastic. We also found that osc-modCs were associated with the aging epigenome, where the osc-modCs preceded age-dependent DNA modification changes, and osc-modC amplitude strongly correlated with the magnitude of the aging effect. These findings indicate that epigenetic aging outcomes, to some extent, can be predicted from the circadian epigenomic parameters at a younger age. Finally, we performed a re-analysis of five epigenome-wide association studies, including schizophrenia and obesity. We detected that differentially modified cytosines in disease were significantly associated with human neutrophil osc-modCs (odds ratio ranged from 1.2 to 8.1). In summary, circadian DNA modifications provide a new perspective on several fundamental questions in biomedical research. In the broad sense, circadian rhythmicity, one of key adaptations of life on Earth, seems to also be mediating an organism's demise.

A. Petronis: None.

S12 Retinal diseases

S12.1
The non-coding morbid genome in inherited retinal diseases

E. De Baere

Ghent University and Ghent University Hospital, Ghent, Belgium

The integration of genome and transcriptome sequencing reveals an increasing number of non-coding mutations in Mendelian disease such as inherited retinal disease (IRD). IRD represents a major cause of early-onset blindness, having an overall prevalence of ~1/3,000. Whole exome (WES) and genome sequencing (WGS) revealed the underlying genetic cause in over 260 disease genes (RetNet) in ~60% of cases, with few novel disease genes identified in the remaining cases. The largest part of missing heritability of IRD is assumed to reside in non-coding regions, harboring cell-specific functional elements. The likelihood of finding non-coding variants increases in IRD subtypes with recognizable phenotypes and without or - in case of autosomal recessive disease - monoallelic coding variants in a known disease gene. Of the non-coding pathogenic variants, the majority are deep-intronic splicing variants, often leading to pseudo-exon inclusion and amenable to antisense oligonucleotide-mediated rescue, as demonstrated for *CEP290*, *USH2A* and more recently for *ABCA4*. Thus far, non-coding mutations in *cis*-regulatory elements (CREs) are more scarce, with only a few examples in choroideremia (*CHM*), Leber congenital amaurosis (*LCA5*, *NMNAT1*), North Carolina Macular Dystrophy (*PRDM13*, *IRX1*) and Stargardt disease (*ABCA4*). Particularly interesting CREs are ultraconserved non-coding elements (UCNEs) that are clustered in genomic regulatory blocks (GRBs) and that may act as distant enhancers. A recent search for RetNet genes and GRBs revealed genes in 12 GRBs. In three of these genes (*CHM, PRDM13, USH2A*), non-coding mutations have already been reported. In general, GRBs strongly coincide with topologically associating domains (TADs), a basic unit of chromosome folding playing key roles in gene regulation. The generation of epigenomic data in retinal cells and its integration with genomic data will be important to understand the non-coding morbid genome of IRD and of other diseases.

E. De Baere: None.

S12.2
Monosymptomatic and syndromic childhood-onset severe retinal dystrophies: News and Views

J. ROZET, S. MECHAUSSIER, I. BARNY, X. GERARD, J. KAPLAN, I. PERRAULT

INSERM UMR1163- Institute of genetic diseases, IMAGINE. Laboratory genetics in Ophthalmology, PARIS, France

Yearly, in Europe, approximately three hundred and fifty individuals are born blind despite a normal eye globe, a normal eye fundus and no other apparent heath issue, supporting a retinal dysfunction. Some of these individuals will experience spontaneous improvement of all or part of their visual function, but the majority of them is affected with a photoreceptor-neuron degenerative diseases of dramatically poor prognosis known as Leber congenital amaurosis (LCA). The initial presentation of LCA is invariable and the disease has long been regarded as homogenous. But, there exist a variability of the retinal outcome ranging from a stationary disease with dramatically poor visual performances to a progressive, yet very severe disease with low but measurable visual acuity in the first two decades of life. Occasionally, the same or similar retinal findings can be seen as the initial symptom in a range of systemic diseases involving in particular, the bone, kidney, liver, ear, cerebellum and/or the central nervous system. Some associations of symptoms are not uncommon and describe well defined syndromes but some others are exceptional, questioning their classification as syndromes. The characterization of the molecular bases of monosymptomatic and systemic diseases sharing severe visual deficiency at, or near birth has identified large numbers of genes and functions along with some strong genotype-phenotype correlations. This knowledge has considerably modified the care of neonates born apparently blind by disclosing potential therapeutic targets, some of which undergo intensive studies, and by providing some reliable markers predictive of the visual and extraocular outcomes. In addition, now that high throughput molecular diagnostic testing has become of common practice, it proved highly valuable to sort disease-causing mutations from polymorphisms, to detect non-coding mutations and sometimes to redirect diagnoses. This presentation will review these advances and will discuss their limitations. Some novel associations of symptoms, candidate genes and pathways will be discussed as well.

J. Rozet: None. **S. Mechaussier:** None. **I. Barny:** None. **X. Gerard:** None. **J. Kaplan:** None. **I. Perrault:** None.

S12.3
Seeing disease through stem cells: using patient iPSC to understand disease mechanisms and test therapies

M. Cheetham

UCL Institute of Ophthalmology, London, United Kingdom

The ability to reprogramme human cells into induced pluripotent stem cells (iPSC) and then differentiate them into a wide range of different cell types has revolutionized our ability to study human disease and offers great potential for regenerative medicine. We have used iPSC derived from inherited retinal dystrophy (IRD) patients to study the mechanisms of disease and to test potential therapies. In particular, I will describe how differentiating iPSC to retinal pigment epithelium (RPE) and 3D retinal organoids that contain photoreceptors can explain the retinal specificity associated with some inherited changes, revealing why retinal cells are more susceptible to disease than other cells that also express the disease gene. Furthermore, iPSC derived retinal cells are ideal for testing gene and mutation specific therapies that cannot be tested on knock-out animal models. This technology also offers the potential for quick translation to the clinic by showing not only efficacy, but also safety in the target human cells.

M. Cheetham: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ProQR. F. Consultant/Advisory Board; Modest; ProQR.

S13 Genome editing

S13.1
High-resolution interrogation of functional elements in the noncoding genome

N. Sanjana

New York Genome Center & NYU, New York, NY, United States

Although the noncoding genome plays a major role in gene regulation and harbors many common disease genetic variants, we have lacked tools for rapid identification and manipulation of functional elements in the noncoding genome. The easy programmability of new genome engineering tools like CRISPR (clustered regularly interspaced short palindromic repeats)-associated nucleases suggests a new way to interrogate genome function in a targeted fashion at the DNA level.

In addition to genome-scale screens targeting protein-coding genes, we have recently developed techniques for adapting CRISPR screens into noncoding regions of the genome. By examining drug resistance to a targeted chemotherapy in human melanoma, we find that mutations at specific noncoding elements lead to changes in transcription factor occupancy and in the local epigenetic landscape and that these changes are coincident with modulation of nearby gene expression. We also find that functional elements harbor predictive hallmarks of noncoding function, such as physical interaction (3C), open chromatin (DNase I hypersensitivity, ATAC-seq), cross-species evolutionary conservation, transcription factor binding (ChIP-seq), and post-translational histone

modifications associated with enhancer elements. This expands the potential of pooled CRISPR screens into noncoding regions for fundamental genomic discovery, gene regulation, and therapeutic development to overcome drug resistance.

N. Sanjana: None.

S13.3
Gene Therapy for Preventing Heritable Diseases

S. Mitalipov

Center for Embryonic Cell & Gene Therapy, Oregon Health & Science University, Portland, OR, United States

Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"-Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family: "Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} In vitro fertilization (IVF) represents a successful cell therapy approach for treatment of infertility. However, IVF is increasingly utilized for preimplantation genetic diagnosis (PGD) followed by embryo selection to prevent the transmission of heritable human diseases. Our Center is actively investigating novel germ line gene therapy approaches that would allow to repair gene defects in mutant gametes or early preimplantation embryos. We are focused on answering important safety and efficacy questions regarding techniques that could one day be useful in preventing thousands of inherited genetic disorders that affect millions of people worldwide. The focus of this lecture is to inform on applications of gene editing and gene replacement strategies in preclinical and clinical studies demonstrating feasibility, efficacy and long-term safety of germ line gene therapy. <!--EndFragment-->

S. Mitalipov: E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Mitogenome Therapeutics.

S14 Cellular heterogeneity in health and disease

S14.2
Dissecting the spatiotemporal subcellular distribution of the human proteome

E. Lundberg

KTH Royal Institute of Technology, Stockholm, Sweden

Compartmentalization of biological reactions is an important mechanism to allow multiple cellular reactions to occur in parallel. Resolving the spatial distribution of the human proteome at a subcellular level increases our understanding of human biology and disease. We have generated a high-resolution map of the subcellular distribution of the human proteome as part of the open access Human Protein Atlas database. We have shown that as much as half of all proteins localize to multiple compartments. Such proteins may have context specific functions and 'moonlight' in different parts of the cell, thus increasing the functionality of the proteome and the complexity of the cell from a systems perspective. I will present how this spatial data can complement quantitative omics data for improved functional read-out. Furthermore, I will present unpublished data on the extent of single cell variations of the human proteome, in correlation to cell cycle progression and other deterministic factors, as well as the overlap with observed variations at the RNA level. In summary, I will demonstrate the importance of spatial proteomics data for improved single cell biology.

E. Lundberg: None.

S14.3
Evolutionary selection of oncogenic mutant clones in normal epithelia

P. Jones[1,2]

[1]*Hinxton, United Kingdom,* [2]*Wellcome Sanger Institute, Cambridge, United Kingdom*

Somatic mutations accumulate in healthy cells throughout life. Most of these mutations do not alter cell behavior and accumulate passively, but occasionally a key gene is altered in a way that provides mutant cells with a competitive advantage. This leads to the formation of large clones that persist long term in normal tissues. However, detecting such clones is challenging due to the difficulties of identifying mutations present in small numbers of cells. The most highly mutated normal tissue reported to date is sun-exposed human skin. Most mutations are caused by ultraviolet light and it is unclear whether aged sun-exposed skin represents a special case due to a lifetime exposure to a powerful mutagen. This motivated us to investigate the mutational landscape of a similar tissue, the lining of the oesophagus, which also consists of layers of keratinocytes. As in the skin, cells are continually shed from the tissue surface and replaced by proliferation throughout life. However, the exposure to mutagens and the tissue environment in an internal epithelium led are very different from the skin, leading us to hypothesize that normal oesophagus

may have a distinct mutational landscape. We mapped mutant clones in normal oesophageal epithelium from nine donors aged 20-75 using ultra-deep targeted sequencing for 74 genes implicated in epithelial cancers. We found the density of mutant clones increased progressively, rising to over 100/cm^2 by middle age. Mutations were mainly caused by age-related mutational processes. There was strong Darwinian positive selection of clones carrying mutations in 14 genes that are also recurrently mutated in oesophageal cancers. By middle age *NOTCH1* and *TP53* mutant clones cover over 40% and 10% of the epithelium respectively. Remarkably, the prevalence of *NOTCH1*mutations in normal esophagus is several times higher than in oesophageal cancers. We conclude that the normal oesophagus is an evolving patchwork of mutant clones, which colonize the majority of the epithelium as we age.

S15 Understanding non-coding variants

S15.2
Non-coding repeat insertion in human disease

I. Silveira

Porto, Portugal

Non-coding repeat expansions composed of trinucleotide to hexanucleotide repeats cause more than 10 neurodegenerative diseases. I will describe the finding of an $(ATTTC)_n$ insertion, in a polymorphic ATTTT repeat in *DAB1* causing spinocerebellar ataxia (SCA), mapping to *SCA37*. The non-pathogenic ATTTT repeat alleles can reach 400 units in the normal population, whereas the pathogenic alleles have about 200 pentanucleotides with the configuration $[(ATTTT)_{60\text{-}79}(ATTTC)_{31\text{-}75}(ATTTT)_{58\text{-}90}]$. In the *DAB1*-oriented strand, $(ATTTC)_n$ is located in 5'-UTR introns of cerebellar-specific transcripts. They arise from the usage of alternative promotors and are highly expressed during human fetal brain development. We investigated the in vivo deleterious effect of the non-coding repeat by injecting zebrafish embryos with RNA containing the repeat insertion resulting in lethal developmental malformations. These results allowed us to establish the causative role of this non-coding repeat insertion in SCA37.

S16 Human epigenome dynamics

S16.1
Establishing the epigenome during human development: New insights of chromatin states and regulatory networks in pluripotent stem cells

P. Rugg-Gunn

The Babraham Institute, Hinxton, United Kingdom

Human pluripotent stem cells (hPSCs) exist in multiple states that are broadly termed naïve and primed. Both cell states can self-renew and undergo multi-lineage differentiation, but are functionally and molecularly distinct. Naïve hPSCs largely recapitulate the transcriptome and epigenome of pre-implantation embryos, and primed hPSCs are similar to early post-implantation embryos. This is an important distinction because these two developmental stages differ enormously in gene regulation and in epigenetic hallmarks such as X-inactivation status and DNA methylation. The research in my lab is focused on understanding the mechanisms of epigenetic and gene regulatory changes as hPSCs transition between the two states, with the aim of applying that information to more precisely control cell fate decisions and to better understand human development. Recently, we have mapped the transcriptional and epigenetic dynamics during cell state change, discovering that different reprogramming methods drive different trajectories. We have also examined the 3D genome organisation of naïve and primed hPSCs. By applying new network-scale computational approaches, we have interrogated the organisation at multiple genomic scales, ranging from a global overview to local communities that recapitulate architectural domains, down to individual promoter-enhancer interactions. Investigating chromatin topology and activity in human pluripotent states offers new insights into features of gene regulatory control during human development.

P. Rugg-Gunn: None.

S16.2
Epigenetics,aging and age-related disorders

R. F. Perez, J. Tejedor, P. Santamarina, A. F. Fernandez, M. F. Fraga

Cáncer Epigenetics Laboratory, CINN-CSIC, ISPA, IUOPA, Oviedo, Spain

Epigenetics refers to the study of stable genetic modifications that result in changes in gene expression and function without a corresponding alteration in the DNA sequence. Epigenetic mechanisms are essential for embryonic development, but they also play an important role during adult life. In addition to the epigenetic changes that occur that have a specific biological purpose, genomic DNA can also accumulate epigenetic alterations or noise during lifetime. It has been proposed that this process, known as "epigenetic drift", is associated with age-related diseases. Although it is

well-known that cancer is an age-dependent disease, the underlying molecular mechanisms are still poorly understood. In this regard, recent work has shown that aberrant DNA hypermethylation in aging and cancer share similar chromatin signatures. It is also well established that in the processes of both aging and cancer there is a global loss of DNA methylation. However, it remains unknown whether the chromatin signatures of DNA hypomethylation were also similar in aging and cancer. Conceptually, this is an important issue because the similarity of the chromatin signatures of DNA hypermethylation in cancer and aging has led to the proposal that DNA methylation is a molecular link between the two processes. In my talk, I will present recent work from our laboratory showing that: i) the genomic distribution of differentially methylated CpG sites is similar in aging and cancer, ii) there is a considerable tissue-independent component to DNA methylation changes in both aging and cancer and, iii) in contrast to DNA hypermethylation, the chromatin signatures of DNA hypomethylation in aging and cancer are different. These results reveal novel aspects of the relationship between DNA methylation and aging that could be important to understanding the possible role of DNA methylation as a molecular link between aging and cancer.

R.F. Perez: None. **J. Tejedor:** None. **P. Santamarina:** None. **A.F. Fernandez:** None. **M.F. Fraga:** None.

S16.3
Epigenetic and epitranscriptomic regulation of meiosis and the preimplantation embryo

A. Klungland

Institute of Medical Microbiology, BIG CAS-OSLO Genome Research Cooperation, Oslo University Hospital, Oslo, Norway

Post-transcriptional RNA modifications were identified several decades ago, but the reversible nature of RNA modifications, discovered just recently by Chuan He's lab and our group (reviewed in Klungland et al., Nat Meth, 2017). We have identified demethylases specific for mRNA (Zheng et al., Mol Cell, 2013) and tRNA (Liu et al., Cell, 2016). Owing to technological advances, knowledge of epitranscriptomic; the study of functionally relevant posttranscriptional modifications of RNA, and their writers, readers and erasers has recently advanced tremendously. We also study the epigenetic make-up of the oocyte and the preimplantation embryo. The presentation will include an update on reversible modifications in RNA (epitranscriptomic modifications) and their potential for regulating meiosis and embryo development as well as our work on epigenetic regulation in the oocyte and preimplantation embryo.

A. Klungland: None.

S17 ESHG-ASHG Building Bridges Debate: Germline genome editing - joint with EMPAG

S17.1
CRISPR-Cas9: Advances and Challenges

E. Charpentier[1,2]

[1]*Max Planck Institute for Infection Biology, Berlin, Germany,* [2]*Humboldt University, Berlin, Germany*

The discovery of the CRISPR-Cas9 gene editing technology is considered one of the most important biotechnology breakthroughs of our times. Initially described as a bacterial immune system, research in our laboratory ultimately demonstrated that CRISPR-Cas9 works as molecular scissors that can be harnessed as a tool to edit any genetic sequence. Similar to a text editing software, the CRISPR-Cas9 technology can correct typos, delete or exchange letters and sentences in the DNA of living cells. Only five years after our discovery, CRISPR has transformed the world of life sciences. Thousands of researchers are further developing the technology into innovative applications in biotechnology, agriculture and medicine. It has triggered high hopes for the development of therapies against cancer, HIV and serious genetic diseases. In agriculture, the CRISPR-Cas9 technology is already waiting in the wings to be marketed, e.g. to make crops more resistant against the effects of climate change or certain types of fungi and parasites. Most certainly, we will witness a lot of new developments to apply the CRISPR-Cas9 technology in our everyday lives in the future - but not all of them are positive. CRISPR has brought forward extensive ethical discussions around one central question: How far can we go with the technology in its applications in humans?

E. Charpentier: None.

S17.2
Human germline genome editing: the ASHG position statement

K. Ormond

Stanford, CA, United States

Between 2015 and 2017, the American Society of Human Genetics (ASHG) workgroup developed a policy statement, which was approved by the ASHG Board in March 2017 and published in August 2017. The statement, which was

endorsed by professional societies in North America, Europe, Asia, South Africa and Australasia, includes the positions below: 1. At this time, given the nature and number of unanswered scientific, ethical and policy questions, it is inappropriate to perform germline gene editing that culminates in human pregnancy. 2. Currently, there is no reason to prohibit in vitro germline genome editing on human embryos and gametes, with appropriate oversight and consent from donors, to facilitate research on the possible future clinical applications of gene editing. There should be no prohibition on making public funds available to support this research. 3. Future clinical application of human germline genome editing should not proceed unless, at a minimum, there is a) a compelling medical rationale, b) an evidence base that supports its clinical use, c) an ethical justification, and d) a transparent public process to solicit and incorporate stakeholder input.

S17.4
Societal opportunities and challenges of genome editing

A. Charo

Madison, WI, United States

Because genome editing makes genetic engineering easier and less expensive, it has the potential to be used for both profoundly important benefits, such as disease treatment and prevention, as well as less urgent - one might say even frivolous - uses. These range from so-called human enhancement to de-extinction to engineering animals as a form of art. It also may usher in a new era for genetically engineered foods, which have in the past been the subject of controversy and mixed governmental and consumer acceptance. Regulation in most countries concerns itself with ensuring a reasonable balance between physical risks and benefits to health and the environment. On the other hand, objections grounded in concerns about possible effects on culture, religion, social structures, and inter-generational equity are handled in very different ways among nations. It is in these variations that one may find the greatest challenge to harmonization of governance policies for this emerging area of biotechnology.

S18 Regulatory sequence functions and elements

S18.1
The gene expression consequences of mammalian regulatory evolution

C. Berthelot[1,2], D. Villar[3], D. T. Odom[3], P. Flicek[2]

[1]Institut de Biologie de l'Ecole Normale Supérieure, Paris, France, [2]European Molecular Biology Laboratory - European Bioinformatics Institute, Hinxton, United Kingdom, [3]CRUK-CI, University of Cambridge, Cambridge, United Kingdom

Tissues and cell types have largely conserved gene expression programs across mammals, enabling extrapolation from animal studies to human medical conditions. These gene expression patterns are controlled by collections of regulatory elements combining promoters and tissue-specific enhancers. However, it is now well-documented that regulatory elements have experienced extensive divergence and turn-over during mammalian evolution. How stable gene expression is maintained by rapidly evolving regulatory landscapes remains a fundamental question in evolutionary genetics, as well as a crucial prior to the use of animal models for human functional genetics.

We report here on an in-depth analysis of gene expression and regulation across mammalian evolution. Using liver as a representative organ, we profiled gene expression as well as histone marks typical of active promoters and enhancers in 20 different mammals. We used this data to quantify how promoters and enhancers evolve in mammals, and how this evolution affects gene expression downstream. This analysis revealed a complex relationship between gene expression and regulatory evolution. First, we show that promoters and enhancers are under significantly different evolutionary pressures: promoters remain largely active across mammals while enhancers are much more evolutionarily labile. Second, regulatory elements exhibit a broad range of evolutionary plasticity from those active across most mammals to those found in only one or a few species. We show how these degrees of conservation correlate with local gene functions, gene expression levels, and gene expression stability. Importantly, we report that highly expressed and stable genes are associated with complex arrays of promoters and enhancers, which can exhibit significant turnover across species as long as the overall landscape complexity is maintained. Our results underscore how functional redundancy in regulatory programs allows for resilience at the gene expression level in mammalian genomes.

C. Berthelot: None. **D. Villar:** None. **D.T. Odom:** None. **P. Flicek:** None.

S18.2
Regulatory principles governing enhancer function during animal development

E. K. Farley, G. A. Jindal, F. Lim, J. L. Grudzien, B. Song

UC San Diego, San Diego, CA, United States

The human genome contains on the order of a million enhancers. These segments of the DNA act as switches to regulate where and when the approximately 20,000 genes are expressed. As such, enhancers provide the instructions for tissue specific gene expression, thus enabling successful development and cellular integrity. Numerous studies have demonstrated that mutations in enhancers can alter tissue specific expression and cause phenotypic variation and disease. For example, a single mutation in a limb bud enhancer leads to aberrant expression of the gene SHH and results in extra fingers and toes. In an enhancer for the membrane protein Duffy, a point mutation results in malarial resistance. Computational analysis suggests that the majority of mutations associated with disease are located within enhancers. Despite the fundamental importance of enhancers for successful development and their discovery over 30 years ago, we lack a broad understanding of how enhancer sequence encodes tissue specific expression. As a result we do not understand which changes in enhancer sequence are simply inert variation between individuals and which mutations lead to phenotypic diversity and disease. These fundamental questions remain unsolved because we cannot relate enhancer sequence to gene expression patterns and phenotype on a scale sufficient to identify the overarching regulatory principles. The two main challenges in deciphering the relationship between enhancer sequence and tissue specific gene expression are: 1) the complexity of enhancers, and 2) the complexity of organisms. To address these problems, we have developed high-throughput functional assays to test millions of enhancer variants in millions of whole developing embryos. The model organism that enables such in-depth functional approaches is the marine chordate *Ciona intestinalis*. I will discuss our recent findings using these high-throughput functional approaches to identify regulatory principles governing enhancer function. I will also discuss how violations in these principles can help us pinpoint mutations associated with disease.

E.K. Farley: None. **G.A. Jindal:** None. **F. Lim:** None. **J. L. Grudzien:** None. **B. Song:** None.

S18.3
Ultraconserved enhancers are required for normal development

D. Dickel

Berkeley, CA, United States

More than 450 regions in the human genome have perfect sequence conservation between human and rodents, and these "ultraconserved" sites have intrigued biologists in the decade since they were first described. While it is known that many of these sequences are distant-acting enhancers, the drivers of such extraordinary evolutionary constraint remain unclear. Surprisingly, initial deletion studies showed that the loss of individual ultraconserved enhancers in mice has no obvious impact on viability or fertility. To explore this apparent discrepancy between extreme evolutionary constraint and lack of obvious phenotypes in more depth, we examined the in vivo consequences of loss of a series of ultraconserved enhancers near the essential neuronal transcription factor *Arx*. The *Arx* locus has an unusually high density of ultraconserved sites, including the three longest perfectly human-mouse-rat conserved sequences in the genome. Four ultraconserved regions near *Arx* drive gene expression in the developing forebrain, in patterns that cumulatively recapitulate the gene's expression domains. Single-cell transcriptome sequencing from transgenic embryos confirmed that the enhancer activity is specific to *Arx*-expressing neuronal subpopulations. We engineered mice missing these four enhancers singly, as well as pairwise for enhancers that display overlapping in vivo activity patterns. While the loss of any single or pair of ultraconserved enhancers resulted in viable and fertile mice, detailed phenotyping revealed neurological or growth abnormalities in nearly all cases, including substantial deficits of cholinergic neurons, altered densities of cortical interneuron populations, and abnormalities of the dentate gyrus. Our results demonstrate the functional importance of ultraconserved enhancers and highlight that extreme sequence conservation may result from evolutionary selection against fitness deficits that appear subtle in a laboratory setting.

S19 New nanotechnologies: the DNA Origami

S19.2
DNA nanostructures as innovative vehicles for smart drug delivery

M. Kostiainen, V. Linko

Aalto University, Espoo, Finland

DNA molecules can be assembled into custom predesigned shapes via hybridization of sequence-complementary domains. The folded structures have high spatial addressability and a tremendous potential to serve as platforms and active components in a plethora of bionanotechnological applications. DNA is a truly programmable material, and its nanoscale engineering thus opens up numerous attractive possibilities to develop novel methods for therapeutics. The tailored molecular devices could be used in targeting cells and triggering the cellular actions in the biological

environment. This presentation focuses on the DNA-based assemblies - primarily DNA origami nanostructures - that can perform complex tasks in cells and serve as smart drug-delivery vehicles in, for example, cancer therapy, prodrug medication, and enzyme replacement therapy.

M. Kostiainen: None. **V. Linko:** None.

Educational Sessions

E02 Hereditary cancer

E02.1
From Li-Fraumeni syndrome to TP53-related inherited cancers: update on molecular basis and clinical management

T. Frebourg

Department of Genetics, Rouen University Hospital, Inserm U1245, Centre for Genomic and Personalized Medicine, Rouen, France

Li-Fraumeni syndrome is a remarkable predisposition to cancer due to germline mutations of the *TP53* suppressor gene and characterized by early-onset tumours and a wide tumour spectrum. Diagnosis criteria have sequentially been updated in order to cover the 4 different clinical presentations associated with germline *TP53* mutations: Familial presentation: Proband with a LFS spectrum tumour (soft-tissue sarcomas (STS), osteosarcoma, adrenocortical carcinoma (ACC), central nervous system (CNS) tumours or female breast cancer) prior to age 46 years AND at least one first- or second-degree relative with a LFS tumour before the age of 56 years or with multiple tumours. (2) Multiple tumours: Patient with multiple malignancies, two of which belonging to the LFS spectrum, the first being developed before 46 years or with a second tumour developed within a radiotherapy field; (3) Rare tumours: Patients with ACC, choroid plexus tumours (CPT) or embryonal anaplastic subtype rhabdomyosarcoma (anRMS); (4) Breast cancer before age 31 years. Therefore, familial history is not mandatory to consider the presence of a germline *TP53* mutation in a patient. Independently of the familial history, the mutation detection rate in children presenting with ACC, CPT or anRMS is approximately 50%, and in females with breast carcinoma before 31 years 6%. The contribution to LFS of *de novo* mutations has recently been estimated to be at least 14% and approximately 1/5 of these de *novo* mutations occur during embryonic development, resulting therefore in mosaics. *TP53* being included in cancer gene panels, germline *TP53* mutations are now more frequently identified in patients who have developed only adult cancers. The penetrance of *TP53* mutations is not known and heterogeneous and depends both from the type of alteration and modifier factors. Dominant negative missense mutations are usually highly penetrant and associated with the severe forms of LFS characterized by childhood cancers. Null mutations are predominantly identified in families with only adult cancers and have a lower penetrance. Germline *TP53* mutations acting as permissive mutations to oncogenic stresses, their phenotypic expression is also probably dependent on environmental factors. The recently published surveillance protocols are heavy and are based, from the first year of life, on abdominal ultrasound every 4-6 months, annual total body MRI, annual brain MRI, and in females from 20 years on annual breast MRI. Considering the diversity of the clinical severity associated to germline *TP53* mutations, the benefits of such heavy protocols regardless to the psychological impacts should be carefully analyzed and discussed in each family. *TP53* mutation carriers have an exceptionally high risk of developing multiple primary tumours, estimated at least to 40%. Several lines of evidence show, in agreement with the key role of p53 in response to DNA damage, that radiotherapy and genotoxic chemotherapies contribute to the development of secondary tumours in LFS. Therefore, in patients with clinical presentations strongly suggestive of LFS, analysis of *TP53* should be performed before the initiation of treatment and in mutation carriers, radiotherapy should be, only if possible, avoided, surgical treatment prioritized and non-genotoxic treatments, such as immunotherapies, considered in the future.

T. Frebourg: None.

E02.2
Prostate Cancer Predisposition: Implications for Early Detection and Treatment

P. Nelson[1,2]

[1]-, Seattle, WA, United States, [2]Fred Hutchinson Cancer Research Center, Seattle, WA, United States

Prostate cancer is among the most heritable of all human malignancies. Risk alleles range from a large number of relatively common, but low penetrance polymorphisms to a small number of rare, high-penetrance gene variants. With respect to these latter variants, prostate cancer is now recognized as a cancer type associated with familial cancer syndromes driven by DNA repair gene mutations including mismatch repair (Lynch syndrome) and homology directed repair (BRCA1/2-Related Breast/Ovarian Cancer Syndrome). Notably, family history often fails to identify individuals with heritable mutations. This presentation will describe current knowledge with respect to inherited predisposition to prostate cancer with implications for

screening/early detection strategies and therapeutic interventions for advanced disease.

E03 Resources for gene function analysis

E03.1
Analysis of mammalian gene function through mouse phenotyping

D. Smedley, on behalf of the International Mouse Phenotyping Consortium

London, United Kingdom

Over the last decade, new sequencing technologies have enabled the discovery of numerous new disease-associated genes. However, for many genes we still know very little about their function and potential role in disease and many patients remain undiagnosed. Model organisms facilitate the exploration of genotype to phenotype associations and public databases of this knowledge allow us to assess genes with no previous involvement in human disease. Towards this goal, the International Mouse Phenotyping Consortium (IMPC) is building the first complete functional catalogue of the mammalian genome by producing and phenotyping a knockout mouse strain for every protein coding gene. Standardised phenotyping is performed on over 250 parameters covering all the major body systems. Over 5,000 knockout mouse lines, many for poorly annotated genes, have already been produced, phenotyped, and made available for distribution to the research community. In addition, over 1,000 embryonic lethal and subviable mouse lines have been analyzed in a specialized embryonic development pipeline that uses high-resolution 3D imaging. Analysis of this data has already led to novel publications on the wide-ranging prevalence of sexual dimorphism, the correlation between essential genes and disease, novel genes associated with deafness and metabolic disease, as well as the identification of hundreds of new animal models for human disease. In my lecture I will describe the IMPC and the key findings from these studies, as well as how the resource is being used today for prioritisation and validation of variants in large-scale disease sequencing projects such as the 100,000 Genomes Project.

E03.2
An atlas of human long non-coding RNAs

P. Carninci

RIKEN Center for Integrative Medical Sciences, Yokohama, Japan

Mapping 5'-ends of RNAs is the key to understand the gene regulation as they identify promoters, as well as long non-coding RNAs (lncRNAs) with functions. In order to comprehensively understand regulatory elements, we developed the Cap Analysis of Gene Expression (CAGE) technology, which enables to identify transcription start sites (TSSs) and quantitatively measure their activity throughout the genome at high-throughput. In the RIKEN Functional Annotation of the Mammalian Genome 5 (FANTOM5) project, we created a very broad map of the promoterome and regulatory networks by simultaneously mapped mRNAs and lncRNAs TSSs and measured their expression at each different promoters with CAGE, on a comprehensive panel of human and mouse primary cells and other tissues. The study revealed the existence of 223,428 and 162,264 promoters and 65,423 and 44,459 enhancers, in human and mouse respectively, which are often tissue specific (Forrest et al. Nature 507, 462, 2014, Andersson et al. Nature 507, 455, 2014). Using CAGE, we also built an atlas of human lncRNAs with accurate 5'-ends (Hon et al. Nature 543, 199, 2017). Classification of lncRNAs revealed that most intergenic lncRNAs are derived from enhancer-like regions rather than classic promoters and GWAS trait-associated SNPs enriched at lncRNA loci were specifically expressed in cell-types relevant to the specific diseases, suggesting their roles in diseases. Ongoing FANTOM6 project is aiming at creating the broadest database of functional lncRNAs, as a valuable resource in the community. Furthermore, we are pursuing a strategic collaboration with the International Human Cell Atlas (HCA) project, which is aiming at the creation of a comprehensive map of all human cell types and states at single cell level, with our newly developed single cell CAGE.

P. Carninci: E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; TranSINE Technologies Co. Ltd.

E04 Pharmacogenomics

E04.1
Preemptive pharmacogenomic testing for preventing adverse drug reactions

H. Guchelaar

Leiden Genome Technology Center, Leiden, Netherlands

Pre-emptive pharmacogenomic testing for preventing Adverse Drug Reactions

Pharmacogenomics (PGx), the study of genetic variability affecting an individual's response to a drug, holds the promise to lead to more efficacious, safer and cost-effective

drug therapy. In recent years, guidelines with therapeutic recommendations have become available to guide clinicians how to interpret and adjust drug therapy based upon a pharmacogenomic test result. Indeed, for some drug-gene pairs (such as capecitabine – DPYD; mercaptopurine - TPMT) pre-therapeutic testing has been implemented in routine clinical care in some centers but there remain barriers for widescale implementation of pre-therapeutic testing.

Studies have shown that at least 90-95% of patients have an actionable genotype when tested for a panel of pharmacogenes including CYP2C9, CYP2C19, CYP2D6, CYP3A5, DYPD, SLCO1B1, TPMT, HLA-B, UGT1A1 and VKORC1. Therefore, pre-emptive testing (that is: multiple pharmacogenomic variants are collected prospectively and embedded into the patient's medical record) of a panel of genetic variants seems a promising and cost-effective approach.

We have performed a pilot study in 200 primary care patients with pre-emptive testing of a PGx panel with the aim to reduce ADRs. In addition, the EU Horizon2020 funded U-PGx consortium was established and investigates a pre-emptive genotyping approach of a panel of important PGx variants as a new model of personalised medicine. To meet this goal, 94 existing pharmacogenomics guidelines of the Dutch Pharmacogenomics Working Group (DPWG) of the Royal Pharmacists Association (KNMP) are combined with novel IT solutions. Implementation is conducted at a large scale in seven European health care environments (The Netherlands, UK, Spain, Italy, Austria, Greece and Slovenia) for a total of 8,100 patients and accounts for the diversity in health system organisations and settings. The multicenter randomized study is open since Q1 2017. Feasibility, health outcome and cost-effectiveness are investigated; the study is powered to show a reduction of 30% of the incidence of grade 2 or higher adverse drug events. The U-PGx consortium (www.upgx.eu) ultimately aims to formulate European strategies for improving clinical implementation of pharmacogenomics.

H. Guchelaar: None.

E04.2
From pharmacogenomics testing to point-of-care clinical decision support

M. J. Ratain

The University of Chicago, CHICAGO, IL, United States

While there are a myriad of publications reporting an association between germline variants and drug response - both safety and efficacy - demonstration of the clinical utility of pharmacogenomic testing has been challenging. Even more challenging is to demonstrate that such testing is cost-effective, particularly in the context of on-demand testing required for safe prescribing of a specific drug. Specific examples of variants of known clinical importance will be discussed. An alternative approach is pre-emptive genotyping for a panel of variants that inform prescribing of one or more drugs. This strategy ensures that the prescriber will have the genetic information at the time of prescribing, which can be delivered using point-of-care decision support. This strategy has been implemented at University of Chicago Medicine as "The 1200 Patients Project", which is led by Peter O'Donnell, MD, using a genomic prescribing system (GPS). Results to date have demonstrated that pharmacogenetic results impact prescribing, and that patient satisfaction and recall of medication recommendations are enhanced. Ease of use of the GPS continues to be an issue for busy practitioners in the outpatient setting. Ongoing studies aim to assess the feasibility and impact of the GPS for hospitalized patients, as well as those undergoing elective surgery. However, larger randomized studies will be required to measure the impact of the GPS (and other similar approaches) on clinical endpoints, such as serious adverse events and treatment failure.

M.J. Ratain: E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; pending patents for genomic prescribing systems.

E05 Bone Density: High and Low

E05.1
Decreased Bone Density: From Gene to Pathways

O. Mäkitie

University of Helsinki, Helsinki, Finland

Genetic discoveries in patients and families with early-onset osteoporosis have increased our understanding of the molecular mechanisms and cellular pathways governing normal bone health. Several forms of monogenic bone fragility disorders, directly or indirectly related to type I collagen, have been described. Importantly, other forms also exist, often with unique skeletal and extra-skeletal features and with variable inheritance patterns. The most common monogenic bone fragility disorder is osteogenesis imperfecta (OI) in which dominantly inherited mutations in the genes encoding type I collagen (*COL1A1*and *COL1A2*) are responsible for approximately 90% of the cases. Several rare autosomal recessive forms of OI have also been described. Recent discoveries have further elucidated the genetic determinants of early-onset skeletal fragility and several forms not related to type I collagen have

been identified. The discovery of *LRP5* mutations in osteoporosis-pseudoglioma syndrome and in early-onset osteoporosis first indicated that the WNT signaling pathway plays an important role in bone mass accrual. Several other studies thereafter, including our discovery of *WNT1* mutations in early-onset osteoporosis, have further highlighted the pathway's significance in various disorders of low and high bone mass and provide evidence for the potential of WNT-targeted therapies in osteoporosis treatment. X-chromosomal osteoporosis caused by *PLS3*gene mutations is another example of novel monogenic forms of osteoporosis. PLS3 osteoporosis affects especially males and leads to severe progressive spinal osteoporosis; even females carrying the mutation may develop symptomatic osteoporosis. PLS3 may play a role in bone mineralization but the pathogenetic mechanisms are not fully understood. Several other monogenic forms of osteoporosis are under investigation. These highlight the complexity of molecular mechanisms governing normal bone homeostasis and underscore the need for individualized treatment protocols.

O. Mäkitie: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Kyowa Kirin, Alexion, Shire, Strakan International.

E06 Statistics in Genetic Research and Diagnostics

E06.1
The Importance of Reproducible Research in High-Throughput Biology

K. Baggerly

UT MD Anderson Cancer Center, Houston, TX, United States

Modern high-throughput biological assays let us ask detailed questions about how diseases operate, and promise to let us personalize therapy. Our intuition about what the answers "should" look like in high dimensions is very poor, so careful data processing is essential. When documentation of such processing is absent or incomplete, we must apply "forensic bioinformatics" to work backwards from the raw data and the reported results to infer what the methods must have been. Such explorations occasionally reveal errors. The most common errors we uncover are simple ones, often involving mislabeling of rows, columns, or variables. These errors are easy to make, but if documentation is adequate, they may be easy to fix. Incomplete documentation is, however, pervasive in much of the scientific literature. We give examples illustrating the potentially severe implications of such mistakes for patient care, and discuss steps journals and US funding agencies are taking in response. Fortunately, new tools (many from the open-source community) have been introduced in the past few years which make documentation much easier.

K. Baggerly: None.

E06.2
Statistics in genetic diagnostics

C. Azencott

Paris, France

This lecture will address some of the current statistical challenges encountered when attempting to build predictive models from genomics data.

Technological progress allows us to gather large amounts of molecular measurements for cohorts of increasing sizes. However, these data sets have characteristics that make them very different from the "big data" on which recent years have witnessed so many methodological advances. In particular, they countain orders of magnitude more features than samples, which creates strong statistical difficulties. The lecture will illustrate these challenges on simple examples, and describe solutions from the statistics and machine learning fields.

More specifically, we will discuss sparsity and how to use prior knowledge, in particular in the form of networks, to constrain the models and alleviate the statistical burden. The lecture will also present how multitask approaches can, by building several related models simultaneously, alleviate the small sample size issue.

To conclude we will describe additional challenges and opportunities for the use of machine learning in building predictive models from omics data.

E07 Organoids

E07.1
Applications scenarios of Organoids

A. Manfrin

-, Lausanne, Switzerland

In the past ten years, the convergence of developmental and stem cell biology, combined with technical innovations in cell culture methods, has resulted in the emergence of a new generation of in vitro models. These models, referred to as organoids, recapitulate at a very basic level some of the complexity of real tissues and organs, and as such promise to bridge the gap between in vitro and in vivo models. Organoids are believed to play an important role in the future of both basic and applied research. In this

presentation, I will summarize some of the main features of organoids, such as their generation by poorly understood self-organization and similarities with real tissues. I will then focus on examples of organoid applications as models for embryonic development and organogenesis, as well as for disease modelling, drug testing, personalized and regenerative medicine. Finally, I will describe our recent effort in combining bioengineering and stem cell biology to extrinsically steer self-organization of human Pluripotent Stem Cell-derived embryonic organoids.

A. Manfrin: None.

E07.2
On the self-engineering of embryonic stem cells

A. Martinez-Arias

Cambridge, United Kingdom

Embryonic Stem (ES) cells are clonal derivatives from the blastocysts of mammalian embryos which have the potential to give rise to all lineages of the embryo and the ability to self renew this ability. Mouse ES cells have proven an excellent system in which to study developmental events, in particular the mechanisms of cell fate decisions. However, while it is easy to coax them into elements of different tissues in culture, they do not organize themselves as they do in embryos. Non adherent culture leads them to form aggregates in which some cell types appear in clusters but there is no overall organization.

Recently we have developed a non adherent culture system in which small aggregates of mouse ES cells undergo symmetry breaking, polarized gene expression and growth and gastrulation like movements in vitro. Using a variety of reporter ES cell lines and comparison with the embryo we observe organization of Wnt, Nodal and FGF signaling that mirrors events in the embryo and have shown that they can develop in culture until an equivalent of embryonic day 9 (E9). We observe that these embryonic organoids develop anteroposterior, dorsoventral as well as bilateral asymmetries. Analysis of pattern formation in these aggregates suggests that they do not develop through standard self organization but that there is a strong genetic components to what otherwise would be simple thermodynamically driven process. These issues will be discussed in course of the talk.

E08 Congenital vasculopathies

E08.1
Etiology of vascular malformations: A question of place and timing

M. Vikkula

Brussels, Belgium

Vascular malformations are *localized* errors of vascular development. They are often identified on the skin as "birthmarks" of various sizes and shapes. They usually slowly grow with the growth of the child. They may also be encountered in other organs, such as the liver, intestine and the brain. The lesions are consisted of tortuous vascular channels of various types, with continuous endothelium surrounded by various numbers of support cells. Most of these lesions occur sporadically, yet sometimes as part of a syndrome or as an inherited disorder. Genetic studies of such families and patients have led to the identification of a number of genes that cause various vascular malformations.

The pathway to unravel the pathophysiological mechanisms of vascular anomalies started by the discovery that inherited TIE2/TEK mutations (an endothelial receptor tyrosine kinase) are responsible for hereditary mucocutaneous venous malformations ("cavernous hemangioma"). Further studies to explain the multifocality of lesions in these patients made realize the importance of tissular second-hits for lesion development in this autosomal dominant disease. This directed the search for causes of sporadic cases towards tissular mutations, and opened a new era, in conjunction with the advent of NGS, for somatic genetic screens in vascular anomalies and other development disorders.

Genetic mutations take place during each cell division. The earlier they occur, the larger the cell population that carries the mutation is. Most of the mutations in vascular anomalies occur in endothelial cells, and they have allowed generation of in vitro and in vivo models, another major step towards development of novel therapies for these chronic, debilitating diseases. As most of the mutations activate well-known oncogenes and signaling pathways, this has opened an era for repurposing of cancer-drugs to vascular anomaly patients. (miikka.vikkula@uclouvain.be; https://www.deduveinstitute.be/human-genetics).

E08.2
Clinical management of vascular malformations

L. M. Boon[1], E. Seront[1], N. Revencu[1], M. Vikkula[2]

[1]Cliniques universitaires Saint Luc, Brussels, Belgium, [2]De Duve Institute, Brussels, Belgium

Vascular anomalies are classified according to clinical and histopathological characteristics into tumors, the most common being infantile hemangioma, and malformations, that are subsequently subdivided according to the affected

vessel type(s). Treatment of vascular anomalies depends on the affected vessel type, the location of the lesion, and the symptoms. As many lesions are extensive, patients should be aware that a complete cure is often not possible. A multidisciplinary approach is mandatory to obtain the best result with the least complications and/or recurrence. Treatments can vary from active follow-up to medication, laser, interventional radiology and/or surgical resection. Identification of several signaling pathways involved in various types of vascular malformations allowed us to target new molecular therapies for vascular malformations, such as mTor inhibitor for slow-flow vascular malformations and anti-angiogenic medication for fast-flow lesions. Clinical trials are currently on going with promising results.

L.M. Boon: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Pierre Fabre. **E. Seront:** None. **N. Revencu:** None. **M. Vikkula:** None.

E09 Iron in the brain - joint session with the European Society of Neurology

E09.2
NBIA - new angles

A. Rötig

Imagine Institut, Paris, France

Neurodegeneration with brain iron accumulation (NBIA) is a genetically heterogeneous condition due to iron accumulation in basal ganglia, manifesting as a progressive extrapyramidal syndrome with dystonia, rigidity and choreoathetosis. Mutations in eleven genes have been hitherto identified. Only two genes are reportedly involved in iron metabolism, *FTL* and *CP*, respectively encoding the light subunit of ferritin, and ceruloplasmin. How the other NBIA proteins involved in lipid metabolism, lysosomal activity and autophagic processes disturb iron metabolism remains poorly understood. Thanks to whole exome sequencing in patients with NBIA we identified mutations in *REPS1* gene that allowed us to better understand iron homeostasis deregulation in NBIA. REPS1 is involved in endocytosis of membrane receptors, including transferrin receptor (TfR1) and vesicle transport. Studying fibroblast cells lines from subjects carrying biallelic mutations in REPS1 and known genes (*PANK2, PLA2G6, C19ORF12* and *FA2H*), we ascribe iron overload to abnormal recycling of TfR1 and reduction of TfR1 palmitoylation in NBIA. Moreover, we describe palmitoylation as a hitherto unreported level of post-translational TfR1 regulation. A widely used antimalarial agent, artesunate, rescued abnormal TfR1 palmitoylation in cultured fibroblasts of NBIA subjects. These observations suggest therapeutic strategies aimed at targeting impaired TfR1 recycling and palmitoylation in NBIA for which no therapy is yet available.

A. Rötig: None.

E10 Genetics of infertility

E10.1
Genetic basis of male reproductive disorders

C. Krausz

Dept. of Experimental and Clinical Biomedical Sciences "Mario Serio"; University of Florence, Florence, Italy

Couple infertility concerns 15% of couples and male reproductive anomalies are present in about half of the cases, either as the main reason or as a cofactor. Male infertility is a multifactorial complex pathological condition in which genetic factors play a relevant role. Azoospermic men are at highest risk for being carriers of a genetic anomaly (25%).The genetic landscape of this condition is highly complex as semen and testis histology phenotypes are extremely heterogeneous and at least 2,000 genes are involved in spermatogenesis (Krausz et al. 2015). Known genetic factors can be screened in all etiologic categories of male infertility (hypothalamic-pituitary axis dysfunction, quantitative and qualitative alterations of spermatogenesis, ductal obstruction/dysfunction). Genetic testing is relevant for its diagnostic value and clinical decision-making and for appropriate genetic counselling (Krausz et al. 2018). Anomalies in sex chromosomes have major role in severe spermatogenic impairment: i) Y chromosome microdeletions (AZF deletions) are the most frequent molecular genetic causes of severe oligo/azoospermia. Carriers of complete AZFa and AZFb deletions have virtually zero chance for successful sperm recovery through Testicular Sperm Extraction, hence this genetic test has also a pre-TESE prognostic value (Krausz and Casamonti, 2017); ii) X chromosome-linked, *TEX11* mutations are novel causes of meiotic arrest. Over the last 20 years, two approaches have been used for the discovery of novel genetic factors: i) array based analyses (SNP and CNV); ii) exome analysis. Results from whole-genome association studies suggest a marginal role for common variants as causative factors. Studies on Copy number variations (CNVs) demonstrated a considerably higher CNV load in infertile patients compared with normozoospermic controls. This phenomenon may well be an expression of a more generalized genomic instability, which could be one of the explanations for the epidemiological observations indicating lower life

expectancy and higher morbidity (including cancer) in infertile men. Recently a genetic link between late-onset Fanconi Anemia due to *FANCA* mutations and SCOS has been identified which further link infertility to a cancer prone disease. Whole-exome analysis has proved a highly successful diagnostic tool in patients belonging to consanguineous families. It allowed the identification of novel candidate genes (mainly autosomal) for central hypogonadism, monomorphic teratozoospermia/asthenozoospermia, congenital obstructive azoospermia and quantitative spermatogenic disturbances. Despite efforts, the aetiology of infertility remains unknown in about 40% of patients and the discovery of novel genetic factors in idiopathic infertility is a major challenge for the field of andro-genetics. The large majority of idiopathic patients are affected by quantitative spermatogenic disturbances and are not familial cases (sporadic). The development of a diagnostic gene panel based on exome data originating from familial cases is highly limited, since these are due to recessive inheritance (i.e.one mutated allele is tolerated). In sporadic cases, hemizygous mutations and heterozygous autosomal mutations with dominant negative effect or haploinsufficiency are the most obvious candidates.While the diagnostic interpretation of a pathogenic mutation on the sex chromosomes is relatively easy, the causative link for a heterozygote mutation is rather complex. It is expected that ongoing, large, consortium-based, whole-exome and whole-genome studies will accelerate the discovery of the missing genetic aetiology of idiopathic male infertility. References: -Krausz C, Cioppi F, Riera-Escamilla A. Testing for genetic contributions to infertility: potential clinical impact. Expert Rev Mol Diagn. 2018 Mar 22:1-16. -Krausz C, Casamonti E. Spermatogenic failure and the Y chromosome. Hum Genet. 2017 May;136(5):637-655. -Krausz C, Escamilla AR, Chianese C. Genetics of male infertility: from research to clinic. Reproduction. 2015 Nov;150(5):R159-74.

C. Krausz: None.

E12 Undiagnosed disease and matchmaking initiatives

E12.2
A User Guide to Matchmaking - the value of data-sharing in rare disease exemplified by DECIPHER and Matchmaker Exchange

H. Firth

Wellcome Sanger Institute, Cambridge, United Kingdom

Analysis of whole genome sequences in the 1000 genomes project identified 4-5 million variants in every genome. Establishing the consequence of these variants for human health and development is a global challenge. This requires differentiating those variants that contribute to healthy variation and resilience of populations to disease from those that have adverse consequences contributing to disease and developmental disorders in individuals

Whole exome and whole genome sequencing for patients with rare disease often yields novel variants that cannot be confidently interpreted in isolation. DECIPHER (https://decipher.sanger.ac.uk) is a node in the federated MME system. It is a major global platform for the visualization of phenotypic and genomic relationships with a mission is to map the clinically relevant elements of the genome and understand their contribution to human disease and development. DECIPHER has facilitated >1500 publications in the peer-reviewed scientific literature; a testament to the importance of match-making in rare disease.

Database silos inhibit diagnosis and discovery in rare disease. The 'Matchmaker Exchange (MME)' project was launched in 2013 to address this barrier to progress and is now a demonstration project for the Global Alliance for Genomics and Health (GA4GH). MME facilitates the matching of cases with similar phenotypic and genotypic profiles (matchmaking) through standardized application programming interfaces (APIs) and procedural conventions for the following connected databases: DECIPHER, Phenome Central, MatchBox, Patient Archive, MyGene2 and GeneMatcher. The GA4GH Matchmaker Exchange increases the power for discovery and diagnosis by users of its component nodes by enabling searching for matching cases in a much larger data-set.

H. Firth: None.

E13 Brain abnormalities in fetal life

E13.1
Malformations of the corpus callosum

C. Depienne[1,2]

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The corpus callosum is the main cerebral commissure in placental mammals with a key role in the communication between the brain hemispheres. Agenesis of the corpus callosum (ACC) corresponds to the complete or partial absence of the corpus callosum and is one of the most frequent brain malformations in humans. The incidence of ACC is 1/4,000 in newborns but rises to 3-5% in patients with intellectual disability (ID). ACC is a highly

heterogeneous condition: complex ACC is commonly associated with other cerebral or extracerebral anomalies and/or variable degrees of ID. In contrast, isolated ACC has been associated with a 75% chance of a normal or subnormal developmental outcome, although specific cognitive processes such as reasoning, verbal and social abilities may be impaired. ACC is usually diagnosed prenatally by ultrasound from the second trimester of pregnancy. Prognostication is difficult due to the widely variable neurodevelopmental outcomes and ACC is a relatively common cause of late pregnancy termination in European countries. ACC associated with ID is frequently caused by mutations in genes encoding transcription factors or chromatin remodelers, such as subunits of the SWI-SNF complex. On the contrary, the genetic factors responsible for isolated ACC remain poorly understood. Recently, we have shown that mutations in *DCC*, encoding the receptor for netrin 1, cause isolated ACC with incomplete penetrance.

C. Depienne: None.

E13.2
Canary in the coal mine: the cerebellum as a sentinel for developmental brain disorders

W. Dobyns[1], K. A. Aldinger[2]

[1]Seattle, WA, United States, [2]Seattle Children's Research Institute, Seattle, WA, United States

The cerebellum is often overlooked in assessing fetuses and children with developmental brain disorders because of multiple patterns of malformation that are inconsistently defined, lack of experience in recognizing these patterns, variable severity including non-penetrance, occasional co-occurrence of atrophy, and limited understanding of the underlying causes. After excluding two well-known groups of autosomal recessive disorders with recognizable patterns of malformation (Joubert syndrome and pontocerebellar hypoplasia), cerebellar malformations have been consistently observed with only two copy number variants (deletion 3q24 or 6p25.3) and a few genes (CASK, OPHN1 and FOXC1). Recent experience has shown that prenatal events such as late 2nd-early 3rd trimester posterior fossa and cerebellar bleeds and (less often) cerebellar ischemia, can cause cerebellar injuries that mimic cerebellar malformations. Accordingly, genetic studies have shown a lower rate of abnormalities than other developmental disorders such as agenesis of the corpus callosum, intellectual disability and autism. We have performed SNP microarrays in ~250 children and whole exome sequencing data in ~100 children with cerebellar malformations. Our analysis suggests that cerebellar hypoplasia is a variable feature in many genetic developmental brain disorders, that prenatal injuries to the cerebellum are common and can often be recognized based on the pattern of abnormality, and that these two processes may co-occur.

K.A. Aldinger: None.

E15 Disorders of sexual development

E15.1
Disorders of sex development: genetics, diagnostics and clinical management

A. Sinclair

Murdoch Children's Research Institute, Melbourne, Australia

Disorders of sex development (DSD) represent a major pediatric concern and clinical management of these conditions can be difficult. Uncertainty about a child's gender can be traumatic for the individual and their family. It may also carry profound psychological and reproductive consequences. Most often the underlying cause of DSD is a variant in a gene or genes regulating gonadal/genital or steroidogenic pathways. Providing a molecular diagnosis for patients with a DSD and their families can serve multiple purposes: naming the underlying cause contributes to acceptance, reduces stigma or blame, and provides crucial clues and guidance for clinical management, including information on the malignancy risks associated with some types of DSD. A diagnosis is integral to genetic counseling and family planning and yet it has been found that currently as few as 13% of patients with a DSD will receive a clinical molecular genetic diagnosis.

To address this we have developed a massively parallel sequencing targeted DSD gene panel which allows us to sequence 64 known diagnostic DSD genes and 1000 candidate genes simultaneously. Using this we have analyzed DNA from the largest reported international cohort of patients with DSD (278 patients with 46,XY DSD and 48 with 46,XX DSD). We found variants in a total of 28 diagnostic genes highlighting the genetic spectrum of this disorder. Sequencing revealed 93 previously unreported DSD gene variants. Overall, we identified a likely genetic diagnosis in 43% of patients with 46,XY DSD. In patients with 46,XY disorders of androgen synthesis and action the genetic diagnosis rate reached 60%. Surprisingly, little difference in diagnostic rate was observed between singletons and trios. In many cases our findings were informative as to the likely cause of the DSD, which will facilitate clinical management. The clinical utility of this targeted DSD genetic screen will be discussed as will the additional approaches we are taking to fill the diagnostic gaps that still exist.

A. Sinclair: None.

E15.2
Reproductive and adrenal development: Insights from nuclear receptor gene mutations

J. Achermann

UCL GOS Institute of Child Health, London, United Kingdom

The nuclear receptor (NR) superfamily plays a crucial role in development as well as diverse aspects of endocrine, reproductive, neurological and metabolic function. Humans have 48 nuclear receptors and to date, defined conditions have been reported for almost half of them. Initially, phenotypes were predicted based on the known physiology of classic ligand-dependent NRs (e.g thyroid receptor beta, vitamin D receptor, androgen receptor, estrogen receptor, mineralocorticoid receptor and glucocorticoid receptor). Subsequently, defects in "orphan" nuclear receptors were found based on genetic mapping strategies or phenotypes predicted from expression/mouse models (e.g. DAX-1/NR0B1, SF-1/NR5A1, PPAR gamma, FXR alpha). More recently, exome/genome sequencing has uncovered novel NR-associated conditions that would not immediately be predicted clinically (e.g. COUP-TFI & II, ROR gamma).

In this educational session I will provide an overview of the clinical features, inheritance and biology of nuclear receptor gene mutations with a focus on genes involved in reproductive and adrenal development and function:

1) Classic ligand-dependent NRs. This includes the role of the a) androgen receptor (NR3C4) in complete and partial androgen insensitivity syndrome (AIS) (OMIM: 300068, 312300), as well as CAG trinucleotide repeats in X-linked spinal and bulbar muscular atrophy (313200); b) estrogen receptor alpha (ESR1/NR3A1) in estrogen resistance (615363); c) glucocorticoid receptor (NR3C1) in glucocorticoid resistance (615962); and d) mineralocorticoid receptor (NR3C2) in pseudohypoaldosteronism type I (177735).

2) Orphan NRs. This includes a) DAX-1/NR0B1 in X-linked adrenal hypoplasia (with hypogonadotropic hypogonadism and infertility) (OMIM: 300200); and b) steroidogenic factor-1 (SF-1)/NR5A1 in combined adrenal/gonadal dysgenesis (612965) (rare), reproductive anomalies in 46, XY individuals (DSD, hypospadias, male factor infertility) (612965, 613957) (common) or primary ovarian insufficiency (POI) in 46,XX women (612964).

3) Recent discoveries. The role of a) SF-1/NR5A1 (617480) in (ovo)testis development (46,XX); b) COUPTF-II/NR2F2 in (ovo)testicular development (46,XX) with cardiac defects; and c) estrogen receptor beta (ESR2/NR3A2) in syndromic and non-syndromic 46,XY DSD.

Understanding the genetic basis of these conditions is important for counselling families, predicting associated features and long-term history, and sometimes for personalising approaches to management.

J.C.A. is a Wellcome Trust Senior Research Fellow in Clinical Science (grant 098513/Z/12/Z, 209328/Z/17/Z) with research support from Great Ormond Street Hospital Children's Charity (grant V2518) and the NIHR GOSH BRC (IS-BRC-1215-20012).

Achermann JC, Schwabe J, Fairall L, Chatterjee K. Genetic disorders of nuclear receptors. J Clin Invest. 2017 Apr 3;127(4):1181-1192.

J. Achermann: None.

E16 Genetics with a Bite

E16.1
Genetics of early tooth development and dental disorders

O. D. Klein

UCSF, San Francisco, CA, United States

Teeth are unique to vertebrates and have played a central role in their evolution, and the tooth is an important model system for many areas of research. Clinically, dental anomalies are common congenital malformations that can occur either as isolated findings or as part of a syndrome. I will use genetic causes of abnormal tooth development as a point of entry to discussion of the molecular pathways and morphogenetic processes involved in tooth development. Developmental biologists have exploited the clear distinction between the epithelium and the underlying mesenchyme during tooth development to elucidate reciprocal epithelial/mesenchymal interactions during organogenesis. The preservation of teeth in the fossil record makes these organs invaluable for the work of paleontologists, anthropologists, and evolutionary biologists. In addition, with the recent identification and characterization of dental stem cells, teeth have become of interest to the field of regenerative medicine. In this talk, I will review some of the major research areas and studies in the development and evolution of teeth, including morphogenesis, genetics and signaling, evolution of tooth development, and dental stem cells.

O.D. Klein: None.

E16.2
GenoDENT: A targeted next-generation sequencing assay for the molecular diagnosis of genetic disorders with orodental involvement

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Rare genetic disorders are often challenging to diagnose. Anomalies of tooth number (hypodontia, oligodontia, anodontia), shape, size, mineralized tissue structure (i.e. enamel (amelogenesis imperfecta), dentin (dentinogenesis imperfecta, dentin dysplasia), cement, alveolar bone), eruption, and resorption may exist as isolated symptoms or diseases, but are often part of the clinical synopsis of numerous syndromes. A vast number of genes are implicated in these disorders. It is difficult to distinguish syndromic versus non-syndromic diseases, as the same genes are often involved in both categories. In addition, subclinical or non-observed phenotypes might be underestimated. Concerning amelogenesis imperfecta, mutations in a number of genes have been reported to cause isolated enamel defects, including *AMELX, ENAM, KLK4, MMP20, FAM83H, WDR72, C4orf26, SLC24A4*, and *LAMB3*. In addition, many other genes such as *DLX3, CNNM4, ROGDI, FAM20A, STIM1, ORAI1*, and *LTBP3* have been shown to be involved in developmental syndromes with enamel defects. The clinical presentation of the enamel phenotype (hypoplastic, hypomineralized, hypomature, or a combination of severities), alone does not allow a reliable prediction of possible causative genetic mutations. Our team has documented orodental phenotypes which are described in the D[4]/phenodent Diagnosing Dental Defects Database registry (www.phenodent.org), which now comprises more than 4000 patients spanning 185 diseases linked to biological sample collection. Understanding the potential genetic cause(s) of rare diseases is critical for overall health management of affected patient. One effective strategy to reach a genetic diagnosis is to sequence a selected gene panel chosen for a determined range of phenotypes. Here we describe the results and problems with a specific gene panel for orodental diseases, GenoDENT, that targets 239 known and 271 candidate genes. The overall diagnostic rate for 300 patients was 60%. This panel, often offered definitive diagnosis, modified previous diagnosis, identified digenic inheritance, allowed genotype/phenotype correlations, orientated patients towards genetic counseling, targeted the clinical procedures according to the disease type, identified new diseases/genes, and facilitated the multidisciplinary management of rare diseases patients.

Conclusions: We have developed a novel targeted NGS assay for the efficient diagnosis of a variety of isolated and syndromic oro-dental diseases. This panel includes novel candidate genes, hence is a tool for gene discovery. RARENET (http://www.rarenet.eu/en/) is a French-German-Swiss cross-border cooperative project, in the framework of INTERREG V, EU (ERDF) funded program, which has the objective of improving the management and health of patients with complex rare diseases.

A. Bloch-Zupan: None. **T. Rey:** None. **V. Laugel-Haushalter:** None. **C. Stoetzel:** None. **M. Prasad:** None. **M. Kawczynski:** None. **S. Troester:** None. **J. Muller:** None. **H. Dollfus:** None. **B. Gérard:** None. **J. Chelly:** None.

Concurrent Sessions

C01 Precision and Predictive Medicine

C01.1
Polygenic risk score can replace clinical risk scores in predicting diabetic complications and their response to therapy

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Background: There is growing interest in the use of genetic variants to predict the risk of diseases but they are considered ineffective compared to clinical risk scores. Objective: To develop polygenic risk scores (PRS) with high predictive value for complications of diabetes and for distinguishing patients responding to ADVANCE intensive antihypertensive therapy.

Methods: Genetic variants of diabetes complications were selected from GWAS publically available catalog. PRS were generated by weighting risk alleles by their effect size.

Results: A genetic model composed of 620 SNPs, sex, age and geno-ethnicity had an area under the curve of the receiver operating characteristics of 0.72 for cardiovascular death in 4098 Caucasian of ADVANCE, comparable to ADVANCE clinical score of 0.70 and exceeding Framingham risk engine. When individuals were divided into tertiles of genetic risk, a significant gradient for incident and prevalent outcomes was demonstrated from 1.55 for heart failure to over 3 fold for macroalbuminuria, myocardial infarction and stroke. For events such as albuminuria and cardiovascular death, ADVANCE blood pressure medication could be administered as preventive measure in the highest tertile of genetic risk model. Cox cumulative hazard plots revealed that individuals with the highest genetic risk had a decrease in mortality by ADVANCE antihypertensive treatment, persisting in ADVANCE-ON post-trial observation. Conclusion: PRS identified diabetic patients with increased risk for outcomes prior to the penetrance of actual risks, increasing its clinical utility over clinical risk score. The highest benefit of treatment was confined to the highest genetic risk category.

P. Hamet: None. **M. Haloui:** None. **F. Harvey:** None. **R. Tahir:** None. **F. Marois-Blanchet:** None. **C. Long:** None. **R. Attaoua:** None. **P. Simon:** None. **J. Chalmers:** None. **M. Marre:** None. **S. Harrap:** None. **M. Woodward:** None. **J. Tremblay:** None.

C01.2
Returning cardiovascular disease risk prediction back to individuals motivate beneficial lifestyle changes: Preliminary results from the GeneRISK-study

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While numerous genetic loci impacting the risk for common complex disease have been identified, their clinical use for disease prevention has remained minimal. To facilitate the translation of genomic research and to empower individuals to undertake risk-reducing interventions, we tested the effects of returning individual cardiovascular disease (CVD) risk information to 7,328 randomly sampled middle-aged individuals from Southern Finland. To communicate the personalized risk information, we developed an interactive graphical interface which utilizes both traditional and genomic risk data (polygenic risk score (PRS) from ~49,000 common genetic variants). While the follow-up of the full cohort is underway, we here report prospective results for 1,022 subjects.

When reassessed by an e-questionnaire and a clinical visit 1.5 years after the baseline 88.4% of participants said that their personal risk information had inspired them to take better care of their health. 13.7% had achieved sustained weight loss (-3 kg) and 17.0% of smokers had quit smoking compared to 4% annual cessation rate in the general population. The propensity for risk-reducing interventions (weight loss, smoking cessation or visiting a physician), was 32.4% in subjects with predicted 10-year risk of CVD >10% and 18.4% in subjects at lower risk. An increased PRS associated with successful intervention particularly in the high risk-group ($p < 0.001$). Our preliminary data show that integrating genomic and traditional health information for common chronic disease prevention and communicating this information back to individuals can support lifestyle changes. By the time of the conference, we estimate to have follow-up data available on >3000 individuals.

E. Widen: None. **I. Surakka:** None. **N. Mars:** None. **P. Pöllänen:** None. **K. Hotakainen:** None. **J. Partanen:** None. **J. Aro:** None. **S. Ripatti:** None.

C01.3
Development of a point of carepharmacogenetic test to avoid antibiotic related hearing loss in neonates

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Introduction: In conjunction with a beta-lactam the UK National Institute for Health and Care Excellence (NICE) recommends the use of gentamicin, an aminoglycoside, as the first-choice antibiotic for empirical treatment of sepsis in the neonatal period. The mitochondrial m.1555A>G variant, with a prevalence of 1 in 500, predisposes to ototoxicity after aminoglycoside administration. This relationship is particularly profound in early childhood, with a single dose causing irreversible profound sensorineural hearing loss. Current genetic testing can take 3 days, an unacceptable delay in the acute setting. We aimed to develop a point of care test (POCT) to identify the variant in a clinically relevant timeframe.

Methods: We used a Genedrive® POCT platform to genotype m.1555A>G. An asymmetric polymerase chain reaction amplifies the target region before identification of

the genotype via melt-curve analysis. The assay was optimised using buccal cell samples from the inner cheek and the system is designed to extract DNA, genotype using the above methodology and provide an actionable readout of the variant.

Results: This POCT identifies the m1555A>G genotype in less than 40 minutes with sensitivity and specificity comparable to the validated laboratory methodology.

Conclusions: Using this system we can identify the m.1555A>G variant in a clinically relevant timeframe. The assay is portable, non-invasive and will allow for tailored antibiotic prescribing in the acute setting to avoid neonatal hearing loss. A feasibility study introducing the POCT into neonatal units is starting in summer 2018, representing the first pharmacogenetic assay for use in the acute setting.

J.H. McDermott: None. **S. Ainsworth:** A. Employment (full or part-time); Significant; Genedrive. **M. Szynkiewicz:** A. Employment (full or part-time); Significant; Genedrive. **G. Miele:** A. Employment (full or part-time); Significant; Genedrive. **W. Newman:** None.

C01.4

From genetics to therapy: successful one-year eculizumab treatment of protein-losing enteropathy caused by loss of the complement regulator CD55

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Introduction. Protein-losing enteropathy (PLE) manifests as hypoalbuminemia, edema, and malabsorption due to intestinal or lymphatic disruption. We studied an extended consanguineous Muslim-Arab family comprising of six patients diagnosed with PLE and hypercoagulability.

Methods. Whole exome sequencing (WES) was performed on three patients and a healthy mother. Flow cytometry was used to test for CD55 and complement overactivation. Three patients were treated by off-label compassionate eculizumab therapy, a humanized anti-C5 monoclonal antibody.

Results. WES analysis revealed a homozygous frameshift variant in *CD55* (c.43del; p.Leu15Serfs*46), supported by two-point LOD score of 4.43 and co-segregation analysis in the extended family ($n = 32$). Absence of CD55 was confirmed by flow cytometry. Furthermore, membrane attack complex (MAC) and iC3b depositions on WBCs were significantly increased in patients compared to age-matched controls ($p < 0.001$). Significant clinical and laboratory improvement was observed in patients treated with eculizumab, including resolution of diarrhea, normalization of serum albumin, and 60% reduction in MAC deposition on WBCs within two months, and further improvement within a year.

Discussion. *CD55* encodes a membrane-bound complement-regulatory protein that inhibits the C3/C5 convertases and protects cells against complement-induced self-injury. The genetic findings and evidence of abnormal complement activation prompted successful off-label compassionate therapy with eculizumab, a terminal complement inhibitor. The treatment led to rapid improvement and ultimately achieved complete amelioration of symptoms in three treated patients. This path from genetic analysis to tailored therapy warrants studying the roles of complement and CD55 in other intestinal disorders that may benefit from similar treatment.

A. Kurolap: E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Submitted patent with Alexion, without royalties. **O. Eshach-Adiv:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Submitted patent with Alexion, without royalties. **T. Hershkovitz:** None. **T. Paperna:** None. **A. Mory:** None. **D. Oz-Levi:** None. **Y. Zohar:** None. **H. Mandel:** None. **J. Chezar:** None. **D. Azoulay:** None. **S. Peleg:** None. **E.E. Half:** None. **V. Yahalom:** None. **L. Finkel:** None. **O. Weissbrod:** None. **D. Geiger:** None. **A. Tabib:** None. **R. Shaoul:** None. **D. Magen:** None. **L. Bonstein:** None. **D. Mevorach:** None. **H.N. Baris:** None.

C01.5

A pharmacogenetic study implicates *NINJ2* in the response to IFNbeta in Multiple Sclerosis patients

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Introduction: Several therapies are available for Multiple sclerosis (MS), however clinical response is heterogeneous and a more personalized approach is needed to maximize treatment efficacy. We aim to confirm and test the functional role of rs7298096, a polymorphism located upstream to the *NINJ2* (nerve injury-induced protein 2) gene in a putative enhancer region, in the clinical response to IFNβ in Multiple Sclerosis (MS).

Materials: a total of 928 IFNβ-treated MS patients have been recruited across 4 centers in Italy, Spain and Australia. We also run eQTL analysis between rs7298096 and *NINJ2* expression using available GTEx data and GEO datasets, and we performed a luciferase reporter assay.

Results: an association between $rs7298096_G$ and time to first relapse (TTFR) after drug start was observed in the Italian discovery cohort ($P_{discovery} = 0.032$) and confirmed in two additional cohorts ($P_{replication} = 0.031$ and 0.025), with $rs7298096_G$ being associated with a longer TTFR ($P_{meta\text{-}analysis} = 2x10^{-4}$; HR: 1.51). Furthermore, we discovered that the SNP has an eQTL effect on *NINJ2* expression in whole blood ($P = 7.0x10^{-6}$) according to GTEx data, and it is able to modulate gene expression in vitro using a luciferase assay ($P < 0.01$).

Conclusions: This study validates the role of rs7298096 in the clinical response to IFNβ in MS patients. Ex vivo and in vitro experiments suggests that the SNP is able to influence the expression of NINJ2, an adhesion molecule involved in inflammation and endothelial cell activation, supporting its role in relapse occurrence and inflammatory activity in MS.

S. Peroni: None. **M. Sorosina:** None. **S. Malhotra:** None. **F. Clarelli:** None. **L. Villar:** None. **V. Martinelli:** None. **C. Guaschino:** None. **L. Citterio:** None. **J. Lechner-Scott:** None. **X. Montalban:** None. **G. Comi:** None. **F. Esposito:** None. **M. Comabella:** None. **F. Martinelli Boneschi:** None.

C01.6

Genome-wide association study of Pandemrix-induced narcolepsy in Sweden - a possible role for glial cell line-derived neurotrophic factor (*GDNF*)

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Background: Narcolepsy is an autoimmune disease characterized by an inability to control sleep and wakefulness. The number of young diagnosed with narcolepsy rose sharply in Sweden following vaccination with Pandemrix against swine influenza 2009-2010. The most frequent form, narcolepsy type I, is caused by a loss of hypocretin neurons, and is strongly associated with *HLA-DQB1*0602*. However, only 0.02% of *HLA-DQB1*0602* carriers developed narcolepsy following vaccination, and it is probable that a combination of other genetic and external risk factors are required.

Method: 43 adjudicated cases of Pandemrix-induced narcolepsy from the Swedish adverse drug reaction biobank Swedegene were compared with 4891 controls from TwinGene. Genotyping was performed on Illumina arrays. The merged dataset contained 600K single nucleotide polymorphisms (SNPs), and after phasing and imputation 8.6 million SNPs. We corrected for principal components 1-4. The genome-wide significance p-value threshold was set to $p < 5x10^{-8}$.

Results: Pandemrix-induced narcolepsy was significantly associated with *HLA-DQB1*0602*, odds ratio (OR) 6.4 [95% confidence interval (CI) 4.2, 9.8], $p = 1.4x10^{-17}$. After correction for *HLA-DQB1*0602*, the strongest association

was with *GDNF-AS1* (OR = 8.6 [95% CI 4.2, 17.6], p = $2.6x10^{-9}$).

Conclusion: We found an association with the non-coding RNA gene *GDNF-AS1*, which is located head to head with *GDNF* and potentially regulates this gene. *GDNF* encodes an essential neurotrophic factor that supports neuronal survival and has been associated with Alzheimer's disease. This finding may increase the understanding of disease mechanisms underlying narcolepsy.

Grants: Swedish Research Council (Medicine 521-2011-2440, 521-2014-3370 and 521-2017-00641); Swedish Medical Products Agency; Clinical Research Support (ALF) at Uppsala University.

M. Wadelius: None. **N. Eriksson:** None. **H. Smedje:** None. **Q.Y. Yue:** None. **P.K.E. Magnusson:** None. **P. Hallberg:** None.

C02 Syndrome updates 1

C02.1

Lethal and non-lethal *GLIS1* related malformation syndromes

P. Prontera[1], C. Le Caignec[2], J. Philippe[3], D. Martin-Coignard[4], C. S. Gunn[3], P. Lindenbaum[5], C. Bokobza[3], R. Redon[5], E. Sallicandro[1], A. Mencarelli[1], D. Rogaia[1], C. Gradassi[1], M. Schippa[1], R. Romani[1], C. Ardisia[1], G. Merla[6], S. Troiani[7], G. Stangoni[1], E. E. Davis[3]

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GLI-similar (Glis)1-3 proteins constitute a subfamily of the Krüppel-like zinc finger transcription factors that are closely related to the Gli family. Glis1-3 play critical roles in the regulation of a number of physiological processes and, to date, mutations in *GLIS2* and *GLIS3* have been implicated in human pathologies (nephronophthisis, an autosomal recessive cystic kidney disease; neonatal diabetes and congenital hypothyroidism, respectively). The role of *GLIS1* in human embryogenesis and disease is unknown, and its function is still poorly understood. We performed whole exome sequencing in two unrelated families, each with two affected siblings, and identified ultra-rare, recessive missense mutations in *GLIS1* that segregate with disease. The variations modified highly conserved amino acids and were predicted to be deleterious by 3 different bioinformatics tools. Comparison of the clinical features of the four individuals harboring *GLIS1* mutations shows phenotypic overlap, including IUGR, arthrogryposis, short ribs, vertebral anomalies, and microretrognathia and microstomia as the predominant craniofacial dysmorphisms. Notably, individuals in these two families display a different degree of severity: lethal (resembling the Crane-Heise syndrome) and non-lethal (similar to Bhoring-Opitz syndromes), respectively. To establish the relevance of *GLIS1* loss of function to disease, we have developed F0 *glis1* zebrafish mutants and transient loss of function models. These animals display significant defects in craniofacial cartilage patterning in comparison to controls. Together, our clinical and genetic findings as well our preliminary modeling studies, indicate a likely role for *GLIS1* in early developmental processes in humans, and potentially represent differing aspects of an allelic spectrum of severity.

P. Prontera: None. **C. Le Caignec:** None. **J. Philippe:** None. **D. Martin-Coignard:** None. **C.S. Gunn:** None. **P. Lindenbaum:** None. **C. Bokobza:** None. **R. Redon:** None. **E. Sallicandro:** None. **A. Mencarelli:** None. **D. Rogaia:** None. **C. Gradassi:** None. **M. Schippa:** None. **R. Romani:** None. **C. Ardisia:** None. **G. Merla:** None. **S. Troiani:** None. **G. Stangoni:** None. **E.E. Davis:** None.

C02.2

The Study of Adults and Adolescents with Silver-Russell syndrome: evaluating the adult phenotype of Silver-Russell syndrome

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Clinical features of Silver-Russell syndrome (SRS) may become less apparent over time and the adult consequences are unknown. Small-for-gestational-age (SGA) birth is associated with adult metabolic syndrome but relevance to SRS (a cause of SGA) is unclear. Educational attainment in adults with SRS has not been assessed. We, therefore, sought to determine the phenotype, cardio-metabolic profile and academic attainment in adults with SRS and propose a scoring system.

Methods: UK participants aged ≥18 years with SRS were invited to a study appointment involving a medical history, clinical examination and investigations.

Results: 33 individuals (18 females) aged 13.32-69.71 years (median 29.58) were recruited. Loss of methylation at H19/IGF2 was diagnosed in 81.8%; maternal uniparental disomy for chromosome 7 in 18.2%. Median height standard deviation score (SDS) was -2.67; weight SDS 1.72; body mass index SDS -0.53; and head circumference SDS -0.95. Short stature (height SDS≤-2) was present in 60.6%. Asymmetry and relative macrocephaly were present in 66.7% and 57.6% respectively. Congenital anomalies were present in 45.5%. In those aged ≥18 years (n = 25), GCSEs were gained by 84%; university degrees by 48%. Type 2 diabetes mellitus (n = 3), hypertension (n = 2), and hypercholesterolaemia (n = 2) were observed.

Conclusions: the clinical features show similarities and differences to childhood reports suggesting a change over time. Educational attainment in this cohort is above the UK average but cardio-metabolic problems appear similar. We will propose a system for targeting epigenetic testing in adults with short stature. Funding:NIHR RfPB grant PB-PG-1111-26003.

O. Lokulo-Sodipe: None. **E.L. Wakeling:** None. **H.M. Inskip:** None. **C.D. Byrne:** None. **D.J.G. Mackay:** None. **J.H. Davies:** None. **I.K. Temple:** None.

C02.3

Mutations in the homeobox gene GSX2 cause hypoplasia/agenesis of the basal ganglia and the olfactory bulbs and diencephalic-mesencephalic junction dysplasia

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Introduction: Basal ganglia (BG) are subcortical gray nuclei which play essential roles in controlling voluntary movements, cognition and emotion. While BG dysfunction is observed in many neurodegenerative or metabolic disorders, congenital malformations are rare. Dysplastic BG are typical of tubulinopathies but their agenesis/marked hypoplasia has never been reported to date.

Material and methods: We ascertained two unrelated sporadic girls presenting with spastic tetraparesis and severe intellectual impairment, sharing a unique malformation characterized by agenesis/hypoplasia of BG (mainly putamen and globus pallidus) and olfactory bulbs, and hypothalamic-mesencephalic fusion. WES identified two novel homozygous variants, c.26C> A (p.Ser9*) and c.752A> G (p.Gln251Arg) in the *GSX2* gene, a member of the family of homeobox transcription factors, which are key regulators of embryonic development. *GSX2* is selectively expressed in the lateral ganglionic eminence, a protrusion of the ventral telencephalon from which, along with the medial ganglionic eminence, the BG and olfactory tubercles originate.

Results: The truncating variant resulted in complete loss of protein expression. The missense variant affects a highly conserved residue of the homeobox domain (HD) and is consistently predicted as pathogenic. Molecular dynamics showed impaired HD structural stability and weaker interaction with DNA. Expression studies on both patients' fibroblasts demonstrated reduced expression of *GSX2* itself, likely due to altered transcriptional self-regulation, as well as significant expression changes of target genes *ASCL1* (downregulated) and *PAX6* (upregulated).

Conclusions: We report for the first time the clinical phenotype and molecular basis associated to BG agenesis in humans. Funding: ERC Starting Grant 260888; Ricerca Finalizzata NET-2013-02356160.

R. De Mori: None. **M. Severino:** None. **M. Mancardi:** None. **D. Anello:** None. **S. Tardivo:** None. **T. Biagini:** None. **M. Breuss:** None. **E. Lorefice:** None. **A. Gamucci:** None. **B. Illi:** None. **A. Micalizzi:** None. **M. Ginevrino:** None. **A. Casella:** None. **R. Rosti:** None. **T. Mazza:** None. **J. Gleeson:** None. **A. Rossi:** None. **E. Valente:** None.

C02.4

Loss of function mutations in *TCF12* cause autosomal dominant Kallmann syndrome and reveal network-level interactions between causal loci

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Dysfunction of gonadotropin-releasing hormone (GnRH) causes a range of reproductive disorders resulting from defects in the specification, migration and/or function of GnRH neurons. To identify new molecular components of this system, we performed a systematic genetic interrogation via whole exome sequencing of families with isolated GnRH deficiency (IGD). We report 10 families with an anosmic form of IGD (Kallmann Syndrome; [KS]) harboring autosomal dominant loss-of-function mutations in *TCF12*, a transcription factor also known to cause syndromic craniosynostosis. We observed no distinction in mutation localization on the *TCF12* locus for KS versus the reported craniosynostosis alleles. Additionally, 3/10 families display both KS and craniosynostosis indicating that allelism at the driver gene alone is insufficient to explain the phenotypic variability. To dissect this phenomenon further, we show that loss of *tcf12* in zebrafish perturbs GnRH neuronal patterning with concomitant attenuation of the expression of several potentially downstream genes that are both mutated in other syndromic forms of IGD and map to a TCF12 affinity network. Finally, rescue of *tcf12* loss of function GnRH phenotypes was achieved by mRNA corresponding to one of these loci, *STUB1*. In addition to extending the rapidly evolving genetic architecture of IGD, these studies begin to assemble one of the functional networks that regulate the ontogeny of GnRH neurons and potentially modulate phenotype. These findings also highlight an emerging class of pleiotropic genes including *FGFR1, SMCHD1, CHD7*, and now *TCF12* that contribute to both IGD and distinct craniofacial abnormalities. Funding: US NIH P50HD028138 (E.E. D., W.F.C., N.K.); K23HD077043 (R.B.).

E.E. Davis: None. **R. Balasubramanian:** None. **Z.A. Kupchinsky:** None. **D. Keefe:** None. **L. Plummer:** None. **B. Meczekalski:** None. **K.E. Heath:** None. **V. Lopez-Gonzalez:** None. **M.J. Ballesta-Martinez:** None. **G. Margabanthu:** None. **S. Price:** None. **J. Greening:** None. **M.E. Wierman:** None. **W.F. Crowley:** None. **N. Katsanis:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Rescindo Therapeutics, Inc.

C02.5

Predictors of all-cause mortality in adults with 22q11.2 deletion syndrome

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Background: Relatively little is known about late outcomes in genomic disorders. Elevated risk of premature death has been reported for 22q11.2 deletion syndrome (22q11.2DS), however the effect of key 22q11.2DS-associated features on all-cause mortality is unknown.

Methods: We studied 1265 adults (≥17 years): 290 with 22q11.2DS (135 male; median age 29.7, range 17.8-68.6, years), their 444 unaffected siblings and 531 unaffected parents, followed for up to 23 years. We compared survival between groups using Kaplan-Meier estimates and Cox regression (hazard ratio (HR) and 95% confidence interval (CI)) to investigate the relationship between all-cause mortality and potential predictor variables including major congenital heart defects (CHD), psychotic illness, and intellectual disability.

Results: Adults with 22q11.2DS had lower ($p < 0.0001$) survival than unaffected siblings or parents. The 22q11.2 deletion (HR 10.3, 95% CI 3.2-32.5) and major CHD (HR 4.7, 95% CI 2.1-10.6) were significant predictors of

mortality. Within 22q11.2DS, major CHD (HR 3.0, 95% CI 1.2-7.5) remained a significant risk predictor when controlling for other factors. There were 31 (10.7%; 13 M) deaths of individuals with 22q11.2DS at median age 46.4 (range 18.1-68.6) years.

Conclusions: Individuals with 22q11.2DS who survive childhood have diminished life expectancy, attributable largely to the 22q11.2 deletion, with major CHD as an additional significant contributor. Median age at death was nearly 5 years older than when examined ~10 years ago in a smaller cohort. A substantial minority of patients outlive both parents. Further longitudinal studies are needed to determine the underlying mechanisms, and potential modifying effects of anticipatory care.

A.S. Bassett: None. **T. Heung:** None. **E. Ng:** None. **J. Graffi:** None. **S. Van Mil:** None. **S. Malecki:** None. **E. Chow:** None. **D. Andrade:** None. **E. Boot:** None. **E. Breetvelt:** None. **C. Silversides:** None.

C02.6

Pathogenesis and treatment of esophageal dilation and gastric epithelial hyperplasia in a mouse model for cardio-facio-cutaneous syndrome

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Germline mutations in *BRAF* are a major cause of cardio-facio-cutaneous (CFC) syndrome, which is characterized by heart defects, characteristic craniofacial dysmorphology and dermatologic abnormalities. Patients with CFC syndrome also commonly show gastrointestinal dysfunction, including feeding and swallowing difficulties and gastroesophageal reflux. We have previously found that knock-in mice expressing a *Braf* Q241R mutation exhibit CFC syndrome-related phenotypes, such as growth retardation, craniofacial dysmorphisms, congenital heart defects, learning deficits and ectodermal abnormalities. However, it remains unclear whether $Braf^{Q241R/+}$ mice exhibit gastrointestinal dysfunction. Here, we report that $Braf^{Q241R/+}$ mice have neonatal feeding difficulties and esophageal dilation. The esophagus tissues from $Braf^{Q241R/+}$ mice displayed incomplete replacement of smooth muscle with skeletal muscle and decreased contraction response to carbachol, an acetylcholine receptor agonist. In contrast, achalasia-like loss of neuronal cells was not observed in the lower esophageal sphincter. The $Braf^{Q241R/+}$ mice also showed epithelial hyperplasia, hyperkeratosis and a thickened muscle layer in the forestomach, showing the increased expression of genes related to cell adhesion, epithelium development and keratin. Treatment with MEK inhibitors, including PD0325901, MEK162 and AZD6244, partly ameliorated the growth retardation, esophageal dilation, hyperkeratosis and thickened muscle layer in the forestomach in $Braf^{Q241R/+}$ mice. The esophageal dilation and aberrant skeletal-smooth muscle boundary were ameliorated in $Braf^{Q241R/+}$ mice treated with the histone H3K27 demethylase inhibitor, GSK-J4. Our results provide clues to elucidate the pathogenesis and possible treatment of gastrointestinal dysfunction and failure to thrive in patients with CFC syndrome.

S. Inoue: None. **S. Takahara:** None. **T. Yoshikawa:** None. **T. Niihori:** None. **K. Yanai:** None. **Y. Matsubara:** None. **Y. Aoki:** None.

C03 Multi-omics 1

C03.1

Host genetics and microbial impact on plasma metabolites is linked to the cardiovascular risk

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The gut microbiome is an attractive therapeutic target for metabolic health, however the functional links between the microbiome and host metabolism is missing. Our study aims to investigate the relationships between gut bacteria and pathways, host genetics, and the fasting plasma metabolome, and understand their relevance to metabolic risk of cardiovascular disease (CVD). For 1,370 individuals ($n = 1073$ from population cohort, and $n = 297$ from obesity cohort) we collected extensive phenotype data, genome-wide genotyping, metagenome sequencing data and plasma metabolomics profiling of 231 metabolomic traits. We investigated associations between host genetics, the gut microbiome and metabolism, using discovery and replication analysis, and linked metabolism-related microbial factors to cardio-metabolic phenotypes in the obesity cohort. Next, we identified bacterial pathways associated to metabolic risk score of CVD. Finally, we examined microbe-diet-metabolism-immune interaction through integration analysis with 78 dietary factors, 12 inflammatory

cytokines, and stool levels of 5 short-chain fatty acids. Both host genetics and microbiome features were associated with host metabolism. 54 bacterial pathways were linked to individual metabolic risk scores for CVD; these microbial effects are largely mediated by BMI, diet and inflammatory cytokines. In particular, bacterial metabolism of L-methionine was consistently associated to higher level of glycoprotein N-acetyls in plasma, and to atherosclerotic plaques in obese individuals. L-methionine is associated to higher metabolic risk score of CVD, higher inflammatory cytokines and lower fruit intake. These findings point to the potential use of bacterial L-methionine biosynthesis and other microbial pathways as a therapeutic target of metabolic diseases and CVD.

A. Zhernakova: None. **A. Kurilshikov:** None. **I. van den Munckhof:** None. **E. Slagboom:** None. **K. Schraa:** None. **L. Joosten:** None. **R. Xavier:** None. **F. Kuipers:** None. **M. Netea:** None. **C. Wijmenga:** None. **J. Fu:** None.

C03.2

Single-cell multi-omics sequencing to understand the nature, extent and biology of cellular heterogeneity in breast cancer

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Single-cell sequencing techniques allow the study of the subclonal architecture of tumors and reveal the co-occurrence of (driver) mutations as well as their order of acquisition over molecular pseudo-time. Recently novel single-cell multi-omics methods have been developed. Importantly, such technologies now enable us to study the diversity of cancer cell states (determined by the interplay of their genome, epigenome and transcriptome) that arises within a tumor, at its most fundamental level, the cell. One example is the genome and transcriptome sequencing (G&T-seq) method, where DNA and RNA of the same single cell can be sequenced in parallel.

480 single cells of a patient with unifocal breast cancer were sequenced and we were able to computationally separate and identify normal and cancer cells based on the genomic and transcriptome profiles. The single-cell DNA copy number landscapes disclosed clear genetic alterations present in subclonal populations of cells. We identified biologically relevant marker genes from the transcriptomic profiles with genes involved in the negative regulation of apoptosis, metastasis, RET signaling and/or increasing cell motility. In addition, ERBB2 was identified as a marker gene in accordance with the HER2+ molecular classification of the tumor.

Furthermore, in this experiment, we were able to unambiguously study for the first time the effect of copy number state on the transcriptome in breast tumors using the G&T-seq technique.

Grants:

FWO strategic basic research grant (1/1/18-1/1/22)

Stichting Tegen Kanker grant 2014-145.

S. Vanuytven: None. **A. Sifrim:** None. **M. Teng:** None. **D. Brown:** None. **L. Mora Bitria:** None. **E. Fernandez Gallardo:** None. **K. Theunis:** None. **S. Majjaj:** None. **H. Duvilliers:** None. **F. Rothe:** None. **C. Sotiriou:** None. **P. Van Loo:** None. **C. Desmedt:** None. **T. Voet:** None.

C03.3

Unraveling the *cis* and *trans* genetic regulatory map in over 1,500 induced pluripotent stem-cells lines

M. J. Bonder[1], C. Smail[2], D. Jakubosky[3], The i2QTL CONSORTIUM, C. Brown[4], S. Montgomery[2], E. Smith[3], K. Frazer[3], O. Stegle[1]

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Genome-wide association studies have yielded a compendium of genetic variants that are associated with human diseases. However, the majority of these variants are in intergenic region, and understanding their function remains challenging. One strategy for linking these risk variants to genes are expression quantitative trait loci (eQTL) studies. To date, most large scale eQTL studies have been performed in LCLs, blood, or post-mortem collected tissues. However, disease-causing variants often have tissue-specific effects. To understand effects manifesting in pluripotent and dedifferentiated cells, we mapped eQTLs in the largest panel of human induced pluripotent stem-cells (iPSCs) considered to date.

Using the data collected in the i2QTL CONSORTIUM we mapped eQTLs in a set of ~1,500 iPSC-lines, derived from over 1,000 donors. Gene expression was quantified using RNA-sequencing, enabling the mapping of eQTLs to genes, exons, transcripts, splicing and UTR usage. We identified significant *cis*-eQTLs for over 63% of expressed genes (FDR < 5%), 5% of which have not been observed in other studies. Our integrative analysis identified hundreds of *trans*-acting regulatory variants, including ~100 GWAS

variants. One example is the *trans*-acting SNP near the transcription factor *ELF2* which leads to expression changes in genes, downstream of *ELF2*. Another interesting example is the observed link between a GWAS variant to a *trans*-egene (*MED19*), both previously implicated in prostate carcinoma, but not previously linked. This highlights the relevance of this map for cancer and pluripotency. Currently, we are including both rare- and structural-variants to try and make a definitive map of *cis*- and *trans*-eQTLs in iPSCs.

M.J. Bonder: None. **C. Smail:** None. **D. Jakubosky:** None. **C. Brown:** None. **S. Montgomery:** None. **E. Smith:** None. **K. Frazer:** None. **O. Stegle:** None.

C03.4

Integration of ~10,000 metabolite features with genotype data and immune phenotypes reveals genetic determinants and common regulatory modules

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Blood metabolites are involved in important biological pathways such as immune homeostasis, but can also be used as biomarkers for human disease. As part of the Human Functional Genomics Project we aim to characterize the genetic determinants of baseline metabolites levels to better understand how these changes relate to the previously observed inter-individual variation in immune response.

We conducted a metabolite quantitative trait locus (mQTLs) mapping for 10,431 baseline metabolic features measured by three profiling approaches (Brain Shake metabolites, general metabolites and untarget metabolites) in 500 healthy Dutch individuals. In total, we mapped >200 independent genome-wide significant loci as being influential on 1229 metabolic features. Pathway analysis on the mQTL genes show a significant enrichment in metabolic processes. We found 116 nonsynonymous mQTL SNPs out of which 17 SNPs show a deleterious effect on metabolic enzyme function. Interestingly, >10 mQTLs shared common co-regulation with Crohn disease or Celiac disease.

We further systematically associated these metabolites features with 9 categories' host factors consisting of baseline immune parameters (including 73 immune cell subpopulation frequencies), molecular profiles (including transcriptome, gut microbiome) and immune response (cytokine production capacity upon stimulation). In general, metabolites show higher correlation with monocyte-derived cytokines than with lymphocyte-derived cytokines. By combining genetic markers and metabolite measurements we achieved a significant improvement in predicting cytokine production when compared to solely using genetics (p value < 0.05).

In summary, this study reveals novel genetics factors regulating blood metabolite levels and highlights the importance of baseline metabolites in immune response.

X. Chu: None. **M. Jaeger:** None. **O.B. Bakker:** None. **R. Aguirre-Gamboa:** None. **M. Oosting:** None. **S.P. Smeekens:** None. **S. Withoff:** None. **R.T. Netea-Maier:** None. **H.J.P.M. Koenen:** None. **I. Joosten:** None. **R.J. Xavier:** None. **L. Franke:** None. **L.A.B. Joosten:** None. **S. Sanna:** None. **V. Kumar:** None. **C. Wijmenga:** None. **M. G. Netea:** None. **Y. Li:** None.

C03.5

Plasma protein levels - a link between host microbiome, genetics, metabolites and disease-related phenotypes

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Blood circulating proteins are often measured as biomarkers or risk factors for various diseases, including cardiovascular disease (CVD). However, causal relationships between proteins and clinical phenotypes are not fully understood. Here, we used genetics as an instrumental variable and assessed causality between circulating proteins, gut microbiome, metabolic traits and complex diseases in 1,294 individuals from the LifeLines-DEEP cohort, which has genetics, proteomics, gut microbiome data and over 2,000 phenotypes available.

In this study, we focused on 92 CVD-related proteins and first tested their association with genetic variation and microbiome. Then we performed a two-directional Mendelian randomization analysis to assess causal relationships between proteins and microbiome, metabolic traits and clinical phenotypes. To do so, we constructed genetic risk scores (GRS) for 73 proteins, 42 microbial factors, over 1000 complex traits and diseases based on our dataset and published GWAS studies. We found 327 protein-GRS associations significant at FDR < 0.05. The most significant proteins include GRN, SELE, PECAM-1 and ICAM-2, potentially relevant to CVD. Around one third of the associated GRS involve metabolic traits. We also see associations with GRS for lipids and immune cell traits. We detected numerous associations between GRS of proteins with microbiome and clinical traits.

Our study suggests bi-directional causal relationships between circulating proteins, gut microbiome and complex traits and diseases. We prioritize proteins as potential targets for therapeutic treatment if they are causal risk factors, and proteins as biomarkers to monitor disease progression if their alteration is a consequence of a specific disease.

D.V. Zhernakova: None. **U. Võsa:** None. **T.H. Le:** None. **A. Kurilshikov:** None. **B. Atanasovska:** None. **M. Bonder:** None. **S. Sanna:** None. **A. Claringbould:** None. **P. Deelen:** None. **R.A. de Boer:** None. **F. Kuipers:** None. **M.G. Netea:** None. **M. Hofker:** None. **C. Wijmenga:** None. **L. Franke:** None. **A. Zhernakova:** None. **J. Fu:** None.

C03.6

A high-resolution, genome-scale promoter 'interactome' in human T follicular helper cells implicates novel effector genes at SLE GWAS loci

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Genome-Wide Association Studies (GWAS) have implicated >60 loci in the susceptibility to Systemic Lupus Erythematosus (SLE). However, it is known that GWAS only reports genomic signals and not necessarily the precise localization of culprit genes. Chromatin conformation capture technologies that detect contacts between distant regions of the genome offer an opportunity to physically map disease variants to effector genes. To move beyond analyzing one locus at a time, and to improve upon the low resolution of available Hi-C datasets, we developed a massively parallel, high-resolution Capture-C based method to characterize the genome-wide interactions of all human promoters in any cell type. We designed a custom Agilent SureSelect RNA library targeting *DpnII* restriction fragments overlapping 36,691 promoters of protein-coding, noncoding, antisense, snRNA, miRNA, snoRNA and lincRNA genes. We applied our method of SPATIaL-seq (genome-Scale, Promoter-focused Analysis of chromaTIn Looping) to primary human T follicular helper (TFH) cells from healthy tonsil, a cell type relevant to SLE. These sub-1kb datasets were intersected with ATAC-seq maps of TFH open chromatin and SLE proxy SNPs from the 63 loci, resulting in an assessment of accessible variants at 48 of these loci. Some 'nearest' genes to the sentinel SNP were supported e.g. *STAT4* and *IKZF3*, while at other loci more distant genes were implicated e.g. *LCLAT1* at the '*LBH*' locus, and the master TFH transcription factor *BCL6* at the '*LPP-TPRG1*' locus. In conclusion, high-resolution, three-dimensional promoter interactions with accessible, disease-associated SNPs in disease-relevant tissue can connect key variants to relevant effector genes with high accuracy.

S.F.A. Grant: None. **M.E. Johnson:** None. **E. Manduchi:** None. **C. Le Coz:** None. **M.E. Leonard:** None. **S. Lu:** None. **K.M. Hodge:** None. **N. Romberg:** None. **A. Chesi:** None. **A.D. Wells:** None.

C04 Epigenetics and Gene Regulation

C04.1

A comprehensive study comparing on- and off-target levels of the most common forms of CRISPR/Cas9 guide RNAs

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The CRISPR/Cas9 system is a powerful tool for manipulating mammalian genomes. Genome editing requires delivery of both the Cas9 nuclease and the targeting guide RNA (gRNA). The gRNA can be generated in multiple ways. Here we compare the efficiency of editing, the on- and off-target repair profiles, and the innate immune stimulation of gRNAs delivered as a plasmid-expressed single guide RNA (sgRNA), an in-vitro transcribed (IVT) sgRNA, a chemically-synthesized sgRNA and a chemically-synthesized bipartite complex (crRNA + tracrRNA) in HEK293 cells and human primary CD34+ hematopoietic stem and progenitor cells. We show that the editing repair profiles generated from delivery of the different gRNA constructs are identical. However, our results show that the chemically-modified crRNA + tracrRNA complex or sgRNA enable the highest genome editing with lowest toxicity. Furthermore, studies performed using human PBMCs revealed high levels of IFNα following delivery of IVT sgRNAs, with no detectable immune activation using chemically-modified gRNAs. A comprehensive analysis of the off-target events associated with the delivery of the common gRNA forms is presented. In addition, the specificity of the gRNA forms is compared when delivered into cells stably expressing Cas9 or when delivered as a ribonucleoprotein complex with wild-type or high-fidelity (HiFi) Cas9 nuclease. The off-target profiles for each class of gRNA were first compared using the unbiased GUIDE-seq approach and quantified using rhAmpSeq™, a multiplexed, amplification-based, target enrichment next-generation sequencing approach. Data suggest delivery of a chemically-modified gRNA precomplexed to the Alt-R HiFi Cas9 nuclease results in the lowest off-target activity.

A. Jacobi: A. Employment (full or part-time); Significant; Integrated DNA Technologies. **G. Rettig:** A. Employment (full or part-time); Significant; Integrated DNA Technologies. **M. Schubert:** A. Employment (full or part-time); Significant; Integrated DNA Technologies. **J. Shapiro:** None. **O. Iancu:** None. **A. Tovin:** None. **E. Cedrone:** None. **M. Dobrovolskaia:** None. **A. Hendel:** None. **M. Behlke:** A. Employment (full or part-time); Significant; Integrated DNA Technologies.

C04.2
The MEF2C regulatory network is disrupted in patients with Rett-like characteristics

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Introduction: Myocyte enhancer factor 2C (MEF2C) is a core transcription factor in neurodevelopment. MEF2C haploinsufficiency has been associated with a Rett-like syndrome, characterized by severe intellectual disability, seizures and stereotypic movements. So far, 11 deletions, eight translocations and one inversion involving 5q14.3 in Rett-like patients have been described, that do not directly affect the MEF2C coding sequence, suggesting that disruption of MEF2C regulatory elements could result in this Rett-like syndrome. The MEF2C regulatory network, however, is yet to be deciphered.

Material & Methods: We performed Circularized Chromosome Conformation Capture (4C) sequencing and characterized the activity of putative, neuronal MEF2C enhancers using in vitro and in vivo enhancer assays.

Results: Extensive 4C sequencing in a neuronal cell line revealed an intricate interaction network, in which the MEF2C promoter physically contacts distal putative enhancers located in the region affected in Rett-like patients. We confirmed enhancer potential for 10 out of 15 selected candidate elements using luciferase assays. Moreover, eight candidate enhancers exhibited in vivo neuronal activity in zebrafish. Six of these were active in the forebrain, of which three also displayed additional activity in specific neurons above the eye and two in the notochord. Further, one enhancer displayed mid- and hindbrain activity and one was specifically active in the notochord.

Conclusions: In summary, we have begun to unravel the complex regulatory network governing neuronal MEF2C transcription, involving multiple distal enhancers. Disrupting this regulatory structure is likely detrimental to normal neurodevelopment and can give rise to neurodevelopmental disorders such as Rett-like syndrome.

E. D'haene: None. **R. Bar-Yackov:** None. **I. Bariah:** None. **L. Vantomme:** None. **S. Van Loo:** None. **F. Avila Cobos:** None. **R. Eshel:** None. **R. Alatawna:** None. **B. Menten:** None. **R. Birnbaum:** None. **S. Vergult:** None.

C04.3
Personalized co-expression networks reveal genetic risk factors that change the regulatory wiring of cells

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Expression quantitative trait locus (eQTL) analyses have taught us that genetic risk factors for disease often affect gene expression in a cell type-specific way. However, much less is known about their effect on the regulatory wiring of individual cells. In order to study how genetic variants can affect regulatory networks, we generated personalized co-expression networks for 45 different individuals by generating single-cell RNA-seq (scRNA-seq) data for approximately 29.000 peripheral blood mononuclear cells.

We first validated our approach by replicating 261 *cis*-eQTLs that were previously identified in bulk RNA-seq data, and subsequently ascertained whether genetic variants can also alter the co-expression between genes (so-called 'co-expression QTLs'). We identified several of these co-expression QTLs at a false discovery rate of 0.05 and replicated these in large bulk RNA-seq datasets. For instance, we found 93 significant co-expression QTLs for the type I diabetes (T1D) variant rs11171739. This SNP affects the co-expression between *RPS26* and 93 other genes, many of which are known to be involved in mRNA translation, which suggests that this T1D variant might dysregulate this particular pathway.

In conclusion, this study shows the feasibility of using scRNA-seq data for eQTL and co-expression QTL analysis. With the expected growth in scRNA-seq eQTL datasets in the near future, we expect many more genetic variants will be identified that affect the regulatory wiring within cells, and which could be used to infer personalized regulatory networks using solely genotype data.

Funding: M.S.: ZonMW-VIDI 917.164.455, L.F.: ZonMW-VIDI 917.14.374, ERC Starting Grant 637640, BIOS Consortium: NWO 184.021.007

M.G.P. van der Wijst: None. **D.H. de Vries:** None. **H. Brugge:** None. **P. Deelen:** None. **M.A. Swertz:** None. **L. Franke:** None.

C04.4

Integrated analysis of transcriptional regulation in PLN R14del cardiomyopathy

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Introduction: The activity of DNA regulatory regions, such as promoters and enhancers, is a crucial factor in transcriptional regulation. They contain hotspots for transcription factor binding sites and are considered candidate regions harboring disease-related non-coding mutations. However, there is lack of information about how chromatin transcriptional regulation differs between health and (cardiac) disease. Here we focus on cardiac material of a homogeneous Dutch founder cohort of genetic cardiomyopathy due to same *PLN* R14del mutation as compared to controls.

Materials and Methods: We performed H3K27ac ChIPseq, RNA sequencing, and 4C on several tissues/cell lines related to PLN R14del cardiomyopathy and controls.

Results: Based on H3K27ac ChIPseq data, 2,107 differentially acetylated peaks and 1,302 topologically associating domains (TADs) have been detected. Using a window of +/-5kb from TSS, we have annotated 863 genes in their proximity. Enrichment of binding motifs related to 200 transcription factors (TFs) inside these regions have been found. Next, we show a correlation between H3K27ac promoter occupancy and the level of RNA expression. Furthermore, we detected a condition specific fingerprint by comparing the differentially active regions in *PLN* R14del to acetylation profiles of additional patients with ischemic cardiomyopathy and unrelated non-ischemic cardiomyopathy. iPSC-derived cardiomyocyte cell lines of PLN R14del patient and control were used for 4C-based annotation of most significant differentially acetylated regions.

Conclusions: Integrative chromatin analysis based on H3K27ac ChIP-seq, RNAseq and 4C identifies the major effector processes involved in PLN R14del end-stage cardiomyopathy, including many novel candidates.

J. Pei: None. **M. Harakalova:** None. **E. Nagyova:** None. **J.M.I.H. Gho:** None. **S. Sepehrkhouy:** None. **J. van Dinter:** None. **A. van Mil:** None. **M.P. Buijsrogge:** None. **N. de Jonge:** None. **M.M. Huibers:** None. **E.E.S.**

Nieuwenhuis: None. **H.M. den Ruijter:** None. **G. Pasterkamp:** None. **P.A. Doevendans:** None. **R. Hajjar:** None. **M. Mercola:** None. **J.P.G. Sluijter:** None. **R.A. de Weger:** None. **C. Cheng:** None. **A. Vink:** None. **M. Mokry:** None. **F.W. Asselbergs:** None.

C04.5

Alteration of *HDAC9* exons that also function as enhancers leads to *TWIST1* haploinsufficiency that result in limb and craniofacial phenotypes

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The transcription factor *TWIST1* plays a vital role in mesoderm development, particularly in limb and craniofacial formation. *TWIST1* haploinsufficiency during development could lead to craniosynostosis and limb malformation such as Saethre-Chotzen syndrome. However, the transcriptional regulatory mechanism that controls *TWIST1* expression during development is yet to be elucidate. Here, we characterized active enhancers in the *TWIST1-HDAC9* locus that control transcription in the developing limb and branchial arches. Using p300 and H3K27ac ChIP-seq data, we identified 12 enhancer candidates encompass protein coding exons of *Histone deacetyase 9 (HDAC9)*. Using zebrafish and mouse enhancer assays, we showed that 8 candidates have limb/fin and branchial arch enhancer activity that recapitulate *Twist1* expression. Each enhancer showed discrete activity pattern that together compile a spatiotemporal transcriptional regulation of *Twist1* in the developing limb/fin and branchial arches. Using 4C-seq, we showed that *Twist1* promoter interacts with Hdac9 exons that function as enhancers in the limb bud and branchial arch of mouse embryos at day 11.5. Alteration of these enhancers lead to *Twist1* haploinsufficiency and polydactyly in mouse models. Furthermore, *TWIST1* enhancers are regulated by limb-expressed transcription factors, including LMX1B and TFAP2 that bind and regulate their activity. Deletion of the LMX1B and TFAP2 binding sites altered *TWIST1* enhancer activity. Thus, our study elucidated essential components of *TWIST1* transcriptional machinery, suggesting that alteration of coding exons of HDAC9 could lead to *TWIST1* haploinsufficiency and a similar phenotypic outcome as *TWIST1* coding mutations.

N. Hirsch: None. **F. shemuluvich:** None. **T. Kaplan:** None. **D.G. Lupiáñez:** None. **R.Y.B. Birnbaum:** None.

C04.6

Treating Retinitis Pigmentosa with transcriptional-based therapeutics

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CRISPR/Cas9, transcription activator like effectors (TAL) and zinc-finger-proteins (ZF), are emerging as remarkably potent and popular DNA targeting platforms for genome editing and transcriptional regulation (gene activation and repression) in research applications. Here we investigated the design and development of therapeutics based on transcriptional modulation. In particular, as experimental models to establish the proof of concept of transcriptional repression as therapeutic, we used a RHODOPSIN (RHO) gene gain-of-function mutant transgenic mouse and the preclinical pig retina. RHO is the gene most commonly involved in the blinding disease autosomal dominant retinitis pigmentosa (adRP). In vivo retinal gene transfer by Adeno-associated virus (AAV) vectors of transcriptional repressors based on synthetic ZF DNA-binding proteins with (ZF6-R) or without (ZF6-DB) canonical repressor domain, enable the complete RHO transcriptional silencing and in turn therapy in the P347S mouse model of RHO adRP. In addition, we showed that the AAV mediated ectopic expression of the endogenous transcription factor (TF) KLF15 similarly generate robust RHO silencing. In the pig retina, comparison of specificity by RNA-sequencing, showed that ZF6-R and ZF6-DB perturbed 220 and 19 differentially expressed genes (DEGs), respectively. Surprisingly, the endogenous ectopic expression of the TF KLF15, showed similar high selectivity with 156 DEGs and therefore a safe profile. Efficacy assessed by retinal functional analysis in the adRP mouse model, show remarkable prevention of retinal degeneration. Collectively, these data support transcriptional modulation as a novel paradigm to generate transcriptional-based therapeutics for the treatment of human inherited disorders by gene therapy supporting their use in translational medicine.

S. Botta: None. **E. Marrocco:** None. **E.M. Surace:** None.

C05 Neurological and Neuromuscular Disorders

C05.1

SMPD4 loss-of-function mutations cause cerebral malformations and arthrogryposis through endoplasmic reticulum stress and autophagy induced by dysregulation of sphingolipid metabolism

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Introduction: Deregulation of sphingolipid homeostasis is an important mechanism in the pathogenesis of several metabolic, neuronal and proliferative diseases.

Materials and Methods: Through whole exome and RNA sequencing, we studied three unrelated families, presenting at birth with congenital arthrogryposis, microcephaly with consistent simplified gyral pattern and mild cerebellar hypoplasia. The only child who survived up to six years developed also anemia and diabetes. We used electronic microscopy (EM) and flow citometry to evaluate ultrastructural and cell cycle anomalies in patients' fibroblast cultures. In addition, we searched for similar cellular alterations within the Connectivity library Map from Broad Institute.

Results: In all patients, we identified biallelic loss-of-function mutations in *SMPD4*, encoding a putative neutral sphingomyelinase without a clearly defined role, localized in the endoplasmic reticulum. Analyses on patients' fibroblasts revealed a drastic reduction of normal *SMPD4* transcript levels, a dilated rough ER with aggregates of misfolded proteins and excess of lysosomes and late autophagic vacuoles. In addition, cell division alterations were observed. Connectivity mapping of *SMPD4* deficient gene expression signatures revealed highest similarity to *SPHK2* knock down. SPHK2 is one of the enzymes controlling the balance between ceramide and sphingosine-1-phosphate, which is important for regulation of cell stress response and cell growth. *SMPD4* mutant patient fibroblasts were more sensitive to the SPHK2 inhibitor ABC294640 compared to control cells.

Conclusions: Our data provide a mechanistic link between defects in sphingolipid homeostasis and a congenital microcephaly through dysregulation of ER stress and autophagy.

Funding: Stichting PPQA to GMM, ZonMW-TOP #91217045 to MF&GMM).

P. Magini: None. **L. Vandervore:** None. **R. Schot:** None. **M. Columbaro:** None. **M. van der Ent:** None. **L. Iommarini:** None. **M. Lequin:** None. **A.M. Porcelli:** None. **P. Govaert:** None. **F. Palombo:** None. **M. Dremmen:** None. **M.C. de Wit:** None. **M. Severino:** None. **M. Hoogeveen-Westerveld:** None. **M.T. Divizia:** None. **P. van den Berg:** None. **F. Verheijen:** None. **P. van der Spek:** None. **A. Jansen:** None. **G. Mirzaa:** None. **W.B. Dobyns:** None. **M. Seri:** None. **T. Pippucci:** None. **M. Fornerod:** None. **G.M.S. Mancini:** None.

C05.2

Resolving the diagnostic odyssey for young patients with rare genetic muscle disease through the application of extended exome sequencing technologies

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Introduction: Muscular dystrophies are rare genetic disorders characterised by progressive skeletal muscle weakness and atrophy. The diseases can be exceptionally debilitating and can directly precipitate premature mortality. Many patients remain undiagnosed even after several years of state-of-the-art clinical work-ups. With a view to resolving this diagnostic odyssey, the MYO-SEQ project applied targeted whole exome sequencing (WES) to over 1,000

European patients with suspected genetic muscle disease. Here, we detail the results for those patients with a reported age at disease onset of ≤15 years ($n = 470$).

Materials and methods: WES was performed using Illumina exome capture (38 Mb target) and a Picard-based pipeline. The variant call set was uploaded onto *seqr* and 429 neuromuscular disease genes examined for point mutations, small indels and copy number variations (CNVs).

Results: Pathogenic variants were identified in 59% ($n = 278$) of patients across 70 genes. The average age at the time of WES diagnosis was 29 years (median 26 years, range 4-77 years). LGMD2A (*CAPN3*) and sarcoglycanopathies (*SGCA*, *SGCB SGCD* and *SGCG*) were the most commonly diagnosed diseases, together accounting for a third of the solved cohort. Through an extended and modified analysis of the WES data, CNVs were detected in 8% of the solved patients and ranged in size from 0.11 kb to 1,047 kb.

Conclusions: We have advanced the utility of whole exome sequencing technologies to pioneer an accessible pathway that efficaciously addresses the diagnostic odyssey faced by rare disease patients.

K. Johnson: None. **A. Töpf:** None. **M. Bertoli:** None. **L. Phillips:** None. **A. Blain:** None. **M. Ensini:** None. **M. Lek:** None. **L. Xu:** None. **D.G. MacArthur:** None. **V. Straub:** None.

C05.3
Cis D4Z4 repeat duplications associated with facioscapulohumeral muscular dystrophy type 2

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Introduction: Facioscapulohumeral muscular dystrophy, known in genetic forms FSHD1 and FSHD2, is associated with D4Z4 repeat chromatin relaxation and somatic derepression of *DUX4* located in D4Z4. A complete copy of *DUX4* is present on 4A chromosomes, but not on the D4Z4-like repeats of chromosomes 4B or 10. Normally, the D4Z4 repeat varies between 8-100 units, while in FSHD1 it is only 1-10 units. In the rare FSHD2, a combination of a 4A allele with a D4Z4 repeat size of 8-20 units and heterozygous variants in the chromatin modifier SMCHD1 causes *DUX4* derepression and disease. We identified FSHD2 patients with unusually large 4A alleles of 21-70 D4Z4 units. Recently, *in cis* D4Z4 repeat duplications were described in ~2% of FSHD cases irrespective of *SMCHD1* mutation status, which prompted us to investigate FSHD2 patients with unusually long D4Z4 repeats for this duplication.

Materials and Methods: Genetic analysis of D4Z4 by Southern blotting and molecular combing of 251 non-FSHD families, 351 FSHD1 families, 62 FSHD2 and 52 FSHD-like families.

Results: In 6/10 FSHD2 families with a 4A allele of 21-70 D4Z4 units, we identified a small D4Z4 repeat duplication associated with *DUX4* expression. The duplication allele frequency was significantly higher than in typical FSHD2 families (2.0%), FSHD1 (2,3%) or non-FSHD families (2.6%). Interestingly, we identified duplication alleles in 7/52 (13.5%) FSHD-like families.

Conclusions: *Cis* duplications explain *DUX4* expression and disease presentation in FSHD2 families with unusual long D4Z4 repeats on 4A chromosomes. Funded by National Institutes of Health and Prinses Beatrix Spierfonds.

R.J.L.F. Lemmers: None. **P.J. van der Vliet:** None. **J. P. Vreijling:** None. **J. Balog:** None. **D. Henderson:** None. **B. van Engelen:** None. **F. Baas:** None. **S. Sacconi:** None. **R. Tawil:** None. **S.M. van der Maarel:** None.

C05.4
Novel biallelic mutations in *VPS13D* cause spastic ataxia and lead to mitochondrial dysfunction

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Introduction: Spastic ataxia is a clinically and genetically highly heterogeneous neurological disease. Despite a wealth of known genes causing this syndrome, many cases remain etiologically unexplained, suggesting that further genetic heterogeneity remains to be discovered.

Results: Exome sequencing in a German family with two affected sisters with spastic ataxia with onset in the third decade of life revealed compound-heterozygous variants in *VPS13D* [c.5409C>A [Tyr1803Ter] and c.12629C>T [Ala4210Val]) in both patients and heterozygous variants in their unaffected parents. Sanger sequencing of cDNA revealed lower expression of the nonsense mutated allele compared to the missense mutated allele, resulting in a ~50% reduction in the total mRNA level of *VPS13D* in the patients compared to non-mutation carriers. This reduction was caused by nonsense-mediated mRNA decay as demonstrated by cycloheximide treatment in cultured cells. *VPS13D* encodes a large protein, paralogs of which cause

other neurological disorders including choreoacanthocytosis (*VPS13A*), Cohen syndrome (*VPS13B*), and Parkinsons's disease (*VPS13C*). Since *VPS13D* disruption in *Drosophila* leads to mitochondrial abnormalities, we investigated mitochondrial morphology and function in patient-derived fibroblasts. While the typical mitochondrial network of elongated organelles and branches was observed in two controls, patients' fibroblasts showed rounded mitochondria and disrupted mitochondrial interconnectivity, which was confirmed by decreased mitochondrial GRP-75 protein levels. These structural mitochondrial changes were accompanied by a reduced ATP production rate.

Conclusions: Our study demonstrated that biallelic mutations in VPS13D cause a movement disorder along the ataxia-spasticity spectrum. Analyses of patient-derived fibroblasts suggest that mutations in this new ataxia/spasticity gene impact on mitochondrial structure and function.

M. Dulovic: None. **J. Trinh:** None. **A. Münchau:** None. **C. Klein:** None. **N. Brüggemann:** None. **K. Lohmann:** None.

C05.5

Mutations in the thioredoxin related gene *TMX2* cause primary microcephaly, polymicrogyria and severe neurodegeneration with impaired mitochondrial energy metabolism

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The endoplasmic reticulum (ER) plays a crucial role in redox and apoptosis regulation, through the ER stress response. Mutations in genes regulating ER-stress can cause microcephaly and abnormal cortical development, e.g. EIF2AK3 (OMIM#226980), and IER3IP1 (OMIM#614231) mutations.

We observed a 14 days old newborn with primary microcephaly and diffuse polymicrogyria at MRI, and confirmed at autopsy, who died of untreatable epilepsy. WES data revealed biallelic compound heterozygous mutations in *TMX2*. Six similarly affected individuals from four additional unrelated families were found to have biallelic *TMX2* mutations. Thioredoxin(TRX)-Related Transmembrane protein 2 (TMX2) is a putative oxidoreductase enriched at the mitochondria-associated membrane of the ER and known to protect against ER stress and to regulate neuronal apoptosis.

Under ER stress conditions, exchange of calcium between ER and mitochondria is impaired, which leads to mitochondrial accumulation of calcium and secondary mitochondrial OXPHOS failure. Mitochondrial energy metabolism in fibroblasts from two unrelated patients showed deficient OXOPHOS reserve capacity (which measures mitochondrial tolerance to stress) and an increase of glycolytic activity in basal conditions, suggesting a mechanism to compensate bioenergetics defect. We hypothesize that mutations in *TMX2* cause defects in bioenergetics metabolism and ER stress-induced apoptosis, leading to primary microcephaly, polymicrogyria and severe neurodegeneration. While mutations of mitochondrial thioredoxin 2 (*TXN2, TRX2*, OMIM #616811) and of cytosolic thioredoxin reductase 1 (*TXNRD1*, OMIM #60112) have been related to early onset neurodegeneration and epilepsy, this is to our knowledge the first observation linking one of the ER-TMX family members to abnormal neurodevelopment and secondary OXPHOS deficiency.

R. Schot: None. **C. Milanese:** None. **L. Vandervore:** None. **A. Fry:** None. **N. Bahi-Buisson:** None. **B. Keren:** None. **C. Nava:** None. **A. Afenjar:** None. **F. Renaldo:** None. **S. Coury:** None. **W. Tan:** None. **M. Fornerod:** None. **P. Mastroberardino:** None. **G.M.S. Mancini:** None.

C05.6

Genome wide detection of somatic mutations in human muscle stem cells

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Somatic mutations are beginning to be explored as a pathological cause of a number of conditions, including aging. Here we investigated the whole genome sequence of single muscle stem cells from healthy individuals (21-79 years of age) to identify age-associated mutation processes and their functional consequences. Stem cell whole genome sequences were obtained from cultured single cell clones (n = 29), circumventing disadvantages associated with single cell sequencing. This strategy, together with functional experiments on cultured clones, allowed us to discover age-specific differences and to highlight their functional relevance in driving age-related muscle decline. We show that aging affects the number of mutations and their genome distribution. In particular, while transcribed genes of young cells were protected from mutations, old cells had lost this protection, possibly as a result of impaired transcription-coupled repair. In addition, old stem cells also showed an increased non-synonymous/synonymous mutation ratio compared to young cells, supporting impaired negative selection against cells with mutations in functional regions at older ages. Using ddPCR, to detect low frequency mutations in tissue, we validated somatic mutations in the muscle bulk tissue. In summary, our results support somatic mutagenesis in muscle stem cells as a contributor to the age-related decline of muscle function.

I. Franco: None. **A. Johansson:** None. **K. Olsson:** None. **P. Vrtačnik:** None. **P. Lundin:** None. **H.T. Helgadottir:** None. **M. Larsson:** None. **G. Revêchon:** None. **C. Bosia:** None. **A. Pagnani:** None. **P. Provero:** None. **T. Gustafsson:** None. **H. Fischer:** None. **M. Eriksson:** None.

C06 Internal Organs

C06.1

Description Osteo-Oto-Hepato-Enteric (O2HE) syndrome, a new recessive autosomal syndrome secondary to loss of function mutations in the UNC45A gene

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Introduction: Combination of congenital diarrhea and hereditary cholestasis is rare in children, and molecular bases of numerous patients remains unknown. Recently, certain genes that were identified in hereditary cholestasis were found in families with congenital diarrhea, and vice versa (*MYO5B*, explained the role of this gene in the polarization of hepatocytes and enterocytes, *ABCB11*, *TTC37* and *SKIV2L)*.

Materials and Methods: We used exome sequencing to study four girls from 3 separate families, aged 4 to 23 years, presenting phenotypic constellation including cholestasis, congenital diarrhea, deafness, brittle bones and learning delays, who didn't correspond to an existing diagnostic framework.

Results: None of the four patients presented identical clinical signs, and mode of entry was severe congenital diarrhea with microvilli resulting in the need for long-term exclusive parenteral nutrition for two patients, and cholestasis for the two others. Compound heterozygote or homozygote variants - truncation or missense - of the Unc-45 Myosin Chaperone A (*UNC45A)* gene were identified in three families, which allowed researchers to group the families and describe the different expression of a newly discovered syndrome. UNC45A hadn't been previously associated with a condition in humans. Research on the C. elegans UNC-45 ortholog has shown the role of this gene in motility disorders. In vitro and in vivo functional studies on a zebrafish model confirmed the involvement of UNC45A.

Conclusions: These results show the importance of next generation sequencing for identification of new genes responsible for heterogeneous clinical presentation, and highlight value of data-sharing in research.

L. Faivre: None. **C. Esteve:** None. **L. Francescatto:** None. **P.L. Tan:** None. **A. Bourchany:** None. **C. Delafoulhouze:** None. **E. Marinier:** None. **P. Bourgeois:** None. **C. Brochier-Armanet:** None. **A. Bruel:** None. **A. Delarue:** None. **Y. Duffourd:** None. **E. Ecochard-Dugelay:** None. **O. Goulet:** None. **P. Gauchez:** None. **E. Gonzales:** None. **C. Guettier-Bouttier:** None. **F. Huet:** None. **M. Komutora:** None. **G. Hery:** None. **C. Lacoste:** None. **R. Maudinas:** None. **K. Mazodier:** None. **Y. Rimet:** None. **J. Rivière:** None. **B. Roquelaure:** None. **J. Sarles:** None. **S. Sigaudy:** None. **E. Savajols:** None. **X. Stephenne:** None. **C. Thauvin-Robinet:** None. **J. Thevenon:** None. **N. Levy:** None. **C. Badens:** None. **J. Hugot:** None. **N. Katsanis:** None. **A. Fabre:** None.

C06.2

Targeted NGS in primary ciliary dyskinesia: expanding mutation spectrum and novel dynein-related gene discovery

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Introduction: Primary ciliary dyskinesia (PCD, MIM: 244400) is a rare motile ciliopathy with high genetic and phenotypic heterogeneity. Symptoms include recurrent respiratory tract infections, laterality defects and adult subfertility. Disease-causing mutations have been identified in 37 genes that still only account for around 70% of cases, with additional genes still to be identified.

Subjects and Methods: 175 unrelated families from various ethnicities were recruited. We screened patients for mutations using a multi-gene panel including all the known PCD genes and other potential candidates. All prioritized variants were confirmed in the affected individuals and segregated in available family members using Sanger sequencing. To functional characterize novel candidate genes, we analysed mutated cilia structure and the consequences of gene silencing in the unicellular aquatic ciliate, *Paramecium*.

Results: We identified 167 disease-causing variants in known PCD genes in 128 families (79%), revealing striking population stratification and high impact recurrent alleles. 50% of mutations were not previously reported. Variants in candidate genes not previously linked to PCD were identified in 14 families. Mutational modelling in *Paramecium* and other functional characterization of the novel *C11orf70* and *DNAH9* genes confirmed their role in dynein-related cilia motility, showing that loss-of-function mutations lead to ultrastructural ciliary defects and consequent motile cilia dysfunction.

Conclusions: Targeted NGS yields a high success rate for PCD diagnosis, overcoming pitfalls of other diagnostic measures. It reveals clinically important mutation stratification and valuable potential for novel gene discovery. Gene silencing in *Paramecium* is effective for modelling the involvement of novel candidate genes in PCD.

M.R. Fassad: None. **A. Shoemark:** None. **J. Hayward:** None. **M. Patel:** None. **P. le Borgne:** None. **F. Koll:** None. **N. Rumman:** None. **H. Morsy:** None. **W.I. Shoman:** None. **N. Fasseeh:** None. **L. Pereira:** None. **C. Constant:** None. **A. Pinto:** None. **S. Lopes:** None. **M. Lemullois:**

None. **A. Aubusson-Fleury:** None. **A. Tassin:** None. **H.M. Mitchison:** None.

C06.3

Targeted exon skipping of a *CEP290* mutation is able to rescue cellular and ciliary phenotypes in vitro and in vivo

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Introduction: Mutations in *CEP290* cause ciliopathy syndromes including the cerebello-retinal-renal syndrome known as Joubert syndrome.

Materials and Methods: We established a secondary culture of human urine-derived renal epithelial cells (hURECs) from a boy aged 14 years with a Joubert syndrome phenotype caused by a homozygous nonsense mutation in exon 41 of *CEP290*. These cells were treated with a morpholino antisense oligonucleotide designed to induce targeted in-frame skipping of *CEP290* exon 41 (ex41-*CEP290* MO). A $Cep290^{LacZ/LacZ}$ gene trap mouse that displays a mild cystic kidney phenotype was used to study in vivo delivery following tail vein injection of an octaguanidine dendrimer-modified morpholino oligonucleotide designed to splice out the *LacZ* gene trap (vivo-*LacZ* MO).

Results: When compared to healthy control, primary cilia of patient's hURECs appeared tortuous and abnormally extended in length. A similar ciliary phenotype was observed in cystic epithelia of $Cep290^{LacZ/LacZ}$ mouse. Treatment of patient's hURECs with ex41-*CEP290* MO led to a rescue of ciliary phenotype. In vivo delivery of vivo-*LacZ* MO to $Cep290^{LacZ/LacZ}$ mice resulted in wild type mRNA transcripts and rescue of Cep290 protein expression in renal tissue. Consistent with this was the observed rescue of both the ciliary length and the cystic kidney disease phenotype in vivo.

Conclusions: These data support the feasibility of personalised approaches of induced exon skipping to treating cystic kidney disease secondary to *CEP290* mutations and show that intravenous delivery of morpholino oligonucleotide to murine renal tissue is efficacious. This research was supported by a MRC grant (MR/M012212/1) to J.A.S. and C.G.M.

E. Molinari: None. **S.A. Ramsbottom:** None. **S. Srivastava:** None. **C.G. Miles:** None. **J.A. Sayer:** None.

C06.4

Single cell RNA sequencing of T cells in Crohn's disease identifies tissue specific drug targets

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Introduction: Crohn's disease (CD) is a chronic intestinal inflammatory disorder, for which 200 genetic risk loci are known. CD risk loci are enriched for genes involved in T-cell signaling.

Characterization of mucosal T-cells in a disease- and tissue-specific context at a single cell resolution is crucial to get insight in the underlying pathomechanisms of CD. We used single-cell-RNA-sequencing (scRNA-seq) of T-cells to increase insight in CD pathomechanisms and to identify potential novel drug targets.

Methods: We performed scRNA-seq of 5,292 CD3+ T-cells from peripheral blood (PBL) and from ileal mucosal biopsies of CD patients. Biopsies were dissociated into T-cells from the epithelium (IEL) and the lamina propria (LPL). scRNA-seq was performed with an adapted SmartSeq2 protocol, using 3'-end library generation and unique molecular identifiers, sequenced on the Illumina NextSeq500.

Results: Cytotoxic T-cells (CTL) dominated the IEL, while Th17 cells were the largest population in the LPL. PBLs were mainly composed of quiescent T-cells. Th17 cells and CTLs show the highest proportion of differentially expressed known CD risk genes. The biological Etrolizumab, but not Vedolizumab, specifically appears to target mucosal CTLs. Two potential drug repositioning targets for mucosal Th17 cells, are MSX-122, an anti-tumor CXCR4-antagonist, and Rivenprost, which targets *PTGER4*, and has been tested in UC patients.

Conclusion: We have conducted the first detailed transcriptomic characterization of disease- and tissue-specific effector cells in Crohn's disease. We show that CD risk genes are significantly overexpressed in ileal mucosal Th17 and CTLs and provide promising targets for future cell-specific therapies in CD patients.

M.D. Voskuil: None. **W.T.C. Uniken Venema:** None. **A. Vich Vila:** None. **R.K. Weersma:** None. **E.A.M. Festen:** None.

C06.5

Mutations in *BNC2* Lead to Autosomal-Dominant Lower Urinary Tract Obstruction (LUTO)

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Introduction: Congenital "lower urinary tract obstruction" (LUTO) is defined by a decrease in the free passage of urine through the urethra. About three out of 10,000 pregnancies are affected, the etiology is so far unknown.

Materials and Methods: Whole exome sequencing (WES) in a family with 4 affected from 3 generations was performed. 258 LUTO patients were screened for further variants in the identified candidate gene BNC2 using Sanger sequencing. Functional studies comprised in situ hybridization (ISH) studies in mouse embryos, translational knockdown (KD) in developing zebrafish larvae (zfl) using Morpholino oligonucleotides (MO) and 'rescue' experiments by co-injection of human BNC2 mRNA with the Bnc2-MO in zfl.

Results: Filtering of WES Data revealed a nonsense mutation (c.2554C>T; p.Arg852*) in BNC2 (basonuclin 2). Out of 258 LUTO patients one additional family (affected father and son) carrying a novel missense mutation (c.2663A>G, p.H888R) in BNC2 could be identified. Functional characterization of BNC2 using ISH showed expression at E13.5 in developing mouse urethra. KD of bnc2 by injection MO in zfl caused cloacal obstruction with formation of a vesicle at the distal end of pronephric duct in 10% of zfl and zystic dilated deformed glomeruli and dilated pronephric ducts in 50%. Co-injection of human BNC2 mRNA with Bnc2-MO in zfl showed rescue of the phenotype confirming the specificity of the Bnc2-MO.

Conclusions: Human genetic and developmental biology models suggest BNC2 as the first monogenic cause of LUTO. Funding: BONFOR

C.M. Kolvenbach: None. **S. Frese:** None. **G.C. Dworschak:** None. **A. Japp:** None. **J.M. Schmidt:** None. **M. Zaniew:** None. **W. Newman:** None. **G. Beaman:** None. **H. Stuart:** None. **A. Woolf:** None. **R. Cervellione:** None. **W. Rösch:** None. **S. Weber:** None. **W. Merz:** None. **F. Hildebrandt:** None. **M. Feldkötter:** None. **B. Hoppe:** None. **H. Thiele:** None. **J. Altmüller:** None. **C. Berg:** None. **M. Ludwig:** None. **P. Grote:** None. **H. Reutter:** None. **B. Odermatt:** None. **A.C. Hilger:** None.

C06.6

Large-scale trans-ethnic genome-wide association study reveals novel loci, causal molecular mechanisms and effector genes for kidney function

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Chronic kidney disease (CKD) is a major public health burden and affects nearly 10% of the global population. We assembled published and de novo genome-wide association studies of estimated glomerular filtration rate (eGFR), a measure of kidney function used to define CKD, in up to 192,346 individuals of diverse ancestry. We identified 56 loci attaining genome-wide significant evidence of association with eGFR ($p < 5x10^{-8}$), including 10 mapping outside those previously reported for kidney function. Across loci, we identified 78 distinct association signals at locus-wide significance ($p < 10^{-5}$), including four in the region mapping to *SLC22A2*. Allelic effects on eGFR of index variants were consistent across populations, with no evidence of heterogeneity due to ancestry ($p_{HET} < 6.4x10^{-4}$, Bonferroni correction). Integration with genomic annotation revealed that eGFR association signals were jointly enriched in coding sequence, and binding sites for ATF3 and HDAC8. ATF3 expression has been shown to be elevated

in animal models of proteinuria and diabetic nephropathy, whilst class I histone deacetylases (including HDAC8) are required for embryonic kidney gene expression, growth and differentiation. Annotation-informed fine-mapping revealed 12 variants accounting for >80% of the probability of driving eGFR association signals, including coding alleles *GCKR* p.Leu446Pro (rs1260326), *CPS1* p.Thr1406Asn (rs1047891), *CACNA1S* p.Arg1539Cys (rs3850625), and non-coding expression quantitative trait loci in kidney for *RGS14* (rs35716097), and *UMOD* and *GP2* (rs77924615). These results define novel causal molecular mechanisms underlying kidney function association signals, and highlight genes through which their effects are mediated, offering a potential route to clinical translation and CKD treatment development.

A.P. Morris: None. **A. Akbarov:** None. **M. Tomaszewski:** None. **N. Franceschini:** None.

C07 NGS diagnostics

C07.1
Reanalysis of unsolved WGS clinical cases from the NIHs undiagnosed diseases network (UDN)

E. A. worthey[1], D. Brown[1], C. Birch[1], M. Gajapathy[1], L. Handley[1], M. Holt[1], N. Sosonkina[1], B. Wilk[1], M. Wilk[1], J. Lazar[1], M. Schroeder[1], D. Bick[1], H. Jacob[2], M. Members of the Undiagnosed Disease Network[1]

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The UDN works to provide patients with previously undiagnosed disease access to a cross-disciplinary network of investigators determined to explore their disease pathology, physiology, and genetics. Patients accepted undergo comprehensive clinical evaluation and phenotyping. Qualifying patients receive either whole exome sequencing at Baylor or whole genome sequencing at HudsonAlpha. Of 300 WGS cases analyzed, 88.9% had received prior genetic testing (WES = 78.1%, other = 10.8%). Primary Findings were for 51% of the analyzed genomes and for 49% of cases with an existing WES. Secondary findings were reported, for adults only, in 28% of cases. A wealth of new information (gene discovery, disease-phenotype expansion, etc.) has become available since 2015. At the end of 2017 we therefore began reanalysis of all unsolved cases making use of newly published diseases and phenotypes and novel tools. This review also seeks to identify variants not meeting clinical reporting criteria including damaging variants in Genes of Uncertain Significance. Through novel secondary analysis we identified chromosomal abnormalities, mosaicism, repeat expansions, intermediate sized structural variants, and translocations, which explain disease in 19 (6.5%) of patients. Based on enhanced tertiary analyses and in depth review we identified 38 candidate variants in GUSs; these are reported to the clinical sites and functional cores for follow up. We have made 5 definitive new diagnoses. In total, through application of novel methods and in depth review, we have provided findings of note for ~14.4% of our participants and families. Continued efforts on this front will contribute to the advancement of knowledge of rare diseases.

E.A. worthey: None. **D. Brown:** None. **C. Birch:** None. **M. Gajapathy:** None. **L. Handley:** None. **M. Holt:** None. **N. Sosonkina:** None. **B. Wilk:** None. **M. Wilk:** None. **J. Lazar:** None. **M. Schroeder:** None. **D. Bick:** None. **H. Jacob:** A. Employment (full or part-time); Significant; AbbVie. **M. Members of the Undiagnosed Disease Network:** None.

C07.2
Finding missing diagnoses in exome sequence data

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Systematic re-analysis of existing genomic data in light of new knowledge and analytical techniques is expected to increase the diagnostic yield. Using the UK-wide Deciphering Developmental Disorders (DDD) Study, we tested this hypothesis by re-analysing exome sequence data from the first 1133 parent-offspring trios three years' after our initial analysis. Through a combination of detecting new variant classes and analysing disease-associated genes that were discovered since our initial analysis, we have been able to increase our diagnostic yield from 27% to 40%. Around 70% of our new diagnoses were in genes not known to be associated with developmental disorders at the time of our initial analysis, highlighting the importance of regularly revisiting variant filtering and interpretation protocols. Nearly 5% of our new diagnoses were mosaic variants inherited from an unaffected parent, of which half were previously wrongly classed as constitutively inherited and therefore excluded from our variant filtering pipeline, and half were previously wrongly classed as *de novo* and would have resulted in inaccurate counselling of parents about recurrence risk. We were unable to detect any significant phenotypic differences

between probands with and without a diagnosis, suggesting that the diagnostic yield should continue to increase in future as a result of similar repeated reanalyses.

C.F. Wright: None. **J. McRae:** None. **G. Gallone:** None. **S. Aitken:** None. **E. Prigmore:** None. **D. Rajan:** None. **M. Hurles:** Other; Modest; Congenica. **D. FitzPatrick:** None. **H. Firth:** None.

C07.3
Inferring compound heterozygotes from large-scale exome sequencing data

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Short-read sequencing technologies have enabled sequencing of the exomes and genomes of hundreds of thousands of people. However, most standard sequencing technologies do not readily provide phase, that is, assigning variants to individual haplotypes. The absence of phase information particularly complicates the identification of compound heterozygous variants in the diagnosis of recessive disorders, where mutations of both copies of a given gene are necessary to develop disease. While the phase of common variants can be accurately inferred using imputation, phasing rare variants is challenging, especially from exome sequencing due to the sparse coverage of each haplotype.

Here, we leverage large-scale whole-exome sequencing data from the genome aggregation database (gnomAD) which contains 123,136 individuals, to infer haplotype frequencies between pairs of rare variants (allele frequency < 1%) located in the same gene. We demonstrate that these frequencies can be used to infer compound heterozygote status for variants within a gene with high accuracy (92.3%) from whole-exome sequencing data using 1,494 trios for which true phase can be determined by allele transmission. Notably our approach performs very well even with a single observation of each allele in gnomAD (allele frequency ~10^{-5}).

Applying our approach to the 123,136 individuals in gnomAD, we compare the burden of rare protein-altering compound heterozygous variants and homozygous variants in each gene. We show the clinical utility of our method when applied to several rare disease cases where we could either exclude or prioritize putative causative compound heterozygous variants (validated by sequencing the proband's parents).

L.C. Francioli: None. **M.H. Guo:** None. **K.J. Karczewski:** None. **B.B. Cummings:** None. **M. Lek:** None. **V. Thaker:** None. **M.J. Daly:** None. **J.J. Hirschhorn:** None. **D.G. MacArthur:** None.

C07.4
Next Generation Children Project: Whole genome sequencing for rapid diagnosis of severely ill children in intensive care

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Children in neonatal or paediatric intensive care units (ICU) are at risk of having an underlying genetic condition. Achieving a rapid diagnosis augments clinical decision-making, suggests more appropriate treatments, and may reduce length of inpatient care. The objective of the Next Generation Children Project (NGC) is to rapidly identify clinically relevant rare genetic variants in the ICU within the UK National Health Service (NHS). Proband/parent trios were recruited for whole genome sequencing (WGS). To date, 112 probands (neonate - 15 years) have been sequenced and analysed, 85% with both parents (319 samples at ~38 X coverage). Sequencing has been reduced to ~10 days on average, bioinformatics and variant interpretation takes ~3 days and clinical confirmation and NHS reporting takes <7 days. A likely diagnosis was reported in 15% of cases, including encephalopathies, myopathies, skeletal dysplasias and rare syndromes. Despite extensive phenotyping (~17 HPO terms/proband), a third of reported cases showed 2 or fewer terms overlapping known gene terms. Preliminary findings of the NGC Project show feasibility of implementing rapid-turnaround WGS within an NHS clinical practice context. They highlight i) the utility of WGS that does not rely on gene panels; ii) the ability to diagnose diseases earlier increases the phenotypic spectrum and iii) fewer features may present early for some conditions. The reduced correlation between genotype and phenotype in intensively ill children suggests the importance of further research to

improve utility of rapid-turnaround WGS assessment in the ICU setting.

This project is supported by the Rosetrees Trust and NIHR BioResource.

C.E. French: None. **A. Sanchis-Juan:** None. **I. Delon:** None. **H. Dolling:** None. **E. Dewhurst:** None. **S. Agrawal:** None. **T. Austin:** None. **R. Armstrong:** None. **G. Belteki:** None. **M. Bohatschek:** None. **S. Bowdin:** None. **R.G. Branco:** None. **S. Broster:** None. **A. D'Amore:** None. **R. Chaudhary:** None. **C. Costa:** None. **H. Firth:** None. **J. Hague:** None. **J. Harley:** None. **R. Kayani:** None. **W. Kelsall:** None. **S. Mehta:** None. **R. O'Donnell:** None. **A. Ogilvy-Stuart:** None. **S. Park:** None. **M. Prapa:** None. **A. Sammut:** None. **K. Schon:** None. **K. Spike:** None. **A. Taylor Tavares:** None. **D. Wari-Pepple:** None. **C.G. Woods:** None. **NIHR BioResource - Rare Diseases:** None. **S. Abbs:** None. **D. Rowitch:** None. **F.L. Raymond:** None.

C07.5

Rapid Whole Genome Sequencing Improves Clinical Utility and Cost Effectiveness of Acutely Ill Children admitted to Neonatal Intensive Care Units

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Introduction: Studies have shown that rapid whole genome sequencing (rWGS) in neonatal intensive care units has resulted in reduced time to diagnosis and improved diagnostic rates compared to the current standard of care. Children with illness, often due to genetic disease, represent 70% of healthcare costs. Thus, this testing holds potential to impact healthcare economics worldwide. We sought to investigate the clinical utility of rWGS in infant inpatients at Rady Children's Hospital.

Methods: After parental consent, blood samples were collected from patients and parents when available. DNA was isolated, PCR-free Illumina genomic library preparation was performed, and Illumina WGS was undertaken to ~45X coverage. Phenotypic features of the proband were translated into Human Phenotype Ontology (HPO) terms.

Results: After 12 months of testing, 94% of families approached enrolled. rWGS was interpreted in 340 families, yielding diagnostic information in 115 families (~34%). On average, diagnosis occurred within 96 hours (fastest 37 hours). Changes in management as a result of diagnosis were identified in 77 families (~67% of diagnosed patients). The changes in management ranged from specific, changes in surgical interventions, to palliative care guidance. Among the first 42 infants, rWGS provided over $1.3M in net cost savings over projected standard care.

Conclusion: Consistent with other studies, rWGS has a high diagnostic yield and reduces time to diagnosis. This study demonstrates that early diagnosis changes acute management. rWGS improves clinical care, preventing disability and unnecessary procedures, while simultaneously reducing acute care costs among a broad cohort of quaternary childrens hospital inpatient infants.

S. Nahas: None. **S. Chowdhury:** None. **D. Dimmock:** None. **S. Kingsmore:** None.

C07.6

Experiences of the Dutch diagnostic data share consortium; limits of the current 5-tier classification system

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The use of genomic information in both research and clinic has expanded enormously the last decade. Yet, the interpretation of the obtained NGS data remains a huge challenge. Since sharing of data improves patient care and facilitates harmonizing patient reports, the Dutch genome diagnostic laboratories created a national database with connections to each of the labs for automated bi-directional sharing. Data are stored separately for variant classifications and variant frequencies, using the Open Source MOLGENIS and VARDA systems. The shared variant classification data is used in daily practice in each labs' NGS analysis software to improve the variant interpretation. Currently the classification database contains >70,000 DNA variants in 4,500 genes of which 66% have been classified as (likely) benign, 18% as VUS, 14% as (likely) pathogenic and 2% unsolved. We developed a classification comparison tool and procedure for the variants classified by multiple labs (n>10,000). There was consensus for 8828 classifications. These valuable curated consensus data already has been shared beyond diagnostic laboratories with the HVP share database (LOVD3) and their submission to ClinVar is underway. The 1248 variants without classification consensus are currently under discussion. In trying to resolve these discrepancies, we encountered the limits of the 5-tier classification system, which is currently a mix of functional and clinical classifications. In 30 cases a variant had clear functional consequences but an association with a low-risk disease, which resulted in opposite classifications. This could potentially

lead to misdiagnosis. An international discussion is proposed to harmonize the classifications of these variants.

M.E. van Gijn: None. **J. Laros:** None. **M.A. Swertz:** None.

C08 Population Genetics

C08.1

Genome-wide gene-based analyses identifies *ANK1* as a modulator of weight loss in obese patients

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Introduction: Hundreds of genetic variants have been associated to obesity through genome-wide association studies (GWAS) using observational cohorts. However, the genetic contribution to efficient weight loss in response to dietary intervention remains unknown.

Materials and Methods: We performed a GWA in two large low-caloric diet intervention cohorts of obese participants: the Canadian Optifast900® meal replacement program (n = 1166 obese subjects from Ottawa, LCD with Optifast 900kcal/d); and the DiOGenes clinical study (n = 789 overweight/obese subjects from eight European countries, LCD with Modifast 800kcal/d).

Results: To improve statistical power, we performed multi-marker (gene-based) analyses and took advantages of recent development in Bayesian linear mixed effect models. Two loci close to the ANK1 and RBSG4 genes respectively were identified in the Canadian discovery cohort (genome-wide FDR < 5%) and replicated in the DiOGenes sample (replication FDR < 5%). Joint-modelling of the association signal together with large-scale epigenome datasets (450 annotations) restricted further the signals to two individual SNPs : rs6981587 for the ANK1 locus and rs873822 for the RBSG4 locus. Full body-knockdown in Drosophila melanogaster of the ANK1 ortholog (Ank) yielded a non-lethal strain with 75% mRNA reduction, and these animals exhibited significantly increased body mass (+20%) and decreased triglyceride levels (-23%) compared to controls. Knock-down of the RBSG4 gene was not possible, as this gene is not conserved in fly.

Conclusions: Our results demonstrate, for the first time, an effect of genetic variants to the efficacy of weight loss in obese subjects and identify a role for ANK1 in weight control.

A. Valsesia: A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **Q. Wang:** None. **N. Gheldof:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **J. Carayol:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **V. Shenton:** None. **G. Lefebvre:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **S. Metairon:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **C. Chabert:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **O. Walter:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **P. Mironova:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **P. Lau:** None. **N. Viguerie:** None. **D. Langin:** None. **P. Descombes:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA. **M. Harper:** None. **G. Neely:** None. **A. Astrup:** None. **W. Saris:** None. **R. Dent:** None. **J. Hager:** A. Employment (full or part-time); Significant; Nestlé Institute of Health Sciences SA.

C08.2

Insights from the largest genetic study of sexual orientation

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Solid evidence about the genetic determinants of sexual orientation is lacking. Moreover, the reduced number of offspring in individuals engaging in non-heterosexual behavior (NHB) presents a Darwinian paradox: why are variants that predispose to NHB are maintained in the population despite apparent selection against them? We report the results from the largest study of sexual orientation by focusing on two main phenotypes: NHB, defined as having had at least one same-sex partner (N = 28,486 cases and 469,427 controls) and number of partners in

heterosexual individuals (NSP;N = 362,993 individuals) in a meta-analysis of UK Biobank and 23andMe. We identified 4 and 41 loci significantly ($P < 5x10^{-8}$) associated with NHB and NSP, respectively. 12 loci were sex-differentiated and the genetic correlation between males and females was significantly < 1 ($rg = 0.67, P = 4x10^{-14}$ for NHB). By studying the association between significant loci and 2,500+ GWAS results we highlighted pleiotropic effects with smoking behavior and hormone-related phenotypes (e.g. balding). Genetic correlations indicated a shared genetic component between NHB and risk-taking ($P = 5.1x10^{-9}$), substance use (e.g. cannabis use,$P = 3.6x10^{-6}$) and several psychiatric traits (e.g. schizophrenia,$P = 1.1x10^{-4}$). A significant heritability enrichment in brain-expressed genes was observed for NSP, but not for NHB. The strong genetic correlation between NHB and NSP ($rg = 0.47, P = 9.5x10^{-25}$), which replicated in an external study of adolescents ($P = 0.007$), is consistent with antagonistic pleiotropy, suggesting that variants predisposing to NHB increase mating success (as measured by NSP) in heterosexuals. Overall, our results depict NHB as a complex, heritable behavioral phenotype, partially sharing a genetic component with risk-taking/substance use, partially with hormonal-related phenotypes.

A. Ganna: None. **G. Beecham:** None. **F. Day:** None. **E. Martin:** None. **R. Meier:** None. **B. Neale:** None. **M. Nivard:** None. **J. Perry:** None. **A. Sanders:** None. **K. Verweij:** None. **R. Wedow:** None. **B. Zietsch:** None.

C08.3

Genome-wide association of bone mineral density in the UK Biobank full release identifies 301 novel loci and implicates *DAAM2* in osteoporosis

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Bone mineral density (BMD) is the most clinically-relevant predictor of fracture and osteoporosis, a common, aging-related disease. Previously, an estimated heel BMD (eBMD) genome-wide association study (GWAS) in 142,487 individuals identified 203 loci, explained 12% of the trait variance and identified novel determinants of osteoporosis. Here, we performed an eBMD GWAS in 426,824 White-British UK-Biobank participants (55% female). Using statistical fine-mapping and functional genomics, we prioritized genes for whole-animal model experiments and focused on dishevelled-associated activator of morphogenesis-2 (*DAAM2*), a novel gene involved in Wnt-signalling. We identified 1,106 conditionally-independent signals ($P < 6.6x10^{-9}$) mapping to 518 loci (301 novel) and explained 20% of the trait variance. We prioritized 538 protein-coding genes (247 novel) strongly enriched for bone cell expression and known osteoporosis drug target and bone disorder genes. We identified plausibly causal *DAAM2* coding SNPs and therefore generated homozygous (*Daam2*$^{-/-}$) and heterozygous knockout mice for skeletal phenotyping with X-ray microradiography, microtomography, and biomechanical testing. *Daam2*$^{-/-}$ mice had non-significant reductions in femur length, and reduced femur and vertebral bone mineral content. Structural trabecular and cortical bone parameters did not differ between wild-type and *Daam2*$^{-/-}$ mice. Strikingly, despite normal parameters, maximum load was greatly reduced in *Daam2*$^{-/-}$ femurs and vertebrae (2.14 standard deviations below predicted strength), suggesting Daam2-deficiency impaired bone quality. Here, we increased the number of associated loci 2.5-fold, the variance explained to 20%, provided an efficient strategy to prioritize genes, and identified a novel determinant of bone quality, *DAAM2*. These results provide further insight into novel, potentially druggable, genes contributing to osteoporosis.

J.A. Morris: None.

C08.4

Low pass genomes of 141,431 Chinese reveal patterns of viral infection, novel phenotypic associations, and the genetic history of China

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We analyze whole-genome sequencing data from 141,431 pregnant Chinese women generated for non-invasive pregnancy testing (NIPT), and identify 9 million genetic variants with a 0.2% false positive rate and a high imputation accuracy at 2.1 million variable sites. We characterize the population genetic structure in China and discover that the present day distribution of alleles is a function of both ancient processes of migration, such as the Silk Road trade, and very recent, large-scale, population movements within the last 70 years. We identify novel phenotype-genotype associations, including several replicated associations with height and BMI, an association between maternal age and a variant near *HCN1* and *EMB*, and between multiple fetuses (e.g. twins) and *NRG1*, a gene modulating thyroid function. Finally, we characterize the distribution of circulating viral DNA at the individual and population level, and identify a unique pattern of high prevalence of hepatitis B and other clinically relevant maternal infections. A GWAS for viral infections identifies an exceptionally strong association between high abundance integrated herpesvirus 6A/6B and two genes, one of which, *MOV10L1*, affects piRNA processing and PIWI protein function. Our results demonstrate the NIPT data can be effectively repurposed for large-scale virological and population genomic studies.

S. Liu*: None. **S. Huang*:** None. **F. Chen*:** None. **L. Zhao*:** None. **Y. Yuan:** None. **S.S. Francis:** None. **L. Fang:** None. **Z. Li:** None. **L. Lin:** None. **R. Liu:** None. **Y. Zhang:** None. **H. Xu:** None. **S. Li:** None. **Y. Zhou:** None. **Q. Liu:** None. **R.G. Walters:** None. **K. Lin:** None. **J. Ju:** None. **T. Korneliussen:** None. **M.A. Yang:** None. **Q. Fu:** None. **J. Wang:** None. **L. Zhou:** None. **A. Krogh:** None. **H. Zhang:** None. **W. Wang:** None. **Z. Chen:** None. **Y. Yin:** None. **H. Yang:** None. **M. Mao:** None. **J. Shendure:** None. **J. Wang:** None. **A. Albrechtsen:** None. **X. Jin:** None. **R. Nielsen:** None. **X. Xu:** None.

C08.5

Imputation and de novo variant discovery from low-pass whole genome sequencing data for cost-effective and scalable trait mapping

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The identification of genetic variants that influence multifactorial traits in humans requires both large sample sizes and comprehensive variant discovery. Historically, this has posed a challenge-- the only cost-effective technology for profiling large numbers of individuals has been genotyping arrays, which measure a small fraction of genetic variation; sequencing-based technologies, which enable more comprehensive profiling of genetic variation, remain too expensive for routine usage. To bridge this gap, we developed a low-pass sequencing assay (in which less than 1x coverage of a target genome is sequenced) along with software for genotyping imputation and de novo variant discovery. To evaluate the performance of this assay, we sequenced 83 individuals to 1x genome coverage and genotyped the same samples on the Affymetrix Axiom Precision Medicine Research Array. We then downsampledthe sequencing data to 0.8, 0.6, 0.4, and 0.2x coverage. At common single nucleotide polymorphisms (SNPs), the mean imputation accuracy from the genotyping array was 90%, which was comparable to the imputation accuracy from 0.4x (88%) to 0.6x (91%) sequencing. We then performed de novo discovery of SNPs from the sequencing data, to test the possibility to discover previously unseen genetic variation. With 1x coverage sequencing we identify over 95% of genetic variants in the samples that are present in at least 10 copies, and with 0.6x coverage we identify over 95% of the genetic variants that are present in at least 20 copies. The method presented allow for a path to association studies that are truly "genome-wide".

J. Pickrell: A. Employment (full or part-time); Significant; Gencove, Inc. **T. Berisa:** A. Employment (full or part-time); Significant; Gencove, Inc. **K. Wasik:** A. Employment (full or part-time); Significant; Gencove, Inc. **D. Fraser:** A. Employment (full or part-time); Significant; PAREXEL Genomic Medicine. **C. Cox:** A. Employment (full or part-time); Significant; GlaxoSmithKline.

C08.6

Prioritising genes of interest from whole genome sequences to maximise diagnostic yield; the experience of the 100,000 genomes project

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Prioritising variants for interpretation is paramount in whole genome sequencing. Patients and clinical teams benefit from efficient prioritisation of relevant genes, considering the original clinical question. The rare disease arm of the 100,000 genomes project will sequence ~60,000 individuals, analysing each genome against a set of curated gene panels; enhancing diagnostic yield from the current knowledge base. To optimise efficiency and reproducibility an automated, yet individualised, approach has been designed. Genomics England PanelApp (https://panelapp.genomicsengland.co.uk) is an international gene curation resource, which captures collective expertise; documenting gene:phenotype relationships. 170 curated gene panels are available for use, covering a wide range of clinical phenotypes. A computer algorithm (PanelAssigner), supervised by clinicians experienced in rare disease diagnosis, prioritises genes tailored to the clinical phenotype through: (i) identifying similarities between the participant's phenotype and HPO data models defining a disease pre-assigned to a specific gene panel (ii) identifying panels similar to a dynamically generated gene panel using participant's HPO terms (iii) applying clinician-developed rules dependent on recruited disease category, HPO terms and age of onset. In excess of 8,000 families, representing around 18,000 participants, have undergone panel assignment using the PanelAssigner led strategy. 24% of the confirmed diagnoses to date resulted from additional panel allocation, beyond the core panel associated with the recruited disorder. Following clinical scientist review of the data, all positive diagnoses are used to inform iteration of the PanelAssigner algorithm. Pre-population of panel assignment streamlines clinical input and opens systems to those less aware of the breadth of panels available.

H.K. Brittain: None. **E.R.A. Thomas:** None. **A. Tucci:** None. **E. Baple:** None. **E.M. McDonagh:** None. **A. Rueda-Martin:** None. **L. Daugherty:** None. **R. Foulger:** None. **S. Leigh:** None. **O. Niblock:** None. **E. Williams:** None. **A. Rendon:** None. **M.J. Caulfield:** None. **R.H. Scott:** None. **D. Smedley:** None.

C09 Mendelian chromatin disorders

C09.1
Mutations in the BAF-complex subunit DPF2 are associated with Coffin-Siris syndrome

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Variants affecting the function of different subunits of the BAF chromatin-remodelling complex lead to various neurodevelopmental syndromes, including Coffin-Siris syndrome. Furthermore, variants in proteins containing PHD fingers, motifs recognizing specific histone tail modifications, have been associated with several neurological and developmental-delay disorders. Here, we report eight heterozygous *de novo* variants (one frameshift, two splice site, and five missense) in the gene encoding the BAF complex subunit double plant homeodomain finger 2 (DPF2). Affected individuals share common clinical features described in individuals with Coffin-Siris syndrome, including coarse facial features, global developmental delay, intellectual disability, speech impairment, and hypoplasia of fingernails and toenails. All variants occur

within the highly conserved PHD1 and PHD2 motifs. Moreover, missense variants are situated close to zinc binding sites and are predicted to disrupt these sites. Pull-down assays of recombinant proteins and histone peptides revealed that a subset of the identified missense variants abolish or impair DPF2 binding to unmodified and modified H3 histone tails. These results suggest an impairment of PHD finger structural integrity and cohesion and most likely an aberrant recognition of histone modifications. Furthermore, the overexpression of these variants in HEK293 and COS7 cell lines was associated with the formation of nuclear aggregates and the recruitment of both wild-type DPF2 and BRG1 to these aggregates. Expression analysis of truncating variants found in the affected individuals indicated that the aberrant transcripts escape nonsense-mediated decay. Altogether, we provide compelling evidence that *de novo* variants in *DPF2* cause Coffin-Siris syndrome and propose a dominant-negative mechanism of pathogenicity.

G. Vasileiou: None. **S. Vergarajauregui:** None. **S. Endele:** None. **B. Popp:** None. **C. Büttner:** None. **A.B. Ekici:** None. **M. Gerard:** None. **N.C. Bramswig:** None. **B. Albrecht:** None. **J. Clayton-Smith:** None. **J. Morton:** None. **S. Tomkins:** None. **K. Low:** None. **A. Weber:** None. **M. Wenzel:** None. **J. Altmüller:** None. **Y. Li:** None. **B. Wollnik:** None. **G. Hoganson:** None. **M. Plona:** None. **M.T. Cho:** A. Employment (full or part-time); Modest; Gene Dx. **C.T. Thiel:** None. **H. Lüdecke:** None. **T.M. Strom:** None. **E. Calpena:** None. **A.O.M. Wilkie:** None. **D. Wieczorek:** None. **F.B. Engel:** None. **A. Reis:** None.

C09.2

Novel neurodevelopmental syndrome due to *de novo* mutations in chromatin remodeler CHD3 in 35 patients

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Chromatin remodeling is of crucial importance during neurodevelopment. CHD3, a member of the CHD subfamily of chromatin remodeling ATPases and subunit of the NuRD complex, has recently been shown to be specifically involved in late neural radial migration and layer specification in the developing cortex. Unlike other members of the CHD subfamily, pathogenic alterations in the *CHD3* gene have not yet been implicated in a human neurodevelopmental phenotype. Upon whole genome sequencing of a cohort of children with rare speech disorders, we discovered an individual with a *de novo* missense variant in *CHD3*. Through a genotype-driven approach to identify more variants disrupting the gene, we collected 35 individuals with *de novo CHD3* mutations. The vast majority of individuals had missense mutations. These mutations showed a significant clustering within the characteristic ATPase/helicase domain of the encoded protein, in and around conserved SNF2-motifs. We used functional assays to demonstrate the effect of the missense mutations on the ATPase activity and remodeling activity of the CHD3 protein. We implicate *de novo* mutations of *CHD3* in a new syndrome that is characterized by intellectual disability, macrocephaly, impaired speech and language and a characteristic facial phenotype.

L. Snijders Blok: None. **J. Rousseau:** None. **J. Twist:** None. **S. Ehresmann:** None. **H. Venselaar:** None. **M. Takaku:** None. **J.D. Roberts:** None. **R.M. Petrovich:** None. **R. Pfundt:** None. **P. Deriziotis:** None. **T. Kleefstra:** None. **H.G. Brunner:** None. **P.A. Wade:** None. **S.E. Fisher:** None. **P.M. Campeau:** None.

C09.3

Germline mutations on the histone H4 core cause a developmental syndrome by affecting DNA damage response and cell cycle control

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Covalent modifications of histones have an established role as chromatin effectors, as they control processes such as DNA replication, transcription and repair or regulate nucleosomal structure.

Here we report monoallelic missense mutations affecting Lysine 91 in the histone H4 core (H4K91) in three individuals with a syndrome of growth delay, microcephaly and intellectual disability.

The human genome contains fifteen histone H4 genes, all differing at the nucleotide level but encoding an invariant H4 protein. RNA sequencing analysis of patient cells showed that ±8% of H4 cDNA molecules carried the

mutated allele. Analysis of the chromatin fraction of patient fibroblasts by mass spectrometry revealed that 1-2% of histone H4 molecules contained the mutated residue.

Expression of the H4 mutants in zebrafish embryos recapitulate the developmental anomalies seen in the patients. We show that the H4 mutations cause genomic instability, resulting in increased apoptosis and cell cycle progression anomalies during early development. Mechanistically, our findings indicate an important role for the ubiquitination of H4K91 in genomic stability during embryonic development.

Our results highlight the functional importance of the histone core and establish H4K91 and its modifications in the realm of human genetic disorders. Loss of Lysine 91 on histone H4 acts in a genetically dominant manner. On a biological level, our data presented here point at a mechanism involving inherent DNA damage accumulation and early perturbed cell cycle through which missense mutations affecting K91 are causative for an identifiable syndrome consisting of dysmorphic features and intellectual disability.

F. Tessadori: None. **J. Giltay:** None. **J. Hurst:** None. **M. Massink:** None. **K. Duran:** None. **H.R. Vos:** None. **R. M. van Es:** None. **R. Scott:** None. **K. van Gassen:** None. **J. Bakkers:** None. **G. van Haaften:** None.

C09.4

Examination of the landscape of histone lysine methylases and demethylases in human developmental disorders leads to identification of novel syndromes

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Introduction: Histone lysine methylation underpins gene regulation. Mutations in 11 out of 51 histone lysine methylases (KMT) and demethylases (KDM) encoding genes are known to cause autosomal dominant or X-linked human developmental disorders (DDs). We hypothesised that mutations in additional KMT/KDMs may cause novel DDs.

Methods: Using gene attributes and variant frequencies in general populations, we prioritised KMT/KDMs as candidate genes for dominant or recessive DDs. We then systematically interrogated databases of exome and array-CGH of individuals with DDs.

Results: We identified 22 KMT/KDMs as additional candidates for dominant DDs. We detected significant enrichment of de novo (DN) heterozygous or bi-allelic protein truncating variants (PTVs) for specific genes in individuals with DDs against controls. Detailed phenotyping of affected individuals uncovered several novel disorders. These included a range of neurodevelopmental phenotypes caused by KMT2C or ASH1L haploinsufficiency and a dysmorphic overgrowth DD caused by heterozygous KMT5B PTVs. We also identified a recognizable dysmorphic DD caused by bi-allelic KDM5B mutations, first autosomal recessive syndrome in this pathway. We also expanded the phenotype spectrum of KMT2B DN PTVs. Overall, we found that pathogenic KMT/KDM mutations underlie the diagnoses in >1.5% individuals with DDs.

Conclusions: Collectively, these results emphasize the significance of histone lysine methylation in normal and abnormal human development. Our results demonstrate that systematic clinically oriented pathway-based analysis of genomic data can accelerate the discovery of rare genetic

disorders. This work enables phenotype and molecular aetiology-based sub-classification of this important group of chromatin disorders.

Grants: CONICYT(72160007)

V. Faundes: None. **W.G. Newman:** None. **L. Bernardini:** None. **N. Canham:** None. **J. Clayton-Smith:** None. **B. Dallapiccola:** None. **S.J. Davies:** None. **M.K. Demos:** None. **A. Goldman:** None. **H. Gill:** None. **R. Horton:** None. **B. Kerr:** None. **D. Kumar:** None. **A. Lehman:** None. **S. McKee:** None. **J. Morton:** None. **M.J. Parker:** None. **J. Rankin:** None. **L. Robertson:** None. **I.K. Temple:** None. **S. Banka:** None.

C09.5

De novo germline variants in *Histone 3 Family 3A (H3F3A)* and *Histone 3 Family 3B* (*H3F3B*) associated with a severe neurodegenerative disorder with unique functional effect different from somatic mutations

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Histones are nuclear proteins that associate with DNA packaged it into condensed chromatin. They are dynamically decorated with post-translational modifications (PTMs), which regulate processes like DNA repair, gene expression, and mitosis/meiosis. The specific Histone 3 Family 3 histones (H3.3), encoded by *H3F3A* and *H3F3B*, mark active genes, maintain epigenetic memory, and maintain heterochromatin and telomeric integrity. Specific somatic mutations in *H3F3A/B* have been associated with pediatric tumors, but no germline mutations have been described. Here we report 24 patients, ages 4 months to 32 years, with *de novo* missense germline mutations in *H3F3A* or *H3F3B* who share a core phenotype of progressive neurologic dysfunction and congenital anomalies, but no malignancies yet. All patients have mild to profound developmental delay, and some also have seizures, developmental regression, congenital heart disease, and craniosynostosis. These 16 mutations in 24 patients, are all *de novo* and not found in large population datasets. We analyzed histones from several patients by mass spectroscopy (MS) and demonstrated that the mutant histone proteins are present at a level similar to that of wild-type H3.3. We quantified PTMs on mutant histones and demonstrated strikingly aberrant patterns of local, but not global, dysregulation of histone PTM. This suggests the pathogenic mechanism of germline histone mutations is distinct from that of the global dysregulation of cancer-associated somatic histone mutations. RNA-Seq on patient lymphoblast and fibroblasts showed a statistically significant upregulation of genes related to mitosis and cell division. Patient fibroblast lines showed increased proliferative capacity, which may contribute to the phenotype.

E.J. Bhoj: None.

C09.6

De novo mutations in the SET nuclear proto-oncogene (SET), encoding a component of the inhibitor of histone acetyltransferases (INHAT) complex in patients with non-syndromic intellectual disability (ID)

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Introduction: The role of disturbed chromatin remodelling in the pathogenesis of ID is well established and is illustrated by *de novo* mutations found in a plethora of genes encoding for proteins of the epigenetic regulatory machinery in this disorder. Here we describe mutations in the SET gene, encoding a component of the "inhibitor of acetyltransferases" complex involved in transcriptional silencing, as novel cause for ID.

Materials and Methods: Whole exome sequencing (WES) was used to detect mutations in the SET gene in four ID patients and in an affected mother and son. Literature revealed four additional patients with *de novo* SET mutations.

Results: WES identified three *de novo* loss-of-function (LoF) mutations and two *de novo* missense mutations. Besides ID the majority of patients had severe speech delay, but other clinical features were inconsistent. The mutations affected all four known alternative transcripts of the SET gene. In contrast, LoF mutations in SET are exceedingly rare in the normal population and if present they affect only one of the four SET transcripts.

Conclusions: Our study identifies SET as a new component of the epigenetic regulatory modules underlying human cognitive disorders and establishes *de novo* mutations in this gene as novel cause for non-syndromic ID. The pivotal role of SET in neurogenesis is evident from numerous previous in vitro and animal studies. The SET protein interacts with several proteins encoded by known autosomal dominant ID genes, i.e. EP300, CREBBP,

SETBP1, KMT2A, RAC1 and CTCF, most of which are involved in histone modification.

S.J.C. Stevens: None. **V. van der Schoot:** None. **M.S. Leduc:** None. **T. Rinne:** None. **S.R. Lalani:** None. **M.M. Weiss:** None. **J.M. van Hagen:** None. **A.A.M. Lachmeijer:** None. **S.G. Stockler-Ipsiroglu:** None. **A. Lehman:** None. **H.G. Brunner:** None.

C10 Best Poster Session 1

P15.05A
Comprehensive prediction of responses to chemotherapies by biochemically-inspired machine learning

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Chemotherapy response varies significantly among cancer patients, and drug resistance is responsible for significant amount of this mortality. The patterns of gene expression and copy number changes in the tumour can predict treatment outcomes after chemotherapy by supervised machine learning (ML)[1-3]. Computational models based on transcriptome gene signatures of tumor cell lines were used predict chemosensitivity and resistance for 25 cancer drugs. This learned set of genes was used to predict clinical outcomes from tumor transcriptomes. Gene signatures have been derived for 5-fluorouracil, bortezomib, carboplatin, cisplatin, docetaxel, doxorubicin, erlotinib, epirubicin, etoposide, gefitinib, gemcitabine, hydroxycyclophosphamide, imatinib, irinotecan, Ixabepilone, methotrexate, oxaliplatin, paclitaxel, pemetrexed, rapamycin, sorafenib, tamoxifen, topotecan, vinblastine, and vinorelbine. These are comprised of 3 - 17 biochemically-relevant genes, and have misclassification rates from 0% to 26.1%. Validation is performed in patients with breast, bladder, ovarian, and colon cancers. Signatures derived from tumour cell lines predicted complete remission from paclitaxel treatment in 84% of breast cancer patients (10% more accurate than differential expression analysis). Expression of MAPT correlated with survival in paclitaxel-treated breast cancer patients. Cisplatin and hydroxycyclophosphamide-resistance were respectively predicted with 71% and 66% accuracy in bladder and breast cancer patients. Analysis of a comprehensive set ML-based gene signatures for a panel of drugs in primary tumours would be feasible to carry out prior to treatment, and could influence selection of therapy. If current treatment plans are not adequate, ML-based genomic profiling may also offer alternative tailored strategies for adjuvant chemotherapies. [1]Mol.Oncol. 10:85-100, 2016; [2]F1000Res. 5:2124, 2017; [3]bioRxiv. https://doi.org/10.1101/231712. Funding:NSERCDiscovery RGPIN-2015-06290.

P.K. Rogan: Other; Significant; CytogGnomix. **E.J. Mucaki:** None. **J.Z.L. Zhao:** None. **J.H.M. Knoll:** Other; Significant; CytoGnomix.

P15.27C
Establishment of tumor-derived organoids: an approach to personalized medicine

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Introduction: Organoids are three-dimensional in vitro grown structures derived from induced pluripotent stem cells, adult stem cells or embryonic stem cells capable of self-renewal and self-organization, that exhibit the same organ functionality as the original tissue. They present organ-specific differential cells types and tissue compartmentalization. Additionally, they can be used to detect genetic alterations in patients. In this work we studied the potential of this type of culture for modeling cancer.

Materials and Methods: Organoids from fresh tumor tissues of 40 patients with several types of cancer (colon (CRC), endometrial, ovary, kidney, lung, head and neck squamous cell carcinoma) were established. Tumor tissues were dissociated into functional units, seeded in Matrigel and cultured with the appropriate medium.

Results: We established organoids from six types of cancer and determined their culture conditions. The cytokines needed for each cell type were different but all of them include EGF, Noggin, Y-27632 and Wnt-3A. For organoids derived from CRC, R-spondin, FGF10, FGF2, prostaglandin E2, nicotinamide, A83-01 and gastrin are needed. Organoids derived from kidney cancer, also need VEGF. Those derived from lung cancer require VEGF and progesterone and β-estradiol are necessary for organoids derived from gynecological cancers.

Conclusions: Organoids can be obtained from different cancer tissues. For their development, it is essential to choose the right cytokines to grow them in. These cultures have shown to mimic three-dimensional structure of the origin tissue and could be a perfect source to determine genetic alterations in these patients.

This project was funded by PI16/01920

M. Ovejero-Sánchez: None. **J. Fernández-Mateos:** None. **P. Vázquez-Cárdenas:** None. **R. González-Sarmiento:** None.

P15.03C

Correction of splice mutation in COL6A1 gene with novel antisense oligonucleotides as prototype for other orphan geneticdiseases

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Premessenger RNA splicing is a necessary step in the production of a functional protein product. Many genetic variants lead to aberrant splicing and cause genetic diseases. One prominent technique for correcting splicing-related mutations is through the use of splice-switching antisense oligonucleotides (AONs). The antisense drug Nusinersen for SMA, recently approved by the FDA, is one notable example. Cummings et al. recently identified a highly recurrent pathogenic splice variant (c.904 +189C>T) in *COL6A1* as a genetic cause of Collagen VI-related dystrophies (COL6-RD). The variant, located in intron 11, leads to the insertion of a dominantly-acting pseudoexon that disrupts the gene's critical motif. They estimate that ~25% of all cases with COL6-RD, but negative by exon sequencing are due to this mutation. This variant was found in a *de novo* pattern in over 30 patients, and we recently identified it in our lab in a 4 year old girl with COL6-RD. As part of *in silico* machine learning tools we are developing in our lab, we designed 16 AONs with 2'-OMe modifications and a phosphorothioate backbone to correct the c.904+189C>T variant. The most effective AONs were able to reduce the mutant allele by 95% in a dose dependent manner. The AONs designed in this work, as well as those designed in the independent work by Bolduc et al., may offer a treatment for children suffering from the collagen VI-like dystrophy. We expect the knowledge gained in this project to be applicable to a wide range of splice mutations. Grant support: Thrasher Research Fund.

D. Yagel: None. **Y. Anikster:** None. **A. Veber:** None. **M. Shohat:** None.

P15.11C

Modulation of cGMP and cAMP as a new therapeutic target for Fragile X Syndrome

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Fragile X syndrome (FXS), the most common form of inherited intellectual disability and a leading cause of autism spectrum disorder (ASD). It is due to the functional deficiency of the Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein involved in translational regulation of many proteins having key roles in synaptic morphology and plasticity. No specific and effective treatment for FXS is available. We searched for FMRP targets by HITS-CLIP during early development of multiple mouse brain regions (hippocampus, cortex and cerebellum) at a time when FMRP is most highly expressed and synaptogenesis peaks. Our data point out one specific phosphodiesterase mRNA as a prominent target of FMRP which negatively modulates its translation and intracellular transport. Since the abundance of this protein and activity are increased in *Fmr1*-KO cortex and hippocampus impacting the homeostasis of cAMP/cGMP, we propose here a new therapeutic approach for FXS, based on the specific pharmacological inhibition of this protein. We will present data showing pharmacological inhibition of this enzyme rescues some behavioral deficits in newborn and adolescent *Fmr1*-null mice such as social communication, discrimination and interaction. Importantly, chronic blockade in newborn *Fmr1*-KO mice, followed by a wash-out interval, results in the rescue of the altered social behavior in adolescent mice, showing that the beneficial effects of early pharmacological blockade of our target are long-lasting.

B. Bardoni: None. **V. Trezza:** None. **S. Martin:** None. **P. Vincent:** None. **L. Ciranna:** None. **M. Jarjat:** None. **S. Delhaye:** None. **S. Castagnola:** None. **F. Melancia:** None. **T. Maurin:** None.

P15.41A

800 exomes for rare disease research: outcomes of the transnational BBMRI-LPC WES call in collaboration with EuroBioBank and RD-Connect

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The 2016 BBMRI-LPC WES Call offered a unique free-of-charge opportunity to genetically diagnose rare disease patients with biological samples deposited within the EuroBioBank network. 800 whole exomes were sequenced from 17 distinct projects, each having 2-3 principal investigators from different countries. The projects spanned a wide range of rare disease phenotypes, including neuromuscular disorders, inborn errors of metabolism, albinism, and sudden cardiac depth, amongst others, and informed consent had to permit data sharing for research purposes through controlled access repositories such as the EGA (https://ega.crg.eu/) and RD-Connect (http://rd-connect.eu/). Clinical and phenotypic information for every case was collected in RD-Connect's customised PhenoTips instance, using standards such as the Human Phenotype Ontology (HPO), OMIM and Orpha codes, and sequencing undertaken at the CNAG in Spain, and the WTSI in the UK. Sequencing data was processed using the RD-Connect standard analysis pipeline and results made available through the RD-Connect Genome-Phenome Analysis Platform (https://platform.rd-connect.eu/) once all call requirements had been met. The Platform allows researchers to analyse and interpret their genotype:phenotype data privately for up to 6 months before it is shared with other authorised users. It also facilitates anonymised data sharing through APIs from initiatives such as the GA4GH/IRDiRC MatchMaker Exchange (http://www.matchmakerexchange.org/) and Beacon Network (https://beacon-network.org). We report on the challenges and lessons learned from conducting such a complex transnational collaborative initiative and present an up-to-date diagnostic yield of the project as a whole, with some illustrative success stories.

S. Laurie: None. **S. Beltran:** None. **M. Bayes:** None. **B. Fuste:** None. **M. Gut:** None. **L. Matalonga:** None. **D. Piscia:** None. **J. Dawson:** None. **R. Thompson:** None. **E. López-Martín:** None. **M. Posada:** None. **L. Monaco:** None. **C. Wang:** None. **G. B. van Ommen:** None. **S. Sims:** None. **E. Zeggini:** None. **H. Löchmuller:** None. **I. Gut:** None.

P17.06B

Inhibition of histone deacetylation up-regulates the repressed paternal allele of the imprinted *Kcnk9* gene and improves the behavioral phenotype of a mouse model of Birk-Barel syndrome

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Kcnk9/KCNK9 is a maternally expressed imprinted gene, whose mutations are causative for the maternally inherited Birk-Barel mental retardation syndrome. It encodes a K2P-channel that controls resting membrane potential and excitability of neurons.

By analyzing WT, *Kcnk9*KO^{mat} and *Kcnk9*KO^{hom} mice in a behavioral test battery during the light phase, our data shows that the absence of *Kcnk9* leads to impaired working memory, reduced acoustic startle response and abnormal sensorimotor gating. Investigations of circadian rhythms revealed selectively increased locomotor activity during the dark phase in *Kcnk9*KO^{hom} and, to a significantly smaller extent, in *Kcnk9*KO^{mat} mice compared to controls. Using Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP) and Allele-Specific RT-qPCR in wildtype (C57BL/6xCast/Ei)F1 hybrid mice, biallelic *Kcnk9* expression from the repressed paternal allele (1-17% of transcripts) was observed in all analyzed brain regions and was particularly strong in the locus coeruleus (LC). Slice patch-clamp recordings revealed wildtype-like pacemaker activity during the dark phase in LC neurons from *Kcnk9*KO^{mat} but not from *Kcnk9*KO^{hom} mice, which discharged at significantly higher frequencies. The neuronal data are in line with the locomotor phenotype and demonstrate the functional relevance of paternal *Kcnk9* expression.

Through epigenetic manipulation with CI994, a specific histone deacetylase inhibitor, we could induce a significant up-regulation of the paternal *Kcnk9* allele in several analyzed brain regions after injections in *Kcnk9*KO^{mat} mice. Together with this we observed a significant behavioral improvement of *Kcnk9*KO^{mat} mice after CI994 treatment. This novel approach shall open new avenues for treatment of cognitive dysfunctions in Birk-Barel syndrome and other imprinting disorders.

A. Cooper: None. **S. Jagannath:** None. **T. Butto:** None. **M. Linke:** None. **F. Lesage:** None. **K. Radyushkin:** None. **J. Roeper:** None. **S. Schweiger:** None. **U. Zechner:** None.

P11.017A

Efficient CrispR/Cas9-based nucleotide editing to model cardiovascular anomalies of Cantú syndrome in zebrafish

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Cantú Syndrome (CS) is a rare genetic disorder caused by gain-of-function (GOF) mutations in genes encoding the pore-forming (Kir6.1, *KCNJ8*) and regulatory (SUR2, *ABCC9*) subunits of an ATP-sensitive potassium (KATP) channel. CS is characterized by facial anomalies, hypertrichosis, extensive cardiac abnormalities and dilated cerebral blood vessels. CS is debilitating with no specific therapy available. Hence, we applied a novel CrispR/Cas9-based genome editing approach to create CS zebrafish models for therapeutic drug screening.

We efficiently introduced three CS-specific point mutations in *abbc9* and *kcnj8* of zebrafish by combining the CrispR/Cas9 system with a short template oligonucleotide harboring the site of mutation. To demonstrate functional validity, we performed live high-speed video-imaging of zebrafish heart and cardinal vein to assess cardiovascular function at 5 days post fertilization. Additionally, cerebral blood vessels were examined for dilations in live *kcnj8* mutants in a Tg(*kdrl:GFP*) transgenic background.

Analogous to CS patients, knock-in fish reveal significantly enlarged ventricles with enhanced cardiac output, contractility and development of pericardial edema. A significantly reduced vein blood flow velocity can be associated with diminished vascular tone reported in patients. Additionally, *kcnj8* mutant fish display distinct cerebral vasodilation in a structure resembling the human circle of Willis.

We developed a novel technique to establish CS-specific zebrafish that closely model cardiovascular features and therefore open the possibility of phenotyping-based drug screening potentially repurposing sulfonylureas already clinically applied to inhibit GOF KATP channels involved in neonatal diabetes. Consequently, future studies in our model will improve understanding and clinical management of CS.

E-Rare grant I-2101-B26

H.I. Roessler: None. **F. Tessadori:** None. **S.M. Savelberg:** None. **J. Bakkers:** None. **M.M. van Haelst:** None. **G. van Haaften:** None.

P17.58B

A CTCF- dependent chromatin interaction ensures robust enhancer - promoter communication at the *Shh* locus

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Sonic Hedgehog (*Shh*) is expressed in the distal-posterior part of developing limb buds and controls digit growth, number and identity. The ZRS enhancer, which regulates *Shh* transcription in developing limbs, is located in the intron 5 of the constitutively transcribed gene *Lmbr1* and has been involved in numerous patient cases with limb malformations. *Shh* and the ZRS communicate through a 1Mb-large stable chromatin interaction. However the mechanism facilitating this long-range contact is not known yet. Using a series of CRISPR/Cas9 engineered alleles and 4C-seq experiments in mouse embryos, we aim to elucidate the role of the *Lmbr1* constitutive transcription as well as CTCF in the establishment of the *Shh*-ZRS chromatin interaction. Deletion of the *Lmbr1* promoter abolishes the transcription over the ZRS, yet 4C-seq experiments showed that the contact frequency between *Shh* and its enhancer remains unchanged in these embryos allowing for normal *Shh* expression. In contrast, the removal of CTCF binding sites around and within the ZRS results in the emergence of compensatory CTCF binding sites as well as in altered 3D chromatin architecture, diminished *Shh* transcription and skeletal abnormalities. Our results suggest that CTCF acts to support a robust and permissive enhancer-promoter interaction that ensures normal *Shh* expression. This CTCF-dependent regulatory mechanism provides a framework to understand the pathomechanism of unsolved structural variants described at the *Shh* locus in patients with skeletal abnormalities.

C. Paliou: None. **P. Guckelberger:** None. **I. Jerković:** None. **V. Heinrich:** None. **S. Haas:** None. **S. Mundlos:** None. **G. Andrey:** None.

P17.22B

chromatin landscape of D4Z4 repeat interactome unveils a muscle atrophy signature in facioscapulohumeral dystrophy

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Despite increasing insights in genome structure organization, the role of DNA repetitive elements, accounting for more than two thirds of the human genome, remains elusive.Facioscapulohumeral Dystrophy (FSHD) is associated with deletion of D4Z4 repeat array below 11 units at 4q35.2. It is known that the deletion alters chromatin structure *in cis*, leading to gene upregulation. Here we show a genome-wide role of 4q-D4Z4 array in modulating gene expression via 3D nuclear contacts. We have developed an integrated strategy of 4q-D4Z4 specific 4C-seq and chromatin segmentation analysis, showing that D4Z4 3D interactome and chromatin states of interacting genes are impaired in FSHD; in particular, genes which have lost the D4Z4 interaction and with a more active chromatin state are enriched for muscle atrophy signature. Among these, we further characterized the muscle atrophy marker *Atrogin 1* (8q24), showing by 4C-seq and 3C strengthened enhancer-promoter chromatin loops at the locus and transcriptional upregulation during FSHD myogenic differentiation. Expression level of these genes is restored by an ectopic wild type 4q-D4Z4 array, suggesting that the repeat directly modulates the transcription of contacted targets.

Our study provides insight into the epigenetic role of DNA repeats in fine-tuning gene transcription by orchestrating the crosstalk between chromatin folding and structure, which deregulation maybe central in human genetic diseases pathophysiology.

This work has been supported by the following grants to B.B.: EPIGEN Italian flagship program, Italian Ministry of Health, Association Française contre les Myopathies (AFM).

A. Cortesi: None. **M. Pesant:** None. **S. Sinha:** None. **F. Gregoretti:** None. **L. Antonelli:** None. **G. Oliva:** None. **G. Soldà:** None. **B. Bodega:** None.

P18.34B

Genetic landscape of kidney function: results from a trans-ethnic genome-wide association meta-analysis of >750,000 individuals

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Introduction. Chronic kidney disease (CKD) is a public health threat, which affects >10% adult population in Western countries and is associated with increased risk of end-stage renal disease, cardiovascular disease, and all-cause mortality. In the absence of therapies for CKD prevention, understanding the biological regulators of glomerular filtration rate (GFR), the key element that defines CKD, is of the highest relevance.

Materials and methods. Within the CKDGen Consortium, 121 studies applied standardized protocols and scripts to run genome-wide association scans (GWAS) of estimated GFR (eGFR) on ~9 million high-quality SNPs, imputed on the 1000 Genomes ph3v5 or Haplotype Reference Consortium datasets. We performed fixed-effect meta-analysis of GWAS data on >750,000 participants of European, East-Asian, South-Asian, Hispanic, and African ancestries. Effect heterogeneity due to ancestry was investigated using trans-ethnic genome-wide meta-regression as implemented in MR-MEGA.

Results. Genomic inflation was negligible: lambda = 1.05 and LD score regression intercept = 1.04. Across ethnicities, we identified 308 1-Mb segments containing ≥1 SNP associated with eGFR (p value ≤ 5E-08): 228 of these loci were novel and 80 validated previously identified signals. Trans-ethnic meta-regression revealed limited heterogeneity correlated with ancestry for most genomic regions. Approximate conditional analysis of the European-ancestry dataset identified 269 independent variants, which together doubled the eGFR variance explained compared to previous estimates.

Conclusions. This study represents the largest screen of kidney function genetic loci to date. The large number of identified loci will contribute to increase our understanding of kidney function's biology.

C. Pattaro: None.

P18.12D

Multi-phenotype genome-wide meta-analysis of lipid levels and BMI in 64,736 Europeans suggests shared genetic architecture

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Serum lipid levels and obesity share biochemical pathways, suggesting overlapping causal genetic factors. Genome-wide association studies (GWAS) of correlated phenotypes have been developed to identify such shared genetic effects with increased power.

We performed a multi-phenotype GWAS (MP-GWAS) on three blood lipids (high-/low-density lipoprotein cholesterol and triglycerides, HDL-C/LDL-C/TG) and body-mass index (BMI) in 22 European-ancestry studies (N = 64,736) imputed to the 1000 Genomes reference panel (Phase 1). We fitted a "reverse regression" model between each single-nucleotide variant (SNV) and the linear combination of HDL-C/LDL-C/TG/BMI using the SCOPA software, i.e. for the ith variant $SNV_i = \beta_{1i} \times HDL\text{-}C + \beta_{2i} \times LDL\text{-}C + \beta_{3i} \times TG + \beta_{4i} \times BMI + \varepsilon_i$, where $\varepsilon_i \sim N(0,\sigma^2)$. Study-specific effect sizes and variance-covariance matrices for each variant were combined in a meta-analysis using the META-SCOPA software.

We identified 14 novel and 41/9 established lipid/BMI loci, respectively, at genome-wide significance ($P < 5x10^{-8}$). Nine novel (*SDC1*, *SLC8A1*, *EPHA6*, *SPATA4*, *MAGI2*, *CTSB, BC014119, SMCO4* and *CNTN5*) and 32 established loci showed effects on both BMI and lipids in the joint model, suggesting shared genetic architecture. This observation was supported through hierarchical cluster analysis, which resulted in six clades representing a mixture of lipid- and BMI-associated variants. We detected significant eQTL effects in whole blood, subcutaneous/visceral fat and liver at 16 of the identified loci, and enrichment of association signals at HDAC6 binding sites, indicating a critical role of associated loci in various cellular events.

The MP-GWAS is a powerful approach to detect shared genetic effects on correlated phenotypes, as demonstrated by our analysis of lipids and BMI.

Funding: EU-FP7-MARVEL (PIEF-GA-2013-626461), WT205915.

M. Kaakinen: None. **R. Mägi:** None. **V. Lagou:** None. **A. Claringbould:** None. **K. Gaulton:** None. **K. Fischer:** None. **A.P. Morris:** None. **I. Prokopenko:** None.

P18.25A

The eQTLs Catalog and LinDA browser: a platform for prioritising target genes of GWAS variants

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The expression Quantitative Traits Loci (eQTLs) are genetic polymorphisms associated with changes in gene expression levels. They have been successfully used to prioritize the target genes of the variants associated with complex traits and diseases (GWAS variants). Up to date a few eQTLs databases exist and they collect only a small portion of the available datasets.

We thus planned to build the largest publically available catalog of eQTLs, coupled with a browser, to optimize and simplify their interrogation.

We collected and manually curated 51 eQTL public studies ranging from 2007 to date, corresponding to more than 100 sample types and 25 human populations for a total of 259176 cis-eQTLs and 32929 genes with at least one cis-eQTL (cis-eGenes). Most of the eQTLs studies were conducted in blood samples from healthy individuals of European ancestry. We found that for 93% of the known protein-coding genes were eGenes, 20% of them intersecting ($r^2 \geq 0.8$) with the NHGRI-EBI GWAS Catalog and 26% of whom considered as druggable. Furthermore, for those GWAS variants for which an eGene was known, we found that the NHGRI-EBI GWAS Catalog proposed the same gene as candidate target only for the 60% of the times.

Our eQTL-Catalog can be used as a reference to measure the degree of novelty for future eQTLs studies; it is provided within a platform with a web interface (LinDA) that we plan to implement with other types of quantitative traits (i.e. epigenetic, proteomic, metabolomics and microbiota) to better dissect the pleiotropy of the GWAS variants.

S. Onano: None. **F. Cucca:** None. **M. Pala:** None.

P18.48D

Predicting rare allele carriers from genotyping-array data using whole genome sequencing data in the Estonian population

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Introduction: Genetic imputation works well with frequent alleles (minor allele frequency>1%), however, its predictive accuracy drops for rare genetic variants which can also play a role for eventual development of diseases. Exceptions to such limitations are endogamous populations like Estonia. Currently, 50,000 participants of Estonian biobank have been genotyped, with additional 100,000 being collected within next year, and their genetic profiles will be added to electronic health records. We performed a study to predict and verify rare mutation carriers for severe disease predispositions such as familial breast cancer (in genes *BRCA1* and *BRCA2*) and familial hypercholesterolemia (*APOB*) using long range haplotyping (LRH) and "surrogate parent" theory.

Materials and Methods: We used whole genome sequence data of 2,244 and genotyped data of 15,416 participants of the Estonian biobank. Whole genome

sequencing (WGS) with 30x coverage was carried out at Broad Institute using Illumina HiSeq xTen platform.

Results: WGS identified 14, 4 and 6 mutations carriers for *BRCA1*, *BRCA2* and *APOB*, respectively. We identified 16 mutations carriers for *BRCA1* and 3 for *BRCA2*, and 5 carriers for *APOB* using LRH among genotyped samples. We failed to find carriers for additional 6 out of 9 mutations, highlighting these as recent mutations and only present in limited historical lineages.

Conclusions: LRH is a cost-effective approach to predict additional rare mutation carriers for different disease predispositions from genotyped data in endogamous populations and will be important in the process of reporting clinically relevant mutations to Estonian biobank participants.

Grants: PUT1660 & IUT20-60 (Estonian Research Council)

T. Sikka: None. **M. Palover:** None. **T. Nikopensius:** None. **M. Alver:** None. **M. Nelis:** None. **A. Metspalu:** None. **N. Tõnisson:** None. **T. Esko:** None.

P18.77A

Deletions at 63 GWAS catalog loci based on genome-wide 1000 Genomes project CNV-tagging SNPs

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Background: Genome-wide association studies (GWAS) successfully exploit the variability of most abundant DNA variants, namely single nucleotide polymorphisms (SNPs), but frequently fail to provide information about causal mutations. Copy number variation (CNV) impacts phenotype variability and disease susceptibility and is one of the sources for the so-called "missing heritability". Despite notable genomic effects of both CNVs and SNPs, the correlation between them is understudied, and the role of CNVs in SNP-based phenotypic effects is not established.

Methods: We estimated linkage disequilibrium (LD) between CNVs and SNPs in protein-coding genes using the 1000 Genomes project sequencing data (1000G) from phase 3. We defined CNV-tagging SNPs for variants reported in the GWAS catalog for disease/phenotype associations (July, 2017) and for recently published DIAGRAM consortium type 2 diabetes (T2D) 1000G reference panel-imputed meta-analysis in Europeans (PMID:28566273).

Results: We replicated established CNV-tagging SNP effects at ten loci, including *NEGR1, LCE3A/B, CFHR1-3* for obesity, psoriasis and nephropathy, respectively. We revealed 31 novel CNVs (length 275bp to ~ 6kb), all but one being deletions. Among novel CNVs fifteen are <1kb, tagging lead breast cancer and T2D/lupus erythematosus SNPs at *CHST9* and *JAZF1* loci among others. Novel CNVs covered drug-target genes, such as *HTR3D/C/E, PLEKHA1*, and *MGST1* and tagged SNPs associated with major depressive disorder, age related macular degeneration, and visceral fat, respectively.

Conclusion: This is the most detailed CNV-tagging SNPs catalog to date, which will help in dissecting the functional impact of SNP-trait associations and could drive the development of new drugs.

Funding: WT205915

E. Loizidou: None. **E. Bellos:** None. **L. Coin:** None. **M. Johnson:** None. **I. Prokopenko:** None.

C11 Metabolic and Mitochondrial Disorder

C11.1

De novo mutations in *SLC25A24* cause a disorder characterized by early aging, bone dysplasia, characteristic face, and early demise (Fontaine syndrome)

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Mitochondria play a critical role in aging. Secondary mitochondrial dysfunction was reported in several progeroid syndromes, caused by alterations in components of the nuclear envelope (Hutchinson-Gilford progeria) or by impaired DNA repair (Cockayne syndrome). We present evidence that a progeroid Fontaine syndrome is caused by a primary mitochondrial dysfunction.

Four unrelated cases are reported, sharing congenitally decreased subcutaneous fat tissue and sparse hair, bone dysplasia of the skull and fingers, a distinctive face, growth retardation, and early demise. Exome sequencing showed that all carried *de novo* missense variant c.649C>T (p.Arg217Cys) or c.650G>A (p.Arg217His) in *SLC25A24*, coding for calcium-binding mitochondrial carrier protein (SCaMC-1, SLC25A24). SLC25A24 allows an electroneutral and reversible exchange of ATP-Mg and phosphate between the cytosol and mitochondria, required for optimal adenine nucleotide levels in the mitochondrial matrix. Molecular dynamic simulation studies predict that p.Arg217Cys and p.Arg217His narrow the substrate cavity of the protein and disrupt transporter dynamics. *SLC25A24*-mutant fibroblasts and cells expressing p.Arg217Cys or p.Arg217His variants showed altered mitochondrial morphology, a decreased proliferation rate, increased mitochondrial membrane potential, and decreased ATP-linked mitochondrial oxygen consumption.

Our findings identify *SLC25A24* mutations affecting codon 217 as the underlying genetic cause of human progeroid Fontaine syndrome and suggest that the *SLC25A24* mutations lead to impaired mitochondrial ATP synthesis and cause hyperpolarization and increased proton leak in association with an impaired energy metabolism. Fontaine syndrome creates a clinical spectrum with Gorlin-Chaudhry-Moss syndrome, which is caused by the same mutations, shares several clinical features, but has apparent normal lifespan.

K. Writzl: None. **A. Maver:** None. **L. Kovačič:** None. **P. Martinez-Valero:** None. **L. Contreras:** None. **J. Satrustegui:** None. **M. Castori:** None. **L. Faivre:** None. **P. Lapunzina:** None. **A.B.P. van Kuilenburg:** None. **S. Radović:** None. **C. Thauvin:** None. **B. Peterlin:** None. **A. del Arco:** None. **R.C. Hennekam:** None.

C11.2

miR-181a and miR-181b Downregulation Protects From Mitochondria-associated Neurodegeneration by enhancing mitochondrial biogenesis and mitophagy

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Introduction: Mitochondrial dysfunction underlies the pathogenesis of a variety of human neurodegenerative disorders, either directly, e.g., in the case of mitochondrial diseases, or indirectly, e.g. in the case of Parkinson's disease. The complexity of these disorders has so far prevented the development of effective therapeutic strategies.

Results: We demonstrate that the microRNAs miR-181a and miR-181b (miR-181a/b) regulate key genes involved in mitochondrial biogenesis and function. We also show that these miRNAs are involved in the global regulation of mitochondrial turnover in the central nervous system through the simultaneous and fine tuning modulation of different gene pathways. We sought to verify whether the modulation of these miRNAs could be therapeutically exploited in neurodegenerative conditions associated to impaired mitochondrial activity. We show that miR-181a/b downregulation strongly protects neurons from cell death and significantly ameliorates the disease phenotype in in vivo models of both primary and secondary mitochondria-mediated neurodegeneration, such as Microphthalmia with Linear Skin Lesions, Leber hereditary optic neuropathy and Parkinson's disease.

Conclusions: Altogether our results indicate that miR-181a/b act as hubs in mitochondrial homeostasis in the central nervous system and represent novel gene-independent therapeutic targets for a wide-range of neurodegenerative diseases caused by mitochondrial dysfunction.

Supported by the Italian Fondazione Telethon (TGM16YGM02) and by a grant from Compagnia di San Paolo and University of Naples "Federico II" (Bando STAR, 16-CSP-UNINA-048).

S. Carrella: None. **A. Indrieri:** None. **A. Romano:** None. **F. Golia:** None. **M. Pizzo:** None. **R. Tammaro:** None. **E. Marroco:** None. **N. Giordano:** None. **A. Carboncino:** None. **A. Spaziano:** None. **L. Ciampi:** None. **J. Henao-Mejia:** None. **A. Williams:** None. **R. Flavell:** None. **S. Banfi:** None. **B. Franco:** None.

C11.3

The genetic landscape of mitochondrial disease: a study of 1116 exomes

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Introduction: Mitochondrial diseases pose a diagnostic challenge due to clinical and genetic heterogeneity. 306 disease-associated genes implicated in mitochondrial energy metabolism are currently recognised and this number continues to grow. This has propelled whole exome sequencing (WES) into the clinical setting, achieving high diagnostic yields.

Methods: The WES data of 1116 paediatric and adult individuals investigated under clinical and/or biochemical suspicion of mitochondrial disease and collected over a 5-year period, were systematically analysed. Each solved exome was coupled to clinical (HPO), biochemical and experimental data.

Results: Genetic diagnosis was established in 446 (40%) individuals and included discovery of 40 novel disease genes. 345 (31%) individuals harboured pathogenic variant (s) within 122 mitochondrial disease-associated genes. Defects in *ACAD9, ECHS1, FBXL4* and *MT-ATP6* occurred most frequently. Defects in 45 of the 122 genes occurred only in single cases. 29 patients with molecularly-verified mitochondrial disease didn't demonstrate respiratory chain complex defects in muscle and/or fibroblasts. 56 diagnosed-cases are amenable to treatment strategies, highlighting the importance of WES. Difficulty in mitochondrial disease definition was reflected by 101 patients harbouring disease-causing variant(s) in non-primarily mitochondrial disease-associated genes (84 genes including *TANGO2, CAD, IARS*, *SLC52A2*) and 118 individuals with unsuspected but genetically-proven mitochondrial disease diagnosed in the same time-period.

Conclusions: This study further elucidates the heterogeneous genetic landscape of mitochondrial disease, prompting careful consideration of diagnostic criteria by highlighting discrepancies between clinical, biochemical and genetic means of diagnosis. Our data demonstrate the need consider this entire body of evidence in aggregate for the diagnosis of mitochondrial disease.

S.L. Stenton: None. **B. Alhaddad:** None. **C. Chang:** None. **T. Haack:** None. **S. Wortmann:** None. **J.A. Mayr:** None. **B. Büchner:** None. **M. Hempel:** None. **F. Distelmaier:** None. **P. Freisinger:** None. **C. Makowski:** None. **D. Rokicki:** None. **R. Taylor:** None. **K. Murayama:** None. **D. Ghezzi:** None. **C. Lamperti:** None. **A. Rötig:** None. **T. Strom:** None. **T. Klopstock:** None. **T. Meitinger:** None. **H. Prokisch:** None.

C11.4

Novel genes associated with severe mitochondrial disorders

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Introduction: Mitochondrial disorders are genetically and phenotypically heterogeneous caused by mutations in mitochondrial DNA (mtDNA) or nuclear genes. Their diagnosis and treatment remains challenging. The prevalence of mitochondrial disorders in India is more than the projected data available in the literature. In India, with a huge number of endogamous ethnic groups, the transmission rate of mitochondrial disorder is more.

Materials and Methods: We used targeted exome and Sanger sequencing in 425 patients with mitochondrial disorders to identify pathogenic mutations.

Results: We have identified mutations in mtDNA and common nuclear genes in 27% of the cases. However, the majority of cases (>70%) remain genetically undetermined. Therefore, we performed whole exome sequencing to identify novel genes responsible for mitochondrial disease. We sequenced whole exome of 30 patients with mitochondrial disease and identified several novel, reported mutations and novel genes. Of the novel genes identified, ALKBH1/ABH1 gene mutations cause severe autosomal recessive mitochondrial disease in early adulthood; hence we have sequenced complete ALKBH1 in 275 patients with mitochondrial disease, found biallelic ALKBH1 mutations in 6 patients from four different families. All mutations in a compound-heterozygous state, disrupted the function of the gene. These mutations showed complete co-segregation with the disease phenotype and absent in 650 control chromosomes. Knockdown of ALKBH1 in zebrafish using morpholino oligonucleotides (MOs) showed severe developmental abnormalities and multiple mitochondrial respiratory chain complex defects.

Conclusion: Our study shows the usefulness of exome sequencing for identification of novel genetic causes of inherited mitochondrial disease.

P. Arumugam: None. **M. Angamuthu K.:** None. **S. Gampa:** None. **V. Challa:** None. **T. Kumarasamy:** None.

C11.5

A homozygous two exon deletion in *UQCRH*: matching mouse and human phenotype

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Introduction: The ubiquinol:cytochrome c oxidoreductase hinge protein (UQCRH) plays an important role in the assembly of the complex III (CIII) of the oxidative phosphorylation (OXPHOS) system. A disease linked to *UQCRH* has not yet been described.

Methods: Whole exome sequencing and autozygosity mapping were performed on two male first cousins from a consanguineous family with recurrent episodes of severe ketoacidosis, excess blood ammonia and hypoglycaemia and signs of encephalopathy. Brain MRI's showed no abnormality and between episodes health was entirely normal. OXPHOS protein levels and complex assembly were analysed by western blot and blue native PAGE. A mouse model was created in C57BL/6N mice by deletion of exons 2 and 3 of *UQCRH*. Standard full screening of the mouse phenotypes was performed. Moreover, enzymatic activity and protein expression of OXPHOS complexes were investigated in mouse tissue.

Results: A homozygous *UQCRH* deletion of exons 2 and 3 was detected. Cultured patients' primary fibroblasts showed incomplete assembly and decreased steady-state protein levels of CIII subunits and low CIII enzyme activity. The murine presentation was more severe with progressive functional impairment and premature death at the age of 6-8 weeks. Enzyme activity assays revealed a significant decrease in CIII activity in heart, brain and liver tissues.

Conclusions: Here we describe the first patients with biallelic mutations in *UQCRH*. Notably, biallelic variants in *UQCRC2* and *UQCRB*, two other subunits of complex III, result in a similar episodic clinical presentation.

Funding: BMBF (Infrafrontier grant 01KX1012), E-Rare project GENOMIT (01GM1207), Wellcome Centre for Mitochondrial Research (203105/Z/16/Z).

S. Vidali: None. **J. Urquhart:** None. **J. Rozman:** None. **K. Thompson:** None. **C. Sanders:** None. **E. Jamson:** None. **C. Breen:** None. **B. Rathkolb:** None. **P. da Silva-Buttkus:** None. **S. Marschall:** None. **O.V. Amarie:** None. **J. Aguilar-Pimentel:** None. **J. Calzada-Wack:** None. **L. Becker:** None. **Y. Cho:** None. **L. Garrett:** None. **S.M. Hölter:** None. **T. Klein-Rodewald:** None. **P. Mayer-**

Kuckuk: None. **I. Treise:** None. **A. Zimprich:** None. **K. Gampe:** None. **S. Leuchtenberger:** None. **K. Pfannes:** None. **C. Stöger:** None. **H. Maier:** None. **J. Graw:** None. **W. Wurst:** None. **K. Höfig:** None. **R.G. Feichtinger:** None. **U. Gärtner:** None. **M. Szibor:** None. **I. Wittig:** None. **J.A. Mayr:** None. **W. Newman:** None. **H. Fuchs:** None. **R.W. Taylor:** None. **V. Gailus-Durner:** None. **H. Prokisch:** None. **M. Hrabě de Angelis:** None.

C11.6
Mutationsin phosphopantothenoylcysteine synthetase (PPCS) cause dilated cardiomyopathy

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Introduction: Coenzyme A (CoA) is an essential cofactor which transfers acyl groups among molecules. Cells synthesize CoA *de novo* from pantothenate through five consecutive enzymatic steps. Dysfunction of two enzymes, PANK2 and COASY, leads to Neurodegeneration with Brain Iron Accumulation. To our knowledge mutations in *PPCS*, another enzyme of the pathway, have so far not been associated with human diseases.

Subjects and Methods: Five patients of two unrelated families presented with dilated cardiomyopathy of varied severity and no neurodegeneration. Identification of variants was performed by WES; pathogenicity of identified mutations evaluated by functional complementation in yeast and modeling in *Drosophila;* biochemical consequences of PPCS deficiency assessed in patients' fibroblasts.

Results: WES identified rare biallelic variants in *PPCS*. The pathogenicity of identified variants was assessed by functional complementation of a yeast model null for *PPCS* (*yPPCS*). Ablation of *yPPCS* is lethal; *yPPCS*-null mutants transformed with the human wild-type *PPCS* were able to grow, whereas mutants transformed with variant-carrying *PPCS* constructs had clear growth defects, confirming the deleterious nature of the variants. *Drosophila PPCS* mutants showed a significant increase in heart rate, heart wall shortening and arrhythmia index, and a decrease in systolic length, indicating that homozygous variants in *PPCS* are associated with cardiac dysfunction.

Conclusions: Identification of biallelic variants in *PPCS* in individuals with cardiomyopathy, linked for the first time CoA synthesis with a cardiac phenotype. Since CoA biosynthesis can occur with pantethine as a source independent from PPCS, pantethine can be envisaged as therapeutic options for the patients still alive.

A. Iuso: None. **M. Wiersma:** None. **H.J. Schüller:** None. **B. Pode-Shakked:** None. **D. Marek-Yagel:** None. **T.B. Haack:** None. **T. Meitinger:** None. **H. Prokisch:** None. **D. Haas:** None. **O.C.M. Sibon:** None. **Y. Anikster:** None.

C12 Skin and Bones

C12.1
Functional analysis of large numbers of non-coding variants from WGS studies by massively parallel cis-regulatory assays

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NGS technologies enable the simultaneous investigation of the entire genome. However, 40% of patients remain without molecular diagnosis despite that fact that on average ~80 *de novo* SNVs per patients are identified. The sheer number of these variants, overhwhelmingly non-coding, make classical functional workup strategies impractical. Here we performed whole genome sequencing of 50 trios affected with congenital limb malformations, and followed this with functional characterization of all observed non-coding *de novo* SNVs via massively parallel reporter assays

(MPRAs). All patients included were array CGH and exome negative. In total we identified 3,396 *de novo* mutations in the 50 patients. 5 *de novo* mutations were located in predicted enhancer regions based on epigenetics marks. For two of these predicted enhancers we could show positive in vivo enhancer activity in transgenic mouse reporter assays. Next we used microarray-based DNA synthesis to create 230 bp oligonucleotides containing all 3,396 *de novo* non-coding variants and the corresponding wild-type sequences and perform lentivirus-based MPRAs in human chondrocyte cells and primary mouse limb bud cells. We experimentally measured the *cis*-regulatory activity of 3,396 *de novo* non-coding mutations in a single, quantitative experiment. We identified 48 variants that showed significant differential expression of the reporter gene. The positive candidates showed up to 5-fold enrichment for ENCODE TF binding motifs indicating that the mutations are likely to change TF binding and thereby contribute to disease. Our study provides a conceptual framework for the experimental assessment of the large number of *de novo* non-coding mutations from WGS studies.

M. Spielmann: None. **M. Kircher:** None. **B. Kragsteen:** None. **M. Mensa:** None. **R. Flöttmann:** None. **M. van de Vorst:** None. **L. Wiel:** None. **J. Veltman:** None. **S. Mundlos:** None. **C. Gilissen:** None. **J. Shendure:** None.

C12.2

Identification of somatic activating PIK3CA mutations in patients with generalized lymphatic anomaly

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Introduction: Generalized lymphatic anomaly (GLA) is a rare vascular disorder characterized by diffuse or multifocal lymphatic malformations (LMs), associated with osteolysis and/or involvement of skin, soft tissues, and viscera. Here we tested the hypothesis that, although the genetic cause is not known, the tissue distribution of the clinical manifestations in GLA seems to follow a pattern of somatic mosaicism.

Materials and Methods: We performed targeted high-throughput sequencing on paired blood/tissue samples from 10 GLA patients. All variants detected were confirmed using at least one orthogonal method. We isolated LECs from fresh tissue samples obtained from three GLA patients that were also used for high-throughput sequencing. Finally we used a Cre-loxP system in mice LECs (Prox1-CreERT2; LSL-Pik3caH1047R) to characterize the effect of excessive PI3K signalling on the structure and function of lymphatics.

Results: We identified four different mosaic *PIK3CA* variants in six of ten patients. The variants were detected in LMs and in LECs isolated from affected tissues. These same *PIK3CA* variants occur in patients with *PIK3CA*-related overgrowth spectrum and cause hyperactivation of the PI3K signalling pathway. We show that excessive PI3K signalling in LECs causes mice lymphatic hyperplasia, non-functional vessels, and induces the formation of lymphatics in bone.

Conclusions: We describe for the first time the presence of somatic activating *PIK3CA* mutations in patients with GLA. This will help to better define the phenotypic consequences of this pathology, and has consequences in the diagnosis, monitoring and treatment of patients. This work was founded by The Lymphatic Malformation Institute (LMI), USA.

L. Rodriguez Laguna: None. **N. Agra:** None. **K. Ibañez:** None. **G. Oliva-Molina:** None. **G. Gordo:** None. **N. Khurana:** None. **D. Hominick:** None. **G. Herranz:** None. **J. Torres Canizalez:** None. **R. Rodriguez Pena:** None. **E. Vallespín:** None. **R. Martín-Arenas:** None. **Á. del Pozo:** None. **C. Villaverde:** None. **A. Bustamante:** None. **C. Ayuso:** None. **P. Lapunzina:** None. **J. Lopez-Gutierrez:** None. **M. Dellinger:** None. **V. Martinez-Glez:** None.

C12.3

A mutant ATP6V1E1 zebrafish model recapitulates the human cutis laxa syndrome

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A mutant ATP6V1E1 zebrafish model recapitulates the human cutis laxa syndrome

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We recently described a novel autosomal recessive cutis laxa (CL) syndrome, caused by missense mutations in

subunit V1E1 of vacuolar H^+-ATPase (ATP6V1E1). The condition is clinically characterized by loose redundant skin folds, facial characteristics, lipodystrophy, hypotonia and cardiopulmonary involvement including pneumothorax, hypertrophic cardiomyopathy and aortic root dilatation. Similarly, to *ATP6V02*-related cutis laxa, impaired retrograde Golgi transport, glycosylation abnormalities, and a dysfunctional secretory pathway have been implicated in the pathogenesis, but the pleiotropic manifestations remain largely unexplained. We use a zebrafish model with a proviral insertion in the 5'UTR of *atp6v1e1*. Homozygous mutant embryos show decreased pigmentation. Decreased and delayed spontaneous hatching of the homozygous *atp6v1e1b* mutants suggests hypotonia, which could be quantitatively assessed by a reduced touch-evoked escape response. All homozygous mutant fish die by 8 days post fertilization (dpf) due to severe cardiovascular edema and insufficient cardiovascular circulation. Quantification of cardiac function at 3 dpf indicates a significantly decreased cardiac output and stroke volume, but normal heart rate in homozygous mutant embryos, and a similar, but not significant trend in heterozygous embryos. Moreover, blood flow is severely reduced in mutants compared to heterozygous and wild-type fish.

Conclusion, our zebrafish model recapitulates several important clinical features including hypotonia and cardiomyopathy. This model provides an important asset to unravel the molecular mechanism causing *ATP6V1E1*-related cutis laxa and to evaluate potential therapeutic strategies.

L. Pottie: None. **P. Sips:** None. **P. Coucke:** None. **B. Callewaert:** None.

C12.4
Recessive spondylocarpotarsal syndrome due to compound heterozygosity for variants in *MYH3*

S. P. Robertson[1], S. Cameron-Christie[1], C. F. Wells[1], M. Simon[2,3], M. Wessels[3], C. Z. N. Tang[1], W. Wei[1], R. Takei[1], C. Aarts-Tesselaar[4], S. Sandaradura[5], D. O. Sillence[5], M. Cordier[6], H. E. Veenstra-Knol[7], E. Trevisson[8], D. M. Markie[1], Z. A. Jenkins[1]

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Spondylocarpotarsal Syndrome (SCTS) is characterised by vertebral fusions and fusion of the carpal and tarsal bones. Bi-allelic mutations in *FLNB* cause SCTS in some families, while monoallelic variants in the gene encoding embryonic heavy chain myosin 3, *MYH3*, have been implicated in dominantly inherited forms. Here five *FLNB* mutation-negative cases from three families were hypothesised to have a recessive form of SCTS on account of sib recurrence. Whole Exome Sequencing (WES) showed that all five were heterozygous for one of two splice-site variants in *MYH3*. Despite 3 of the 5 cases sharing two allelic haplotypes over *MYH3*, no second variant could be identified by WES. Subsequent WGS demonstrated a variant altering a conserved splice donor site in the 5'UTR of *MYH3*, a region of the gene not captured by exome capture platforms. Expanding the case cohort to 16 unsolved SCTS cases, 9 had truncating mutations transmitted by unaffected parents, with 6 inheriting the same 5'UTR variant in *trans*, an observation at variance with the allele frequency for this variant in population databases. This variant disrupts splicing of exons 1-3 in the 5' UTR but is still permissive of *MYH3* translational initiation, albeit with reduced efficiency. Although some *MYH3* variants cause dominant SCTS, these data indicate that many truncating variants do not lead to the disease except when in *trans* with a second hypomorphic allele. These observations make genetic diagnosis and counselling challenging in the context of simplex presentations of SCTS especially using a genotype-first methodology.

S.P. Robertson: None. **S. Cameron-Christie:** None. **C. F. Wells:** None. **M. Simon:** None. **M. Wessels:** None. **C.Z. N. Tang:** None. **W. Wei:** None. **R. Takei:** None. **C. Aarts-Tesselaar:** None. **S. Sandaradura:** None. **D.O. Sillence:** None. **M. Cordier:** None. **H.E. Veenstra-Knol:** None. **E. Trevisson:** None. **D.M. Markie:** None. **Z.A. Jenkins:** None.

C12.5
Mutations in the Epithelial Cadherin p120 Catenin ComplexCause Mendelian Non-Syndromic Cleft Lip and Palate

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States, [4]New South Wales Health Pathology, Prince of Wales Hospital, Randwick, Australia, [5]5. Department of Orthodontics & the Iowa Institute for Oral and Craniofacial Research, University of Iowa,, Iowa, IA, United States, [6]Department of Pediatrics, University of Iowa, Iowa, IA, United States, [7]Birth Defects Research Laboratory, University of Washington, Seattle, WA, United States, [8]Division of Medical Genetics, Department of Medicine, University of Washington, Seattle, WA, United States, [9]Division of Genetic Medicine, Department of Pediatrics, University of Washington, Seattle, WA, United States, [10]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States, [11]Department of Biological Sciences, University of Delaware, Newark, DE, United States, [12]Department of Genome Sciences, University of Washington, Seattle, WA, United States, [13]Northwest Clinical Genomics Laboratory, Center for Precision Diagnostics, University of Washington, Seattle, WA, United States, [14]Division of Craniofacial Medicine, Department of Pediatrics, University of Washington, Seattle, WA, United States, [15]Centre for Molecular and Biomolecular Informatics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [16]Fundación Clínica Noel, Medellin, Colombia, [17]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [18]South Australian Clinical Genetics Service, Adelaide, Australia, [19]Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [20]UVA Center for Advanced Medical Analytics, School of Medicine, University of Virginia, Virginia, VA, United States, [21]Lidral Orthodontics, Rockford, MN, United States

Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"-Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family:Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin; mso-ansi-language:EN-US;} Non-syndromic cleft lip/palate (NS-CL/P) is one of the most common human birth defects and is generally considered a complex trait. Despite numerous loci identified by genome-wide association studies, the effect sizes of common variants are relatively small with much of the presumed genetic contribution remaining elusive. We report exome sequencing results in 209 people from 72 multi-affected families with pedigree structures consistent with autosomal dominant inheritance and variable penetrance. We describe four new NS-CL/P genes encoding components of the p120-catenin complex *(CTNND1, PLEKHA7, PLEKHA5*) and an epithelial splicing regulator (*ESRP2*), in addition to *CDH1*. The findings were validated in a second cohort of 497 NS-CL/P patients comprising small families and singletons. Pathogenic variants in these genes were found in 14% of multi-affected families and 2% of the replication cohort of smaller families. Enriched expression of each new gene/protein in human and mouse embryonic oro-palatal epithelia, demonstration of functional impact of *CTNND1* and *ESRP2* variants, and recapitulation of the CL/P spectrum in *Ctnnd1* knockout mice support a causative role in CL/P pathogenesis. These data show that primary defects in regulators of epithelial cell adhesion are the most significant contributors to NS-CL/P identified to date and that inherited and *de novo* single gene variants explain a substantial proportion of NS-CL/P. <!--EndFragment-->

T. Roscioli: None. **L.L. Cox:** None. **T.C. Cox:** None. **L. M. Moreno Uribe:** None. **Y. Zhu:** None. **C.T. Richter:** None. **N. Nidey:** None. **J.M. Standley:** None. **M. Deng:** None. **E. Blue:** None. **J.X. Chong:** None. **Y. Yang:** None. **R.P. Carstens:** None. **D. Anand:** None. **S.A. Lachke:** None. **J.D. Smith:** None. **M.O. Dorschner:** None. **E. Kirk:** None. **A.V. Hing:** None. **H. Venselaar:** None. **L. Consuelo Valencia Ramirez:** None. **M.J. Bamshad:** None. **I.A. Glass:** None. **J.A. Cooper:** None. **E. Haan:** None. **D.A. Nickerson:** None. **H. van Bokhoven:** None. **H. Zhou:** None. **K. Krahn:** None. **M.F. Buckley:** None. **J.C. Murray:** None. **A.C. Lidral:** None.

C12.6
Somatic activating mutations in *MAP2K1* cause melorheostosis

H. Kang[1], S. Jha[2,3], Z. Deng[4], N. Fratzl-Zelman[5], W. A. Cabral[1], A. Ivovic[6], F. Meylan[7], E. P. Hanson[8], E. Lange[9], J. Katz[9], P. Roschger[5], K. Klaushofer[5], E. W. Cowen[10], R. M. Siegel[7], T. Bhattacharyya[2], J. C. Marini[1]

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Melorheostosis is a benign bone overgrowth condition, with radiographic appearance of "dripping candle wax". Because melorheostosis occurs sporadically, somatic mutations were hypothesized, but bone tissue was not previously investigated. Paired biopsies of affected and contralateral unaffected bone of 15 melorheostosis patients were utilized for DNA extraction, osteoblast cultures and histology. Using WES and ddPCR, we identified somatic mutations in *MAP2K1*, encoding the kinase MEK1, in affected, but not unaffected, bone of 8 melorheostosis patients. Mosaicism was supported by immunohistochemistry of ERK1/2 activation in bone tissue and two populations of cultured osteoblasts with distinct p-ERK1/2 levels on flow cytometry. These activating mutations (p.Q56P, p.K57E, p.K57N) cluster in the MEK1 negative regulatory domain. Osteoblasts from affected bone displayed increased MEK1-ERK1/2 signaling, which enhanced osteoblast proliferation and stimulated bone remodelling, while inhibiting osteoblast differentiation and bone mineralization. This coincided with the histology of melorheostosis, featuring exuberant deposition of parallel layers of lamellar bone, followed by intense bone remodelling with elevated cellularity. Melorheostosis provides the first demonstration that the *MAP2K1* oncogene is important to human bone formation. Interestingly, germline mutations in the catalytic core of *MAP2K1* and the negative regulatory domain of *MAP2K1* and *MAP2K2* were identified in Cardio-Facio-Cutaneous syndrome (CFC). CFC and other RASopathies are associated with variable cardiac, facial and neurodevelopmental defects, but not with bone overgrowth. Somatic *MAP2K1* mutations in melorheostotic osteoblasts and others recently reported in extracranial arteriovenous malformations apparently have consequences distinct from germline mutations. We conclude that outcomes of *MAP2K1* mutations depend on mutation timing and location.

H. Kang: None. **S. Jha:** None. **Z. Deng:** None. **N. Fratzl-Zelman:** None. **W.A. Cabral:** None. **A. Ivovic:** None. **F. Meylan:** None. **E.P. Hanson:** None. **E. Lange:** None. **J. Katz:** None. **P. Roschger:** None. **K. Klaushofer:** None. **E.W. Cowen:** None. **R.M. Siegel:** None. **T. Bhattacharyya:** None. **J.C. Marini:** None.

C13 Prenatal and Reproductive Genetics

C13.1
Implementing NIPT as part of a national prenatal screening program: The Dutch TRIDENT studies

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In the Netherlands, Non-Invasive Prenatal Testing (NIPT) has been implemented as part of the TRIDENT studies. TRIDENT-2 aims at offering NIPT to all pregnant women (~174,000/year) within the national governmentally supported prenatal fetal aneuploidy screening program. Since April 2017 women can choose NIPT as a contingent test

after first-trimester combined screening (FTS), but may also choose NIPT as first-tier test. The TRIDENT-2 study evaluates implementation and women's perspectives.

All women are offered screening and are counselled by certified counsellors, generally midwives. A first-tier NIPT costs women € 175, comparable to FTS (~€ 168). NIPT is performed by three Dutch university clinical genetic laboratories using an in-house validated test. Women can choose to have analysis of chromosomes 21, 18, and 13 without or with a report of incidental findings (findings other than trisomy 21, 13, 18) on the remaining autosomes, respectively using the 'targeted' or 'whole genome' WISECONDOR pipeline. Sex chromosomes are not analyzed.

After eight months of study, 48,234 tests have been performed (first-tier NIPT uptake 40%) and 98.3% reports were successfully issued. Mean turnaround time was 7 working days. 80% of women chose to have all autosomes analyzed. A total of 152 cases of T21 (0.3%), 32 cases of T18 (0.1%), 41 cases of T13 (0.1%) and 158 (0.3%) other chromosomal aberrations were found. First year results (and available follow-up) will be presented.

This 3-year study aims to provide all necessary information for a successful introduction of NIPT within the Dutch National prenatal screening program.

Grant: ZonMw Netherlands

M.M. Weiss: None. **R.H. Galjaard:** None. **E.A. Sistermans:** None. **C.J. Bax:** None. **M.N. Bekker:** None. **C.E. M. de Die-Smulders:** None. **I. Feenstra:** None. **M.J.V. Hoffer:** None. **N.S. den Hollander:** None. **M.F.C.M. Knapen:** None. **I.M. van Langen:** None. **K.D. Lichtenbelt:** None. **P.M. Lombardi:** None. **M.C. van Maarle:** None. **K.R.M. van der Meij:** None. **M.J. Pieters:** None. **G.H. Schuring-Blom:** None. **E. Sikkel:** None. **S.J. Stevens:** None. **R.F. Suijkerbuijk:** None. **A.J.E.M. van der Ven:** None. **D. Van Opstal:** None. **L. Henneman:** None. **M.V. Macville:** None.

C13.2

Rapid Prenatal Diagnosis through Targeted Exome Sequencing: A Cohort study

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Background: Prenatal genetic diagnosis provides information for pregnancy and perinatal decision-making and management. Prenatal exome sequencing approaches have identified genetic diagnoses when conventional tests (karyotype and microarray) were not diagnostic. However, prenatal phenotyping limitations, counselling challenges regarding variants of uncertain significance, incidental and secondary findings, and technical problems pose unique challenges in the prenatal use of exome sequencing. Here we use a multidisciplinary approach to explore the utility of rapid targeted fetal exome sequencing for prenatal diagnosis, using skeletal dysplasias as an exemplar.

Methods: Pregnant women who had had, or who were undergoing, an invasive procedure to exclude chromosomal abnormalities following ultrasound detection of fetal abnormalities suggestive of a skeletal dysplasia in UK Fetal Medicine units were identified prospectively. After multidisciplinary review by local Fetal Medicine teams and Clinical Geneticists with expertise in fetal dysmorphology, parents were consented for targeted exome trio-sequencing. Variant interpretation focused on a virtual panel of 240 genes known to cause skeletal dysplasias.

Results: Definitive molecular diagnosis was made in 15/18 (83%) cases. In some cases fetal ultrasound findings alone were of sufficient severity for parents to opt for termination. In others, molecular diagnosis informed accurate prediction of outcome, improved parental counselling and informed parental decisions and perinatal management.

Conclusions: Trio-sequencing with expert multidisciplinary review for case selection and data interpretation yields timely, high diagnostic rates in fetuses presenting with unexpected skeletal abnormalities. This can result in improved parental counselling and pregnancy management.

F. Faravelli: None. **N. Chandler:** None. **S. Best:** None. **J. Hayward:** None. **S. Mansour:** None. **E. Kivuva:** None. **D. Tapon:** None. **A. Male:** None. **C. DeVile:** None. **L. Chitty:** None.

C13.3

Temporal dynamics of placental gene expression

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Introduction: Placenta has a principal role in affecting fetal and maternal physiology. Since pregnancy complications

coincide with changes in placental transcriptome, knowledge of normal placental gene expression dynamics across gestation is crucial to understand alterations in the placental transcriptome that may lead to pregnancy complications. The aim of the study is to describe a profile of placental temporal gene expression dynamics over the course of human pregnancy.

Materials and Methods: Analyzed placental samples covered the entire human gestational period: 1st trimester (7-10 gestational weeks; n = 8), 2nd trimester (17-21 g.w.; n = 8) and term (37-41 g.w.; n = 8). RNA-Seq protocol and basic bioinformatics have been reported by Reiman et al. (2017). The current study focused on grouping placental genes based on their highly correlated expression dynamics across pregnancy trimesters. Enrichment analysis for functional pathways was conducted for each gene-group using g:Profiler (https://biit.cs.ut.ee/gprofiler/).

Results: Among 14,920 genes expressed in the placenta, roughly a quarter exhibited a clear temporal expression pattern over the course of pregnancy. Enrichment analysis showed that genes overexpressed in the first trimester are involved in cell cycle and transcription regulation; whereas mid-gestation specific genes are associated with fetal development, cell communication and differentiation of tissues.

Conclusions: We show that a large proportion of genes expressed in the human placenta follow a tightly correlated temporal expression profile. We hypothesize that disturbances of this 'gestational clock' may lead to placental and fetal developmental abnormalities and pregnancy complications.

Reiman et al. FASEB J. (2017): 201601031RR

Grants: IUT34-12 (Estonian Research Agency), Happy Pregnancy (SA Archimedes).

M. Reiman: None. **S. Sõber:** None. **M. Laan:** None.

C13.4

Assessing the landscape of selfish *de novo* mutations in human testes

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Low level mosaicism, attributed to clonal expansion of spontaneous mutations, is prevalent in somatic tissues such as skin and blood. We have previously shown that specific 'selfish' mutations spontaneously arising in male germline stem cells (spermatogonia) lead to clonal expansion resulting in elevated mutation levels in sperm over time. This process, termed selfish spermatogonial selection, explains the high spontaneous birth rate and strong paternal age effect of disorders such as achondroplasia, Apert, Noonan and Costello syndromes, with direct evidence for this process occurring at specific positions in six genes (*FGFR2*, *FGFR3*, *RET*, *PTPN11*, *HRAS* and *KRAS*). Here we perform a discovery screen to identify novel mutations and genes under selection in the male germline by performing massively-parallel simplex PCR targeting using RainDance technology to interrogate mutational hotspots in 71 genes (66.5kb in total) in 276 biopsies of testes from 5 men (mean age: 73 years). Ultra-deep sequencing (~22,000x), a custom low-frequency variant prioritization pipeline and targeted validation identified 59 distinct variants with frequencies as low as 0.06%, including 53 variants not previously directly associated with selfish selection. The majority (80%) of variants identified were previously implicated in either developmental disorders and/or oncogenesis, including mutations in six newly-associated (*BRAF, CBL, MAP2K1*, *MAP2K2, NF1* and *RAF1*) genes, all of which encode components of RAS/MAPK signalling. Our findings extend the link between mutations causing dysregulation of RAS/MAPK signalling and selfish selection and suggest that the ageing male germ line is a repository for such deleterious mutations. Supported by grants from the Wellcome Trust.

G.J. Maher: None. **H.K. Ralph:** None. **Z. Ding:** None. **N. Koelling:** None. **H. Mlcochova:** None. **E. Giannoulatou:** None. **P. Dhami:** None. **G. McVean:** None. **A.O.M. Wilkie:** None. **A. Goriely:** None.

C13.5

X-chromosome exome sequencing in highly selected idiopathic azoospermic patients: identification of novel and recurrent genetic factors for early spermatogenic failure

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Background: Despite the well known enrichment of the X chromosome in genes specifically expressed in the testis, so far only two X-linked genes are diagnostic targets in non obstructive azoospermia (NOA). The apparent paucity of X-linked NOA is likely to be the consequence of the lack of comprehensive, whole X chromosome targeting studies.

Material and Methods: X-chromosome exome sequencing in 50 idiopathic NOA patients with known testis histology. Variants were filtered and prioritized according to their minor allele frequency (MAF≤0.01) and their predicted pathogenicity. RNA interference was used to determine the role of *Caf1-55* (the human *RBBP7* ortholog) in *Drosophila* spermatogenesis.

Results: We identified 77 rare and predicted as pathogenic variants in 42/50 NOA patients. 76 variants were private mutations whereas one was found in two unrelated patients. Nine genes (six of them with testis specific expression or overexpression in the testis) were recurrently mutated in 16 different patients. Two patients affected by spermatogonial arrest presented pathogenic mutations in the *RBBP7* gene. Conditional *Caf1-55* KO showed that male mutants had tiny testis, no spermatozoa and were sterile.

Conclusions: This is the first X chromosome exome analysis in highly selected NOA patients. Our approach was relatively successful in identifying candidate genes for the NOA phenotype. Up to now, we performed functional analysis only for the *RBBP7* gene demonstrating that the protein is essential for *Drosophila* spermatogenesis hence we propose it as a novel genetic factor for early spermatogenic failure.

Funding: Instituto Carlos III (FIS/FEDER: PI14/01250) and GEMINI Consortium

A. Riera-Escamilla: None. **D. Moreno-Mendoza:** None. **L. Nagirnaja:** None. **J. Rusch:** None. **E. Ruiz-Castañé:** None. **E. Ars:** None. **D.F. Conrad:** None. **C. Krausz:** None.

C13.6
Dysfunctional SEMA3G signalling underlies familiar hypogonadotropic hypogonadism & defective GnRH neuron migration

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Hypogonadotropic hypogonadism (HH) is a rare genetic disorder that impairs sexual reproduction. It can be caused by a defective development or function of Gonadotropin-releasing hormone (GnRH)-neurons. Most HH cases is still idiopathic, despite recent advances in sequencing technologies. Here we have combined *in silico* with in vitro and in vivo mouse models to identify and functionally characterise the role of a novel gene in the etiopathogenesis of HH. Specifically, we have applied homozygosity mapping together with exome sequencing and computational modelling to identify a shared point mutation of the Semaphorin 3G (*SEMA3G*) gene in two brothers with HH, born from consanguineous parents. Further, we have shown that the mutation impairs GnRH neuron migration and Akt activation in vitro, and that mice lacking *Sema3g* show a reduced number of hypothalamic GnRH neurons and reduced fertility. Together, these results indicate that SEMA3G signalling is required for GnRH neuron migration and its insufficiency contributes to the pathogenesis of HH.

Grants; telethon GP13142 for AC,

A. Cariboni: None. **A. Lettieri:** None. **R. Oleari:** None. **S. Tahir:** None. **K. Hussain:** None.

C14 Cancer genetics

C14.1
Tet1 and *Tdg* suppress intestinal tumorigenesis by downregulating the inflammatory and immune responses in the Apc^{Min} mouse model

R. Tricarico[1], J. Madzo[2], G. Scher[1], J. Jelinek[2], J. Ingram[3], I. Peshkova[3], W. Chang[4], E. Nicolas[5], Y. Zhou[6], K. Devarajan[6], S. Maegawa[2], V. Doneddu[7], L. Bagella[7], H. Cooper[8], S. Balachandran[3], M. Clapper[4], S. Grivennikov[4], E. Koltsova[3], J. Issa[2], A. Bellacosa[1]

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Introduction: Aberrant DNA methylation is frequently observed in colorectal cancer (CRC), but the underlying mechanisms are poorly understood. Ten-Eleven Translocation (TET) dioxygenases and DNA repair enzyme Thymine DNA Glycosylase (TDG) are involved in active DNA demethylation by generating and removing, respectively, novel oxidized cytosine species. Mutations of *TET1* and *TDG*, and alterations of the levels of oxidized cytosine species have been identified in human CRC cases, but the biological significance of the TET-TDG demethylation axis in intestinal tumorigenesis is unclear.

Materials and Methods: We generated *Apc*^{Min} mice with additional inactivation of *Tet1* and/or *Tdg*, and characterized the methylome and transcriptome of intestinal adenomas by DREAM and RNA sequencing, respectively.

Results: *Tet1*- and/or *Tdg*-deficient *Apc*^{Min} mice show enhanced intestinal tumorigenesis in comparison to wild type *Tet1* and *Tdg Apc*^{Min} mice. Specifically, *Tet1* and/or *Tdg*-deficient *Apc*^{Min} adenomas manifested increased size or features of erosion and stroma activation. Methylome analysis revealed progressive loss of global DNA hypomethylation in colonic adenomas from *Tet1*- and *Tdg*-deficient *Apc*^{Min} mice, and hypermethylation of CpG islands in *Tet1*-deficient *Apc*^{Min} mice. In addition, RNA sequencing showed upregulation of genes in inflammatory and immune response pathways in *Tet1*- and *Tdg*-mutant colonic adenomas compared to control *Apc*^{Min} adenomas.

Conclusions: Taken together, these findings demonstrate the important role of active DNA demethylation mediated by TET-TDG in reducing intestinal tumor formation, by modulating the epigenome and inflammatory/immune responses. This study highlights a novel mechanism of epigenetic deregulation during intestinal tumorigenesis with diagnostic, therapeutic and prognostic implications.

R. Tricarico: None. **J. Madzo:** None. **G. Scher:** None. **J. Jelinek:** None. **J. Ingram:** None. **I. Peshkova:** None. **W. Chang:** None. **E. Nicolas:** None. **Y. Zhou:** None. **K. Devarajan:** None. **S. Maegawa:** None. **V. Doneddu:** None. **L. Bagella:** None. **H. Cooper:** None. **S. Balachandran:** None. **M. Clapper:** None. **S. Grivennikov:** None. **E. Koltsova:** None. **J. Issa:** None. **A. Bellacosa:** None.

C14.2

Lynch syndrome families with heritable constitutional epimutation reveal the diversity of genetic events associated with methylation of *MLH1* promoter

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Introduction: Constitutional epimutations are an alternative to genetic mutations in the etiology of genetic diseases. These epimutations can be primary, defined as purely epigenetic events labile in the germline. They can also be secondary, caused by a *cis*-acting genetic defect transmitted following a Mendelian inheritance pattern, with re-establishment of the epigenetic change in the offspring. In Lynch syndrome, a few families with a heritable *MLH1* epimutation have been reported so far, with various genetic bases including single nucleotide variations and copy number variations.

Materials and Methods: We designed a long-range PCR next-generation sequencing strategy to screen *MLH1* entire gene and applied it to 4 French families with heritable epimutations and 10 additional patients with no proven transmission of their epimutations.

Results: This strategy successfully detected the insertion of an *Alu* element in *MLH1* coding sequence in one family. Two previously unreported *MLH1* variants were identified in other epimutation carriers: a nucleotide substitution within intron 1 and a single-nucleotide deletion in the 5'-UTR. A partial duplication of *MLH1* gene was detected in another family. We demonstrated the segregation of these variants with *MLH1* methylation and studied the functional consequences of these defects on transcription.

Conclusions: This study represents the largest cohort of patients with *MLH1* secondary epimutations associated with a broad spectrum of genetic defects. It provides further insight into the complexity of molecular mechanisms leading to heritable epimutations. This work was supported by Lille University Hospital *(Fonds hospitalier d'aide à l'émergence et la structuration des activités et équipes de recherche).*

J. Leclerc: None. **C. Flament:** None. **T. Lovecchio:** None. **L. Delattre:** None. **E. Ait Yahya:** None. **S. Baert-Desurmont:** None. **N. Burnichon:** None. **M. Bronner:** None. **O. Cabaret:** None. **S. Lejeune:** None. **R. Guimbaud:** None. **G. Morin:** None. **J. Mauillon:** None. **P. Jonveaux:** None. **P. Laurent-Puig:** None. **T. Frébourg:** None. **N. Porchet:** None. **M. Buisine:** None.

C14.3

Oxidative modification of cell-free DNA fragments promotes their penetration into stem and cancer cells and activates adaptive response

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Introduction: In pathologies such as cancer and under damaging conditions (e.x. radiation) fraction of cells undergoes cell death and releases DNA that becomes part of the circulating cell-free DNA (cfDNA) pool. CfDNA can be oxidized under oxidative stress. We have shown that in case of cancer the level of oxidation in cfDNA increases.

Materials and Methods: Model genetic construction was obtained by insertion of poly-G DNA fragments in a vector with GFP gene and subsequent oxidation with UV-radiation or H_2O_2. Penetration was confirmed with fluorescent microscopy (comparison with penetration using TurboFect (Thermo, Germany)). *mRNA* was isolated from MSCs and MCF-7 using RNeasy Mini kit (Qiagen, Germany). Gene expression level was assessed using RT-PCR (TBP as internal standard). Protein levels were measured with fluorescent antibodies using flow cytometry. Statistics was performed using Statgraphics software.

Results: Oxidation of cfDNA transforms cfDNA fragments into biologically active molecules that regulate level of expression of number of genes. This was shown on a model genetic construction containing poly-G insertion that is prone to oxidation. Plasmids with oxidized poly-G insertions penetrate MCF-7 and MSCs and cause rapidly-repaired DNA breaks. In concentration 10 - 200 ng/ml oxidized plasmids activate DNA reparation processes and induce antioxidant signaling pathways regulated by NRF2, thus, inhibiting apoptosis and causing adaptive response. In concentrations >350 ng/ml oxidized plasmids cause apoptosis.

Conclusions: Oxidation of cfDNA plays an important role in its penetration into cancer and stem cells and in adaptive response development. The study was supported by RFBR grants № 16-04-01099A, № 16-04-00576A.

V. Sergeeva: None. **E. Malinovskaya:** None. **V. Veiko:** None. **E. Ershova:** None. **L. Kameneva:** None. **M. Konkova:** None. **N. Veiko:** None. **A. Kalyanov:** None. **M. Abramova:** None. **E. Savinova:** None. **S. Kostyuk:** None.

C14.4

Accurate functional classification of thousands of *BRCA1* variants with saturation genome editing

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Introduction: Variants of uncertain significance (VUS) limit the utility of genetic information. The challenge of VUS is epitomized by *BRCA1*, a tumor suppressor gene integral to DNA repair and genomic stability. Germline *BRCA1* loss-of-function variants predispose women to early-onset breast and ovarian cancers. Although *BRCA1* has been sequenced in millions of people, the risk associated with most newly observed variants cannot be definitively assigned. To date, functional studies of *BRCA1* VUS have been limited in scope and performed outside of the gene's endogenous context.

Methods: We employ saturation genome editing to assay 96.5% of all possible single nucleotide variants (SNVs) in 13 exons that encode critical domains of BRCA1. Our multiplex, CRISPR/Cas9-based assay leverages targeted deep sequencing to measure variants' effects on cellular fitness and mRNA expression in human cells dependent on *BRCA1* function.

Results: We obtain function scores for 3,893 SNVs. Scores are bimodally distributed and nearly perfectly concordant with established assessments of pathogenicity (sensitivity = 97%; specificity = 98%). Of 256 VUS, 25.0% score as non-functional, as do 15.9% of 3,140 SNVs currently unobserved clinically. RNA measurements delineate mechanisms by which SNVs result in loss of *BRCA1* function. Hundreds of missense SNVs detrimental to protein function are identified, as well as dozens of exonic and intronic SNVs that compromise *BRCA1* function by disrupting splicing.

Conclusions: We predict this data will be directly useful for the clinical interpretation of cancer risk based on *BRCA1* sequencing. Furthermore, we propose that this paradigm can be extended to many additional clinically-actionable genes.

G.M. Findlay: None. **R. Daza:** None. **B.K. Martin:** None. **M.D. Zhang:** None. **A.P. Leith:** None. **M. Gasperini:** None. **J.D. Janizek:** None. **L.M. Starita:** None. **J. Shendure:** None.

C14.5
A whole-exome case-control study of soft-tissue sarcoma

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To evaluate the contribution of rare genetic variation to the development of soft-tissue sarcoma, we conducted a whole-exome case-control study in 219 cases and 3,507 controls of European ancestry. We controlled for technological stratification biases using XPAT and conducted gene-based association tests using VAAST 2.1. Our cases consisted of samples from TCGA with one of six soft-tissue sarcoma subtypes: leiomyosarcoma (LMS), dedifferentiated liposarcoma (DLPS), myxofibrosarcoma (MFS), undifferentiated pleiomorphic sarcoma (UPS), malignant peripheral nerve sheath tumors (MPNST), and synovial sarcoma (SS). We observed nominally significant association signals for three well-established sarcoma susceptibility genes, *NF1* ($p = 1 \times 10^{-5}$), *TP53* ($p = 0.0025$), and *RB1* ($p = 0.028$). *MSH2*, an HNPCC gene, was also nominally significant ($p = 0.0085$); previous studies have suggested that sarcomas may be within the spectrum of HNPCC tumors. In general, the effect size estimates for variants in these genes were large relative to other cancer types. For likely-gene disrupting variants, the ORs were 39.4 (95% CI: 7.1 – 219.3) and 33.0 (95% CI: 2.4 – 462.5) for *NF1* and *MSH2*, respectively. For predicted damaging missense variants, the ORs were 12.0 (95% CI: 2.4 – 59.1) and ∞ (95% CI: 6.7 – ∞) for *RB1* and *TP53*, respectively. In subtype-specific analyses, all four genes were significantly associated with LMS, the subtype with the largest sample size. Three of the four genes were significantly associated with at least one additional subtype. Our results indicate that rare coding variants can confer substantial increases in risk for multiple soft-tissue sarcoma subtypes.

F. Hu: None. **Y. Yu:** None. **J. Chen:** None. **P. Scheet:** None. **C.D. Huff:** F. Consultant/Advisory Board; Modest; Living DNA.

C14.6
Rare variants in the Aicardi-Goutières syndrome genes *ADAR* and *RNASEH2B* and a type I interferon signature in glioma and prostate carcinoma risk and tumorigenesis

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In search of novel germline alterations predisposing to tumors, in particular to gliomas, we studied a family with two brothers affected by anaplastic gliomas, and their father and paternal great-uncle diagnosed with prostate carcinoma. In this family, whole-exome sequencing yielded rare, simultaneously heterozygous variants in the Aicardi-Goutières syndrome (AGS) genes *ADAR* and *RNASEH2B* co-segregating with the tumor phenotype. AGS is a genetically induced inflammatory disease particularly of the brain, which has not been associated with a consistently increased cancer risk to date. By targeted sequencing, we identified novel *ADAR* and *RNASEH2B* variants, and a 3- to 17-fold frequency increase of the AGS mutations *ADAR*,c.577C>G;p.(P193A) and *RNASEH2B*, c.529G>A;p.(A177T) in the germline of familial glioma patients as well as in test and validation cohorts of glioblastomas and prostate carcinomas versus ethnicity-matched controls, whereby rare *RNASEH2B* variants were significantly more frequent in familial glioma patients. Tumors with *ADAR* or *RNASEH2B* variants recapitulated features of AGS, such as calcification and increased type I interferon expression.

Patients carrying *ADAR* or *RNASEH2B* variants showed upregulation of interferon-stimulated gene (ISG) transcripts in peripheral blood as seen in AGS. An increased ISG expression was also induced by *ADAR* and *RNASEH2B* variants in tumor cells and was blocked by the JAK inhibitor Ruxolitinib. Our data implicate rare variants in the AGS genes *ADAR* and *RNASEH2B* and a type I interferon signature in glioma and prostate carcinoma risk and tumorigenesis, consistent with a genetic basis underlying inflammation-driven malignant transformation in glioma and prostate carcinoma development.

F. Brand: None. **U. Beyer:** None. **H. Martens:** None. **J. Weder:** None. **A. Christians:** None. **N. Elyan:** None. **B. Hentschel:** None. **M. Westphal:** None. **G. Schackert:** None. **T. Pietsch:** None. **B. Hong:** None. **J.K. Krauss:** D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; St. Jude Medical/AbbVie. F. Consultant/Advisory Board; Modest; Medtronic, Boston Scientific. **A. Samii:** None. **P. Raab:** None. **A. Das:** None. **C.A. Dumitru:** None. **I.E. Sandalcioglu:** None. **O.W. Hakenberg:** None. **A. Erbersdobler:** None. **U. Lehmann:** None. **G. Reifenberger:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Roche, Merck (EMD, Darmstadt). F. Consultant/Advisory Board; Modest; Amgen, Celldex, Medac. **M. Weller:** None. **M.A.M. Reijns:** None. **M. Preller:** None. **B. Wiese:** None. **C. Hartmann:** None. **R.G. Weber:** None.

C15 Syndrome updates 2

C15.1
The ARID1B spectrum: From non-syndromic intellectual disability to Coffin-Siris syndrome

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ARID1B mutations are one of the most frequent causes of ID as determined by large-scale whole exome sequencing studies. Most published clinical data however is obtained from clinically diagnosed Coffin-Siris patients (ARID1B-CSS) and it is unclear whether this data is representative for patients identified through unbiased sequencing techniques (ARID1B-ID). Therefore we sought to determine genotypic and phenotypic differences between ARID1B-ID and ARID1B-CSS.

We asked clinicians to add their patient's information to a web-based survey based on previously reported ARID1B features (www.arid1bgene.com). In total, 144 patients (80 ARID1B-CSS and 64 ARID1B-ID patients) were submitted. All patients had truncating mutations which were distributed throughout the gene. Remarkably, no pathogenic mutations were identified in the small in-frame exon 3 and the first 850 bases of exon 1. All but one patient were reported to have intellectual disability (ID). ARID1B-CSS patients displayed more CSS-associated dysmorphic features ($p < 0.001$) than ARID1B-ID patients. ARID1B-ID patients were more inclined to have myopia (39.5% vs 18.3%, $p = 0.024$) and cryptorchidism (67.6% vs 41.4%, $p = 0.005$). Prevalences of motor (100%) and speech (93.8%) delay, feeding difficulties (69.2%), seizures (29.7%) and agenesis of corpus callosum (41.2%) were comparable in both groups.

Based on our data of 144 patients we conclude that except for dysmorphic features there do not seem to be major differences between ARID1B-ID and ARID1B-CSS patients. There therefore appears to be an ARID1B-spectrum, and patients should be managed similarly regardless of clinical diagnosis.

E.P.J. van der Sluijs: None. **J. Clayton-Smith:** None. **G.W.E. Santen:** None.

C15.2
Novel gene and pathomechanism in Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS) is a rare multisystem developmental disorder caused by mutations in different subunits (*SMC1A*, *SMC3*, *RAD21*) or regulators (*NIPBL*, *HDAC8*) of the cohesin complex. *NIPBL* variants account for

>70% of patients and result in *NIPBL* haploinsufficiency. NIPBL interacts via its N-terminus with MAU2, forming the kollerin complex, responsible for cohesin's loading onto chromatin. By CRISPR/Cas9, we generated cell lines expressing an N-terminal truncated form of NIPBL deficient for complex formation with MAU2. Molecular characterization revealed reduced NIPBL protein levels and loss of MAU2. However, ChIP-sequencing analyses show similar chromatin-bound cohesin and NIPBL in wild type and mutant cells indicating unaffected cohesin loading mechanisms. Interestingly, by genome sequencing of CdLS patients, we identified the first *de novo* variant in *MAU2*. This mutation causes an in-frame deletion of seven amino acids affecting an α-helix important for wrapping the N-terminus of NIPBL within MAU2. Accordingly, in vitro analyses revealed a dramatic reduction of MAU2 heterodimerization with NIPBL. Notably, MAU2 is required for the correct folding of the N-terminus of NIPBL, which becomes unstable in the absence of MAU2. Therefore, it might be possible that the inability of MAU2 to interact with NIPBL results in decreased NIPBL protein levels, confirming NIPBL haploinsufficiency as the major pathogenic mechanism of CdLS. In summary, we described the first *MAU2* mutation in a patient with characteristic CdLS phenotype. Our functional investigations reveal a new pathogenic mechanism relevant for CdLS resulting in decreased NIPBL levels upon functional alteration of its binding partner MAU2.

I. Parenti: None. **S. Ruiz Gil:** None. **J. Pié:** None. **T.M. Strom:** None. **R. Brouwer:** None. **F. Diab:** None. **V. Dupé:** None. **G. Gillessen-Kaesbach:** None. **E. Mulugeta:** None. **W. van I.Jcken:** None. **F. Ramos:** None. **E. Watrin:** None. **K.S. Wendt:** None. **F.J. Kaiser:** None.

C15.3
New models for human diseases from the International Mouse Phenotyping Consortium

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Introduction: The International Mouse Phenotyping Consortium (IMPC) is conducting genome and phenome-wide phenotyping on knockout-mouse strains. Following a viability screen, homozygous-viable mice enter the adult pipeline, whereas for homozygous-lethal strains, heterozygous animals are phenotyped. The latest data release in December 2017 comprises 4,364 genes, which adds ~1,000 genes to those previously published, and identifies new models for human diseases.

Methods: Relevant disease models were identified by computing the similarity between the phenotypes in the mice and the human phenotypes associated to their orthologous loci in Mendelian disease. We also compared the ability of homozygous and heterozygous knockouts to phenocopy human disease, stratifying by mode of inheritance.

Results: 281 new knockouts in rare disease genes have been analysed since August 2016. Approximately 40% of these partially mimic the clinical phenotypes. Homozygous mutants showed higher similarity scores. However, when divided according to mode of inheritance, these differences only remained significant for autosomal recessive genes, which also had better concordance with mouse zygosity. Diseases with no previous mouse models described in the literature by the Mouse Genome Informatics (MGI) group (~10% of new strains) include Spermatogenic Failure-6, an infertility condition caused by mutations in *SPATA16*, now observed in homozygous mice, and Dilated Cardiomyopathy-1BB, associated with *DSG2* and mice displaying increased heart weight, left ventricular dilation, and reduced cardiac contractility.

Conclusions: Novel mouse models covering diverse biological systems continue to be incorporated to the IMPC catalogue. Analysis of their phenotypes is enhancing the range of available models that are relevant for human diseases.

P. Cacheiro: None. **T. Konopka:** None. **D. Smedley:** None. **A. Mallon:** None. **T. Meehan:** None. **H. Parkinson:** None.

C15.4
Thrombocytopenia Microcephaly Syndrome - a novel phenotype associated with ACTB mutations

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Until recently missense germ-line mutations in ACTB, encoding the ubiquitously expressed β-cytoplasmic actin (CYA), were exclusively associated with Baraitser-Winter Cerebrofrontofacial syndrome (BWCFF), a complex developmental disorder. Here, we report six patients with previously undescribed heterozygous variants clustered in the 3'-coding region of ACTB. These patients present with clinical features different from BWCFF, including thrombocytopenia, microcephaly, and mild developmental disability. Patient derived cells are morphologically and functionally distinct from controls. Assessment of cytoskeletal constituents identified a discrete filament population altered in these cells, which comprises force generating and transmitting actin binding proteins (ABP) known to be associated with thrombocytopenia. In silico modelling and molecular dynamics (MD)-simulations support altered interactions between these ABP and mutant β-CYA. Our results describe a new clinical syndrome associated with ACTB mutations with a distinct genotype-phenotype correlation, identify a cytoskeletal protein interaction network crucial for thrombopoiesis, and provide support for the hypomorphic nature of these actinopathy mutations.

S.L. Latham: None. **N. Ehmke:** None. **P.Y.A. Reinke:** None. **M.H. Taft:** None. **M.J. Lyons:** None. **M.J. Friez:** None. **J.A. Lee:** None. **R. Hecker:** None. **M.C. Frühwald:** None. **K. Becker:** None. **T.M. Neuhann:** None. **E. Schrock:** None. **K. Sarnow:** None. **K. Grützmann:** None. **L. Gawehn:** None. **B. Klink:** None. **A. Rump:** None. **C. Chaponnier:** None. **R. Knöfler:** None. **D. Manstein:** None. **N. Di Donato:** None.

C15.5

The Genomic Autopsy Study: using genomics as an adjunct to standard autopsy to unlock the cause of complex fetal and neonatal presentations

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Background: The cause of pregnancy loss and perinatal death remains unexplained in ~25% of cases, despite a high perinatal autopsy rate in Australia. The most common factor contributing to perinatal death is congenital abnormalities. Aim: To use WES and WGS to identify genetic causes of fetal/newborn abnormalities that result in termination of pregnancy, death in utero or in the newborn period, in view to providing families with answers regarding cause and likelihood of recurrence.

Methods: WES and/or WGS is being performed using Illumina sequencing systems, if microarray has not identified a cause. Prospective cases are families referred to the Genetics unit (parent-fetus trios) and retrospective cases are from stored autopsy samples (singletons). High priority cases are consanguineous families, fetuses with multiple malformations, and unexplained fetal/newborn death. Statistical, bioinformatic and experimental laboratory techniques are used to confirm causality of variants.

Results: 43 prospective trios and 60 retrospective singletons have been recruited and sequenced. Of the prospective cohort, 23% of cases have been solved and an additional 26% have a single promising candidate being investigated. In the retrospective cohort, 18% of cases have strong candidates identified. Solved cases include new

disease gene discoveries, new syndrome identification and novel severe fetal presentations of existing rare pediatric disease. The study has contributed directly to the birth of 3 healthy newborns.

Discussion: Our results provide insights into molecular mechanisms of early development and indicate that a genomic autopsy using WES/WGS should be a routine component of the investigation of pregnancy loss and perinatal death.

C.P. Barnett: None. **A.B. Byrne:** None. **P. Arts:** None. **J. Feng:** None. **P.S. Wang:** None. **A. Schrieber:** None. **P. Brautigan:** None. **M. Babic:** None. **W. Waters:** None. **L. Pais:** None. **S. Yu:** None. **J. Lipsett:** None. **L. Moore:** None. **N. Manton:** None. **Y. Khong:** None. **E. Ludding-ton:** None. **E. Thompson:** None. **J. Liebelt:** None. **L. McGregor:** None. **M. Dinger:** None. **D.G. MacArthur:** None. **S. King-Smith:** None. **C. Hahn:** None. **K. Kassahn:** None. **H. Scott:** None.

C15.6
Functional Dysregulation of CDC42 Causes Diverse Developmental Phenotypes

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Exome sequencing has markedly enhanced the discovery of genes implicated in Mendelian disorders, particularly for individuals in whom a known clinical entity could not be assigned. This has led to the recognition that phenotypic heterogeneity resulting from allelic mutations occurs more commonly than previously appreciated. Here, we report that missense variants in CDC42, a gene encoding a small GTPase functioning as an intracellular signaling node, underlie a clinically heterogeneous group of phenotypes characterized by variable growth dysregulation, facial dysmorphism, and neurodevelopmental, immunological, and hematological anomalies, including a phenotype resembling Noonan syndrome, a developmental disorder caused by dysregulated RAS signaling. In silico, in vitro, and in vivo analyses demonstrate that mutations variably perturb CDC42 function by altering the switch between the active and inactive states of the GTPase and/or affecting CDC42 interaction with effectors, and differentially disturb cellular and developmental processes. These findings reveal the remarkably variable impact that dominantly acting CDC42 mutations have on cell function and development, creating challenges in syndrome definition, and exemplify the importance of functional profiling for syndrome recognition and delineation. E-Rare (NSEuroNet), National Institute of Neurological Disorders and Stroke (NINDS)

S. Martinelli: None. **O. Krumbach:** None. **F. Panta-leoni:** None. **S. Coppola:** None. **E. Amin:** None. **L. Pannone:** None. **K. Nouri:** None. **L. Farina:** None. **R. Dvorsky:** None. **F. Lepri:** None. **M. Buchholzer:** None. **R. Konopatzki:** None. **L. Walsh:** None. **K. Payne:** None. **M.E. Pierpont:** None. **S. Vergano:** None. **K. Langley:** None. **D. Larsen:** None. **K. Farwell:** None. **S. Tang:** None. **C. Mroske:** None. **I. Gallotta:** None. **E. Di Schiavi:** None. **M. della Monica:** None. **L. Lugli:** None. **C. Rossi:** None. **M. Seri:** None. **G. Cocchi:** None. **L. Henderson:** None. **B. Baskin:** None. **M. Alders:** None. **R. Mendoza-Londono:** None. **L. Dupuis:** None. **D. Nickerson:** None. **J. Chong:** None. **N. Meeks:** None. **K. Brown:** None. **T. Causey:** None. **M. Cho:** None. **S. Demuth:** None. **M. Digilio:** None. **B. Gelb:** None. **M. Bamshad:** None. **M. Zenker:** None. **M. Ahmadian:** None. **R. Hennekam:** None. **M. Tartaglia:** None. **G. Mirzaa:** None.

C16 Multi-omics 2

C16.1
High throughput characterization of genetic effects on DNA:protein binding and gene transcription

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The majority of the human genome is composed of non-coding regions containing regulatory elements, which control gene expression. Many variants associated with complex traits are in these regions, and contribute to an individual's phenotype by disrupting gene regulatory sequences. Consequently, it is important to not only identify functional regulatory elements, but also to test if a variant within a binding site affects gene regulation. We developed a new streamlined protocol for high-throughput reporter assay, BiT-STARR-seq (Biallelic targeted STARR-seq), to identify allele-specific expression (ASE) while directly accounting for PCR duplicates through unique molecular identifiers incorporation. We tested 75,501 oligos (43,500 SNPs) and identified 2,720 SNPs with significant ASE (FDR 10%). To validate disruption of binding as one of the mechanisms underlying ASE, we performed high throughput EMSA (BUNDLE-seq) for NFKB-p50. We tested the same oligo library used in BiT-STARR-seq and identified 2,951 SNPs with significant allele-specific binding (ASB) (FDR 10%). Of the SNPs with ASB, 173 also had ASE (OR = 1.97, p-value = 0.0006). When we focused on variants associated with complex traits, we identified 1,531 SNPs with ASE in the BiT-STARR-seq and 1,662 SNPs with ASB in the BUNDLE-seq assay. We characterized the mechanism whereby the alternate allele for variant rs3810936 increases risk for Crohn's disease through increased NFKB binding and consequent altered gene expression. In conclusion, combining ASB and ASE in high-throughput assays is a new powerful approach to validate the molecular mechanism for regulatory variants associated with disease traits.

C. Kalita: None. **C. Brown:** None. **A. Freiman:** None. **X. Wen:** None. **R. Pique-Regi:** None. **F. Luca:** None.

C16.2
A pedigree-based estimate of the human germline retrotransposition rate

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Introduction: Mobile elements compose at least half of the human genome, and some mobile element families are still actively inserting in the genome. However, the rate of mobile element retrotransposition has been inferred only indirectly from phylogenetic comparisons. We report the first direct estimate of the retrotransposition rate in a large series of 3-generation human pedigrees.

Materials and Methods: We performed whole genome sequencing at 30x average coverage on blood-derived DNA from 603 CEPH individuals, comprising 34 multi-generation pedigrees. The families were joint-called using Mobile Element Locator Tool (MELT) and RUFUS to identify de novo Alu, LINE1, and SVA elements. All candidate de novo mobile element insertions (MEI) were validated using PCR and Sanger sequencing, and flanking SNPs were typed to determine parent of origin.

Results: The false-negative and false-positive rates of MEI detection were 5% and 15%, respectively. The retrotransposition rate estimates for Alu elements, one in 50, is roughly half the rate estimated using phylogenetic analyses, a difference similar to that observed for single nucleotide variants. The SVA and LINE1 retrotransposition rates are both approximately one in 100-150 births, which is higher than phylogenetic estimates. De novo MEIs were transmitted at a 3-fold higher rate among fathers than mothers, with no apparent age bias.

Conclusions: The directly estimated Alu retrotransposition rate is roughly half of previous phylogenetically based estimates, whereas LINE1 and SVA rates are higher than previously estimated. Future studies will investigate whether a paternal bias is a consistent hallmark of de novo retrotransposition.

Support: NIH GM-59290

J.E. Feusier: None. **W. Watkins:** None. **J. Thomas:** None. **L. Baird:** None. **M. Leppert:** None. **L.B. Jorde:** None.

C16.3
Multivariate analysis of immune phenotypes reveals novel genetic and context specific genetic factors for cytokine production capacity

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Immune responses in healthy individuals show a remarkable inter-individual variation. Previous studies have identified that genetic and non- genetic host factors play an important role in this variation of the immune response. However, a large portion of the variation remains unexplained due to limited power of these studies and to the fact that interactions between genetics and environment are largely ignored. We aim to identify both the genetic and the environment-dependent genetic effects that are shared by multiple immune functions in a large population cohort.

After the measurement of cytokines (protein level) in response to pathogen stimulations in 500 healthy individuals, we firstly grouped cytokines based on biological relevance in monocytes (IL-β, IL-6 and TNF-α) or T cells (IFN-γ, IL17 and IL22) and the pathogen used in stimulation, respectively. Subsequently, we applied a multi-trait modeling strategy, where the biological relevant groups were evaluated at genome-wide level. A total of 12 genome-wide significant loci were detected, of which 6 replicate the previous single-trait mapping and 6 are novel. The 12 loci are enriched for genes implicated in immune response pathways and are co-localized with genetic risk factors associated to immune-mediated diseases like inflammatory bowel disease and rheumatoid arthritis. In addition, several loci were identified to regulate cytokine production differentially between gender and smoking status.

Our results show that integration of multiple immune phenotypes can reveal novel genomic associations and context-specific cytokine QTLs, highlighting the shared genetic basis of cytokine production upon stimulation that contribute to inter-individual diversity of the immune function.

R.A. Aguirre-Gamboa: None. **O. Bakker:** None. **T. Spenkelink:** None. **U. Võsa:** None. **M. Jaeger:** None. **M. Oosting:** None. **S. Smeekens:** None. **R. Netea-Maier:** None. **R. Xavier:** None. **I. Jonkers:** None. **L. Franke:** None. **L.A.B. Joosten:** None. **S. Sanna:** None. **V. Kumar:** None. **C. Wijmenga:** None. **M. Netea:** None. **Y. Li:** None.

C16.4
Time informative markers to date ancient Skeletons

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During the last years, we have witnessed a conspicuous upraise in the sequencing of ancient DNA data, with the proliferation of studies attempting to shed light on human origins and migration routes. Dating the ancient DNA is, therefore, of key importance. However, although radiocarbon dating is a well-established practice, its outcome can be altered by numerous factors, leading to inaccuracies with carbon dates older than 25,000 BP considered highly unreliable. Here, we introduce a DNA admixture-based dating method, which utilizes allele frequencies that vary over time to create dating components. We developed synthetic DNA typical to specific time periods (e.g., 10,500-9,000 BP) and then calculated for each ancient genome the time profile, which consisted of the frequencies of those DNA molecules. Using a dataset of ~300 ancient Eurasians with reliable radiocarbon date ranging from 15,000 BP to 1,500 BP, we demonstrated that our predicted dates are perfectly correlated with radiocarbon dates ($r^2 = 0.9$). The average difference between our predictions and the average radiocarbon dates was 800 years. Our results confirm the usefulness of using time-dependent allelic frequency for biodating and contribute to the understanding of the complexity of migration and admixture events. Grant Info: GH was partially supported by the UK EPSRC Doctoral Training Partnership Grant EP/N509735/1 as a Vacation Bursary Training Project.

U. Esposito: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; DNA Diagnostic Centre. **G. Holland:** None. **E. Elhaik:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; DNA Diagnostic Centre.

C16.5
A homozygous loss-of-function mutation in *C17orf62* causes chronic granulomatous disease

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Introduction: Chronic granulomatous disease (CGD) is a rare primary immunodeficiency that results from defects in the phagocytic NADPH (the reduced nicotinamide adenine dinucleotide phosphate) oxidase complex.

Materials and Methods: We have whole-genome sequenced 37K Icelanders and genotyped 155K, a large fraction of the Icelandic population (11% and 46% of 338K, respectively). As part of this effort, we are performing genetic analysis of rare diseases in the clinical setting. We sequenced the genomes of two Icelandic brothers diagnosed with CGD in an attempt at finding the causal mutation.

Results: We identified a homozygous loss-of-function mutation, p.Tyr2Ter, in *C17orf62* in both brothers, and found six additional homozygous individuals through imputation of the mutation into 155K chip-genotyped Icelanders. Genotype-based recall, and review of hospital-based information, revealed that all eight homozygotes have signs of CGD, manifesting as colitis, severe and recurrent infections, or an impaired neutrophil oxidative burst. Mice deficient in the homolog of *C17orf62* are highly susceptible to infections. Homozygosity for p.Tyr2Ter associates with inflammatory bowel disease ($P = 8.3 \times 10^{-8}$; OR (95% CI) = 67.6 (14.5, 315.5)), and growth retardation ($P = 3.3 \times 10^{-4}$; Effect (95% CI) = -1.24 SD (-1.92, -0.56) / -8.5 cm). We show that homozygosity for p.Tyr2Ter causes complete loss of the C17orf62 protein and a drastic reduction in one of NADPH's subunits, indicating a chaperone role for C17orf62.

Conclusions: By leveraging a large population-specific dataset we have elucidated the role of a previously uncharacterized gene in humans, *C17orf62*, and identified a novel cause of CGD.

G.A. Arnadottir: A. Employment (full or part-time); Significant; deCODE genetics/Amgen Inc. **G.L. Norddahl:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **S. Gudmundsdottir:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **A.B. Agustsdottir:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **S. Sigurdsson:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **B. O. Jensson:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **K. Bjarnadottir:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **F. Theodors:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **S. Benonisdottir:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **E.V. Ivarsdottir:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **A. Oddsson:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **R.P. Kristjansson:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **G. Sulem:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **G. Masson:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **K. B. Orvar:** None. **H. Holm:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **S. Bjornsson:** None. **R. Arngrimsson:** None. **D.F. Gudbjartsson:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **U. Thorsteinsdottir:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **I. Jonsdottir:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **A. Haraldsson:** None. **P. Sulem:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen. **K. Stefansson:** A. Employment (full or part-time); Significant; deCODE genetics / Amgen.

C16.6

The neurodevelopmental 16p11.2 CNVs have, as yet overlooked, mirror effect on sexual development in humans and animal models

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We uncovered that the 16p11.2 BP4-BP5 dosage, one of the most frequent genetic causes of mental disorders, was associated with age at menarche (AAM) in 60,498 UKBB females. Compared to controls AAM was decreased in deletion ($\Delta = -1.5$ years, $p = 0.01$) and increased in duplication carriers ($\Delta = +1.5$; $p = 7.8 \times 10^{-5}$; corrected for BMI and birthyear). We replicated these associations in 129,991 UKBB women, 22,212 EGCUT females and 67 16p11.2 clinical patients. A directionally consistent trend was observed in 16p11.2 UKBB and patient males. These features were accompanied by reproductive tract/fertility disorders, e.g. miscarriages (OR = 2.65; $p = 0.019$), oligo-/amenorrhea (OR = 10.2; $p = 0.01$), cryptorchidism/hypospadias. We validated these results in 16p11.2 mice models by detecting significant changes in timing of first ovulation ($p < 0.01$), estrous cyclicity ($p = 0.03$) and uterine size ($p = 8.1 \times 10^{-3}$). Corroboratively, genes differentially-expressed in 16p11.2 patients and models were enriched for urogenital disease genes.

We found a significant link between the human 16p11.2 dosage, AAM and volume of hypothalamus ($p_{FWE} < 0.05$), a structure associated with sexual development, suggesting that perturbation of the GnRH axis could be responsible for the observed phenotypes. Using Mendelian Randomization we prioritized three potential causal genes for AAM. We challenged them by modulating dosage of all 16p11.2 genes in *gnrh3:egfp* transgenic zebrafish larvae and quantified GnRH neuronal patterning. We confirmed a putative causal effect of *INO80E* and *GDPD3* in the 16p11.2 reproductive axis.

These findings highlight that identification of traits associated with neurodevelopmental CNVs in the general population provides valuable and unbiased insight into disease etiologies in terms of affected genes and pathways, pleiotropy and comorbidities.

K. Mannik: None. **M. Lepamets:** None. **A. Mikhaleva:** None. **K. Lepik:** None. **Z. Kupchinsky:** None. **H. Ademi:** None. **T. Arbogast:** None. **A. Messina:** None. **S. Rotman:** None. **E. Dubruc:** None. **J. Chrast:** None. **S. Martin-Brevet:** None. **T. Laisk-Podar:** None. **Y. Herault:** None. **C.M. Lindgren:** None. **Z. Kutalik:** None. **J.C. Stehle:** None. **N. Katsanis:** None. **S. Nef:** None. **B. Draganski:** None. **E.E. Davis:** None. **R. Magi:** None. **A. Reymond:** None.

C17 Intellectual disability 1

C17.1

De novo mutations in protein kinase genes *CAMK2A* and *CAMK2B* cause intellectual disability

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The alpha- and beta-isoforms of calcium/calmodulin-dependent serine/threonine protein kinase II (CAMK2) play a pivotal role in neuronal function. Although CAMK2 was one of the first proteins shown to be essential for normal learning and synaptic plasticity in mice, its requirement for human brain development has not yet been established. Through a multi-center collaborative study based on a

whole-exome sequencing approach, we identified 20 exceedingly rare *de novo CAMK2A or CAMK2B* variants in 27 unrelated individuals with neurodevelopmental impairment. The individuals all present with intellectual disability, language and speech delay, and virtually all of them show behavioral anomalies. The variants are either in the kinase domain or in the autoregulatory domain, suggesting they may change the kinetic function of the enzyme. Variants were assessed for their effect on CAMK2 function on neuronal migration in the developing nervous of mouse embryos transfected with cDNA constructs by *in utero* electroporation. For both *CAMK2A* and *CAMK2B*, we identified pathogenic variants that decreased or increased CAMK2 auto-phosphorylation at Thr286/Thr287. We further found that all variants affecting auto-phosphorylation also affected neuronal migration, highlighting the importance of tightly regulated CAMK2 auto-phosphorylation in neuronal function and neurodevelopment. Our data establish the importance of *CAMK2A* and *CAMK2B* and their auto-phosphorylation in human brain function, and expand the phenotypic spectrum of the disorders caused by variants in key players of the glutamatergic signaling pathway.

S. Küry: None. **G.M. van Woerden:** None. **T. Besnard:** None. **X. Latypova:** None. **M.T. Cho:** A. Employment (full or part-time); Significant; GeneDx. **S. Sanders:** None. **H.A.F. Stessman:** None. **E.A. Sellars:** None. **J. Berg:** None. **J.L. Waugh:** None. **L.A. Robak:** None. **J.A. Bernstein:** None. **M. Deardorff:** None. **G.E. Hoganson:** None. **D.S. Johnson:** None. **T. Dabir:** None. **A. Sarkar:** None. **G. Lesca:** None. **P.A. Terhal:** None. **T.E. Prescott:** None. **D.K. Grange:** None. **A. van Haeringen:** None. **C. Lam:** None. **G. Mirzaa:** None. **K.L. Helbig:** None. **A. Afenjar:** None. **C. Nava:** None. **A. Vitobello:** None. **L. Faivre:** None. **B. Cogné:** None. **J.A. Rosenfeld:** None. **P. B. Agrawal:** None. **S. Odent:** None. **S. Bézieau:** None. **Y. Elgersma:** None. **S. Mercier:** None.

C17.2

Rotatin mutations impair bipolar mitotic spindle formation leading to a wide spectrum of brain malformations

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Centrosomes are conserved microtubule-based organelles involved in eukaryotic cell division and ciliogenesis. Multiple malformations of cortical development are associated with abnormal centrosomal protein function. Rotatin (RTTN), localized at centrosome and basal body, is a protein previously linked to a wide variety of brain malformations, affecting neuronal proliferation, migration and organization. The molecular disease mechanism(s) have not yet been elucidated. We have gathered patient material from a cohort of 8 patients with homozygous or compound heterozygous *RTTN* mutations. Quantification of *RTTN* mRNA shows residual transcript in all patients, supporting the hypothesis that complete lack of *RTTN* is embryonically lethal in human, as seen in *RTTN-/-* knockout mice. Immunocytochemistry of RTTN in control fibroblasts and derived neurons shows localization at the centrosome. Furthermore, in the cohort of RTTN patients and *siRTTN* fibroblasts, we show that RTTN deficiency leads to abnormal centrosome amplification during cell division, leading to multipolar mitotic spindles and mitotic failure. This cellular phenotype has also been reported in *Drosophila melanogaster* homologue Ana3 knockout neuroblasts. FACS cell cycle analysis showed a highly significant increase of G1 phase cells and decrease in G2/M-phase cells in RTTN deficient fibroblasts. Proteomics analysis shows that RTTN interacts with the non-muscle cellular myosin complex (MYH9-MYH10-MYH14). This complex has already shown to play a role in centrosome amplification, ciliogenesis and nucleokinesis during neuronal migration. Mutational impairment of the RTTN-Myosin binding could explain the cellular phenotype and cortical malformations in our patients. LV was supported by Steunfonds Marguerite-Marie Delacroix, Research Foundation Flanders (FWO) and COST Action CA16118.

L.V. Vandervore: None. **R. Schot:** None. **E. Kasteleijn:** None. **R. Oegema:** None. **F. Verheijen:** None. **A. Gheldof:** None. **K. Stouffs:** None. **R. Poot:** None. **W.B. Dobyns:** None. **N. Bahi-Buisson:** None. **A.C. Jansen:** None. **G. Mancini:** None.

C17.3

Dual molecular effects of dominant *RORA* mutations cause two variants of syndromic intellectual disability with either autistic features or cerebellar ataxia

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Introduction: Through a multi-center international collaboration, we identified 16 individuals with *de novo* or dominant variants in *RORA*, encoding the retinoic acid receptor (RAR)-related orphan nuclear receptor alpha, by whole exome sequencing or chromosomal microarray. The mutational spectrum included four copy number variations (two *de novo* deletions, one dominant deletion and one *de novo* duplication) and nine *de novo* single nucleotide variants. Intellectual disability (ID) was the predominant clinical feature (15/16), accompanied by seizures (11/16), autistic features (5/16) and cerebellar hypoplasia or atrophy (3/16).

Materials and methods: To investigate the relevance of *RORA* disruption to neurodevelopmental phenotypes in humans, we abrogated the *D. rerio* ortholog, *roraa*, through either transient suppression or CRISPR/Cas9 genome editing.

Results: Disruption of *roraa* causes a significant reduction of cerebellar size in developing larvae. Moreover, wild-type human *RORA* mRNA could rescue cerebellar defects caused by *roraa* morpholino knockdown. Through in vivo complementation and titration of variant mRNA, we observed that missense variants had two distinct pathogenic mechanisms of either haploinsufficiency or dominant toxic effects, according to their localization in the ligand- or DNA-binding domains. Notably, ID and autism were hallmark features in individuals with loss of function variants, whereas ID and cerebellar features were the main phenotypes in individuals with variants harboring a dominant toxic effect.

Conclusions: Our combined genetic and functional data point to dual mutational effects that likely determine phenotypic outcome. In sum, our results show that *RORA* variants lead to a neurodevelopmental disorder

characterized by intellectual disability, seizures, autistic features and cerebellar defects.

X. Latypova: None. **C. Guissart:** None. **T.N. Khan:** None. **P. Rollier:** None. **H. Stamberger:** None. **K. McWalter:** A. Employment (full or part-time); Significant; Gene Dx. **M.T. Cho:** A. Employment (full or part-time); Significant; Gene Dx. **S. Kjaergaard:** None. **S. Weckhuysen:** None. **G. Lesca:** None. **T. Besnard:** None. **K. Õunap:** None. **L. Schema:** None. **A.G. Chiocchetti:** None. **M. McDonald:** None. **J. de Bellescize:** None. **M. Vincent:** None. **H. Van Esch:** None. **S. Sattler:** None. **I. Forghani:** None. **I. Thiffault:** None. **C.M. Freitag:** None. **D. Barbouth:** None. **M. Cadieux-Dion:** None. **N.P. Saffina:** None. **L. Grote:** None. **W. Carré:** None. **C. Saunders:** None. **S. Pajusalu:** None. **A. Boland:** None. **D. Hays Karlowicz:** None. **J. Deleuze:** None. **M.H. Wojcik:** None. **R. Pressman:** None. **B. Isidor:** None. **A. Vogels:** None. **W. Van Paesschen:** None. **F. Rivier:** None. **N. Leboucq:** None. **B. Cogné:** None. **S. Sasorith:** None. **D. Sanlaville:** None. **K. Retterer:** A. Employment (full or part-time); Significant; Gene Dx. **S. Odent:** None. **N. Katsanis:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Rescindo Therapeutics, Inc. F. Consultant/Advisory Board; Significant; Rescindo Therapeutics, Inc. **S. Bézieau:** None. **M. Koenig:** None. **L. Pasquier:** None. **E.E. Davis:** None. **S. Küry:** None.

C17.4

Description of novel intellectual disability genes involved in RNA metabolism

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Monogenic forms of intellectual disability (ID) are characterized by an extreme heterogeneity, with more than 700 genes now implicated. The most frequent cause of monogenic ID - the fragile X-syndrome- is due to the absence of the RNA-binding protein (RBP) FMRP. Many other ID genes involved in RNA metabolism have been recently identified. About 5% (40/721) of the ID-associated genes play a role in the post-transcriptional regulation of gene expression by regulating mRNA splicing, nuclear export, degradation or translation into proteins. We describe here two novel ID genes involved in RNA metabolism.

We identified 4 *de novo* frameshift variants in *NOVA2* in patients with Angelman-like ID. This gene encodes a RBP implicated in alternative splicing of axon-guidance genes. The 4 mutations cluster in a small interval suggesting a dominant negative mechanism. This GC and repeat-rich interval is poorly covered in most exomes, and the gene may have thus been missed in large scale projects, such as DDD. We show that the mutations lead to dysfunctional truncated proteins not able to regulate specific splicing events like the NOVA2 wild-type. The inactivation of *NOVA2* by siRNA in differentiated N2A cells causes an increase of neurons with multiple neurites.

We identified 4 *de novo* missense variants in *DDX6*, encoding a protein implicated in mRNA degradation and regulation of translation initiation. These variants alter the interaction between DDX6 and its known partners and its ability to form P-bodies.

These two novel ID genes highlight the important role of RNA metabolism alterations in neurodevelopmental disorders.

F. Mattioli: None. **B. Isidor:** None. **C. Balak:** None. **M. Benard:** None. **E. Schaefer:** None. **M. Hinckelmann-Rivas:** None. **V. Geoffroy:** None. **J. Muller:** None. **A. Lebechec:** None. **J. Deleuze:** None. **A. Boland:** None. **H. Dollfus:** None. **J. Chelly:** None. **K. Ramsey:** None. **F. Tran-Mau-Them:** None. **S. Nambot:** None. **N. Jean:** None. **A. Telegraphi:** A. Employment (full or part-time); Modest; GeneDx. **A. Boughton:** None. **C. Gamble:** None. **M. Cho:** A. Employment (full or part-time); Modest; GeneDx. **Z. Shad:** None. **E. Kaplan:** None. **R. Dineen:** None. **M. Huentelman:** None. **V. Narayanan:** None. **D. Weil:** None. **J. Mandel:** None. **A. Piton:** None.

C17.5

OTUD7A regulates neurodevelopmental phenotypes in the 15q13.3 microdeletion syndrome

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Nalpathamkalam[5], S. Lamoureux[5], J. Wei[5], M. Speevak[5], J. Stavropoulos[5], K. J. Hope[2], B. W. Doble[2], J. Nielsen[6], R. Wassman[4], S. W. Scherer[5], K. K. Singh[2]

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Introduction: Copy number variations (CNVs) are strong risk factors for neurodevelopmental and psychiatric disorders. The 15q13.3 microdeletion syndrome region contains up to 10 genes and is associated with numerous conditions, including autism spectrum disorder (ASD), epilepsy, schizophrenia and intellectual disability; however, the mechanisms underlying their pathogenesis remain unknown. 15q13.3 microdeletion syndrome region impacts ten genes and it is very complex to unravel the critical gene or genes that regulates the phenotype.

Materials and Methods: We combined whole genome sequencing, human brain gene expression (proteome and transcriptome), and a mouse model with a syntenic heterozygous deletion (Df(h15q13)/+ mice) and determined that the microdeletion results in abnormal development of cortical dendritic spines and dendrite outgrowth. Large scale genetic data was integrated to find out the most critical gene (s) within the 15q13.3 microdeletion syndrome region. In addition, we have applied functional analysis in vivo and in vitro to identify phenotypes in mouse and human cell lines.

Results: Analysis of large scale genomic, transcriptomic and proteomic data identified *OTUD7A* as a critical gene for brain function. *OTUD7A* expression was found to localize to dendritic and spine compartments in cortical neurons, and its reduced expression in Df(h15q13)/+ cortical neurons contributed to the dendritic spine and dendrite outgrowth deficits.

Conclusions: Our results reveal *OTUD7A* as a major regulatory gene for 15q13.3 microdeletion syndrome phenotypes that contribute to the disease mechanism through abnormal cortical neuron morphological development.

U. Mohammed: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Al Jalila FOundation, Lineagen, NeuroGen. **B.K. Unda:** None. **V. Kwan:** None. **N.T. Holzapfel:** None. **S.H. White:** None. **L. Chalil:** None. **M. Woodbury-Smith:** None. **K.H. Ho:** None. **E. Harward:** None. **N. Murtaza:** None. **B. Dave:** None. **G. Pellecchia:** None. **L. D'Abate:** None. **T. Nalpathamkalam:** None. **S. Lamoureux:** None. **J. Wei:** None. **M. Speevak:** None. **J. Stavropoulos:** None. **K.J. Hope:** None. **B.W. Doble:** None. **J. Nielsen:** None. **R. Wassman:** None. **S.W. Scherer:** None. **K.K. Singh:** None.

C17.6

Abnormal Social and Cognitive Behavior is associated with Inherited Noncoding Mutations in Human Accelerated Regions (HARs)

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Comparative analyses have identified genomic regions potentially involved in human evolution, but do not directly assess function. Human accelerated regions (HARs) represent conserved genomic loci with elevated divergence in humans compared to other primate and non-primate species. At least some HARs are thought to contribute to neurodevelopmental functions underlying the unique social and behavioral traits of humans. If some HARs regulate human-specific social and behavioral traits, then mutations would likely impact cognitive and social disorders. Recent studies support the role of HARs in complex neurodevelopmental functions and associated disorders including Autism Spectrum Disorder (ASD) and Schizophrenia. Strikingly, through large-scale whole-genome and targeted HAR-ome sequencing we find, for the first time, that rare noncoding mutations in neurally active HARs contribute to simplex, multiplex, and consanguineous ASD cases. These mutations highlight more than 20 HARs with putative essential roles in neural development. Functional validation of candidate HARs and underlying mutations using chromatin interaction sequencing, high-throughput reporter assays, and transgenic mice allowed for further functional characterization of inherited HAR mutations in active regulatory elements for *RBFOX1*, *CUX1, PTBP2, GPC4, CDKL5*, and other genes implicated in neural function, autism spectrum disorder, or both. Our data expand the previous association of HARs in consanguineous families with ASD to include both simplex and multiplex families, providing strong

genetic evidence that specific HARs are essential for normal development, consistent with suggestions that their evolutionary changes may have altered social and/or cognitive behavior.

R.N. Doan: None. **T. Shin:** None. **B. Bae:** None. **B. Cubelos:** None. **C. Chang:** None. **A.A. Hossain:** None. **S. Al-Saad:** None. **N.M. Mukaddes:** None. **O. Oner:** None. **M. Al-Saffar:** None. **S. Balkhy:** None. **G.G. Gascon:** None. **M. Nieto:** None. **C.A. Walsh:** None.

C18 Cardiovascular disorders

C18.1

A novel murine model for arrhythmogenic cardiomyopathy points to a pathogenic role of Wnt/b-catenin signaling and miRNA dysregulation

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Aims: Among the most common inherited cardiomyopathies, arrhythmogenic cardiomyopathy (AC) is characterized by progressive myocardial fibro-fatty replacement. Clinically, AC manifests with ventricular arrhythmias, syncope, and sudden death. Among the known causative genes, those encoding for the desmosomal proteins plakophilin-2 (PKP2), desmoplakin (DSP), and desmoglein-2 (DSG2) are most commonly mutated. Little is known about the molecular mechanism(s) behind AC wide phenotypic variability, although it seems that the causative mutations lead to both structural and epigenetic alterations . We aimed at studying the pathogenic effects of a nonsense mutation in desmoglein-2, both at the structural and epigenetic level.

Methods and Results: We generated transgenic mice with cardiac-specific overexpression of the FLAG-tagged human desmoglein-2 harboring the Q558* mutation. In Tg-hQ mice, desmosome density and length were significantly decreased and Wnt/β-catenin signalling suppressed. RNA-Seq performed in Tg-hQ hearts and non-transgenic hearts revealed 24 miRNAs deregulated in transgenic animals. Further bioinformatic analyses for selected miRNAs suggested that miR-217-5p, miR-499-5p, and miR-708-5p might be involved in AC pathogenesis.

Conclusions: Downregulation of the Wnt/β-catenin signaling might be considered a common key event in AC pathogenesis. We identified the miRNA signature in AC hearts, with miR-708-5p and miR-217-5p being the most upregulated and miR-499-5p the most downregulated miRNAs. All of them were predicted to be involved in the regulation of the Wnt/β-catenin pathway and might reveal the potential pathophysiology of AC, as well as be useful as therapeutic targets for the disease.

Grants: H2020-MSCA-IF-2014; TRANSAC, University of Padua Strategic Grant CPDA133979/13; Veneto Region Target Research.

M. Calore: None. **A. Lorenzon:** None. **L. Vitiello:** None. **G. Poloni:** None. **G. Beffagna:** None. **E. Dazzo:** None. **R. Polishchuk:** None. **P. Sabatelli:** None. **R. Doliana:** None. **D. Carnevale:** None. **G. Lembo:** None. **P. Bonaldo:** None. **L.J. de Windt:** None. **P. Braghetta:** None. **A. Rampazzo:** None.

C18.2

Large-scale meta-analysis of GWAS in over one million individuals identifies more than 1,000 novel independent variants associated with blood pressure

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Introduction: Elevated blood pressure (BP) is the leading cause of cardiovascular death worldwide. We report genetic association analysis of three BP traits in >1 million people.

Methods: We undertook a combination of a one- and two-stage genome-wide association study (GWAS) analysis to test ~7M single nucleotide polymorphisms (SNPs) with minor allele frequency ≥1% for systolic and diastolic BP and pulse pressure. We combined the UK Biobank (UKB) cohort (N = 458,577) with the International Consortium for Blood Pressure GWAS (N = 299,024), totalling 757,601 individuals of European descent in the discovery. Data from the Millions Veterans Program and the Estonian Biobank was used in the two-stage design as replication samples (N=249,262).

Results: We identified 535 novel loci. We also confirmed all 274 published loci and a further 92 loci that had been previously reported but not replicated. Conditional analysis revealed 163 additional independent secondary variants, hence totalling over 1,000 independent signals at 901 loci. There is substantial gain in the percentage of BP variance explained by more than 2-fold. We also observed ~10mmHg higher systolic BP in UKB for the comparison between the upper and lower quintiles of the genetic risk score distribution. Bioinformatics analyses identified new potential therapeutic targets for hypertension.

Conclusions: This is the largest ever BP GWAS that has tripled the number of BP loci to a total of 901, explaining 27% of the estimated heritability of BP. This study reveals many new insights into BP regulation and drug targeting illustrating the potential for improved cardiovascular disease prevention in the future.

E. Evangelou: None. **H. Warren:** None. **D. Mosen-Ansorena:** None. **B. Mifsud:** None. **R. Pazoki:** None. **H. Gao:** None. **G. Ntritsos:** None. **N. Dimou:** None. **J.N. Hellwege:** None. **A. Giri:** None. **T. Esko:** None. **A. Metspalu:** None. **A.M. Hung:** None. **C.J. O'Donnell:** None. **T.L. Edwards:** None. **I. Tzoulaki:** None. **M. Barnes:** None. **L.V. Wain:** None. **P. Elliott:** None. **M. Caulfield:** None.

C18.3

Whole genome sequencing improves genetic testing outcomes in hypertrophic cardiomyopathy

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Background: Whole genome sequencing (WGS) can detect most types of genetic variants. We assessed WGS for hypertrophic cardiomyopathy (HCM) in which prior cardiomyopathy gene-panel sequencing, or exome sequencing, did not establish a molecular diagnosis, i.e. gene-elusive HCM, and as a first-line genetic test.

Methods: WGS was performed on 46 gene-elusive HCM probands, 16 family members, and 12 HCM probands with no prior genetic testing. We searched for coding-region variants and genomic rearrangements in 184 cardiac hypertrophy genes, deep intronic variants that alter RNA splicing, and mitochondrial genome variants. All splice-altering variants were validated using RNA extracted from fresh venous blood, or cardiomyocytes derived from induced pluripotent stem cells, of the patients.

Results: We found a pathogenic or likely pathogenic variant in 10 out of 46 (22%) gene-elusive HCM families. Four families had deep intronic variants in *MYBPC3* that activate splicing of pseudo-exons; one family had a synonymous *MYBPC3* variant shown to disrupt splicing; one family had mitochondrial genome variant; one family had a nonsynonymous *MYH7* variant that was not genotyped during exome sequencing; and three families had variants in genes not included in prior cardiomyopathy gene-panel testing. As a first-line genetic test, WGS identified a pathogenic variant in 5 out of 12 (42%) families with no prior genetic testing.

Conclusions: WGS identified additional genetic causes of HCM over targeted gene sequencing approaches. Genetic screening of deep intronic regions identified pathogenic variants in 9% of gene-elusive HCM. These findings translate to more accurate diagnosis and management in HCM families.

R.D. Bagnall: None. **J. Ingles:** None. **M.E. Dinger:** None. **M.J. Cowley:** None. **S. Barratt-Ross:** None. **A.E. Minoche:** None. **S. Lal:** None. **C. Turner:** None. **A.**

Colley: None. **S. Rajagopalan:** None. **Y. Berman:** None. **A. Ronan:** None. **D. Fatkin:** None. **C. Semsarian:** None.

C18.4

Germline loss-of-function mutations in EPHB4 cause a second form of capillary malformation-arteriovenous malformation (CM-AVM2) deregulating RAS-MAPK signaling

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Purpose: Most AVMs are localized and occur sporadically; however they also can be multifocal in autosomal dominant disorders, such as Hereditary Hemorrhagic Telangiectasia (HHT) and Capillary Malformation-Arteriovenous Malformation (CM-AVM). Previously, we identified RASA1 mutations in 50% of patients with CM-AVM. Herein we studied non-RASA1 patients to further elucidate the pathogenicity of CMs and AVMs.

Methods : We conducted a genome-wide linkage study on a CM-AVM family. Whole exome sequencing was also performed on 9 unrelated CM-AVM families. We identified a candidate-gene and screened it in a large series of patients. The influence of several missense variants on protein function was also studied in vitro.

Results : We found evidence for linkage in two loci. Whole-exome sequencing data unraveled four distinct damaging variants in *EPHB4* in five families that co-segregated with CM-AVM. Overall, screening of *EPHB4* detected 47 distinct mutations in 54 index patients: 27 lead

to a premature stop codon or splice-site alteration, suggesting loss of function. The other 20 are non-synonymous variants that result in amino-acid substitutions. In vitro expression of several mutations confirmed loss of function of EPHB4. The clinical features included multifocal CMs, telangiectasias, and AVMs.

Conclusions : We found *EPHB4* mutations in patients with multifocal CMs associated with AVMs. The phenotype, CM-AVM2, mimics *RASA1*-related CM-AVM1 and also HHT. RASA1 encoded p120RASGAP is a direct effector of EPHB4. Our data highlights the pathogenetic importance of this interaction and indicts EPHB4-RAS-ERK signaling pathway as a major cause for arterio-venous malformations.

N. Revencu: None. **M. Amyere:** None. **R. Helaers:** None. **E. Pairet:** None. **E. Baselga:** None. **M. Cordisco:** None. **W. Chung:** None. **J. Dubois:** None. **J. Lacour:** None. **L. Martorell:** None. **J. Mazereeuw-Hautier:** None. **R. Pyeritz:** None. **D. Amor:** None. **A. Bisdorff:** None. **F. Blei:** None. **H. Bombei:** None. **A. Dompmartin:** None. **D. Brooks:** None. **J. Dupont:** None. **M. González-Enseñat:** None. **I. Frieden:** None. **M. Gerard:** None. **M. Kvarnung:** None. **A. Kwan Hanson-Kahn:** None. **L. Hudgins:** None. **C. Léauté-Labrèze:** None. **C. McCuaig:** None. **D. Metry:** None. **P. Parent:** None. **C. Paul:** None. **F. Petit:** None. **A. Phan:** None. **I. Quere:** None. **A. Salhi:** None. **A. Turner:** None. **P. Vabres:** None. **A. Vicente:** None. **O. Wargon:** None. **S. Watanabe:** None. **L. Weibel:** None. **A. Wilson:** None. **M. Willing:** None. **J. Mulliken:** None. **L. Boon:** None. **M. Vikkula:** None.

C18.5

Association of modifiers and other genetic factors explain Marfan syndrome clinical variability

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p { margin-bottom: 0.25cm; line-height: 120%; }a:link { color: rgb(0, 0, 255); } Marfan syndrome (MFS) is a rare autosomal dominant connective tissue disorder related to mutations in the *FBN1* gene. Prognosis is related to aortic risk of dissection. MFS clinical variability is notable, for age of onset as well as severity and number of clinical manifestations. To identify genetic modifiers, we combined genome-wide approaches in 1070 clinically well-characterized *FBN1* disease-causing variant carriers: 1) an *FBN1* eQTL analysis in 80 fibroblasts of *FBN1* stop variant carriers, 2) a linkage analysis and 3) a kinship matrix association study in 14 clinically concordant and discordant sib-pairs, 4) a genome-wide association study and 5) a whole exome sequencing in 98 extreme phenotype samples. Three genetic mechanisms of variability were found. A new genotype/phenotype correlation with an excess of loss-of-cysteine variants ($p = 0.004$) in severely affected subjects. A second pathogenic event in another thoracic aortic aneurysm gene or the *COL4A1* gene (involved in cerebral aneurysm) was found in 9 individuals. A polygenic model involving at least 9 modifier loci (named gMod-M1-9) was observed through cross-mapping of results. Notably, gMod-M2 which co-localizes with *PRKG1*, in which activating variants have already been described in thoracic aortic aneurysm, and gMod-M3 which co-localized with a metalloprotease (proteins of extra-cellular matrix regulation) cluster. Our results represent a major advance in understanding the complex genetic architecture of MFS and provide the first steps toward prediction of clinical evolution.

M. Aubart: A. Employment (full or part-time); Significant; AP-HP, INSERM. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; DHU FIRE. **S. Gazal:** None. **P. Arnaud:** None. **L. Benarroch:** None. **M. Gross:** None. **J. Buratti:** None. **A. Boland:** None. **V. Meyer:** None. **N. Hanna:** None. **O. Milleron:** None. **C. Stheneur:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Programme Hospitalier de Recherche Clinique CRC07032 and P071009. **H. Zouali:** None. **T. Bourgeron:** None. **I. Desguerre:** None. **M. Jacob:** None. **L. Gouya:** None. **E. Genin:** None. **J. Deleuze:** A. Employment (full or part-time); Significant; Centre National de Genotypage. **G. Jondeau:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Agence Nationale de la Recherche (NONAGES, ANR-14-CE15-0012-01, Fédération de Cardiologie, Société Française de Cardiologie. **C. Boileau:** B.

Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Programme hospitalier de recherche clinique AOM10108 and CRC15014.

C18.6
Nationwide study associates atrial fibrillation with titin-truncating variants

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Introduction: Atrial fibrillation (AF) is the most common cardiac arrhythmia with more than 30 million people affected. AF is a major risk factor for stroke, heart failure and premature death.

Materials and Methods: We performed whole exome sequencing on 24 families with at least three family members diagnosed with AF. To investigate the effect of genetic variants we investigated CRISPR/Cas9 modified zebrafish carrying truncating variant of titin (*ttn.2*).

Results: Four titin-truncating variants (TTNtv) were identified. These variants co-segregated perfectly with disease and were significantly enriched in patients with familial AF ($P = 1.76 \times 10^{-6}$), as compared to the control group ($n = 663$). This association was replicated in an independent cohort of early-onset lone AF patients ($n = 399$; Odd Ratio=36.8; $P = 4.13 \times 10^{-6}$). We studied CRISPR/Cas9 modified zebrafish carrying a titin truncating variant. Using confocal microscopi we observed compromised assembly of the sarcomere in the atria at the early larval stage in homozygous fish suggesting a predisposition for disease of the atria already in the early development of the heart. We investigated the adult heterozygous mutant hearts using Transmission electron microscopy. The heterozygous mutant hearts lacked structurally intact sarcomeres. The z-discs appeared fuzzy and poorly organised, and the M-line was missing from all the sarcomeres in both atria and ventricle. Mutant had significantly shorter sarcomere in atria ($p < 0.001$).

Conclusions: These results indicate that TTNtv are important risk factors for AF that predispose for disease ([OR] = 36.8). CRISPR/Cas9 modified zebrafish heterozygous lacked intact sarcomeres. These analyses add a new dimension to the understanding of the molecular predisposition for AF.

G. Ahlberg: None. **L. Refsgaard:** None. **P.R. Lundegaard:** None. **L.S. Andreasen:** None. **M.F. Ranthe:** None. **N.S. Linscheid:** None. **J.S. Nielsen:** None. **M.S. Melbye:** None. **S. Haunsø:** None. **A. Sajadieh:** None. **S. Olesen:** None. **S. Rasmussen:** None. **A. Lundby:** None. **P.T. Ellinor:** None. **A.G. Holst:** None. **J.H. Svendsen:** None. **M.S. Olesen:** None.

C19 Advanced sequencing technologies

C19.1
Clinical experience with shallow whole genome sequencing as a detection method for Copy Number Variations

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Genomic Copy Number Variations (CNVs) are an important cause of several human genetic disorders. Genomic microarrays are currently widely implemented as the first tier test for CNV detection. Although it has been proven that microarrays perform well for detecting submicroscopic CNVs, throughput is still rather limited, consumable cost is high, and resolution is fixed depending on the probe density on the microarray. We replaced microarrays by shallow whole genome sequencing (sWGS) for the detection of submicroscopic CNVs in a diagnostic setting. Library preparation is largely automated on a liquid handling robot and afterwards samples are sequenced on a HiSeq3000 instrument by means of 50bp single-end sequencing. Depending on the indication, resolution can be adjusted by altering the number of samples per lane and hence the amount of sequence reads per sample. More sequence reads enable a smaller window size and hence a higher resolution. Since July 2017 more than 1000 clinical samples were processed including postnatal blood specimen, leukemic samples (ALL, CLL), tumors, products of conception (POCs) and prenatal samples. The plummeting costs of sequencing and the ability to automation results in a more cost-effective workflow and a higher throughput. CNVseq shows a higher flexibility towards resolution and species under investigation. Moreover, we show that both duplications and deletions are more clearly defined, enabling a better identification of (low-grade) mosaicism. In conclusion, we show that shallow whole genome sequencing is an

appropriate alternative for genomic microarrays for the detection of clinically relevant copy number aberrations.

B. Menten: None. **M. De Smet:** None. **L. Raman:** None. **T. Sante:** None. **N. Van Roy:** None. **A. Dheedene:** None.

C19.2

An international interlaboratory study of complex variant detection by clinical genetic tests

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Background: NGS is a capable technique for detecting SNVs and small indels in tractable parts of a patient's genome. NGS, however, has limitations. In a study presented at the 2017 ESHG meeting, we found that variants of other, technically challenging types comprise between 9 and 19% of the pathogenic variants harbored by patients, depending on clinical indication. These variants include large indels, single-exon CNVs, variants in repetitive regions, etc. This study has since been expanded to include over 180,000 patients with diverse indications, and leads to similar conclusions.

Methods: We developed a synthetic specimen containing 22 challenging pathogenic variants of diverse types in 7 commonly tested genes. This specimen was sequenced using 10 different NGS workflows by an international group of collaborating laboratories. These tests employed different sequencing platforms, library methods, and bioinformatics pipelines.

Results: With one exception, all of the relatively "easy" SNVs and indels were uniformly detected. However, only 10 of the 22 challenging variants were detected by all tests, and just 3 tests detected all 22. Many, but not all of these limitations were bioinformatic in nature.

Discussion: Evaluating the sensitivity of tests for complex variants can be difficult. Recent guidelines [Roy et al. 2017] recommend that at least 59 variants of each type be used in validation, a number that is difficult to achieve for complex variants given the scarcity of positive controls. Methods such as ours may help, and this specific specimen is available to members of the ESHG community and is being expanded.

S. Lincoln: A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Illumina, ThermoFisher. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae. **A. Fellowes:** None. **S. Mahamdallie:** None. **S. Chowdhury:** None. **E. Klee:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Soft Genetics. **J. Zook:** None. **R. Truty:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae. **R. Garlick:** A. Employment (full or part-time); Significant; Seracare. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Seracare. **S. Aradhya:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae. **M. Salit:** None. **N. Rahman:** F. Consultant/Advisory Board; Significant; AstraZeneca. **S. Kingsmore:** None. **R. Nussbaum:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae. **M. Ferber:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Soft Genetics. F. Consultant/Advisory Board; Modest; Oneome. **B. Shirts:** None.

C19.3

A pipeline to detect repeat expansions from whole genome sequencing in the 100,000 Genomes Project

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Introduction: The 100,000 Genomes Project is building the infrastructure to deliver short read whole genome sequencing (WGS) as part of a new genomic medicine service for the NHS in England. One advantage of WGS is its ability to test for a variety of variant types simultaneously. Germline repeat expansions cause over 20 neurological disorders that can be hard to differentiate clinically from each other and from disorders caused by other variant types. We present data on the use of pipeline to identify repeat expansions

from WGS that uses the ExpansionHunter bioinformatics tool.

Materials and Methods: A pilot dataset comprising 4,833 genomes from rare disease patients was used to genotype repeats in 20 loci. Within this cohort, 414 experimental validation results (normal alleles) across 13 loci were available to assess the STR size estimates. An additional cohort of 5,529 patient genomes was then analysed to identify pathogenic expansions.

Results: ExpansionHunter computed precise and accurate estimates of 99.8% normal alleles tested across all the loci analysed. Further, we identified 10 pathogenic expansions including FXS, ALS, HTT, SCA6 and SCA12 that were consistent with the patient phenotype. Of these, 6 were externally validated and the remaining are awaiting validation results.

Conclusions: We describe an accurate pipeline to detect repeat expansions from WGS which is suitable for use in the future NHS genomic medicine service. Additionally we will assess the performance ExpansionHunter at a range of expanded allele sizes though the analysis of 180 positive controls.

K. Ibanez: None. **E. Dolzhenko:** None. **K. R Smith:** None. **R. H Scott:** None. **E. Thomas:** None. **E. Baple:** None. **H. Brittain:** None. **D. Bourn:** None. **P. Brennan:** None. **J. Polke:** None. **H. Houlden:** None. **A. Rendon:** None. **M. J Caulfield:** None. **M. A Eberle:** None. **A. Tucci:** None.

C19.4

Amplification-free, CRISPR-Cas9 targeted enrichment and SMRT Sequencing of repeat-expansion disease causative genomic regions

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Targeted sequencing has proven to be economical for obtaining sequence information for defined regions of the genome. However, most target enrichment methods are reliant upon some form of amplification which can negatively impact downstream analysis. For example, amplification removes epigenetic marks present in native DNA, including nucleotide methylation, which are hypothesized to contribute to disease mechanisms in some disorders. In addition, some genomic regions known to be causative of many genetic disorders have extreme GC content and/or repetitive sequences that tend to be recalcitrant to faithful amplification.

We have developed a novel, amplification-free enrichment technique that employs the CRISPR/Cas9 system to target individual genes. This method, in conjunction with the long reads, high consensus accuracy, and uniform coverage of SMRT Sequencing, allows accurate sequence analysis of complex genomic regions that cannot be investigated with other technologies. Using this strategy, we have successfully targeted a number of repeat expansion disorder loci (*HTT*, *FMR1*, *ATXN10*, *C9orf72*).

With this data, we demonstrate the ability to isolate thousands of individual on-target molecules and, using the Sequel System, accurately sequence through long repeats regardless of the extreme GC-content. The method is compatible with multiplexing of multiple target loci and multiple samples in a single reaction. Furthermore, because there is no amplification step, this technique also preserves native DNA molecules for sequencing, allowing for the direct detection and characterization of epigenetic signatures. To this end, we demonstrate the detection of 5-mC in the CGG repeat of the *FMR1* gene that is responsible for Fragile X syndrome.

J. Ekholm: A. Employment (full or part-time); Significant; PacBio. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; PacBio. **Y. Tsai:** A. Employment (full or part-time); Significant; PacBio. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; PacBio. **T. Hon:** A. Employment (full or part-time); Significant; PacBio. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; PacBio. **B. Bowman:** A. Employment (full or part-time); Significant; PacBio. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; PacBio. **J. Ziegle:** A. Employment (full or part-time); Significant; PacBio. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; PacBio. **B. Schule:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; PacBio. **T. Ashizawa:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; PacBio. **K. McFarland:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; PacBio. **T. Clark:** A. Employment (full or part-time); Significant; PacBio. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; PacBio. **R. Vogelsang:** None.

C19.5

Long-read sequencing - for detecting clinically relevant structural variation

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Current genome sequencing approaches are extremely successful in identifying disease causing mutations. Nonetheless, these methods yield data that is incomplete, and fall short of detecting all clinically relevant genetic variants present in individual genomes. We anticipate that long-read sequencing techniques may augment genetic diagnosis for a clinically well-characterized patient population with intellectual disability that we have studied over the years. Previously analyses with CNV-microarrays, WES (de Ligt, NEJM 2012) and by short-read WGS (Gilissen, Nature 2014) all failed to detect a causal variant. We performed long-read SMRT-sequencing in five such patient-parent trios with coverages between 15-45-fold.

Per individual genome we identify >22,000 SVs between 50bp-50kb in size. More than 65% of SVs are novel compared to high-depth short-read sequencing (Illumina NovaSeq). Using the trio approach we are able to filter the 500-1,000 rare SVs per patient to ~40 SVs that have no parental read support; validations of the best 5 candidate *de novo* SVs per case are ongoing. The high coverage genomes even allow detection of SVs/indels in the 20-50bp range; per genome >25,000 of those are called per genome.

Next to identifying novel SVs our data also confirm that long-read sequencing data provide coverage in >20Mb previously uncovered genome regions. Additionally long-read sequencing data facilitate phasing of *de novo* SNVs and compound heterozygous variants.

In summary, long-read sequencing identifies a significant number of previously hidden SVs. The *de novo* mutation rates as well as the clinical relevance of these previously hidden genetic variants can now be further explored.

A. Hoischen: None. **A.M. Wenger:** A. Employment (full or part-time); Significant; A.M.W., P.B., L.H., J.K., K.C. and J.K. are employees and shareholders of Pacific Biosciences, a company commercializing DNA sequencing technologies. **M. van der Vorst:** None. **M. Kwint:** None. **M. Nelen:** None. **K. Neveling:** None. **P. Baybayan:** A. Employment (full or part-time); Significant; A.M.W., P.B., L.H., J.K., K.C. and J.K. are employees and shareholders of Pacific Biosciences, a company commercializing DNA sequencing technologies. The other authors declare no conflict of interest. **L. Hickey:** A. Employment (full or part-time); Significant; A.M.W., P.B., L.H., J.K., K.C. and J.K. are employees and shareholders of Pacific Biosciences, a company commercializing DNA sequencing technologies. The other authors declare no conflict of interest. **J. Kuijpers:** A. Employment (full or part-time); Significant; A.M.W., P.B., L.H., J.K., K.C. and J.K. are employees and shareholders of Pacific Biosciences, a company commercializing DNA sequencing technologies. The other authors declare no conflict of interest. **J. Korlach:** A. Employment (full or part-time); Significant; A.M.W., P.B., L.H., J.K., K.C. and J.K. are employees and shareholders of Pacific Biosciences, a company commercializing DNA sequencing technologies. The other authors declare no conflict of interest. **K. Corcoran:** A. Employment (full or part-time); Significant; A.M.W., P.B., L.H., J.K., K.C. and J.K. are employees and shareholders of Pacific Biosciences, a company commercializing DNA sequencing technologies. The other authors declare no conflict of interest. **H.G. Brunner:** None. **L.E.L.M. Vissers:** None. **C. Gilissen:** None.

C19.6

A novel approach using long-read sequencing and ddPCR to investigate gonadal mosaicism and estimate recurrence risk in two families with developmental disorders

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Introduction: *De novo* mutations contribute significantly to severe early-onset genetic disorders. Even if the mutation is apparently *de novo*, there is a recurrence risk due to parental germ line mosaicism, depending on in which gonadal generation the mutation occurred.

Materials and Methods: We demonstrate the power of using SMRT sequencing and ddPCR to determine parental origin and allele frequencies of *de novo* mutations in germ cells in two families whom had undergone assisted reproduction.

Results: In the first family, a *TCOF1* variant c.3156C>T was identified in the proband with Treacher Collins syndrome. The variant affects splicing and was determined

to be of paternal origin. It was present in <1% of the paternal germ cells, suggesting a very low recurrence risk. In the second family, the couple had undergone several unsuccessful pregnancies where a *de novo* mutation *PTPN11* c.923A>C causing Noonan syndrome was identified. The variant was present in 40% of the paternal germ cells suggesting a high recurrence risk.

Conclusions: Our findings highlight a successful strategy to identify the parental origin of mutations and to investigate the recurrence risk in couples that have undergone assisted reproduction with an unknown donor or in couples with gonadal mosaicism that will undergo preimplantation genetic diagnosis.

Grant references: Marcus Borgströms Foundation, Magnus Bergvalls Foundation, The Lars Hierta Memorial Foundation, The Royal Physiographic Society in Lund, The Swedish Society of Medicine and Uppsala University Hospital. MW was supported by Swedish Society for Medical Research and SG by the Sävstaholm Foundation.

M. Wilbe: None. **S. Gudmundsson:** None. **J. Johansson:** None. **A. Ameur:** None. **E. Stattin:** None. **G. Annerén:** None. **H. Malmgren:** None. **C. Frykholm:** None. **M. Bondeson:** None.

C20 Intellectual Disability 2

C20.1

De novo missense variants in *RHOBTB2* cause a developmental and epileptic encephalopathy in humans, and altered levels cause neurological defects in *Drosophila*

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While the role of typical Rho GTPases and other Rho-linked proteins in synaptic plasticity and cognitive function is widely acknowledged, the role of atypical RhoGTPases such as RHOBTB2 in neurodevelopment has barely been characterized. We now identified *de novo* missense variants clustering in the BTB-domain encoding region of *RHOBTB2* in ten individuals with a developmental and epileptic encephalopathy, characterized by early onset epilepsy, severe intellectual disability, postnatal microcephaly, movement disorders and in several individuals by (postictal) hemiparesis and secondary MRI anomalies. RHOBTB2 interacts with a cullin-dependent ubiquitin ligase complex and thus regulates auto-ubiquitination and recruits other substrates to the complex. Though direct interaction of mutant RHOBTB2 with CUL3 did not appear to be impaired by co-immunoprecipitation, we observed increased levels of mutant RHOBTB2 compared to wild-type 24 hours after transfection of HEK293 cells. Abolishing this effect by adding a proteasome inhibitor indicates decreased degradation of mutant RHOBTB2 in the proteasome, probably due to impaired ubiquitination. Similarly, elevated levels of the *Drosophila* ortholog RhoBTB in vivo were associated with seizure susceptibility and severe locomotor defects, while knockdown of RhoBTB resulted in no or only very mild phenotypes. Knockdown of RhoBTB in the *Drosophila* dendritic arborization neurons, however, resulted in a significantly decreased number of dendrites, thus suggesting a role of RhoBTB in dendritic development. We establish missense variants in the BTB domain encoding region of *RHOBTB2* as causative for a developmental and epileptic encephalopathy and elucidate the role of atypical RhoGTPase RhoBTB in *Drosophila*

neurological function and possibly for dendrite development.

J. Straub: None. **E.D.H. Konrad:** None. **J. Grüner:** None. **A. Toutain:** None. **L.A. Bok:** None. **M.T. Cho:** None. **H.P. Crawford:** None. **H. Dubbs:** None. **G. Douglas:** None. **R. Jobling:** None. **D. Johnson:** None. **B. Krock:** None. **M.A. Mikati:** None. **A. Nesbitt:** None. **J. Nicolai:** None. **M. Phillips:** None. **A. Poduri:** None. **X.R. Ortiz-Gonzales:** None. **Z. Powis:** A. Employment (full or part-time); Significant; Ambry Genetics. **A. Santani:** None. **L. Smith:** None. **A.P.A. Stegmann:** None. **C. Stumpel:** None. **M. Vreeburg:** None. **D.D.D. Study:** None. **A. Fliedner:** None. **A. Gregor:** None. **H. Sticht:** None. **C. Zweier:** None.

C20.2

Inborn de novo mutations in *NFE2L2* cause a multisystem disorder in children and adolescents: From gene identification to therapy development

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Introduction: In more than half of the children with leukoencephalopathy conventional methods are insufficient to identify the cause of disease. Using NGS we were able to describe a new paediatric neurological disease caused by variants in *NFE2L2*. NRF2, encoded by *NFE2L2*, is the master regulator of defense against stress. Somatic mutations of *NFE2L2* leading to NRF2 accumulation promote drug resistance in cancer cells.

Materials and Methods: We investigated a 9-year old boy with developmental delay, failure to thrive, immunodeficiency and leukoencephalopathy. We applied Mendeliome patient-parent sequencing on the Illumina Miseq platform and filtered for pathogenic variants. GeneMatcher was used to find further patients. NFE2L2 variant was characterized using cell biology and biochemical approaches. As a therapy approach the flavone luteolin was tested.

Results: We identified *de novo* mutations in *NFE2L2* in four children and showed that cancer related mutations as inborn *de novo* mutations cause an early onset multisystem disorder with failure to thrive, immunodeficiency and neurological symptoms. NRF2 accumulation leads to widespread misregulation of gene expression and an imbalance in cytosolic redox balance. Patients' blood show increased activity of the NRF2 regulated enzymes G-6-P-dehydrogenase and glutathione reductase as well as decreased homocysteine level. Treatment with luteolin leads to a reduction of the NRF2 level and decreased expression of NRF2 targets in fibroblasts and an improvement of the course of disease in one patient.

Conclusions: The unique combination of white matter lesions, hypohomocysteinaemia and increased G-6-P-dehydrogenase activity will facilitate early diagnosis and therapeutic intervention of this novel disorder.

Grant: DFG:Ga354/14-1

S. Diegmann: None. **J. Church:** None. **R. Schnur:** None. **M. Krusen:** None. **S. Dreha-Kulaczewski:** None. **W. Kühn-Velten:** None. **A. Wolf:** None. **B. Huppke:** None. **F. Millan:** None. **A. Begtrup:** None. **F. Almusafri:** None. **H. Thiele:** None. **J. Altmüller:** None. **P. Nürnberg:** None. **M. Müller:** None. **J. Gärtner:** None. **P. Huppke:** None.

C20.3

De novo mutations affecting *PPP2CA*, encoding the catalytic Cα subunit of PP2A, cause PP2A dysfunction and a neurodevelopmental disorder

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Reversible protein phosphorylation plays an essential regulatory role in neuronal signaling. Type 2A Protein Phosphatases (PP2A) are highly expressed in brain, and catalyze dephosphorylation of phospho-Ser/Thr residues in diverse substrates. Structurally, PP2A holoenzymes comprise a catalytic C, a scaffolding A, and a regulatory B-type subunit, which determines substrate specificity and physiological function. Recently, *de novo* mutations in genes encoding subunits Aα and B56δ, and less frequently, B56β and B56γ, have been implicated in intellectual disability/developmental delay (ID/DD). Now, we describe 12 individuals with a *de novo* mutation in *PPP2CA*, encoding the catalytic Cα subunit, phenotypically characterized by mild to severe ID/DD, behavioral problems, variable types of epilepsy, hypotonia and brain abnormalities. *PPP2CA* mutations consist of a partial deletion, a frame shift, three nonsense (Gln125*, Arg214*, Arg295*), one single amino acid duplication, four non-recurrent and one recurrent missense mutation (His191Arg). Functional studies showed complete null alleles in the former four cases, hinting towards a haploinsufficiency mechanism. In the eight other cases, functional characterization of the mutants showed mutation-specific biochemical distortions, including decreased binding to the A subunit and/or specific B-type subunits, as well as mildly to severely impaired phosphatase activity. Several mutants showed increased binding to the B"'/STRN subunits, an observation previously also made for ID-associated Aα mutants. Thus, in the latter eight cases, a dominant mechanism could not be excluded. We conclude that PP2A biogenesis and activity are severely compromised in individuals with ID/DD carrying *de novo PPP2CA* mutations, further underscoring the importance of PP2A dysfunction in genetic brain disorders.

S. Reynhout: None. **S. Jansen:** None. **D. Haesen:** None. **S. Van Belle:** None. **S. de Munnik:** None. **E. Bongers:** None. **J. Schieving:** None. **C. Marcelis:** None. **J. Amiel:** None. **M. Rio:** None. **H. McLaughlin:** None. **R. Ladda:** None. **S. Sell:** None. **M. Kriek:** None. **C. Peeters-Scholte:** None. **P. Terhal:** None. **K. van Gassen:** None. **N. Verbeek:** None. **S. Henry:** None. **J. Scott Schwoerer:** None. **S. Malik:** None. **N. Revencu:** None. **C. Ferreira:** None. **E. Macnamara:** None. **B. de Vries:** None. **C. Gordon:** None. **V. Janssens:** None. **L. Vissers:** None.

C20.4

Breaking TADs: an emerging pathogenic mechanism exemplified by Autosomal Dominant demyelinating LeukoDystrophy (ADLD)

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Genomic rearrangements and CNVs are structural aberrations of the human genome which contribute to human disease. It is well established that structural abnormalities spanning coding genes can have pathogenic consequences due to gene dosage effect (coding CNVs). However, the increasing knowledge about regulatory elements, spatial folding of DNA, and the discovery of Topologically Associated Domains (TADs) allowed the identification of rearrangements that modify the regulatory context of disease genes, without affecting their coding region (non-

coding CNVs). Here, we describe an instructive and unique example of a neurodegenerative disorder associated to both coding and noncoding CNVs at the *LMNB1* locus. Autosomal Dominant adult-onset demyelinating LeukoDystrophy (ADLD) is a fatal disorder affecting myelin in the central nervous system. ADLD is due to *LMNB*1 gene duplication, causing its overexpression at mRNA and protein levels. We have collected four patients with clinical and MRI findings suggestive of ADLD, without LMNB1 duplication. Using array-CGH, we have identified four different overlapping deletions located upstream *LMNB1*. The minimal deleted region was 167 kb and span the physiological enhancer of the *LMNB1* gene already characterized by our group. Furthermore, it eliminates a TAD boundary, allowing foreign interactions between the *LMNB1* promoter and four brain-specific enhancers. We demonstrated LMNB1 overexpression in fibroblasts, and in a postmortem brain sample. In conclusion, ADLD is caused by LMNB1 overexpression due to the duplication of the gene or to the alteration of the LMNB1 regulatory landscape. This latter represents an emerging pathogenic mechanism that should be taken into account in clinical practice.

E. Giorgio: None. **M. Spielmann:** None. **B.C. Nmezi:** None. **G. Vaula:** None. **A. Lehman:** None. **A. Brussino:** None. **S. Cavalieri:** None. **M. Ferrero:** None. **E. Di Gregorio:** None. **C. Mancini:** None. **E. Pozzi:** None. **E. Riberi:** None. **Q.S. Padiath:** None. **A. Brusco:** None.

C20.5

AAV9- CRiSPR/Cas9 preclinical trial on patient-derived *FOXG1* mutated cells

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A curative therapy for the congenital variant of Rett syndrome due to *FOXG1* mutations is lacking. Regenerative gene-based medicines aim to cure disease at the molecular level using innovative gene editing technologies, such as CRiSPR/Cas9 technology. Recent studies have shown the efficacy of adeno-associated virus (AAV) vectors which transport guide RNAs (sgRNA) and Cas9 in restoring the expression of altered proteins. Recent works have shown that AAV9 serotype is an efficient vector to preferentially target neuronal cells in-vivo by intravenous injection. We have recently generated iPSC-derived neurons from *FOXG1*-mutated patients, paving the way to testing personalized therapeutic approaches in disease-relevant cells. We have engineered a two-plasmid system to correct specific *FOXG1* mutations, namely c.460dupG - p.Glu154Gly fs*300 and c.688C>T - p.Arg230Cys. The first construct brings mutation-specific sgRNA and donor DNA and an mCherry/GFP reporter system. The second construct harbours a self-cleaving spCas9 able to inactivate itself after cleavage, avoiding off-target cuts. The two plasmids have been encapsidated into AAV9. The efficiency of the AAV system has been tested in multiple cell types in-vitro, indicating that AAV9 has indeed the highest infection efficiency in neurons and fibroblasts while for iPSCs and neuronal precursor cells AAV2 is more efficient. The efficacy of sequence correction has been tested by Next Generation Sequencing (NGS) analysis. Our proposed AAV9-CRISPR/Cas9 strategy can provide the proof-of-principle of the feasibility of a gene therapy strategy, opening up the possibility of in-vivo clinical trials for patients affected by the congenital variant of Rett syndrome.

S. Croci: None. **S. Daga:** None. **F.C. Lorenzetti:** None. **F.T. Papa:** None. **F. Donati:** None. **C. Lo Rizzo:** None. **D. Lopergolo:** None. **L. Pancrazi:** None. **M. Doria:** None. **A. Auricchio:** None. **M. Costa:** None. **S. Conticello:** None. **A. Renieri:** None. **I. Meloni:** None.

C20.6

A recurrent *de novo PACS2* heterozygous missense variant causes neonatal-onset developmental epileptic encephalopathy, facial dysmorphism and cerebellar dysgenesis

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The developmental and epileptic encephalopathies (DEEs) are a group of severe infantile and childhood onset. The identification of pathogenic genetic variants in DEEs remains crucial for deciphering this complex group and for accurately caring affected patients. Using trio whole exome sequencing, we first identified a heterozygous missense variant in *PACS2* in two unrelated patients with DEE and facial dysmorphism. National and international data sharing allowed us to identify a recurrent *de novo PACS2* heterozygous missense variant (p.Glu209Lys) in 12 other unrelated patients. All patients, aged 16 months to 16 years, presented infantile epilepsy starting on the first days or weeks of life and an overall developmental delay or ID with or without behavioral disorders, common cerebellar dysgenesis and facial dysmorphism. *PACS2* encodes a multifunctional sorting protein involved in nuclear gene expression and pathway traffic regulation. PACS2 depletion alters gene expression and causes mitochondria fragmentation, mitochondria uncoupling from the endoplasmic reticulum and disturbs cell metabolism and blocks apoptotic programs. *PACS2* is an important paralog of *PACS1* that has been previously reported in patients with intellectual disability, facial morphological features and/or epilepsy. Both proteins harbor cargo(furin)-binding regions (FBRs) that bind cargo proteins, sorting adaptors and cellular kinase. Functional studies demonstrated that the *PACS2* recurrent variant reduces the ability of the predicted autoregulatory domain to modulate the interaction between the PACS2 FBR and client proteins, which may disturb cellular function. These findings support the causality of this recurrent *de novo PACS2* heterozygous missense in DEEs with facial dysmorphim and cerebellar dysgenesis.

N. Jean-Marçais: None. **H.E. Olson:** None. **E. Yang:** None. **D. Heron:** None. **K. Tatton-Brown:** None. **P.A. van der Zwaag:** None. **E.K. Bijlsma:** None. **B.L. Krock:** None. **E. Backer:** None. **E. Kamsteeg:** None. **M. Sinnema:** None. **M.R.F. Reijnders:** None. **D. Bearden:** None. **R.J. Lunsing:** None. **L. Burglen:** None. **G. Lesca:** None. **L.A. Smith:** None. **B. Sheidley:** None. **P. L. Pearl:** None. **C. Moufawad El Achkar:** None. **A. Poduri:** None. **C.M. Skraban:** None. **A.I. Nesbitt:** None. **D.E. Fransen van de Putte:** None. **C.A.L. Ruivenkamp:** None. **P. Rump:** None. **I. Sabatier:** None. **D.A. Sweetser:** None. **J. L. Waxler:** None. **J. Tarpinian:** None. **K.J. Wierenga:** None. **J. Donadieu:** None. **V. Narayanan:** None. **K.M. Ramsey:** None. **C. Nava:** None. **S.H. Lelieveld:** None. **J. Schuurs-Hoeijmakers:** None. **H.G. Brunner:** None. **B. Keren:** None. **F. Tran Mau-Them:** None. **J. Thevenon:** None. **L. Faivre:** None. **G. Thomas:** None. **C. Thauvin-Robinet:** None.

C21 Statistical Genetics

C21.1
Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases

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Introduction: Mendelian randomization (MR) is an approach to infer causality of an exposure with an outcome. An assumption in MR is that the variant used as the instrumental variable acts on the outcome exclusively through the exposure. Horizontal pleiotropy occurs when the variant has an effect on the outcome outside of the pathway of the exposure. Horizontal pleiotropy can cause bias in the causal estimate in MR. Studies have shown that variants identified from genome-wide association studies are associated with multiple traits and that pleiotropy may be common. However, the extent to which horizontal pleiotropy affects MR is unknown.

Materials and Methods: We have evaluated the role of horizontal pleiotropy in MR. Specifically, we developed a method to detect and correct for horizontal pleiotropic outliers, called the Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test.

Results: After applying MR-PRESSO to pairwise comparisons of 82 complex traits and diseases, we observed significant horizontal pleiotropy in 922 (22%) of 4,250 MR tests in total. Amongst causal relationships, we observed that significant pleiotropic outliers distorted causal estimates in MR between 168% and 189% on average. Finally, we observed that horizontal pleiotropy in MR can be corrected in some but not in all instances.

Conclusions: These findings suggest that horizontal pleiotropy is pervasive and should be carefully considered in order to maintain the validity of MR.

Grants: RD is supported by R35GM124836 and R01HL139865 from the NIH, and 15CVGPSD27130014 from the AHA. B.N. is supported by R01MH094469 and R01MH107649-01 from the NIH.

M. Verbanck: None. **C. Chen:** None. **B. Neale:** F. Consultant/Advisory Board; Modest; Deep Genomics, Avanir, Trigeminal solutions. Other; Modest; Illumina. **D. Ron:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; AstraZeneca, Goldfinch Bio.

C21.2
Mendelian randomization combining GWAS and eQTL data reveals new loci, extensive pleiotropy and genetic determinants of complex and clinical traits

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Interpretation of GWAS results is challenging, as most of the associated variants fall into regulatory regions and overlap with expression-QTLs (eQTLs), indicating their potential involvement in gene expression regulation. To address this challenge, we propose a summary statistics-based Mendelian Randomization (MR) approach that uses multiple SNPs jointly as instruments and multiple gene expression traits as simultaneous exposures. Such an approach should be more robust to violations of MR assumptions than state-of-the-art tools (GSMR, TWAS). When applied to 43 human phenotypes it uncovered 3,233 putative genes causally associated with at least one phenotype resulting in 8,388 gene-trait associations; of note 5,982 of these loci were missed by GWAS. For example, expression of *CRIPT*, previously associated with a Mendelian syndrome with short stature (OMIM:615789), is causally associated with height in the general population. Similarly, expression of the RIDDLE syndrome-associated *RNF168* (OMIM:611943) correlates with educational attainment. Our analysis found 58% (1,866/3,233) of genes having pleiotropic causal effect, impacting up to 20 traits. Notably, *ANKRD55* showed directionally consistent causal effect on rheumatoid arthritis, Crohn's disease and inflammatory bowel disease. Using eQTLs from multiple tissues (GTEx) revealed numerous tissue-specific causal effects, often driven by differences in gene-expression. In line with previous findings, LDL level is driven specifically by *SORT1* liver expression.Our method identifies loci missed by conventional GWAS and pinpoints likely functionally relevant disease genes in known regions. Our findings unravel tissue-specific, causal gene-expression networks shared among a range of phenotypes. They shed light onto key biological mechanisms underlying complex clinically important traits.

E. Porcu: None. **S. Rueger:** None. **F.A. Santoni:** None. **A. Reymond:** None. **Z. Kutalik:** None.

C21.3
Equivalence of LD-score regression and individual-level-data methods

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LD-score (LDSC) regression disentangles the contribution of polygenic signal, in terms of SNP-based heritability, and population stratification, in terms of a so-called intercept, to GWAS test statistics. Whereas LDSC regression uses summary statistics, methods like Haseman-Elston (HE) regression and genomic-relatedness-matrix (GRM) restricted maximum likelihood infer parameters such as SNP-based heritability from individual-level data directly. Therefore, these two types of methods are typically considered to be profoundly different. Nevertheless, recent work has revealed that LDSC and HE regression yield near-identical SNP-based heritability estimates when confounding stratification is absent. We now extend the equivalence;

under the stratification assumed by LDSC regression, we show that the intercept can be estimated from individual-level data by transforming the coefficients of a regression of the phenotype on the leading principal components from the GRM. Using simulations, considering various degrees and forms of population stratification, we find that intercept estimates obtained from individual-level data are nearly equivalent to estimates from LDSC regression ($R^2 > 99\%$). An empirical application corroborates these findings. Hence, LDSC regression is not profoundly different from methods using individual-level data; parameters that are identified by LDSC regression are also identified by methods using individual-level data. In addition, our results indicate that, under strong stratification, there is misattribution of stratification to the slope of LDSC regression, inflating estimates of SNP-based heritability from LDSC regression *ceteris paribus*. Hence, the intercept is not a panacea for population stratification. Consequently, LDSC-regression estimates should be interpreted with caution, especially when the intercept estimate is significantly greater than one.

R. de Vlaming: None. **M. Johannesson:** None. **P.K.E. Magnusson:** None. **M.A. Ikram:** None. **P.M. Visscher:** None.

C21.4
Regional heritability analysis of complex traits using haplotype blocks defined by natural recombination boundaries

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We describe a genome-wide analytical approach that draws its theoretical basis from a variant of the GREML approach that uses both local and genome-wide relationship matrices to provide regional estimates of the heritability across locally defined regions in the genome. The novelty in this approach is that it utilises a relationship matrix that is based on local haplotype blocks defined by recombination boundaries in the genome. Compared with single SNPs, use of haplotypes provides a better strategy for capturing the true genomic relationship amongst individuals in the presence of rare variants and thus provides real benefit over single SNPs in recovering much of the hidden heritability and identifying novel gene variants. We hypothesize that this approach will complement existing GWAS analytical approaches by capturing regions in the genome contributing to the phenotypic variation that existing GWAS methods fail to capture. We implemented this approach on simulated data and explored it in some detail, with results from the simulation study supporting our hypothesis. We further demonstrate that there are real benefits to be gained from this approach by applying it to real data from circa 20,000 individuals from the Generation Scotland: Scottish Family Health Study. We analysed height and major depressive disorder (MDD). We identified four novel regions (p-value between 3.75E-05 and 3.605E-06) and 32 novel regions (p-value between 4.92E-05 and 7.5E-07) harbouring a total of 64 and 1156 SNPs for height and MDD respectively. This approach, therefore, offers an opportunity to capture novel genetic variants in complex traits.

R.F. Oppong: None. **P. Navarro:** None. **C.S. Haley:** None. **S. Knott:** None.

C21.5
Associations of polygenic scores with lipid biomarkers in diverse populations

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Lipid biomarkers are risk factors for cardiovascular disease. The majority of findings to-date stem from genome-wide association studies conducted in cosmopolitan European populations. It is not clear whether the genetic architecture of lipids differs across populations.

We used imputed genotypes from genome-wide arrays and measures of high (HDL), low density lipoprotein-cholesterol (LDL) and triglycerides from UKHLS (N = 9,961), two isolated Greek populations from the HELIC study (N = 1,641 and N = 1,945), Ugandan samples from the APCDR study (N = 4,778). We tested associations of established lipid-associated SNPs identified through European samples and of polygenic scores based on these variants using linear mixed models.

Few of the 140 loci displayed nominally significant associations with their target biomarker in the Greek and Ugandan samples. The polygenic scores were associated

with highly consistent effects across the European populations ($p < 3 \times 10^{-4}$ in each case). For the Ugandan cohort, there were significant associations of the HDL (beta = 0.153 per SD, 95% confidence interval [CI]:0.125-0.182, $p = 4 \times 10^{-26}$) and LDL scores (beta = 0.133, 95%CI:0.105-0.161, $p = 4 \times 10^{-20}$) with slightly attenuated effect sizes compared to the UK samples (beta = 0.216, 95%CI:0.197-0.235, $p = 2 \times 10^{-102}$ and beta = 0.177, 95%CI:0.157-0.196, $p = 5 \times 10^{-69}$, respectively). There was no evidence of association for the triglyceride score in the Ugandan samples (beta = -0.022, 95%CI:-0.050-0.007, p = 0.14). Triglyceride levels were predicted inversely by the HDL score in the UK (beta = -0.032, 95%CI:-0.052--0.012, $p = 1 \times 10^{-3}$) and the Ugandan samples (beta = -0.062, 95%CI:-0.090−0.033, $p = 5 \times 10^{-4}$).

Our findings question the assumption that genetic factors are generally the same across populations for any complex trait. This cautions against generalising findings from studies in samples with European ancestry to non-European populations.

K. Kuchenbaecker: None. **T. Reiker:** None. **A. Gilly:** None. **D. Gurdasani:** None. **B. Prins:** None. **D. Suveges:** None. **L. Southam:** None. **G. Asiki:** None. **J. Seeley:** None. **A. Kamali:** None. **K. Hatzikotoulas:** None. **A. Farmaki:** None. **G. Melloni:** None. **G. Ritchie:** None. **J. Schwartzentruber:** None. **P. Danecek:** None. **B. Kilian:** None. **M. Pollard:** None. **E. Tsafantakis:** None. **M. Karaleftheri:** None. **G. Dedoussis:** None. **M. Sandhu:** None. **E. Zeggini:** None.

C21.6

Two evidence of ongoing epistatic selection against genomic deletions in the human population

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Every human genome harbors several rare potentially deleterious deletions. Previous work has evaluated their impact (in terms of their total length, number of affected genes) on human health. This however fails to account for potential interactions between deleterious variants. To estimate possible epistatic mechanisms we followed two approaches using our annotation of 33,000 heterozygous deletions in a cohort of 350,000 unrelated genetically British individuals from the UK Biobank.

First, we analyzed all pairwise combinations of deletions and observed that some deletion-pairs were identified in the same genome significantly less than expected by chance. We observed that deletions that often co-occur are significantly shorter than those who co-occur rarely. Our results suggest antagonistic epistasis between long deletions, with each additional variant decreasing fitness more drastically than the previous one.

Second, assuming that decreased expression of a gene is an important consequence of a deletion, we expect to see more Gain of Expression (GOE) single-nucleotide regulatory alleles in hemizygous deletion carriers, with potential compensatory effect to partially recover the gene expression level. Indeed, we observe a significant increase in frequency of GOE alleles (defined as cis-eQTLs in GTEx) in deletion carriers versus euploid controls, supporting our hypothesis that cases with non-compensated deleterious variants have increased probability to be selected out .

Altogether our results revealed abundant epistatic interactions both between different deletions and between deletions and single-nucleotide regulatory variants, the determination of which will be important to reliably assess the human mutation load in the personalized health era.

K. Popadin: None. **E. Porcu:** None. **M. Lepamets:** None. **K. Mannik:** None. **M. Garieri:** None. **R. Magi:** None. **Z. Kutalik:** None. **A. Reymond:** None.

C22 Best Poster Session 2

P02.48C

Mutation in the intracellular chloride channel CLCC1 associated with autosomal recessive retinitis pigmentosa

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Retinitis pigmentosa (RP) is an inherited eye disease characterised by photoreceptor death and retinal degeneration, resulting in vision loss. This condition affects ~1:4000 individuals worldwide and is highly clinically and genetically heterogeneous, presenting with variable symptoms and inheritance patterns. We identified a homozygous missense alteration (c.75C>A, p.D25E) in the *CLCC1* gene, which encodes a presumptive intracellular chloride channel highly expressed in the retina, associated with autosomal recessive RP in eight consanguineous families from Pakistani and the UK. The p.D25E alteration decreased CLCC1 channel function accompanied by accumulation of mutant protein in granules within the ER lumen. In keeping with these findings, Clcc1$^{+/-}$ KO mice displayed depressed electroretinogram and photoreceptor number. Together these findings define a single founder gene mutation as a cause of RP in families of Pakistani descent, and strongly suggest that CLCC1 function is crucial for maintaining retinal integrity and function. This work was supported by National Eye Institute Grant R01EY021237-01 (SAR), National Human Genome Research Institute (NHGRI)/ National Heart Lung and Blood Institute (NHLBI) to the Baylor Hopkins Center for Mendelian Genomics (UM1 HG006542, JRL), Medical Research Council UK (G1002279), the Newlife Foundation for Disabled Children (SG/15-16/12), Fight For Sight (Ref 2027), Wellcome Trust 209083/Z/17/Z.

I. D'Atri: None. **L. Li:** None. **X. Jiao:** None. **F. Ono:** None. **R. Nelson:** None. **C. Chan:** None. **N. Nakaya:** None. **Z. Ma:** None. **Y. Ma:** None. **X. Cai:** None. **L. Zhang:** None. **S. Lin:** None. **A. Hameed:** None. **B.A. Chioza:** None. **H. Hardy:** None. **G. Arno:** None. **S. Hull:** None. **M. Khan:** None. **J. Fasham:** None. **G.V. Harlalka:** None. **M. Michaelides:** None. **A.T. Moore:** None. **Z. Akdemir:** None. **S. Jhangiani:** None. **J.R. Lupski:** None. **F.P.M. Cremers:** None. **R. Qamar:** None. **A. Salman:** None. **J.K. Chilton:** None. **J. Self:** None. **F. Kabir:** None. **M. Naeem:** None. **M. Ali:** None. **J. Akram:** None. **P.A. Sieving:** None. **S. Riazuddin:** None. **S. Riazuddin:** None. **J. Hejtmancik:** None. **E.L. Baple:** None. **A.H. Crosby:** None.

P04.05A

Loss of GPNMB causes autosomal recessive amyloidosis cutis dyschromica in humans

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Amyloidosis cutis dyschromica (ACD) is a distinct form of primary cutaneous amyloidosis characterized by generalized hyperpigmentation mottled with small hypopigmented macules on the trunks and limbs. Families and sporadic cases have been reported predominantly in East and Southeast Asian ethnicities; however, the genetic cause has not been elucidated. Homozygous premature nonsense mutations leading to loss of function of GPNMB contribute to the iris pigment dispersion phenotype in mouse pigmentary glaucoma. However, no mutations in *GPNMB* have been identified in human pigmentary glaucoma and pigment dispersion syndrome. We establish that the compound heterozygosity or homozygosity of *GPNMB* truncating alleles is the cause of autosomal recessive ACD. Six nonsense or frameshift mutations were identified in nine individuals diagnosed with ACD. Immunofluorescence analysis of skin biopsies showed that GPNMB is expressed in all epidermal cells, with the highest staining observed in melanocytes. GPNMB staining is significantly reduced in the lesional skin of affected individuals. Hyperpigmented lesions exhibited significantly increased amounts of DNA/keratin-positive amyloid deposits in the papillary dermis and infiltrating macrophages compared with hypo-/depigemented macules. Depigmentation of the lesions was attributable to loss of melanocytes. Intracytoplamic fibrillary aggregates were observed in keratinocytes scattered in the lesional epidermis. Thus, our analysis indicates that loss of GPNMB, which has been implicated in melanosome

formation, autophagy, phagocytosis, tissue repair, and negative regulation of inflammation, underlies autosomal recessive ACD, and provides insights into the etiology of amyloidosis and pigment dyschromia.

C. Yang: None. **S. Lin:** None. **C. Chiang:** None. **Y. Wu:** None. **W. H'ng:** None. **C. Chang:** None. **Y. Chen:** None. **J. Wu:** None.

P06.64D

Substrate reduction therapy approach for Sanfilippo C syndrome: use of iPSC and iPSC-derived neurons from patients as cellular models

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Sanfilippo C syndrome is a rare lysosomal storage disorder caused by mutations in the *HGSNAT* gene, which encodes an enzyme involved in heparan sulphate (HS) degradation. It is characterized by a severe and progressive neurodegeneration for which no effective treatment exists.

Previously, we demonstrated the usefulness of siRNAs targeting *EXTL2* genes (involved in HS synthesis) as an effective short-term substrate reduction therapy (SRT), on Sanfilippo C patients' fibroblasts. Now, we use different lentiviral vectors encoding shRNAs targeting *EXTL2* to analyse their long-term effect. We observe a clear reduction in *EXTL2* mRNA levels sixty days after transduction and an evident decrease of the HS amounts.

Due to the good results obtained, now we are using neurons derived from patients' induced pluripotent stem cells (iPSC) as a cellular model. We are using an established protocol to differentiate those iPSC into neurons within a week. Neurons show mature signatures and functional properties after one month. This technique will provide new insights in the usefulness of this treatment in the main affected cell type. To evaluate this therapeutic option, we are analysing the neurons, focusing on aspects such as the inhibition of the *EXTL2* gene at the mRNA level or the accumulation of HS over time by immunocytochemistry.

Our preliminary results in patients' fibroblasts indicate that shRNAs could be a long-term SRT and open a door for the development of a promising therapeutic approach for Sanfilippo C syndrome.

Fundings: Catalan Government (2014SGR 932), Spanish Government (SAF201456562R), Asoc. Stop Sanfilippo (Spain), MPS España.

N. Benetó: None. **E. Creus-Bachiller:** None. **M. García-Morant:** None. **D. Grinberg:** None. **L. Vilageliu:** None. **I. Canals:** None.

P06.72D

Methylmalonic Aciduria cblB type cellular model: Hepatocyte differentiation from iPSC and pharmacological chaperones evaluation

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The understanding of the cellular and molecular mechanisms underlying inherited metabolic disorders (IMDs) is essential for developing new strategies for their prevention and treatment. Due to the genotype variability of IMDs and the upcoming of personalized medicine has prompted the emergence of developing new models. The aim of this work was the generation of a hepatic model of methylmalonic aciduria cblB type by hepatocyte differentiation of induced pluripotent stem cells (IPSCs) generated by reprogramming of patient-derived fibroblasts. This organic aciduria is caused by the deficiency of ATP: cob(I)alamin adenosyltransferase (ATR) encoded by the *MMAB* gene. Fibroblasts from a patient bearing a hypomorphic destabilizing mutation in this gene (p.Ile96Thr) were reprogrammed using a commercial kit based on Sendai virus vectors. After the molecular and functional characterization of the iPSC line, these cells were differentiated in vitro into definitive endoderm and then incubated with specific factors, aimed at hepatocyte differentiation. IPSC-derived hepatocytes expressed relevant hepatic markers analyzed by immunofluorescence. Finally, the hepatocytes generated were used for evaluation of potential pharmacological chaperones previously described (N-{[(4-chlorophenyl)carbamothioyl] amino]-2-phenylacetamide and 4-(4-(4-fluorophenyl)-5-methyl-1H-pyrazol-3-yl)benzene-1,3-diol)) in combination with hydroxocobalamin, providing evidences of its positive effect on the activity of the mutant ATR hepatocytes. Hence, our findings provide an experimental suitable model for the investigation of the hepatotoxicity of new drugs and the pathogenesis of this severe disease serving also as ex vivo platform for organoids generation and therapeutic applications.

PI13/01239 ISCIII; Fundación Isabel Gemio; LCF/PR/PR16/11110018 Fundación la Caixa

E. Richard: None. **Á. Briso-Montiano:** None. **S. Brasil:** None. **L. R. Desviat:** None. **M. Ugarte:** None. **B. Pérez:** None.

P06.35C

Biallelic mutations in MRPS34 lead to instability of the small mitoribosomal subunit and Leigh syndrome

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The synthesis of all 13 mitochondrial DNA (mtDNA)-encoded protein subunits of the human oxidative phosphorylation (OXPHOS) system is carried out by mitochondrial ribosomes (mitoribosomes). Defects in the stability of mitoribosomal proteins or mitoribosome assembly impair mitochondrial protein translation, causing combined OXPHOS enzyme deficiency and clinical disease. Here we report four autosomal-recessive pathogenic mutations in the gene encoding the small mitoribosomal subunit protein, MRPS34, in six subjects from four unrelated families with Leigh syndrome and combined OXPHOS defects. Whole-exome sequencing was used to independently identify all variants. Two splice-site mutations were identified, including homozygous c.321+1G>T in a subject of Italian ancestry and homozygous c.322-10G>A in affected sibling pairs from two unrelated families of Puerto Rican descent. In addition, compound heterozygous MRPS34 mutations were identified in a proband of French ancestry; a missense (c.37G>A [p.Glu13Lys]) and a nonsense (c.94C>T [p.Gln32*]) variant. We demonstrated that these mutations reduce MRPS34 protein levels and the synthesis of OXPHOS subunits encoded by mtDNA. Examination of the mitoribosome profile and quantitative proteomics showed that the mitochondrial translation defect was caused by destabilization of the small mitoribosomal subunit and impaired monosome assembly. Lentiviral-mediated expression of wild-type MRPS34 rescued the defect in mitochondrial translation observed in skin fibroblasts from affected subjects, confirming the pathogenicity of MRPS34 mutations. Our data establish that MRPS34 is required for normal function of the mitoribosome in humans and furthermore demonstrate the power of quantitative proteomic analysis to identify signatures of defects in specific cellular pathways in fibroblasts from subjects with inherited disease.

N.J. Lake: None. **B.D. Webb:** None. **D.A. Stroud:** None. **T.R. Richman:** None. **B. Ruzzenente:** None. **A.G. Compton:** None. **H.S. Mountford:** None. **J. Pulman:** None. **C. Zangarelli:** None. **M. Rio:** None. **N. Bodaert:** None. **Z. Assouline:** None. **M.D. Sherpa:** None. **E.E. Schadt:** None. **S.M. Houten:** None. **J. Byrnes:** None. **E. M. McCormick:** None. **Z. Zolkipli-Cunningham:** None. **K. Haude:** A. Employment (full or part-time); Significant; GeneDx. **Z. Zhang:** A. Employment (full or part-time); Significant; GeneDx. **K. Retterer:** A. Employment

(full or part-time); Significant; GeneDx. **R. Bai:** A. Employment (full or part-time); Significant; GeneDx. **S. E. Calvo:** None. **V.K. Mootha:** None. **J. Christodoulou:** None. **A. Rotig:** None. **A. Filipovska:** None. **I. Cristian:** None. **M.J. Falk:** None. **M.D. Metodiev:** None. **D.R. Thorburn:** None.

P06.36D

Mutations in *NDUFAF8* cause Leigh syndrome with an isolated complex I deficiency

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Introduction Mitochondrial diseases are clinically and genetically heterogeneous metabolic conditions. Complex I deficiency is the most common biochemical diagnosis, particularly prevalent in paediatric patients. Mitochondrial disease is caused by mutations affecting the mitochondrion's own genome (mtDNA) or one of the ~1150 nuclear-encoded mitoproteome components. The limited genotype: phenotype correlation in mitochondrial disease means next-generation sequencing methodologies are critical for the rapid genetic diagnosis of patients.

Materials and Methods Whole exome and targeted next-generation sequencing was performed for three subjects with suspected mitochondrial disease. Functional evaluation of identified variants was undertaken using subject fibroblasts and/or muscle biopsy, including cDNA studies, complexome profiling, complementation studies and assessment of steady-state levels.

Results We describe three unrelated individuals who harbour biallelic variants in *NDUFAF8*, a recently identified ancillary factor required for assembly of the complex I holoenzyme. We provide functional evidence to support the pathogenicity of these *NDUFAF8* variants and unequivocally establish this gene as a cause of complex I deficiency in association with an exclusively Leigh-like clinical presentation.

Conclusions Functional experimentation including complementation studies and complexome profiling of subject cell lines establishes *NDUFAF8* as the twelfth complex I assembly factor associated with human disease and validates the importance of orphan gene characterisation.

Acknowledgements NIHR doctoral fellowship (NIHR-HCS-D12-03-04); Wellcome Centre for Mitochondrial Research (203105/Z/16/Z); MRC Centre for Neuromuscular Diseases (G0601943); NHS Highly Specialised Service for Rare Mitochondrial Disorders; The Lily Foundation; Deutsche Forschungsgemeinschaft: SFB 815/Z1; BMBF mitoNET: 01GM1113B. The views expressed are those of the author(s) and not necessarily the NHS, NIHR or DoH.

C.L. Alston: None. **M.T. Veling:** None. **J. Heidler:** None. **L.S. Taylor:** None. **L. He:** None. **A. Broomfield:** None. **J. Pavaine:** None. **H. Prokisch:** None. **S. Wortmann:** None. **P.E. Bonnen:** None. **R. McFarland:** None. **I. Wittig:** None. **D.J. Pagliarini:** None. **R.W. Taylor:** None.

P09.001A

Dissecting tissue-specific functional networks associated with 16p11.2 reciprocal genomic disorder using CRISPR engineered human iPS and mouse models

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Reciprocal genomic disorders (RGDs) represent a recurrent class of copy number variants (CNVs) that collectively comprise a major contributor to neurodevelopmental disorders (NDD) and altered anthropometric traits. Here, we systematically dissected the functional networks associated with 16p11.2 RGD from transcriptome analyses of 70 mice with reciprocal CNV of the syntenic 7qF3 region across cortex, striatum, and cerebellum, as well as liver, white and brown adipose tissues in a subset of 16 mice (n = 250 samples). We integrated these data with brain tissues from a *Kctd13* mouse model (a putative driver of 16p11.2 neuroanatomical phenotypes, n = 50), and CRISPR-engineered, isogenic 16p11.2 iPSC-derived NSCs (n = 25) and induced neurons (n = 27). The strongest magnitude of effect sizes from 7qF3 were observed across brain regions by comparison to non-brain tissues (cortex 7qF3 region average p-value = 8.80E-35; non-brain p = 0.0013), reflecting the ~3x higher basal expression changes. Coexpression network analyses isolated a consistent module of 16p11.2 genes, as well as a module that was highly enriched for constrained genes (ExAC pLI≥0.9), autism-associated genes, early fetal development coexpression networks derived from BrainSpan, and neurological phenotypes and processes. Differentially expressed genes (DEGs) were enriched in this 'constrained' module network; moreover, DEGs from the Kctd13 mouse coalesced into this same module (cortex enrichment p = 7.82E-41), suggesting overlap in altered transcriptional networks between full length CNV and deletion of KCTD13 alone. These analyses identify a tissue-specific impact of 16p11.2 RGD that converges on a module of co-expressed genes that are intolerant to genetic perturbation and associated with critical processes in human neurodevelopment.

P. Razaz: None. **D.J. Tai:** None. **S. Erdin:** None. **T. Aneichyk:** None. **T. Arbogast:** None. **A. Ragavendran:** None. **A. Stortchevoi:** None. **B.B. Currall:** None. **C.E.F. Esch:** None. **E. Morini:** None. **W. Ma:** None. **R.J. Kelleher:** None. **C. Golzio:** None. **N. Katsanis:** None. **J. F. Gusella:** None. **M. Talkowski:** None.

P09.098B

Biallelic mutations in the homeodomain of NKX6-2 underlie a severe hypomyelinating leukodystrophy

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Introduction: Hypomyelinating leukodystrophies are genetically heterogeneous disorders with overlapping clinical and neuroimaging features reflecting variable abnormalities in myelin formation. The homeobox protein NKX6-2 is a transcription factor regulating multiple developmental processes with a main role in oligodendrocyte differentiation and regulation of myelin-specific gene expression.

Materials and Methods: Whole-exome sequencing (WES) and homozygosity mapping of selected patients from three unrelated families was undertaken. The variants identified were validated by Sanger sequencing and cosegregation analysis.

Results: Five affected subjects (three unrelated families) were documented to share biallelic inactivating mutations affecting the NKX6-2 homeobox domain. A trio-based whole exome sequencing analysis in the first family detected a homozygous frameshift change [c.606delinsTA; p.(Lys202Asnfs*?)]. In the second family, homozygosity mapping coupled to whole exome sequencing identified a homozygous nucleotide substitution (c.565G>T) introducing a premature stop codon (p.Glu189*). In the third family, whole exome sequencing established compound heterozygosity for a non-conservative missense change affecting a key residue participating in DNA binding (c.599G>A; p.Arg200Gln) and a nonsense substitution (c.589C>T; p.Gln197*), in both affected siblings. The clinical presentation was homogeneous, with four subjects having severe motor delays, nystagmus and absent head control, and one individual showing gross motor delay at the age of 6 months. All exhibited neuroimaging that was consistent with hypomyelination.

Conclusions: The finding of individuals with a severe neurodevelopemental phenotype with hypomyelination associated with biallelic mutations in *NKX6-2* provides direct evidence of the relevant role of NKX6-2 in CNS development in humans.

C. Aiello: None. **I. Dorboz:** None. **C. Simons:** None. **R. Stone:** None. **M. Niceta:** None. **M. Elmaleh:** None. **M. Abuawad:** None. **D. Doummar:** None. **A. Bruselles:** None. **N. Wolf:** None. **L. Travaglini:** None. **O. Boespflug-Tanguy:** None. **M. Tartaglia:** None. **A. Vanderver:** None. **D. Rodriguez:** None. **E. Bertini:** None.

P09.139C

SINEUP, a synthetic antisense non-coding RNA-based technology, as possible new therapeutic tool for haploinsufficiency: Autism Spectrum Disorders (ASD) and Epilepsy as Proof-of-Principle

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Autism spectrum disorders (ASD) and epilepsies are heterogeneous conditions that frequently coexist with other developmental disabilities. Genetic bases are prominent risk factors for both disorders. Among others, loss of function mutations in *CHD8* gene represents a recurrent risk factor for ASD, while *CHD2* is more frequently mutated in epilepsy. Thus, the sole reduction in *CHD8* or *CHD2* expression is able to cause cellular and molecular phenotypes that are key hallmarks to follow and rescue in assessing new therapeutic approaches.

Particularly, we aim to test SINEUPs, a novel class of synthetic antisense long non-coding RNAs - able to increase the translation of target proteins to physiological level without affecting transcription - to rescue the phenotypes caused by *CHD8* or *CHD2* haploinsufficiency.

Since the activity of SINEUP depends on two domains, an effector domain required for translation enhancement and a binding domain conferring target specificity, we designed SINEUP molecules able to recognize the initial and internal methionines of CHD8 and CHD2 proteins. We then proceeded to test the efficacy of different SINEUPs on neural progenitors. From our preliminary observations, SINEUPs targeting internal methionines are more efficient in stimulating CHD8 and CHD2 protein production, thus representing a valid target to be further tested in patients' derived cell lines and in zebrafish, an in vivo model of the disorders.

In conclusion, our studies represents the first step towards the development of new types of RNA-based therapy, with implications for a large repertory of presently incurable genetic diseases.

F. Di Leva: None. **M. Arnoldi:** None. **G. Alvari:** None. **A. Messina:** None. **S. Casarosa:** None. **G.L. Carvill:** None. **S. Zucchelli:** None. **S. Gustincich:** None. **M. Biagioli:** None.

P12.214B

Modeling human hereditary cancer syndromes using CRISPR/Cas9 mediated genome editing in *Xenopus tropicalis*

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CRISPR/Cas9 and TALEN mediated genome editing creates unique and unmatched opportunities for modeling human disease in non-mammalian model organisms. The amphibian *Xenopus tropicalis* is extremely well positioned for this approach. It shares with zebrafish the aquatic habitat and easy manipulations associated with its external development. However, it manifests unique features making it a powerful organism for modeling human genetic disease. (1) unlike zebrafish, *Xenopus tropicalis* has a true diploid genome, precluding redundancy. (2) Its genome shows high synteny with humans, greatly facilitating identification of disease orthologs. (3) Gene manipulations can be restricted to specific tissues and organs via targeted blastomere injection. We have recently generated the first genetic cancer models in *Xenopus tropicalis*. Via mosaic targeting of the tumor suppressor gene *apc* we generated tadpoles that rapidly (< 1.5 months) and efficiently (>90%) developed a range of neoplasia characteristic for Familial Adenomatous Polyposis (Van Nieuwenhuysen et al., Oncoscience 2015). Similarly, we found that *rb1/rbl1* double mosaic mutant tadpoles rapidly develop retinoblastoma (Naert et al. Sci. Rep. 2016). More recent work also indicates the possibility of modeling Li-Fraumeni, T-cell acute lymphoblastic leukemia and pancreatic neuroendocrine cancer. The rapid kinetics of tumor development in *Xenopus* pave the way for their use as pre-clinical model, providing unique possibilities for fast identification of modifier genes and novel drug targets. We present our first promising results with multiplexed gene targeting in desmoid tumors. We believe that these models offer a unique experimental platform to contribute to the field of human cancer and medical genetics.

D. Dimitrakopoulou: None. **T. Naert:** None. **D. Tulkens:** None. **R. Noelanders:** None. **T. Van Nieuwenhuyse:** None. **P. Van Vlierberghe:** None. **K. Vleminckx:** None.

P19.24D

Genetic counselling in hereditary diffuse gastric cancer: economical and psycho-social impact

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Introduction: Hereditary Diffuse Gastric Cancer (HDGC) syndrome is caused by *CDH1*-germline mutations and carriers have high-risk to develop early-onset diffuse gastric cancer (DGC) in both genders and lobular breast cancer (LBC) in females. HDGC is deadly for those expressing clinical phenotype, but presymptomatic testing allows disease prevention or early-diagnosis in mutation-carriers through risk-reduction gastrectomy and yearly breast MRI, discharging ~50% of non-carriers. As Health-Care-Provider of ERN-GENTURIS, we aim to evaluate the economic impact of genetic counselling, presymptomatic diagnosis and multidisciplinary care for the National Health Service (NHS), and the clinical and psycho-social implications for HDGC families.

Material and Methods: We evaluated outputs of structured oncogenetics and high-risk consultations in 111 individuals from 7 HDGC families by consulting clinical/financial records, and assessed its psycho-social impact by applying an emotional-distress-scale (HADS) to 70/111 individuals.

Results: From 2011-2016, we screened 111 individuals, from which 53% tested negative (n = 58;30M:28F) and were discharged from follow-up each costing 200€ to the Hospital. 47% (n = 53;26M:27F) tested positive. From 7 probands, 2 were diagnosed with LBC and remain alive after curative surgery, while 5 diagnosed with DGC are deceased. The hospital expenses with probands range from 30-50K€ independently of cancer type. Costs with carriers opting for prophylactic approaches range from 4-8K€ (higher cost/females), except if disease is identified after the first high-risk consultation, raising the cost to 27K€ but life-saving. Those opting for surveillance, cost ~1K €/patient. Concerning psychologic impact, carriers demonstrate higher levels of distress than non-carriers.

Conclusion: Structured healthcare in HDGC economically benefits NHS, is life-saving for carriers, reassuring non-carriers.

L. Garrido: None. **T. Nércio:** None. **R. Leal:** None. **R. Guimarães:** None. **L. Ferro:** None. **L. Vilarinho:** None. **S. Costa:** None. **A. Magalhães:** None. **A.S. Mesquita:** None. **A.F. Pereira:** None. **I. Gullo:** None. **H. Pinheiro:** None. **S. Sousa:** None. **B. Carvalho:** None. **A.P. Neto:** None. **L. Capela:** None. **C. Teixeira:** None. **A. Fareleira:** None. **V. Devezas:** None. **G. Macedo:** None. **J. Preto:** None. **J. Barbosa:** None. **M. Baptista:** None. **G. Pinto:** None. **M. Damasceno:** None. **J.L. Fougo:** None. **J. Costa-Maia:** None. **S. Fernandes:** None. **F. Carneiro:** None. **S. Castedo:** None. **C. Oliveira:** None.

P20.05A

Raw Genomic Data: Storage, Access and Sharing

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Whole Genome Sequencing (WGS) and Whole Exome sequencing (WES) for diagnostic and research purposes generate a large volume of raw data. Previous ethical and legal discussions concerning genomic data management have mainly focused on concerns related to interpretation of the results and the return of both primary and secondary findings from these tests. Yet to date, issues related to the storage and return of primary sequencing data (such as bam (Binary Alignment) or fastq files (unaligned reads)) that allow both the regeneration of primary results and the reanalysis of data have received far less attention, particularly in the clinical setting. This is despite the huge potential for long-term data storage to lead to future data re-analysis and reinterpretation in light of new evidence. In this paper, we critically appraise three main issues, namely, data storage policies and practices of clinical laboratories, patients' access to raw data, and sharing of raw data by individuals. We argue that there is a need for well-established and transparent raw genomic data retention and returning policies in order to enable patients to practice their rights to access raw genomic data. In addition, professional communities could guide laboratories in adopting best practices for the storage and return of raw data, and introduce uniformity to these practices. As WES and WGS become more embedded in diagnostics, it is timely to consider how current data storage policies align with patients' rights and interests to access raw data, and how these rights can be managed in the clinical setting.

M. Shabani: None. **D. Vears:** None. **P. Borry:** None.

P16.40D

Exploring molecular interactions by clustering analysis of similarity scores from next-generation phenotyping approaches

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Introduction: Recent advances in computer vision and machine learning resulted in the next-generation phenotyping (NGP) tools for syndromology. Deep convolutional neural networks such as DeepGestalt in Face2Gene have been trained on thousands of patient photos with confirmed molecular diagnoses and learned phenotype representations of multiple disorders. These systems of artificial intelligence support clinicians in the diagnostic workup of patients and even excel human experts in certain classification tasks. We therefore wondered whether this unprecedented sensitivity to detect mutation-specific patterns in the facial gestalt could also be used to reveal information about gene function.

Materials and Methods: We designed the knowledge base Deep Phenotyping for Deep Learning (DPDL) as a public resource to compile similarity scores from NGP tools and performed a cluster analysis on currently 1216 cases with monogenic disorders.

Results: A prominent cluster was formed by BRAF, PTPN11, NRAS and other genes of the MAPKinase pathway that result in phenotypes such as Noonan, LEOPARD or cardiofaciocutaneous syndrome and which are considered similar. Likewise, many genes linked to inborn errors of metabolism also clustered, mirroring a higher-level feature that is referred to by clinicians as 'coarse facies'. Furthermore, we also found genes involved in chromatin remodeling to be near neighbors, even if no superordinate joint feature exists in medical terminology to describe the associated diseases.

Conclusions: We were able to reproduce molecular interactions by information encoded in the facial gestalt by using NGP tools. Thus, the phenotype space exploration is a promising subject in the characterization of gene function.

T. Hsieh: None. **N. Hajjir:** None. **J.T. Pantel:** None. **M. Mensah:** None. **M. Zhao:** None. **J. Hertzberg:** None. **M. Schubach:** None. **S. Köhler:** None. **Y. Gurovich:** A. Employment (full or part-time); Significant; FDNA. **N. Fleischer:** A. Employment (full or part-time); Significant; FDNA. **H. David-Eden:** A. Employment (full or part-time); Significant; FDNA. **Y. Hanani:** A. Employment (full or part-time); Significant; FDNA. **T. Kamphans:** None. **D. Horn:** None. **S. Mundlos:** None. **P. Krawitz:** None.

C23 Sensory disorders

C23.1

Antisense therapy for a common corneal dystrophy ameliorates *TCF4*repeat expansion-mediated toxicity

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Introduction: Fuchs endothelial corneal dystrophy (FECD) is a common triplet repeat-mediated disease. Corneal transplantation is the only treatment option available to patients with advanced disease; alternative therapeutic strategies are urgently required.

Methods: A total of 450 FECD patients were recruited to the study; either with clinical signs of FECD or prior corneal transplantation surgery for FECD. The triplet repeat, termed CTG18.1, situated within an intronic region of *TCF4*, was genotyped using a short tandem repeat assay. A corneal endothelial cell (CEC) model was developed to probe disease mechanism and investigate therapeutic approaches. RNA foci were visualised by fluorescence *in situ* hybridisation. Immunocytochemistry was performed to determined MBNL1 and MBNL2 cellular localisation. Differential splicing events were analysed by reverse transcription (RT)-PCR and antisense oligonucleotides (ASOs) were transfected using standard methodologies.

Results: We determine that a non-coding trinucleotide repeat expansion in *TCF4* confers significant risk for FECD

in our large patient cohort (OR = 76.47; 95% CI: 47.45-123.2; $p = 5.69 \times 10^{-71}$). CTG18.1 expansions are associated with nuclear RNA foci, sequestration of splicing factor proteins (MBNL1 and MBNL2) to the foci and altered mRNA processing in the patient-derived CEC model. Antisense oligonucleotide (ASO) treatment led to a significant reduction in the incidence of nuclear foci, MBNL1 recruitment and downstream aberrant splicing events, suggesting functional rescue.

Conclusions: This proof-of-concept study highlights the potential of a targeted ASO therapy to treat the accessible and tractable corneal tissue affected by this repeat expansion-mediated disease.

C. Zarouchlioti: None. **B. Sanchez-Pintado:** None. **N. J. Hafford Tear:** None. **P. Klein:** A. Employment (full or part-time); Significant; ProQR therapeutics. **P. Liskova:** None. **K. Dulla:** A. Employment (full or part-time); Significant; ProQR therapeutics. **M. Semo:** None. **A.A. Vugler:** None. **K. Muthusamy:** None. **L. Dudakova:** None. **H.J. Levis:** None. **P. Skalicka:** None. **P. Hysi:** None. **M.E. Cheetham:** None. **S.J. Tuft:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ProQR therapeutics. **P. Adamson:** A. Employment (full or part-time); Significant; ProQR therapeutics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; ProQR therapeutics. **A.J. Hardcastle:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; ProQR therapeutics. **A.E. Davidson:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; ProQR Therapeutics.

C23.2

NGS and animal model reveal *SLC9A3R1* as a new gene involved in human age-related hearing loss (ARHL)

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Introduction To shed light on the genetics of ARHL (the most common sensory disorder in the elderly) we analysed a large cohort of patients and developed a series of animal models.

Methods 46 genes, selected on the basis of GWAS, animal models and literature updates, were analysed in 464 ARHL patients. After data filtering, the most promising results were validated by either "in vitro" or "in vivo" studies.

Results We identified *SLC9A3R1* as a new ARHL gene. An ultra-rare (MAF: 7,70E-05) missense variant was detected in 2 unrelated male patients showing severe to profound high-frequency hearing loss. The variant, predicted as damaging, was not present in 1071 matched controls and protein modelling confirmed its pathogenic effect on protein's structure. *In situ* hybridization showed *Slc9a3r1* expression in zebrafish inner ear. A zebrafish KI model, using CRISPR-Cas9 technology, was generated, revealing a reduced auditory response at all frequencies in *Slc9a3r1*-/- compared to *Slc9a3r1*+/+ and *Slc9a3r1*+/-. Additional phenotypic analyses demonstrated no abnormalities in the anatomy or number of hair cells or in the volume of the stato-acoustic ganglion, however, a significant variation in the total volume of the saccular otolith which is responsible for sound detection, was observed in *Slc9a3r1*-/- compared to *Slc9a3r1*+/+ (while the utricular otolith, necessary for balance, was not affected, in agreement with the human phenotype).

Conclusions Our data strongly support *SLC9A3R1* role in the pathogenesis of ARHL in both human and zebrafish. Larger cohorts of patients should be further analyzed to understand the overall contribution of *SLC9A3R1* to ARHL.

A. Morgan: None. **M. Brumat:** None. **M. Cocca:** None. **M. Di Stazio:** None. **S. Bassani:** None. **M. La Bianca:** None. **P. Gasparini:** None. **G. Girotto:** None.

C23.3

Congenital Macular Dystrophy is caused by non-coding duplications downstream of the IRXA cluster

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Introduction: Autosomal dominant North Carolina Macular Dystrophy (NCMD) is believed to represent a failure of macular development, impairing central vision. Prior genetic linkage pinpointed the disease locus to chromosome 6q16 and 5p15. The aim was to identify causative variants and further the understanding of the disease mechanism.

Materials & Methods: CRISPR technology was used to reproduce a non-coding duplication between *IRX1* and *ADAMTS16* (chr5) recurrently identified in NCMD families

by whole genome sequencing. Gene expression analysis was verified on the developing eye and limb-bud. Chromosome conformation capture (c-HI-C) and chromatin immunoprecipitation performed on the same tissues, mapped chromatin architectural folding and defined regulatory elements.

Results: A non-coding duplication (chr5) was identified as an ancestral allele in 12 NCMD families. Expression of *PRDM13* (chr6) was substantially elevated in eye but not limb-bud tissue in the mouse model versus control. c-Hi-C at the 5p locus showed unique chromatin interactions in developing wild-type eye versus limb-bud, suggesting distinct tissue-specific regulation.

Conclusions: The eye-specific over-expression of *PRDM13*, a previously suggested candidate, provides the first molecular evidence that elevated *PRDM13* expression may be the origin of the phenotype. It also represents the first molecular evidence to link the two loci biologically. The possible interaction between the putative element and subsequent *PRDM13* expression control remains unknown. Chromatin organization findings highlight the relevance of tissue specific DNA contacts in development and disease. Further characterization of mouse model and exploration of the nine unsolved cases may uncover additional molecular targets for NCMD. Funding: FightForSight; Erasmus; MaxPlanckFoundation.

R.S. Silva: None. **K. Kraft:** None. **G. Arno:** None. **V. Cipriani:** None. **V. Heinrich:** None. **N. Pontikos:** None. **B. Puech:** None. **A. Moore:** None. **V. van Heyningen:** None. **S. Mundlos:** None. **A.R. Webster:** None.

C23.4

Ectopic expression of GRHL2 due to non-coding mutations promotes cell state transition and causes Posterior Polymorphous Corneal Dystrophy 4

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In a large family of Czech origin, we mapped a new locus for an autosomal dominant corneal endothelial dystrophy, Posterior Polymorphous Corneal Dystrophy 4 (PPCD4), to 8q22.3-q24.12. Whole genome sequencing identified a unique variant (c.20+544G>T) in this locus, within a regulatory region in intron 1 of *GRHL2*. Targeted sequencing identified the same variant in three additional unsolved PPCD families, including a *de novo* occurrence that suggests this is a recurrent mutation. Two further unique variants were identified in intron 1 of *GRHL2* (c.20 +257delT and c.20+133delA) in unrelated PPCD families. GRHL2 is a transcription factor that suppresses epithelial-to-mesenchymal transition (EMT) and is a direct transcriptional repressor of *ZEB1*. *ZEB1* mutations leading to haploinsufficiency cause PPCD3. We previously identified promoter mutations in *OVOL2*, a gene not normally expressed in the corneal endothelium, as the cause of PPCD1. OVOL2 drives mesenchymal-to-epithelial transition (MET) by directly inhibiting EMT-inducing transcription factors, such as *ZEB1*. Here, we demonstrate that the *GRHL2* regulatory variants identified in PPCD4 individuals induce increased transcriptional activity in vitro. Furthermore, although GRHL2 is not expressed in corneal endothelial cells in control tissue, we detected aberrant ectopic expression of GRHL2 in the corneal 'endothelium' in PPCD4 patient tissue. *GRHL2* patient corneal 'endothelial' cells also ectopically express the epithelial markers E-Cadherin and Cytokeratin 7 indicating they have transitioned to an epithelial-like cell type. We suggest that mutations inducing MET within the corneal endothelium are a convergent pathogenic mechanism leading to failure of the endothelial barrier and disease.

P. Liskova: None. **L. Dudakova:** None. **C.J. Evans:** None. **K.E. Rojas López:** None. **N. Pontikos:** None. **D. Athanasiou:** None. **H. Jama:** None. **J. Sach:** None. **P. Skalicka:** None. **V. Stranecky:** None. **S. Kmoch:** None. **C. Thaung:** None. **M. Filipec:** None. **M.E. Cheetham:** None. **A.E. Davidson:** None. **S.J. Tuft:** None. **A.J. Hardcastle:** None.

C23.5

Whole genome sequencing in patients with ciliopathies uncovers a novel recurrent tandem duplication in *IFT140*

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Introduction: Ciliopathies represent a wide spectrum of rare diseases with overlapping phenotypes and a high genetic heterogeneity. Among those, *IFT140* is implicated in a variety of phenotypes ranging from isolated retinis pigmentosa to more syndromic cases such as the Bardet-Biedl syndrome.

Materials and Methods: Clinical characterizations, whole genome (4 individuals) and targeted exome sequencing (~350 patients) and bioinformatics analysis were performed on ciliopathy families. Patients' skin fibroblasts were analyzed to assess the pathogenicity of the causative mutations.

Results: Using whole genome sequencing in patients with uncharacterized ciliopathies, we identified a novel recurrent tandem duplication of exon 27 to 30 (6.7 kb) in *IFT140*, c.3454-488_4182+2588dup p.(Tyr1152_Thr1394dup), missed by whole exome sequencing. Pathogenicity of the mutation was assessed on the patients' skin fibroblasts. Several hundreds of patients with a ciliopathy phenotype were screened and biallelic mutations were identified in 11 families representing 12 pathogenic variants of which 7 are novel. Among those unrelated families especially with a Mainzer-Saldino syndrome, 8 carried the same tandem duplication (2 at the homozygous state and 6 at the heterozygous state).

Conclusions: We demonstrated the implication of structural variations in *IFT140* related diseases expanding its mutation spectrum. We also provide evidences for a unique genomic event mediated by an *Alu-Alu* recombination occurring on a shared haplotype. We confirm that whole genome sequencing can be instrumental in the ability to detect structural variants for genomic disorders.

V. Geoffroy: None. **C. Stoetzel:** None. **S. Scheidecker:** None. **E. Schaefer:** None. **I. Perrault:** None. **S. Bär:** None. **A. Kröll:** None. **M. Delbarre:** None. **M. Antin:** None. **A. Leuvrey:** None. **C. Henry:** None. **H. Blanché:** None. **E. Decker:** None. **K. Kloth:** None. **G. Klaus:** None. **C. Mache:** None. **D. Martin-Coignard:** None. **S. McGinn:** None. **A. Boland:** None. **J. Deleuze:** None. **S. Friant:** None. **S. Saunier:** None. **J. Rozet:** None. **C. Bergmann:** None. **H. Dollfus:** None. **J. Muller:** None.

C23.6
Biallelic loss-of-function variants in *DNMBP* cause congenital cataract and visual impairment

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Visual impairment is a heterogeneous group of disorders both in etiology and clinical presentation. Autosomal recessive disorders have a high prevalence in consanguineous populations, due to the extensive genomic regions of homozygosity that include alleles identical by descent. Hence, consanguineous families are the best source to identify novel causative autosomal recessive genes. We have analyzed more than 150 consanguineous families with diverse phenotypes related to visual impairment. We used a strategy that combines exome sequencing of a proband and genotyping of the entire family. In two different families, we found two different homozygous loss-of-function (LoF) variants in the *DNMBP* gene (OMIM#611282); a stopgain (NM_015221: c.811C>T:p.Arg271Ter) in family F385 (nine-affected individuals, LOD score: 5.18 at $\theta = 0$), and a frameshift deletion (NM_015221:c.2947_2948delGA) in family F372 (two-affected individuals; LOD score: 0.85). The phenotypes in both families correspond to a congenital cataract and retinopathy. The expression of the *DNMBP* gene is enriched during eye development in mice, zebrafish and flies. Preliminary analysis using RNAi mediated knockdown of the *sif* gene in *Drosophila* (the DNMBP orthologue) affects the amplitudes of the ERG in 3 day old flies using different drivers. DNMBP regulates the shaping of cell junctions through the local activation of Cdc42

and its effectors in human. RNAi-mediated depletion of DNMBP resulted in modified assembly pattern of junctional F-actin and E-cadherin. Notably, E-cadherin has an important role in lens vesicle separation and lens epithelial cell survival. These results establish that *DNMBP* is a candidate for early onset of severe cataract and visual impairment.

M. Ansar: None. **A. Nazir:** None. **H. Chung:** None. **S. Imtiaz:** None. **M.T. Sarwar:** None. **P. Makrythanasis:** None. **E. Falconnet:** None. **M. Guipponi:** None. **C. Borel:** None. **C.J. Pournaras:** None. **M.A. Ansari:** None. **E. Ranza:** None. **F.A. Santoni:** None. **J. Ahmed:** None. **I. Shah:** None. **K. Gul:** None. **H. Bellen:** None. **S.E. Antonarakis:** None.