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ABSTRACT

Abstracts from the 50th European Society of Human Genetics Conference: Oral Presentations

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The ESHG 2017 marks the 50th Anniversary of the first ESHG Conference which took place in Copenhagen in 1967. Additional information about the event may be found on the conference website: https://2017.eshg.org/

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Plenary Sessions

PL1
50 years of ESHG

PL1.1
A brief history of how we got here

A. Read

Manchester, United Kingdom

This year the European Society of Human Genetics celebrates the 50th anniversary of its first meeting, held here in Copenhagen in 1967. I have been asked, on behalf of the Board and Executive of the Society, to start off this year's conference with a brief look back at how we came to be here. In doing this I have drawn heavily on the historical material assembled by Professor Peter Harper, whose articles should be consulted for more detail.

The story of the ESHG is a story of two halves. The first 24 years, up to 1991, were dominated by one man, Professor Jan Mohr of Copenhagen. When he agreed with a small group of colleagues to start a European Society of Human Genetics his vision was of a stripped-down organisation with minimal administrative tasks. There were no elected officers, just a permanent secretary (himself for all those years) and an unchanging 20-member Board. Virtually their only function was to nominate somebody each year to organise a symposium on some aspect of human genetics. The society gave no financial support for this - it hardly could, with only around 200 members whose subscription was $7.

By 1988 it had become clear that something more was needed if the ESHG was to become a significant force in developing a European human genetics community. Revolutionary moves culminated at the meeting in Leuven in 1991 where a rotating president and officers were elected, and statutes adopted formally incorporating the society under Belgian law. The society's journal, the European Journal of Human Genetics, was established shortly afterwards and the modern ESHG was born. We now have an annual turnover of over 2 million euros, professional administration through Jerome del Picchia and his team at the Vienna Medical Academy, and an important voice in European and international developments in human genetics. Jan Mohr (who died in 2009) might have mixed feelings about some of these developments but surely he would agree that this 2017 meeting is the best human genetics conference ever.

A. Read: None.

PL1.2
How will the present time in genetics be remembered?

H. Brunner[1,2]

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At 50 years, the Society of Human Genetics has confidently assumed its current position as a pillar of modern medicine. Is ours a special time? Have we reached the

high-point of Human Genetics? I don't think that the history of human genetics ends here at all. Rather, it seems that fundamental change is still very much in the air. First, let's celebrate that after more than 100 years, the battle between the Mendelians and Biometricians has finally ended. And it is a draw. Mendelian rare conditions mainly reflect gene disruptions subject to strong selective forces, whereas common complex disease largely reflects common polygenic regulatory variation of gene activity. Sometimes these mix, as for the major neurodevelopmental disorders: Intellectual disability, Autism, and Schizophrenia are mostly due to a mix of polygenic and de novo single gene variants. The Mendelian forms being associated with lower IQ. As Galton predicted in the 19th century, height is generally polygenic and Peter Visscher's work has used this data to show that most of the heritability is not missing but undetected, because the effects of individual loci are too small to pass stringent significance tests. Work from Sardinia shows that some of the alleles that confer short stature affect known Mendelian genes, which have drifted up to appreciable frequencies in this population to create the well-known island effect on height. After a period of frenzied activity following the invention of next generation sequencing, the era of monogenic disease gene discovery has passed its peak. At the same time, our ability to see pathogenic variation rather than infer it, is fundamentally changing medicine and our societies. Rare diseases have become a recognized part of medicine, not just curiosities. One such area where gene-based discoveries are changing medicine fast is cancer therapy. The understanding of the gene composition of the Philadelphia chromosome led to the first targeted cancer drug, Imatinib. Other examples followed such as EGFR mutations in lung cancer. Soon, all women with breast and ovarian cancer will be tested for BRCA1 and BRCA2 mutations, to guide their therapy. Similar testing could happen for male patients with prostate cancer. All this will lead to an enormous increase in the detection of people with genetic predisposition for cancers, which constitutes an interesting example of how Molecular Pathology and Mendelian predisposition genetics come together. Work by Ségolene Ayme and others has led to the recent launch of the European Reference Networks whose purpose is to improve diagnosis and care for rare diseases patients across Europe. NIPT represents another major triumph for the power of next generation sequencing technology. It is almost incomprehensible how NIPT seemed such a distant goal for so many years. The diagnostic rate of exome sequencing and microarrays is between 20 and 60% for most diagnostic situations where Mendelian disease is sought. This experience underlines that the genome is not entirely readable yet. Progress requires single cell techniques for mosaic situations, long read sequencing for repetitive elements and other genomic dark matter, and whole genome sequencing to capture smaller genomic rearrangements. All of these will come. Still, reading the genome does not equal understanding it. Fortunately, Functional Genomics is about to come of age, now that the groundwork has been done with the Hapmap, ENCODE and Blueprint projects. The future of Human Genomics will be radically different as we start to study Traits rather than States, and this at the single cell level and in real time. Multi-omics approaches will arrive soon, as will innovative gene therapy approaches. The future may turn out much more surprising and informative than any of us can now predict.

H. Brunner: None.

PL1.3
The Future: Solved Problems and Persisting Challenges

A. Visel[1,2,3]

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Groundbreaking discoveries by individual researchers, disruptive technological advancements, and massive-scale data collection efforts by large research consortia have fundamentally transformed the field of human genetics. The introduction of powerful data generation and analysis techniques fueled the emergence and rapid growth of the field of genomics as a data-driven science. Researchers now routinely apply genomic approaches to resolve the genetic basis of human diseases in ways that were unimaginable just a few decades ago. Extrapolating from the mind-boggling pace of progress to date, I will attempt to speculate about the future of human genetics and genomics in the decades ahead. Specifically, I will discuss ways in which many current challenges will likely become resolved through foreseeable technological improvements. More importantly, I will describe problems that are expected to present persistent conceptual challenges. In particular, I will discuss the continued conquest to decipher the vast non-coding portion of the human genome, our current difficulties in understanding its function, and barriers to identifying connections between non-coding sequence changes and human disease.

A. Visel: None.

PL2
What's New? Highlights Session

PL2.1
Enhancer composition and dosage control developmental gene expression

A. J. Will[1,2], G. Cova[1,2], M. Osterwalder[3], W. Chan[1,2], N. Brieske[1], A. Visel[3], E. Klopocki[4], D. G. Lupiáñez[1,2], S. Mundlos[1,2]

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Gene expression is controlled by enhancers, cis-regulatory elements that are often located in clusters displaying redundancy in reporter assays and similarities in transcription factor occupancy. Copy number variations (CNVs) of such elements can be associated with disease, induced by yet unclear pathomechanisms. At the *Indian Hedgehog* (*Ihh*) locus, for example, duplications result in craniosynostosis, syndactyly, and polydactyly, phenotypes not previously associated with Ihh function. Here, we dissect the regulation of *Ihh* in vivo and show that a cluster of at least 8 enhancers with individual tissue-specificity regulates *Ihh* expression in the digit anlagen, growth plates, skull sutures and digit tips. To investigate how gene expression responds to systematic variations in the number of regulatory elements, we generated mutants using CRISPR/Cas9 and investigated their phenotypes. Consecutive deletions of enhancer elements show that they function in an additive manner resulting in growth defects of the skull and long bones. Duplications, in contrast, cause tissue-specific upregulation of *Ihh* leading to fusion of sutures in the skull and misexpression in the distal interdigital space causing syndactyly, polydactyly and abnormally shaped phalanges. Chromosome conformation capture (4C) of the region shows that the duplications result in specific regulatory configurations that explain the appearance of the pathogenic phenotypes.

In summary, this study shows that CNVs of non-coding regulatory elements can result in specific regulatory abnormalities including gene over- and misexpression. The composition of individual enhancer elements and their relative dosage within a cluster confer precision of spatio-temporal gene expression.

A.J. Will: None. **G. Cova:** None. **M. Osterwalder:** None. **W. Chan:** None. **N. Brieske:** None. **A. Visel:** None. **E. Klopocki:** None. **D.G. Lupiáñez:** None. **S. Mundlos:** None.

PL2.2
Quantifying the impact of rare coding variation across the phenotypic spectrum

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Whole exome sequencing (WES) studies enable us to determine the impact of rare coding variation on complex traits. Here we assemble WES data on 100,289 individuals from a combination of cohort studies with electronic health records (EHR) and case/control disease studies to evaluate the role that a burden of rare (<0.1% allele frequency) loss of function (LoF) variants in 3,172 highly evolutionarily constrained genes (HC) plays in conferring risk for 13 quantitative traits and 10 diseases. Carriers of at least one HC-LoF variant had increased risk of autism, schizophrenia, bipolar disorder, intellectual disability and ADHD (P-values (p) range: 8×10^{-4}–1×10^{-14}). The effect was stronger for individuals diagnosed with > 1 disorder, but also significant in those without comorbidities. In controls without any aforementioned disorder, we observed a significant association with the broader ICD-10 category of mental/behavioral disorders. Furthermore, carriers of HC-LoF variants tended to be shorter ($p = 2 \times 10^{-4}$), have fewer years of education ($p = 3 \times 10^{-4}$) and tended to be younger ($p = 5 \times 10^{-7}$); the latter observation possibly reflecting reduced survival or study participation. Other gene-sets (ClinVar genes, mice or cell lethal genes) did not show any LoF-burden associations, however GWAS-derived gene-sets implicated in lipids and myocardial infraction showed a significant LoF-burden with the corresponding traits. Finally, using EHRs of 14,709 individuals, we performed a phenome-wide scan and identified a significant association between HC-LoF and chronic kidney failure ($p = 2 \times 10^{-6}$) and with number of hospital visits ($p = 0.0014$). In

conclusion, we describe the signature of rare deleterious coding variants on multiple complex traits.

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PL2.3
De novo gain-of-function mutations in the epigenetic regulator SMCHD1 cause Bosma arhinia microphthalmia syndrome

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Bosma arhinia microphthalmia syndrome (BAMS) is an extremely rare and striking condition characterized by complete absence of the nose (arhinia) with or without ocular defects. Arhinia is presumed to result from a specific defect of the nasal placodes or surrounding neural crest-derived tissues during embryonic development. By exome sequencing we identified missense mutations in the extended ATPase domain of the epigenetic regulator Structural Maintenance of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) as the cause of BAMS in all 14 cases studied. All mutations were de novo where parental DNA was available. ATPase assays using wildtype or mutant versions of purified SMCHD1 protein indicated that the BAMS mutations increase the catalytic activity of the protein. In overexpression assays in Xenopus embryos we observed that injection of SMCHD1 RNA harboring BAMS mutations resulted in more severe frontonasal and eye hypoplasia than injection of wildtype SMCHD1. These functional assays suggest that the BAMS mutations behave as gain-of-function alleles. This is in contrast to loss-of-function mutations in SMCHD1 that have been associated with facioscapulohumeral muscular dystrophy (FSHD) type 2, a disorder with no phenotypic overlap with BAMS. In FSHD type 2, loss of the epigenetic silencing activity of SMCHD1 results in pathogenic misexpression of the transcription factor DUX4 in skeletal muscles. Our results establish SMCHD1 as a key player in nasal development and provide biochemical insight into its enzymatic function that may be exploited for development of therapeutics for FSHD.

C.T. Gordon: None. **S. Xue:** None. **G. Yigit:** None. **H. Filali:** None. **K. Chen:** None. **N. Rosin:** None. **K. Yoshiura:** None. **M. Oufadem:** None. **T. Beck:** None. **C. Dion:** None. **A. Sefiani:** None. **H. Kayserili:** None. **J. Murphy:** None. **C. Chatdokmaiprai:** None. **A. Hillmer:** None. **D. Wattanasirichaigoon:** None. **S. Lyonnet:** None. **F. Magdinier:** None. **A. Javed:** None. **M. Blewitt:** None. **J. Amiel:** None. **B. Wollnik:** None. **B. Reversade:** None.

PL2.4
Genetic variation in the Estonian population: a pharmacogenomic study of adverse drug reactions using electronic health records

K. Krebs[1], T. Tasa[1,2], M. Kals[1], R. Mägi[1], T. Esko[1], A. Metspalu[1], J. Vilo[2], L. Milani[1]

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Introduction: Advances in next generation sequencing (NGS) technologies coupled with electronic health records (EHR) have provided new opportunities for the interpretation of the role of genetic variation in different diseases and traits. Pharmacogenomics applies NGS methods to document genetic variants important in drug response with the ultimate goal of reducing the negative effects of variability in drug response including adverse drug reactions (ADR). Here we performed a large scale study of genes important in drug response of Estonian biobank participants by using comprehensive information from Estonian e-health databases.

Materials and Methods: We sequenced the whole genomes of 2,240 participants of the Estonian biobank using PCR-free sample preparation and 30x coverage at the Broad Institute, and further included all variants imputed for 14,219 genotyped subjects in the Estonian biobank. We identified ADR diagnoses using ICD10 codes, and prescribed drugs by searching the participants' records from the Estonian Health Insurance Fund.

Results: When focusing on 64 genes important in drug response we observed 622 variants in the coding regions and 388 variants in the regulatory regions of these genes. By linking with the EHRs we were able to validate several previously documented genetic variants associated drug induced ADRs. By genome wide analysis we identified a novel gene, CTNNA3, to associate with ADRs among individuals treated with oxicams. The finding replicated in an extended cohort from the biobank.

Conclusion: EHRs together with genotype information and additional thorough validation of ADRs are a way to find individuals potentially at risk for unexpected drug response.

K. Krebs: None. **T. Tasa:** None. **M. Kals:** None. **R. Mägi:** None. **T. Esko:** None. **A. Metspalu:** None. **J. Vilo:** None. **L. Milani:** None.

PL2.5
From pathogenic mechanism to a therapeutic approach in Spinocerebellar Ataxia 38 (SCA38)

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ELOVL5 gene is associated with autosomal dominant Spinocerebellar Ataxia 38 (SCA38, MIM#611805), a rare adult-onset cerebellar neurodegeneration. *ELOVL5* encodes for an elongase, an enzyme with a critical role in regulating the amount of a subset of polyunsaturated fatty acids (PUFAs). We explored pathogenic mechanism of SCA38, studying aberrant ELOVL5-p.Gly230Val protein. In COS7 cells, expressing ELOVL5-p.Gly230Val, we demonstrated mutant protein alters Golgi-ER transport, and accumulates into Golgi apparatus. We also showed unfolded protein response (UPR) activation by a significant increase of CHOP, ATF-4 and XBP1 markers.

Nevertheless, in *Elovl5*-ko mice, we showed a significant motor impairment at 3 months using the balance beam test ($P < 0.001$), and at 6 months using the rotarod test ($P < 0.05$). At 12 months, we identified a reduction in the thickness of the third cerebellar lobule molecular layer ($P < 0.05$). Overall, a dual gain-/loss-of-function mechanism is likely in this disorder.

Based on this assumption and considering that fatty acids produced by ELOVL5 have a feedback negative loop, reducing *ELOVL5* gene expression, we designed double-blind placebo-controlled study involving 10 SCA38 patients using PUFAs. We conducted an initial 16-week trial (600 mg/day DHA vs. placebo, 1:1), followed by 24-week single-blind open-label study. We observed a significant clinical improvement of neurological symptoms in treated subjects as compared to patients under placebo ($P = 0.02$). The effect of DHA treatment was enhanced in the open-label phase, where a significant improvement of both clinical symptoms and cerebellar metabolism was observed.

These findings suggest DHA supplementation is an effective treatment for SCA38.

E. Di Gregorio: None. **M. Ferrero:** None. **M. Manes:** None. **E. Hoxha:** None. **L. Boccone:** None. **L. Orsi:** None. **N. Mitro:** None. **D. Caruso:** None. **A. Alberici:** None. **A. Padovani:** None. **S. Cavalieri:** None. **E. Giorgio:** None. **C. Mancini:** None. **E. Pozzi:** None. **E. Riberi:** None. **R. Gabriele:** None. **I. Balbo:** None. **L. Masante:** None. **V. Zambelli:** None. **M. Maldini:** None. **M. Sallese:** None. **F. Tempia:** None. **B. Borroni:** None. **A. Brusco:** None.

PL2.6
Analysis of de novo mutation clustering identifies candidate disease genes in neurodevelopmental disorders due to likely gain-of-function and dominant-negative mechanisms

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Haploinsufficiency (HI) is the most common mechanism through which mutations exert their effect and cause disease. Typically, these pathogenic mutations are spread throughout the gene, and result in absence of protein product. In contrast, non-haploinsufficiency (NHI) mechanisms, caused by gain-of-function/dominant-negative missense mutations, are often characterized by the spatial clustering of mutations within a gene. Here we exploited this property and developed a method to specifically identify genes with significant spatial clustering patterns for de novo mutations. We applied our method to a dataset of

4,043 de novo missense mutations from published exome studies of patient-parent trios with (neuro)developmental disorders (NDDs). Among the 15 genes that we identified there was a strong enrichment for known NDD genes (12 out of 15, p = 1.65e-04) thereby validating our approach. Strikingly, 11 out of these 12 genes are known to act through a disease mechanisms other than HI. Interestingly, identified genes are significantly less tolerant to population variation than known HI genes (p = 8.59e-03). The 3 genes that were not previously linked to NDDs are involved processes that are known to be disrupted in NDDs. Finally, we performed 3D-modeling of protein structures to show that, unlike known HI genes, clustering mutations are unlikely to affect protein folding and more likely to disturb protein interactions/complex formation. In summary, we show that our method successfully identifies known NDD disease genes and that these are strongly enriched for disease mechanisms other than HI. We identify further 3 genes with similar patterns and propose these as novel NDD candidate genes.

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PL3
ESHG-ASHG Building Bridges Debate: Ethical and Legal Discussions - Past, Present & Future

PL3.1
Reflecting on ethics in genetics: The past, present and future

R. Chadwick

University of Manchester, Manchester, United Kingdom

What is sometimes called 'gen-ethics' has gradually expanded its scope as it has moved out of the genetics clinic to have application in health care in general and in multiple other aspects of life. As the focus of debate has moved from genetic counselling and eugenics to the promises of therapies and enhancements; genomic research and data sharing; prediction and personalisation; the ethical frameworks have also been tested. Traditional thinking on informed consent and privacy has been challenged. A more 'personalised' future may require new thinking about the concept of 'person' itself.

R. Chadwick: None.

PL3.2
Going from perceptions of genetic risk to the balancing of benefits and risks

M. Hansson

Uppsala University, Uppsala, Sweden

Perceptions of genetic risk information and how this kind of information may affect the individual as well as relatives to the index patient have been studied thoroughly since many years in many patient populations. Examples of issues that have been extensively researched are: i) how at risk individuals and patients understand their risk of disease after testing and genetic counselling; ii) their emotional responses to the information; iii) the effects of the information on aspects related to quality of life; iv) the influence of testing on family dynamics and, v) their uptake of recommended risk-reducing strategies. Apart from the last item concerning the relationship between genetic risk information and changes of behaviour, this research field is mature. New technological tools challenges, however, established genetic risk information strategies. WGS will reveal information that is not related to the main condition being investigated, leading to questions on how to best inform about incidental/unsolicited findings. Complex diseases come with both genetic risk and risks related to environmental and life style factors where the individual may play a significant role in modifying the total risk. From a historical perspective we see now a methodological development in genetic risk information where the focus is not only on what matters to the patient or how much it matters but how he/she prefers the trade off between benefits and risk. Methods like Discrete Choice Experiments and Best Worst Scaling may help us to get our hands on not only perceptions of risk but how the patient wants to balance these risks against expected benefits. I will in this presentation briefly describe this development.

M. Hansson: None.

PL3.3
The Evolution of Genetic Counseling: Effectively Meeting Our Clients' Needs

B. B. Biesecker

National Human Genome Resaerch Institute/National Institutes of Health, Bethesda, MD, United States

Over the past forty years the clinical practice of genetic counseling has expanded from prenatal and pediatric

patients to include adults at risk for common disease (oncology, cardiology, neurology, psychiatry) and healthy adults pursuing predictive testing. Across all settings we have responsibilities to help clients make informed choices about use of genetic testing and cope effectively with increased risk or genetic conditions in their families. Our psycho-educational practice comprises teaching genetic concepts alongside providing psychological counseling to enhance decision making and adaptation to genetic risk and conditions. A systematic review of 25 years of randomized controlled trials involving genetic counseling outcomes reveals that telephone counseling is as effective as in-person counseling for patients at increased risk of inherited cancer. An intervention study prompting genetic counselors to attend to topics of interest to their clients led to more targeted and valued sessions within a similar timeframe. These outcomes illustrate ways to streamline services and improve its effectiveness. Yet studies of communication in genetic counseling demonstrate dominant speech by genetic counselors and use of technical language at a high literacy level. This pattern limits clients' understanding in failing to engage them in a productive dialogue. The use of therapeutic counseling interventions is minimal compared to information provision, suggesting a lack of opportunity for shared decision making and enhanced psychological wellbeing—the predominant outcome of randomized trials in genetic counseling. With the onslaught of additional information and uncertainties emanating from genome sequencing we face a challenge that genetic counseling may become further dominated by technical information with less time spent on processing the potential value of the information for the client. New evidence-based practice models are greatly needed to maximize the opportunities of genetic counseling to meet our clients' needs.

B.B. Biesecker: None.

PL3.4
From Medical Genetics to Applied Genomics: Implications for Human Geneticists' Core Goals and Values

E. Juengst

Chapel Hill, NC, United States

As a biomedical field, human genetics since c. 1960 has subscribed to a very distinctive ethos when dealing with genetic health problems. Long before "patient self-determination" and "shared decision-making" became everyday words in other health care fields, clinical geneticists have made a point of respecting their clients' beliefs, values, and goals, even if their choices carried risks for themselves, their families or the wider society. When translational genomic research began to make genetic risk assessment relevant across multiple medical specialties, geneticists took their client-centered ethos along, through the vision of a "personalized genomic medicine" that would both tailor care to patient's molecular profiles and also empower them to take a more active role in their health care. The theoretical goal of "precision medicine" is to take the next step, by integrating genetic risk data with information about patients' specific social histories and environmental exposures, to reveal actionable "epigenetic" factors that influence their risks. Along this route, however, evidence from the human genetics literature suggests that odd things are happening. The genomic variation studies that are necessary to achieve human genetics' molecular goals are reorienting the field from individuals to populations, by suggesting public health applications that depend for their success on individual decisional conformity, not autonomy. As genomic sequencing tools becomes integrated into mainstream medicine, more traditional forms of medical paternalism seem to be influencing genetics, limiting rather than expanding, the patient's role in decision-making about medical information. And as genomic applications move further into anthropology, forensics, national security, international sports, and the marketplace, the field moves further from a health focus, making "Applied Genomics" an apt label for even its human uses. For precision medicine to move from being a scientific dream to a clinical reality, geneticists will have to decide whether these trends reflect healthy ethical evolution or moral compromise.

PL4
Mendel Lecture

PL4.1
Mendel Lecture: Reading and Writing Genomes

G. Church

Boston, MA, United States

By 1863 Gregor Mendel could "read" the state of seven sites in the pea genome by their impact on visible traits and could "write" genomes by mating randomly mutagenized plants. Today, we can routinely read 95% of the 6 billion basepairs of the human genome using and fluorescent or nanopore sequencing and many other "omes" and traits at sub-cellular resolution using in situ sequencing (FISSEQ). Today, we can "write" billions of

basepairs of genomic DNA on chips and change 320 sites in a cell at once to radically change the fundamental triplet genetic code that connects genotypes to phenotypes. We can engineer multi-virus resistant cells, humanized pigs for transplantable organs, and wild animal populations to eliminate Malaria, Lyme disease and invasive species. We already have many genetically modified humans. These technologies are changing exponentially, increasing the need for thoughtful and thorough discussion of the desired trajectories without prematurely dismissing alternative paths as impractical.

PL5
ESHG Award Lecture

PL5.1
ESHG Award Lecture: X-chromosome structure and epigenetic dynamics during X inactivation

E. Heard

Institut Curie, Unité de Génétique et Biologie du Devéloppement, Paris, France

X-chromosome inactivation during early female development is an essential epigenetic process that is required to achieve appropriate dosage for X-linked gene products. We are interested in understanding how the differential treatment of the two X chromosomes in the same nucleus is set up during development and how this differential expression is then maintained, or reversed in certain circumstances such as the inner cell mass of the mouse embryo or in the germ line. The establishment of X inactivation involves the non-coding Xist RNA that triggers chromosome-wide chromatin re-organisation and gene silencing. Recent insights have been made into the nature of these chromosome-wide changes and the factors that mediate them. The inactive X is folded into a unique bipartite, heterochromatic structure that tends to lack topologically associated domains (TADs), except at regions of escape. However little is known about the molecular mechanisms underlying X inactivation and in particular, the degree to which 3D X-chromosome structure is a cause or a consequence of gene expression. Our recent studies have focused on the degree to which organization into TADs (i) influences monoallelic *Xist* regulation and (ii) participates in regional escape from X inactivation on the inactive X chromosome.

E. Heard: None.

Concurrent Symposia

S01
Single cell studies: From technology to biology

S01.1
Single cell RNAseq-based characterisation of adult stem cells

B. Deplancke

Lausanne, Switzerland

This presentation will consist of three mostly unpublished parts. First, I will describe our recent efforts characterizing the heterogeneity of adipose stem cells. Using single cell transcriptomics, we were able to identify three distinct and novel subpopulations. One population is thereby of particular interest since these cells are refractory to fat cell differentiation despite their stem cell-like characteristics and also appear to have repressive capacity. This raises the hypothesis whether a dysbalance in this population may lead to fat cell accumulation and thus obesity. More generally, it reveals the power of single cell genomics in uncovering new, biomedically relevant cell populations. In a second part, I will briefly describe our efforts to generate a novel web tool supporting the automated analysis of single-cell RNA-seq data. This Automated Single-cell Analysis Pipeline (ASAP) can be accessed at https://asap.epfl.ch/ and should greatly facilitate the implementation of single cell genomics in non-expert labs. I will end by presenting our recently developed single cell-based classifier to identify somatic stem and progenitor cells in heterogeneous populations. I will show how this classifier enables the identification of stem-like cells in still ambiguous systems such as the pancreas and the epidermis. In addition, it aids in the exploration of lineage commitment hierarchies, thus facilitating the study of biological processes such as cellular differentiation, tissue regeneration, and cancer.

S01.2
Towards single-cell proteomics: Unraveling cell populations in health and disease by single-cell mass cytometry

S. Chevrier

Institute of Molecular Life Sciences, Zurich, Switzerland

No abstract received.

S. Chevrier: None.

S01.3
Singlecell RNA-seq unveils the molecular diversity of midbrain development in human,mouse and stem cells

G. La Manno

Karolinska Institutet, Unit of Molecular Neurobiology, Stockholm, Sweden

Understanding the embryonic development of human ventral midbrain is of major interest for Parkinson's disease. However, the heterogeneity of cell types developing in this area and the level of similarity between human embryo and rodent models remains unclear. We transcriptomically profiled ventral midbrain development in human, mouse and stem cell cultures, at the single cell level. By the means of unbiased cell type discovery, species comparison and time course analysis we gained quantitative insights into progenitors heterogeneity, cell type conservation and dopaminergic neuron specification. Finally, we used the single-cell data to train a machine-learning algorithm to assess the composition and quality of stem-cell-derived preparations for cell replacement therapy.

G. La Manno: None.

S02
One gene, many phenotypes

S02.1
Filaminopathies

S. P. Robertson

University of Otago, Dunedin, New Zealand

Mutations affecting any one of the three filamin genes (*FLNA, FLNB* and *FLNC*) give rise to a heterogeneous group of no less that 16 different clinical disorders. Since their first description beginning in 2003, a deeper understanding of their clinical presentation has emerged, linking phenotypes with the location of causative mutations and/or their effect on gene function. These linkages assist in planning the clinical evaluation of individuals with potential filaminopathy phenotypes and assigning a likelihood of pathogenicity for some variants of uncertain significance in these genes. Assisting an understanding of the pathogenesis of these disorders has been the discovery of mutations in additional loci resulting in close phenocopies of these conditions, leading to a deepening understanding of how these actin binding cytoskeletal proteins regulate morphogenesis.

S.P. Robertson: None.

S02.2
Laminopathies

N. Levy

Marseille, France

No abstract received.

S02.3
Disruption of Na^+ binding in alpha-3 Na^+,K^+-ATPaseby neurological disease mutations and rescue by second-site mutation

B. Vilsen[1], R. Holm[2], C. P. Rønn[2], M. S. Toustrup-Jensen[2], A. P. Einholm[2], V. R. Schack[2]

[1]Aarhus, Denmark, [2]Aarhus University, Aarhus, Denmark

The Na^+,K^+-ATPase is an ion pump that uses energy liberated by hydrolysis of ATP to exchange intracellular Na^+ for extracellular K^+, thus creating essential gradients for Na^+ and K^+ across the cell membrane. High resolution crystal structures of Na^+- and K^+-bound states have shown where in the protein structure the ions bind. Of the three Na^+ sites (I, II, and III), site III is most specific for Na^+. Missense mutations in the genes encoding the α2- and α3-isoforms of the Na^+,K^+-ATPase, expressed in glia and neurons, respectively, cause neurologic disorders. A high proportion of the α3 disease mutations occur in the transmembrane sector and several seem to affect Na^+ binding at site III. Hence, we have shown that rapid-onset dystonia parkinsonism (RDP) and alternating hemiplegia of childhood (AHC) mutations F780L, D923N, and Y1013dup disturb Na^+ binding selectively without effect on K^+ binding. The CAPOS (Cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss) mutation E818K is likely to disturb Na^+ site III by perturbation of a crucial hydrogen bonding network apparent in the crystal structure. Recently, we found that a secondary mutation rescues the defective Na^+ binding at site III caused by RDP/AHC mutation D923N. Here I also present data showing rescue of the compromised function of additional neurological disease mutants. A perspective is that it may be feasible to develop an efficient pharmaceutical mimicking the rescuing effect, which optimally would rescue the compromised function of a variety of α3-disease mutants with reduced affinity of Na^+ site III.

R. Holm: None. **C.P. Rønn:** None. **M.S. Toustrup-Jensen:** None. **A.P. Einholm:** None. **V.R. Schack:** None.

S03
Novel Treatment Options

S03.1
Emerging targeted drug therapies in skeletal dysplasias

R. Savarirayan

Murdoch Childrens Research Institute, Melbourne, Australia

Quantum advances have occurred in the field of human genetics in the six decades since Watson and Crick fulfilled their "wish to suggest a structure for the salt of deoxyribose nucleic acid." These culminated with the human genome project, and the ability to deliver medical advice, management, and therapy tailored to a specific genetic blueprint (individualised medicine). Advances in molecular diagnostic capabilities have been rapid, to the point where the genome can be sequenced for several thousand dollars within a week. Crucially, this genomic revolution has facilitated the identification of targets for "precision" treatments to combat genetic diseases at their source. Disruptive, pathogenesis-based therapies are now revolutionising the management of inherited disorders of cartilage and bone (skeletal dysplasias), changing their natural history, and giving patients and families new options and outcomes. These include the use of C-natriuretic peptide in achondroplasia and recombinant tissue non-specific alkaline phosphatase in hypophosphatasia. This presentation will review the current status of these emerging therapies in clinical trials and practice, and future potential targeted therapeutic options for skeletal dysplasias.

R. Savarirayan: None.

S03.2
Gene therapy of myotubular myopathy

A. Buj Bello

Genethon / INSERM UMR 951, Evry, France

Mutations in the myotubularin gene (*MTM1*) result in X-linked myotubular myopathy (XLMTM), a pediatric disease of skeletal muscle characterized by small centrally nucleated myofibers containing abnormal mitochondrial accumulations. Patients typically present with severe hypotonia and respiratory failure, and most of them die during early infancy. We have developed a gene therapy approach to treat this disease and shown the efficacy of intravenous injection of a unique dose of a recombinant adeno-associated viral vector expressing myotubularin in mouse and dog models of the disease, leading to long-term correction of the muscle phenotype. Results from a dose escalation study aiming at defining the therapeutic dose in XLMTM dogs will also be presented. These preclinical results support the development of a gene therapy clinical trial in patients with myotubular myopathy.

A. Buj Bello: F. Consultant/Advisory Board; Significant; Audentes Therapeutics.

S03.3
Pronuclear transfer to prevent mitochondrial DNA disease (Mito therapy)

M. Herbert

Newcastle, United Kingdom

No abstract received.

S04
From Association to Causality in complex diseases

S04.2
Efficient fine-mapping of genome-wide association study results

M. Pirinen, C. Benner

Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland

Typically genomic regions pinpointed by GWAS contain hundreds of correlated variants and a key step in transforming GWAS information into biological insights is fine-mapping, narrowing down the set of all variants to a much smaller set of most probable causal variants. This talk outlines recent ideas that have made computational fine-mapping practical in human genomics and demonstrates them through our experience with the FINEMAP algorithm. Key components include: (1) Compressing data to light-weight summaries to avoid logistics and privacy concerns related to complete data sharing and to minimize the computational overhead, (2) Efficient implementation of sparsity assumptions and (3) Efficient search algorithms. An important practical question is to what degree we can rely on linkage disequilibrium estimates from external reference data when fine-mapping large GWAS meta-analyses of traits and diseases using summary statistics.

M. Pirinen: F. Consultant/Advisory Board; Significant; Genomics plc. **C. Benner:** None.

S04.3
Integration of eqtl and gwas to find susceptibility genes for complex traits

B. Pasaniuc

Los Angeles, CA, United States

No abstract received.

S05
3D genome architecture: non-coding variants and human disease

S05.1
A 3D Code in the Human Genome

E. Lieberman Aiden

Houston, TX, United States

Stretched out from end-to-end, the human genome - a sequence of 3 billion chemical letters inscribed in a molecule called DNA - is over 2 meters long. Famously, short stretches of DNA fold into a double helix, which wind around histone proteins to form the 10nm fiber. But what about longer pieces? Does the genome's fold influence function? How does the information contained in such an ultra-dense packing even remain accessible? In this talk, I describe our work developing 'Hi-C' (Lieberman-Aiden et al., Science, 2009; Aiden, *Science*, 2011) and more recently '*in-situ* Hi-C' (Rao & Huntley et al., *Cell*, 2014), which use proximity ligation to transform pairs of physically adjacent DNA loci into chimeric DNA sequences. Sequencing a library of such chimeras makes it possible to create genome-wide maps of physical contacts between pairs of loci, revealing features of genome folding in 3D. Next, I will describe recent work using in situ Hi-C to construct haploid and diploid maps of nine cell types. The densest, in human lymphoblastoid cells, contains 4.9 billion contacts, achieving 1 kb resolution. We find that genomes are partitioned into contact domains (median length, 185 kb), which are associated with distinct patterns of histone marks and segregate into six subcompartments. We identify ~10,000 loops. These loops frequently link promoters and enhancers, correlate with gene activation, and show conservation across cell types and species. Loop anchors typically occur at domain boundaries and bind the protein CTCF. The CTCF motifs at loop anchors occur predominantly (>90%) in a convergent orientation, with the asymmetric motifs "facing" one another. Next, I will discuss the biophysical mechanism that underlies chromatin looping. Specifically, our data is consistent with the formation of loops by extrusion (Sanborn & Rao et al., *PNAS*, 2015). In fact, in many cases, the local structure of Hi-C maps may be predicted *in silico* based on patterns of CTCF binding and an extrusion-based model. Finally, I will show that by modifying CTCF motifs using CRISPR, we can reliably add, move, and delete loops and domains. Thus, it possible not only to "read" the genome's 3D architecture, but also to write it.

S05.2
Long Range regulation of mammalian gene expression

D. Higgs

Weatherall Institute of Molecular Medicine, Oxford, United Kingdom

Our laboratory studies how transcriptional and epigenetic programmes are played out in chromatin spanning the human and mouse globin loci as haematopoietic cells undergo lineage fate decisions and differentiation. Our aim is to understand the principles by which all mammalian genes are switched on and off during cell fate decisions. Globin gene expression is controlled by a group of conserved regulatory elements some of which lie within the introns of an adjacent widely expressed gene (Nprl3) and another lies in intergenic DNA. All of these elements have the chromatin signature of enhancer elements. Using Chromosome Conformation Capture, we have shown that they physically interact with each other and with the globin gene promoters, and together are essential for normal globin gene expression. From genome-wide studies, this configuration appears to be a common feature of highly expressed, lineage-specific genes and such groups of regulatory elements have more recently been called "super-enhancers". Using homologous recombination we have deleted individual elements and combinations of elements to investigate how they may work in concert to regulate gene expression. We find that no element on its own is indispensible for globin gene expression and despite their superficially common features they have radically different effects on nascent transcription. However, removal of two critical elements virtually abolishes globin expression raising the question of what is the role of the other elements in and around the superenhancer? One possibility is that the configuration of the five enhancer-like elements provides some polarity to the enhancer complex. We have tested this by inverting the entire enhancer structure within its natural locus and observing the effect of this on globin expression

in vivo with dramatic effects. In the proposed project we plan to investigate in further detail how these *cis*-acting elements work together within the broader context of the chromosomal environment. We have recently performed Hi-C experiments and have defined the Topologically Associated Domain (TAD) containing the globin gene cluster in erythroid and non-erythroid cells. We will next investigate how activation, deletion and re-orientation of the globin regulatory elements affect expression of other genes within the same TAD and in neighbouring TADs. Importantly, using globin as our model, we will address the general question of the relationship between higher order, long-range chromosomal structure and function.

D. Higgs: None.

S05.3
Structural variants cause 3D confirmational changes

S. Mundlos

Max-Planck-Institut für Molekulare Genetik, Charité, Berlin, Germany

S. Mundlos: None.

S06
Treatment-Focused Genetic Testing in Cancer

S06.1
Circulating tumor DNA in cancer monitoring

E. Heitzer, P. Ulz, J. B. Geigl, M. R. Speicher

Medical University of Graz, Institute of Human Genetics, Graz, Austria

Due to the continuous development of targeted cancer therapies a comprehensive and longitudinal characterization of cancer genomes becomes increasingly important. However, repeated tumor sampling is challenging and therefore, the dynamics of tumor evolution, especially in metastatic cancers, remains incompletely characterized. One possibility to overcome this issue is the analysis of circulating tumor DNA (ctDNA), which is shed into the circulation from primary tumors and metastases. In addition to circulating tumor cells (CTCs), and tumor-derived exosomes, ctDNA enables a serial, non-invasive assessment of the genetic architecture of tumors. We have analyzed several hundred plasma DNA samples from patients with breast, prostate, colon, and lung cancer. Using a variety of targeted (hybridization based capturing and amplicon-based enrichment) and untargeted approaches (plasma-Seq, mFAST-SeqS) we have shown that changing levels of ctDNA correlate with the response to certain treatments. Furthermore, ctDNA analyses can be used to identify resistance mechanisms, e.g. in men with prostate cancer under androgen-deprivation therapy or in individuals with colorectal cancer under anti-EGFR therapy, or to detect novel emerging actionable targets such as *ERBB2*. Moreover, we were able to characterize the dynamics of clonal evolution of tumor genomes derived from plasma DNA. To facilitate the biological and clinical interpretability, we have developed innovative bioinformatics tools for pathway analyses and tumor genome stratification. In this presentation selected cases will demonstrate the potential clinical utility of ctDNA analyses. In addition, this presentation will focus on latest developments in ctDNA analyses. We have recently shown that also functional information can be retrieved from whole-genome sequencing (WGS) data. As plasma DNA is nucleosome protected DNA, the genomic sequencing coverage around transcription start sites (TSSs) shows distinct coverage patterns for expressed genes compared to unexpressed genes. Here examples how expressed oncogenes can be directly inferred from whole-genome sequencing of plasma DNA from breast cancer patients will be presented.

E. Heitzer: None. **P. Ulz:** None. **J.B. Geigl:** None. **M.R. Speicher:** None.

S06.2
CANCELLED - Next-generation sequencing: a change of paradigm in molecular diagnostics of cancer

D. González de Castro

Centre for Cancer Research and Cell Biology, QUB, Belfast, United Kingdom

Next Generation Sequencing (NGS) is playing an ever-increasing role in the diagnosis and patient stratification for precision medicine. Implementation of NGS strategies for the characterisation of cancer specimens in clinical practice requires particular approaches to overcome some of the inherent limitations of the process, including DNA degradation, pathological review of tumour content, tumour heterogeneity, reliable detection of different types of sequence variants and a robust bioinformatics pipeline to identify low level somatic mutations. Different technologies provide benefits and limitations, without a clear single approach that can be applied to all instances. Structural variation and copy number alterations are key genomic aberrations in oncology and pose significant challenges for

some NGS technologies, normally requiring alternative preparations to single nucleotide variation and small indels. Alternative sampling strategies using body fluids can partially overcome some of the limitations, and provide additional information for patient management. Finally, integration of NGS with other methodological approaches is required for accurate patient diagnosis and stratification and, therefore, a multidisciplinary approach with complementary technologies is the key to provide a viable precision medicine solution to cancer.

D. González de Castro: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; AstraZeneca, Roche Molecular Systems. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; AstraZeneca, Roche Molecular Systems, GSK/Novartis. F. Consultant/Advisory Board; Modest; AstraZeneca, Philips.

S06.3
Precision cancer medicine: translating laboratory studies into improvements in patient care

G. Capella

Catalan Institute of Oncology-IDIBELL, Barcelona, Spain

There are a number of prospective and predictive biomarkers routinely used in clinical practice for solid tumors that have an impact on patient care. Predictive biomarkers currently validated include: (i) levels of expression of hormone receptors for the prediction of response to hormone blockade in breast cancer; (ii) *HER2* expression levels for trastuzumab; (iii) *C-KIT* mutation in the prediction of response to imatinib in Gastrointestinal Stromal Tumors; (iv) *EGFR* mutations in the response to TKI inhibitors in non-small cell lung cancer (NSCLC); (v) *EML4/ALK* translocation in the response to crizotinib in NSCLC; (vi) *KRAS* and *NRAS* mutations as negative markers of response to EGFR blockade by cetuximab or panitumumab; (vii) *BRAF* mutations in the response to vemurafenib in advanced melanoma; (vii) *BRCA* germline mutation as predictor to response to PARP inhibitors in breast cancer. Critical issues in the translation of the preclinical findings to the clinical setting include the use of robust methodologies valid in suboptimal samples with clearly defined cut-off values. Regarding the design of the clinical studies a number of characteristics must be hIghlighted: (i) the use of response rate as a preferred endpoint; (ii) the focus in advanced diseases; (iii) preferably based in single-agent studies; (iv) an adequate power (v) a well-defined timing of sample acquisition; and (vi) an independent validation preferably in randomized trials. So far validation of gene signatures or other complex markers such as immune infiltrates has been lagging behind precluding a more widespread use of these technologies in the routine setting. Among the challenges to face in this implementation in the years to come include to accelerate the transition, to modify study design based on the lessons learnt and to promote structural changes favoring a biomarker-based drug development and a reimbursement of cost of biomarker analysis once its usefulness is shown.

G. Capella: None.

S07
Still the golden age of chromosomes

S07.1
The molecular pathogenesis of trisomy 21

S. E. Antonarakis

University of Geneva, Geneva, Switzerland

Trisomy 21 is the model human phenotype for all genomic gain dosage imbalances including microduplications. The functional genomic exploration of the post-sequencing years of chromosome 21 and the generation of numerous cellular and mouse models have provided an unprecedented opportunity to decipher the molecular consequences of the genome dosage imbalance. Transcriptome analyses of cellular models has revealed that the gene expression dysregulation caused by the trisomy 21 affects the entire genome, it is organized in large domains, and may be mediated by chromatin modifications. HiC experiments reveal altered long-range trans chromatin interactions that may be related to the dysregultion of gene expression. Single-cell fibroblast analyses has contributed to the dynamics of allelic expression of chromosome 21 genes, and provided new insights into the gene dosage mechanisms. The understanding of the molecular pathophysiology of Down syndrome may provide innovative treatment opportunities.

S.E. Antonarakis: None.

S07.2
Mosaic loss of chromosome Y (LOY) - not that normal benign phenomenon after all

L. A. Forsberg[1,2,3]

[1]Dept. Immunology Genetics and Pathology, Uppsala University, Uppsala, Sweden, [2]Beijer Laboratory of Genome Research, Uppsala, Sweden, [3]Science for Life Laboratory, Uppsala, Sweden

The life expectancy of men in developed countries is about 6 years shorter compared with women, but the underlying mechanism(s) are not well understood. Men with LOY in the peripheral blood harbor a mixture of cells with and without chromosome Y, as a consequence of post-zygotic mutations. Our findings indicate that LOY might be a key for understanding the sex difference in longevity.

We have investigated LOY in blood cells and its pathological consequences in several prospective as well as case-control studies, through collaborations and data sharing. Associations between LOY in blood cells and various outcomes were evaluated using a set of statistical techniques. Methods to estimate the degree of LOY mosaicism in a sample are based on quantifying a lower than expected abundance of DNA derived from the Y chromosome, in relation to reference loci from other parts of the genome. For example, data-tracks measuring DNA copy number in SNP-array experiments, read-depth in NGS-data and qPCR can be used.

Frequent LOY in hematopoietic cells was first described more than 50 years ago and was long considered neutral. In contrast, our results show that LOY in blood cells is associated with increased risk for all-cause mortality, as well as risk for various non-hematological diseases. For example, in a cohort called ULSAM, encompassing men between 70 and 93 years of age at blood sampling, we found that men with LOY in more than 1/3 of the nucleated blood cells had about two-fold risk for all-cause mortality during 20 years follow-up time. Furthermore, LOY in blood cells was associated with increased risk for mortality in non-hematological cancers as well as risk for diagnosis of Alzheimer's disease in this cohort. Analyses performed by us and others in independent cohorts have successfully reproduced these findings.

The causality behind associations between LOY in blood cells and increased risk for various diseases in other organs remains an open question. One hypothesis is that disrupted immune system function(s) in blood cells with LOY could play a role, leading to reduced protection from disease processes in different tissues of the body. Regardless of underlying mechanism(s), however, we envision that LOY in blood could become a predictive biomarker in future medicine.

LOY is the most common post-zygotic mutation and it accumulates with age. At least 20% of men older than 80 years are affected. Smoking mediates a four-fold increased risk in a dose-dependent and transient manner. Genome-wide analyses identified 19 DNA regions associated with acquired LOY in blood cells, including genes implicated in aneuploidy, genome instability and cancer susceptibility. As a male-specific genetic risk factor, an increased risk for pathology and mortality associated with LOY in blood cells, could help explain why men on average live shorter lives compared to women.

L.A. Forsberg: E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; CRAY Innovation AB.

S07.3
Introducing the emerging era of "Cytogenomics"

M. Talkowski

Boston, MA, United States

A new era of 'cytogenomics' is emerging in which technological advancements are being leveraged for new insights into chromosomal abnormalities at the level of the individual nucleotide. Dr. Talkowski's lab has integrated various molecular and computational genomics methods to evaluate the genomic landscape of balanced chromosomal abnormalities (BCAs). These analyses have revealed the remarkable complexity that can underlie their formation, often mediated by small inversions at the breakpoints, and the extreme examples of chromosome shattering, now recognized as 'chromoanagenesis' and 'chromothripsis', that can occur in the viable human germline and often resolve to a massive yet balanced reorganization of the chromosomes. These studies have also surveyed the contribution of de novo BCAs (translocations, inversions, insertions, and balanced chromoanagenesis) to human congenital anomalies and the remarkable reservoir of novel gene discovery possible from the delineation of BCAs in such individuals, as well as their diagnostics yields. These analyses also observed apparent 'hotspots' of BCA formation and the consequences of apparent alterations of higher order nuclear organization of the chromosomes, such as disruption of a topologically associating domains (TAD) harboring known driver loci of microdeletion syndromes, suggesting alternative regulatory mechanisms of genomic disorders. Ongoing studies have also begun to characterize the diversity of structural variation, including recurrent yet complex forms of inversion variation in the human germline, as well as the contribution of all classes of structural variation to the genetic architecture of autism and other neurodevelopmental disorders in the coding and noncoding regulatory genome. These studies collectively suggest that structural variation represents a major component of the genetic etiology of human developmental anomalies, and that emerging genomics technologies and large scale reference maps will be critical to define, interpret, and accurately quantify the role of this unique class of genomic variation to human disease, both in basic research and clinical interpretation.

S08
New technologies in Neurogenetics

S08.1
3D analysis of commissural systems with light sheet microscopy

A. Chédotal

Institut de la Vision, Paris, France

In most animal species including humans, commissural axons connect neurons on the left and right side of the nervous system. This communication between the two sides of the brain and spinal cord is necessary for a series of complex function, including binocular vision, coordinated locomotor movements, and sound direction localization. In humans, the balance of commissural and non-commissural axons is essential to CNS physiology and to the integration of sensory stimuli/inputs. Abnormal axon midline crossing during development causes a whole range of neurological disorders ranging from congenital mirror movements, horizontal gaze palsy, scoliosis or binocular vision deficits. Partial or complete corpus callosum agenesis are some of the most common brain malformations in children with variable neurological outcomes. I will discuss some of the genetic mechanisms underlying anomalies of midline crossing and present some of our most recent work that challenges the existing dogmas and suggest that commissural axon guidance mechanisms are more diverse across species than previously appreciated. To facilitate the analysis of the organization and evolution of commissural systems in vertebrates, we have developed a new imaging method which combines whole-mount immunostaining or commissural axon tracing, tissue clearing with organic solvents and 3D light-sheet microscopy. I will present new applications of this method in the field of human embryology and our ongoing effort to generate a database and cell atlas of the developing human embryo, normal or pathologic.

A. Chédotal: None.

S08.2
Brain imaging genetics in neurodevelopmental disorders

B. Franke

Radboud University Medical Center and Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands

With more and more common genetic variants for neurodevelopmental disorders like ADHD, schizophrenia, and autism being identified, we find ourselves facing a new bottleneck in understanding the etiology of those disorders: the functions of a variant/gene/locus identified and the way by which it increases disease risk, are often unknown. Brain imaging genetics - or neuroimaging genetics - offers an attractive human in vivo model to investigate the effects of (common genetic) risk factors on the brain. However, after an initial hype, the field of imaging genetics research has gone through a phase of disillusionment in recent years. Too many underpowered candidate gene studies without replication investigating single regions of interest have undermined the trust in this model. I will show that neuroimaging genetics is currently making a convincing come-back. Within the worldwide ENIGMA Consortium, we have performed GWAS to identify the genetic factors that contribute to the regional/global structures of our brain using well-powered samples. Based on those data, combined with the knowledge of the brain regions affected by neurodevelopmental disorders, we investigated the genetic overlap between the variants determining brain structure from the ENIGMA studies and those influencing disease risk from the studies of the Psychiatric Genomics Consortium and iPSYCH. Such studies are performed at the level of the global genetic architecture as well as at the level of single genetic variants. I will show data on both approaches for schizophrenia and attention-deficit/hyperactivity disorder. Based on those recent studies we can conclude that neuroimaging genetics offers a promising model system to understand the effects leading from a genetic variant to an increased risk for the neurodevelopmental disorder. Having gone through a rough start, more recent studies in this field in well-powered samples and employing brain wide/genome-wide approaches offer a glimpse of the actual power of this human in vivo model.

B. Franke: None.

S09
Explaining phenotypic variability

S09.1
Oligogenic inheritance and mutational load

N. Katsanis

Durham, NC, United States

No abstract received.

S09.2
Genetic and epigenetic regulation of repetitive DNA in relation to disease

S. M. van der Maarel

Leiden University Medical Center, Leiden, Netherlands

A major constituent of the human genome is repetitive DNA, including tandem and interspersed repeats. Of the tandem repeats, macrosatellite repeats (MSRs) belong to the largest repeat structures in the human genome typically spanning hundreds of kilobases of genomic DNA. Because of their polymorphic nature, MSRs represent an extreme example of copy number variation. Their structure and function however, is poorly understood, but their association with human disease suggests that tandem repeats such as MSRs are under strict genetic and epigenetic constraint.

Two disorders of epigenetic dysregulation of tandem repeats are Immunodeficiency, Centromere instability and Facial anomalies (ICF) syndrome and FacioScapulo-Humeral Dystrophy (FSHD). ICF syndrome is a primary immunodeficiency caused by mutations in at least four genes, some of them encoding known chromatin modifiers such as DNMT3B and HELLS, while for the remaining genes their role in the epigenetic regulation of the genome is less established. ICF patients show genome wide changes in the epigenetic regulation of pericentromeric and subtelomeric tandem repeats. How this epigenetic dysregulation relates to the clinical symptoms is, however, largely unknown. Conversely, how loss of control over the epigenetic regulation of the subtelomeric D4Z4 MSR causes the muscular dystrophy FSHD is much better defined with documentation of incomplete repression of the germline and early stem cell transcription factor DUX4 in skeletal muscle. Interestingly, there is considerable genetic and epigenetic overlap between these clinically discordant disorders, and with the recently genetically elucidated Bosma ahrinia microphthalmia syndrome (BAMS).

Identifying the commonalities and differences between these chromatin disorders of repetitive DNA will provide us a better understanding of the function and regulation of repetitive DNA.

S.M. van der Maarel: None.

S09.3
Multiple molecular diagnoses underlie some cases of apparent phenotypic expansion

J. E. Posey[1], E. Karaca[1], Z. H. Coban Akdemir[1], X. Song[1], T. Harel[2], S. Jhangiani[1], Y. Bayram[1], V. Bahrambeigi[1], D. Muzny[1], R. A. Gibbs[1], J. R. Lupski[1,3]

[1]Baylor College of Medicine, Houston, TX, United States, [2]Hadassah-Hebrew University Medical Center, Jerusalem, Israel, [3]Texas Children's Hospital, Houston, TX, United States

Multiple molecular diagnoses, characterized by the observation of two (dual) or more unique disease conditions each resulting from variation at a separate locus, represent a particular challenge to ascertain clinically and molecularly. Whole exome sequencing (WES) and the development of bioinformatic tools to identify de novo single nucleotide and small indel variants, as well as copy number variants, has made WES a truly comprehensive genomics assay that does not require pre-supposition of the correct gene-based molecular diagnoses. As such, WES has enabled the identification of multi-locus molecular diagnoses in ~5% of WES-informative cases, resulting in blended phenotypes. We hypothesized that multiple molecular diagnoses might underlie some cases of apparent 'phenotypic expansion'. From a WES-studied cohort of 128 Turkish families with neurodevelopmental phenotypes, we performed a re-analysis of 19 cases with molecular diagnoses initially characterized as having a phenotype that extended beyond that previously described for the reported etiologic disease gene. Of these 19 cases, 12 additional potentially pathogenic variants were newly identified in 9 (47.4%) cases, resulting in 6 dual and 3 multiple molecular diagnoses. Two novel candidate disease genes (*SRR, MAGI3*) and one intragenic deletion (*MPDZ*, exons 3–27) were identified. Two families each with two affected siblings demonstrated intrafamilial phenotypic variability explained by the identification of 3 molecular diagnoses in the more severely affected proband, and one molecular diagnosis in the less severely affected sibling. Absence of heterozygosity (AOH) contributed to molecular diagnoses in 6 cases, including one proband for whom 3 independent molecular diagnoses resulted from 3 homozygous potentially pathogenic variants in the same region of AOH. These findings highlight the

roles of clinical phenotyping and AOH-mediated reduction to homozygosity in enumerating mutational burden. Elucidation of additional highly penetrant alleles in cases with apparent phenotypic expansion may provide a general explanation for a large class of phenotypic variation.

J.E. Posey: Other; Modest; Baylor College of Medicine and Miraca Holdings, Inc. have formed a joint venture with shared ownership of the Baylor Genetics (BG) laboratory. JEP is an employee of BCM. **E. Karaca:** None. **Z.H. Coban Akdemir:** None. **X. Song:** None. **T. Harel:** None. **S. Jhangiani:** None. **Y. Bayram:** None. **V. Bahrambeigi:** None. **D. Muzny:** None. **R.A. Gibbs:** Other; Modest; Baylor College of Medicine and Miraca Holdings, Inc. have formed a joint venture with shared ownership of the Baylor Genetics (BG) laboratory. RAG is an employee of BCM. **J.R. Lupski:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; JRL has stock ownership in 23andMe, and stock options in Lasergen, Inc., JRL is a co-inventor of US and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. F. Consultant/Advisory Board; Modest; JRL is a paid consultant for Regeneron Pharmaceuticals. Other; Modest; Baylor College of Medicine and Miraca Holdings, Inc. have formed a joint venture with shared ownership of the Baylor Genetics (BG) laboratory. JRL is an employee of BCM.

S10
Population and evolutionary genetics

S10.1
Genetic time travel

***J. Krause*[1,2]**

[1]Jena, Germany, [2]Max Planck Institute for the Science of Human History, Jena, Germany

Genomic History of Ice Age Europe

Little is currently known about the genetic history of ancient Europeans before the advent of agriculture ~8,500 years ago. We have analysed genome-wide data from 51 modern humans remains that span around 40,000 years of Eurasian prehistory. Over this time, the proportion of Neanderthal DNA decreased from 3–6% to around 2%, consistent with natural selection against Neanderthal variants in modern humans. Whereas the earliest modern humans in Europe did not contribute substantially to present-day Europeans, all individuals between ~37,000 and ~14,000 years ago descended from a single founder population which forms part of the ancestry of present-day Europeans. A ~35,000-year-old individual from northwest Europe represents an early branch of this founder population which was then displaced across a broad region, before reappearing in southwest Europe during the last ice age ~19,000 years ago. During the major warming period after ~14,000 years ago, a new genetic component related to present-day Near Easterners appears in Europe. These results document how population turnover and migration have been recurring themes of European prehistory.

S10.2
Peopling of the world

E. Willerslev

Copenhagen, Denmark

No abstract received.

S10.3
The origins of Farming

M. Thomas

University College London, London, United Kingdom

Farming and sedentism first become established in southwest Asia during the early Holocene and later spread to neighbouring regions, including Europe and southern Asia, along multiple dispersal routes. The extent to which its spread was mediated by demic expansion of farmers, or by the transmission of farming technologies and lifeways to indigenous hunter-gatherers without a major concomitant migration of people, has been the subject of considerable debate for more than 100. Recent ancient DNA studies indicate a dominant role of migration in the transition to farming in central and northern Europe, with evidence of only limited hunter-gatherer admixture into early Neolithic populations. By analysing DNA from early Aegean farmers we extend the European Neolithic migratory chain all the way back to southwestern Asia. However, Early Neolithic genomes from the Zagros region of Iran reveal a previously uncharacterised population that is neither ancestral to the first European farmers nor has contributed significantly to the ancestry of modern Europeans. They are genetically distinct from all other available prehistoric genomes, but show strong affinities to modern day Iranian, Pakistani and Afghan peoples. These data suggest that multiple hunter-gatherer populations adopted farming in SW-Asia, that early farming technologies and domesticates were

exchanged in a 'federal' Neolithic core zone, and that components of pre-Neolithic population structure were preserved as farming spread into neighbouring regions.

M. Thomas: None.

S11
Cancer immunogenetics

S11.1
Next-generation immunotherapies for colorectal cancer

N. de Miranda

Leiden University Medical Center, Leiden, Netherlands

Following the encouraging clinical responses observed in cancer patients treated with immune checkpoint blockers, immunotherapy shows great promise for the treatment of cancer. The blockade of co-inhibitory pathways in T-cells promotes their activation and triggers anti-tumour immunity. The latter was shown to be driven against tumour-mutated antigens (neo-antigens) and to be dependent on the existence of neo-antigen-specific, activated T-cells, prior to therapeutic intervention. This observation suggests the complementary enhancement of T-cell responses by means of neo-antigen vaccination and/or adoptive transfer of neo-antigen-specific T-cell clones. The accumulated evidence on an association between the occurrence of natural anti-tumour immune responses in colorectal cancers (CRCs) and improved clinical prognosis makes CRC patients excellent candidates to benefit from immunotherapy. We are screening the coding genomes of CRCs by whole-exome and RNA next-generation sequencing (NGS). Somatic mutation profiles (mutanomes) are annotated and neo-antigens corresponding to the transcribed mutations are tested for their ability to induce activation of autologous T-cells derived from tumour infiltrating lymphocytes (TILs) and peripheral blood. The discovery of neo-antigen-specific T-cell clones in CRC patients would support the development of anti-cancer therapies consisting of neo-antigen-based vaccines and/or adoptive transfer of neo-antigen-specific T-cell clones.

Recently, we have also reported on an elusive immune cell population that was specifically associated with CRCs that had escaped T-cell recognition through loss of Human Leukocyte Antigen (HLA) class I. Strikingly, the presence of these cells also implied a good clinical prognosis as they were found in patients without metastatic disease. Nevertheless, these cells have not yet been fully characterized and are only recognized for being CD45/granzyme B + while lacking typical T- and NK-cell markers. We are phenotyping this immune cell population so that their functional characterization is possible. They could constitute a novel immunotherapeutic agent for the treatment of cancers that have escaped T-cell recognition through loss of HLA class I expression.

N. de Miranda: None.

S11.2
Dissecting tumor-immune cell interactions using genomics tools

Z. Trajanoski

Medical University of Innsbruck, Innsbruck, Austria

Recent breakthroughs in cancer immunotherapy and decreasing costs of high-throughput technologies sparked intensive research into tumour-immune cell interactions using genomic tools. The wealth of the generated data and the added complexity pose considerable challenges and require computational tools to process, analyse and visualise the data. Recently, a number of tools have been developed and used to effectively mine tumour immunologic and genomic data and provide novel mechanistic insights.

It this presentation I will show results generated using state-of-the-art computational tools addressing several prevailing questions in cancer immunology including: quantification of tumor-infiltrating immune cells from RNA-sequencing data, identification of determinants of tumor immunogenicity, and immunoediting that tumors undergo during progression or as a consequence of targeting the PD-1/PD-L1 axis.

Z. Trajanoski: None.

S11.3
Adoptive T cell therapy

T. Blankenstein[1,2]

[1]Max-Delbrück-Center for Molecular Medicine, Berlin, Germany, [2]Charité Centrum für Tumormedizin, Institut für Immunologie, Berlin, Germany

Adoptively transferred T cells have been shown to reject large established tumors. In such models, T cells recognize the tumor antigen as foreign. The task is to generate human T cell receptors (TCR) that recognize human tumor-associated (self) antigens as foreign and use these TCRs for gene therapy. We use mice with a humanized TCR repertoire to isolate therapeutic TCRs.

T. Blankenstein: None.

S12
Genetics and Microbiome

S12.1
Microbiome host-pathogen interactions

R. Xavier

Boston, MA, United States

No abstract received.

S12.2
Host-microbe interaction

J. Raes

Leuven, Belgium

No abstract received.

S12.3
Genetics of the microbiome

A. Zhernakova, A. Kurilshikov, M. Bonder, C. Wijmenga, J. Fu

University Medical Center Groningen, Groningen, Netherlands

Gut microbiome plays an important role in human metabolism, immunity, and health. Various factors including diet, medication, and environment have been shown to influence the gut microbiome composition. Recently the role of host genetics in shaping the gut ecosystem has been described in human studies and animal models. In this presentation I will summarize the current state of microbiome research with an emphasis on the effect of host genetics on the gut microbiome composition. In particular, I will focus on results of recent genome-wide association studies of microbiome composition and function, and on the genetic determinants of the host immune system that help shape the gut microbiome.

A. Zhernakova: None. **A. Kurilshikov:** None. **M. Bonder:** None. **C. Wijmenga:** None. **J. Fu:** None.

S13
Next generation clinical genetics

S13.1
Integrating the Phenomic and Genomic Architectures of Developmental Disorders

D. R. FitzPatrick

MRC Human Genetics Unit, University of Edinburgh, Edinburgh, United Kingdom

Clinically-defined syndrome diagnoses have an excellent record in predicting defined sets of causative genotypes. The Deciphering Developmental Disorders (DDD) project is a UK- and Ireland-wide study that aims to develop and use new genetic technology and statistical analyses to make a definitive diagnosis in individuals with severe or extreme developmental disorders. DNA samples are available from ~13,500 affected individuals have been recruited with 10,000 of these also having samples available from both parents.We have recently reported a significant excess of damaging de novo variants in 94 different genes in a cohort of 4294 probands with previously undiagnosed developmental disorders. Genome wide significance in this study was based on human genetic data alone. The overall diagnostic rate for all modes of inheritance within DDD is ~41%. This diagnostic rate involved some element of individual clinicians assessing whether the phenotype matches the genotype. It would be useful to develop computational approaches to this matching as it should allow more rational variant filtering options when dealing with genome-wide sequence data. However, the extent to which clustering in phenotypic space can be used to predict specific diagnostic genotypes is not clear. Here I will explore the use of facial imaging, growth z scores, developmental milestones and HPO terms for diagnostic filtering. The phenotype is likely to become an important component of the computational approach to the analysis of genome wide sequencing data1 and a consistent approach to the collection and utilitation of such information is a vital part of study design **References:** PMID: 28358133; PMID: 28135719; PMID: 25533962; PMID: 24963138

D.R. FitzPatrick: None.

S13.2
The clinical geneticists' perspective on exome sequencing

A. Rauch

Zurich, Switzerland

Finding an etiological diagnosis is a major challenge in most rare and/ or genetically heterogeneous conditions. Exome sequencing is capable of significantly increasing the diagnostic yield, but still leaves many patients undiagnosed and may impose concerns about variants of unknown significance. Data analysis guided by clinical considerations can well improve the interpretation of exome sequencing results and should stimulate the development of better algorithms.

S13.3
Fast-WES for neonates, how useful is it really?

G. W. E. Santen

Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands

Whole exome and genome sequencing (WES/WGS) are currently reshaping clinical genetic practice. Current turnaround times are too slow for widespread implementation, but shorter turnaround times will no doubt stimulate application of WES beyond classical clinical genetics. There have been several studies showing high diagnostic yields (40–60%) of fast WES/WGS in neonatal intensive care unit (NICU) patients. However, many of the syndromes detected in these papers are considered recognizable syndromes (such as CHARGE, Noonan and Kabuki syndromes). In our hospital, as in all larger hospitals in the Netherlands and many in Europe, clinical geneticists routinely perform consultations *before* genetic investigations are requested. In the case of a high confidence clinical diagnosis management is already influenced and fast WES/WGS has only limited added value. As the costs of fast WES/WGS are currently significantly higher (up to 50%) than for standard protocols, the question of whether fast WES/WGS should be the standard tool in the evaluation of neonates in the NICU/PICU becomes relevant.

Therefore, we evaluated the potential usefulness of fast-WES (a 1–2 week full WES protocol) in our clinical practice. We retrospectively evaluated two years of NICU consultations in children <4 months old. In part of this period fast-WES was available and considered in the absence of a clinical diagnosis, when significant procedures were considered, or when the patient's condition was deteriorating unexpectedly.

To our great surprise, we found that fast-WES was only requested four times in the course of this study. Surprisingly, the diagnostic yield of WES (23 regular WES and the 4 fast-WES, mostly trio) was very low (<5%). Part of the explanation of this low yield lies in a high rate of early clinical diagnoses by SNP-array and clinical consultations, thus filtering out many known diagnoses *before* requesting (fast-)WES.

In our experience fast-WES can be a very useful tool but it is not often indicated in our NICU/PICU population, where pre-screening by a genetic consultation and SNP-array has a high diagnostic yield. We have found fast-WES extremely useful in other cases outside the scope of this study, such as in slightly older children and during pregnancy. As costs will continue to decrease we hope to be able to be less restrictive in offering fast-WES to patients, further reducing the time to diagnosis for a subset of patients.

G.W.E. Santen: None.

S14
Organoid models: The Maxi Impact Of Mini Organs

S14.1
Dissecting human and chimpanzee cerebral organoids using single-cell RNA-seq

J. Camp[1], S. Kanton[1], F. Badsha[2], F. Mora-Bermudez[2], S. Pääbo[1], W. Huttner[2], B. Treutlein[1,2,3,3]

[1]Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [3]Technical University Munich, Munich, Germany

Cerebral organoids have emerged as powerful models of human brain development, and offer the potential to study uniquely human brain evolution. However, the extent to which cerebral organoid systems recapitulate fetal gene expression networks remains unclear. Here we use single-cell RNA sequencing (scRNA-seq) to dissect and compare cell composition and progenitor-to-neuron lineage relationships in human and chimpanzee cerebral organoids and fetal human neocortex. We find that human and chimpanzee organoid cortical cells use gene expression programs remarkably similar to those of the fetal tissue in order to organize into cerebral cortex-like regions. We identify genes that are differentially expressed in human progenitors and neurons relative to chimpanzee, and highlight modern human genetic changes that can be studied in organoid

cultures. More broadly, this strategy can be extended to other organoid systems modeling human and chimpanzee development and disease.

J. Camp: None. **S. Kanton:** None. **F. Badsha:** None. **F. Mora-Bermudez:** None. **S. Pääbo:** None. **W. Huttner:** None. **B. Treutlein:** None.

S14.2
Common mechanisms between Zika virus-induced and inherited microcephaly in human brain organoids

J. Gopalakrishnan

Cologne, Germany

No abstract received.

S14.3
Liver organoids for the study of liver biology and disease

M. Huch

Gurdon Institute-Wellcome Trust, Cambridge, United Kingdom

Despite the enormous replication potential of the liver, there are currently no culture systems available that sustain hepatocyte replication in vitro. Hepatocytes can be maintained in culture for a few days. However, they lose their hepatocyte phenotype and function almost immediately, thus precluding its application for cell therapy treatments. Liver stem cells have the potential to selfrenew and differentiate into functional hepatic lineages. Mouse liver stem cells can be indefinitely expanded in vitro (for >1 year), into "liver organoids", in our liver stem cell culture system, in the absence of a mesenchymal niche. The cultured cells express ductal markers and differentiate into functional hepatocytes in vitro and in vivo. We have now further developed our culture system to study human liver stem cells and human liver disease. We describe a culture system that allows the long-term expansion of adult human liver stem cells (> 3 months) from donor biopsies while maintaining their differentiation potential towards functional hepatocytes in vitro. The expanded cells are highly stable at the chromosome and structural level, while single base changes occur at very low rates. The cells can readily be converted into functional hepatocytes in vitro and upon transplantation in vivo. Organoids from α1-antitrypsin deficiency and Alagille Syndrome patients mirror the in vivo pathology. Clonal long-term expansion of primary adult liver stem cells opens up experimental avenues for disease modeling, toxicology studies, regenerative medicine and gene therapy.

M. Huch: None.

S15
ESHG / ESC JOINT Symposium: Polygenic Cardiovascular traits

S15.1
Implications of understanding the genetic basis of coronary artery disease

N. J. Samani

University of Leicester, Leicester, United Kingdom

Over the last decade, large-scale GWAS meta-analyses have identified several dozens of genetic loci associated with risk of coronary artery disease (CAD). This presentation will describe the current state of the discovery process, discuss what we have learnt and how the findings are being clinically translated.

N.J. Samani: None.

S15.2
Genetics of arterial blood pressure: from common to rare variants in the general population

P. Munroe

London, United Kingdom

Over the past 10 years' substantial progress has been made mapping blood pressure loci using genome-wide association studies and deploying bespoke microarrays (most recently the Cardio-Metabochip and Exome chip arrays) in very large sample sizes. There are now over 400 blood pressure loci, providing hundreds of candidate genes, and new insights into pathways that are key in blood pressure regulation. My presentation will discuss some of the results of the most recent analyses and highlighting the key findings.

S15.3
The genetic architecture of type 2 diabetes

P. Froguel

Lille, France

No abstract received.

S16
Autophagy in health and disease

S16.1
Autophagy gets to the bone

***C. Settembre*[1,2]**

[1]Naples, Italy, [2]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy

Autophagy is a lysosomal pathway deputed to the recycling of cellular components. The regulation of autophagy is essential for tissue homeostasis and is controlled by the mammalian target of rapamycin complex 1 (mTORC1) kinase in response to nutrients. My lab has recently demonstrated that autophagy, by regulating collagen levels in cartilages, is necessary during bone growth. However, whether an impairment of autophagy plays a role in the pathogenesis of genetic disorders affecting the skeleton is still unknown. During my talk I will present new data showing that autophagy dysfunction accounts for bone growth retardation in lysosomal storage disorder (LSD) by inhibiting collagen secretion by growth plate chondrocytes, the main regulators of longitudinal bone growth. Genetic inhibition of mTORC1 activity or pharmacological stimulation of the pro-autophagy protein Beclin1 rescued chondrocyte autophagy, collagen levels in cartilages and bone growth retardation in two different LSD mouse models. Taken together, these data unveil a role for mTORC1 and autophagy in the pathogenesis of skeletal disorders and suggest their modulation as new therapy for the treatment of LSDs.

S16.2
Autophagy in metabolic processes

R. Ricci

IGBMC, Illkirch, France

Proper nutrient sensing is crucial in multicellular organisms. In mammals upon feeding, the pancreatic ß cell mainly senses increasing glucose levels and secretes insulin that acts to induce anabolic pathways in different organs. Inversely, the ß cell decreases insulin secretion to suppress these anabolic reactions upon low glucose levels during fasting. As known for many eukaryotic cells, nutrient deprivation is also expected to induce macroautophagy (hereafter referred to as "autophagy"). During autophagy, cellular components are sequestered into double-membrane autophagosomes, which subsequently fuse with lysosomes (autolysosomes), where degradation occurs. Resulting catabolites maintain cells metabolically active ensuring cell survival. We recently were able to demonstrate that, in contrast to other mammalian cells, autophagy in ß cells was not a predominant response upon nutrient deprivation. Instead of autophagy, starved ß cells induced lysosomal degradation of newly generated (nascent) secretory insulin granules in the vicinity of the Golgi, a process we termed starvation-induced insulin granule degradation (SINGD). Keeping autophagy high during starvation and as a consequence generation of intracellular nutrients led to uncontrolled insulin release. As the nascent insulin granules were shown to be preferentially secreted, suppresion of autophagy through SINGD is thus an optimal strategy to counteract insulin secretion, at the same time providing sufficient nutrients for ß cells to survive. The observed positive correlation between autophagy and insulin secretion may suggest an involvement of autophagy in postprandial insulin release probably going beyond the widely established housekeeping role of autophagy. Importantly, we have now evidence that degradation of insulin granules in lysosomes is dramatically enhanced in ß cells of murine and human diabetic islets. We also have preliminary data supporting that lysosomal degradation of insulin contributes to loss of insulin and chronic suppression of autophagy. Autophagy is indeed important to maintain ß cell function, in particular in a situation of high insulin demands such as in type 2 diabetes (T2D). Overall, our mechanism thus represents an important evolutionary adaption for nutrient-deprived ß cells. However, its deregulation may actually contribute to ß cell failure in T2D. Our findings may thus change the current paradigm in which decreased ß cell survival and/or dedifferentiation mainly accounted for insulin loss in T2D.

R. Ricci: None.

S16.3
Autophagy in neurodegeneration and ageing

N. Tavernarakis

Institute of Molecular Biology and Biotechnology, Crete, Greece

Mitochondria, the main energy hub of the cell, are highly dynamic organelles, playing essential roles in fundamental cellular processes. Mitochondrial function impinges on several signalling pathways modulating cellular metabolism, cell survival and healthspan. Maintenance of mitochondrial function and energy homeostasis requires both generation of newly synthesized and elimination of dysfunctional mitochondria. Impaired mitochondrial function and excessive mitochondrial content are major characteristics of ageing and several human pathophysiological

conditions, highlighting the pivotal role of the coordination between mitochondrial biogenesis and mitophagy. However, the cellular and molecular underpinnings of mitochondrial mass homeostasis remain obscure. We found that DCT-1, the Caenorhabditis elegans homolog of mammalian BNIP3 and BNIP3L/NIX, is a key mediator of mitophagy promoting longevity under stress. DCT-1 acts downstream of the PINK-1-PDR-1/Parkin pathway and is ubiquitinated upon mitophagy-inducing conditions to mediate the removal of damaged mitochondria. Accumulation of damaged mitochondria triggers SKN-1 activation, which initiates a bipartite retrograde signaling pathway stimulating the coordinated induction of both mitochondrial biogenesis and mitophagy genes. Taken together, our results unravel a homeostatic feedback loop that allows cells to adjust their mitochondrial population in response to environmental and intracellular cues. Age-dependent decline of mitophagy both inhibits removal of dysfunctional or superfluous mitochondria and impairs mitochondrial biogenesis resulting in progressive mitochondrial accretion and consequently, deterioration of cell function.

N. Tavernarakis: None.

Educational Session

E01
Sequencing, Sponsored by Illumina

E01.1
The Future of Genomic Medicine

E. Mardis

Nationwide Children's Hospital, Columbus, OH, United States

Large-scale biomedical discovery has been tremendously facilitated by the application of next-generation sequencing (NGS) and advanced computational analyses over the past ten years. As a result of these studies, a greatly enhanced understanding of the genomic underpinnings of human disease has been obtained. There is increasing evidence that applying this new knowledge to aid in diagnosis of patients with genetic diseases and cancers can provide additional precision through NGS-based diagnostic assays. My talk will highlight these translational efforts in cancers and constitutional disorders, focusing on case studies to illustrate the precision obtained, and on the remaining challenges that must be overcome to achieve routine use of genomics in clinical diagnosis, prognosis and treatment decision-making.

E. Mardis: None.

E01.2
Deep Sequencing of 10,000 Human Genomes

A. Telenti

Human Longevity, Inc., San Diego, CA, United States

The ability to sequence human genomes at high scale allows the investigation of the landscape of human diversity: the rates of discovery, the characteristics of the non-protein-coding genome, and the hallmarks of conservation and essentiality. Analysis of 10,000 deep sequenced genomes (30X coverage) identifies variation in 1 every 13 nucleotides in the genome. Most of the variations are singletons, and each additional sequenced genome adds over 8000 additional new variants to the database. This density of variation allows the creation of a map of human genetic constraint in the non-coding genome that differs from the conservation map defined by interspecies alignment. There is a strong pattern of coordination of genetic constraint between genes and their regulatory regions up to 2MB. Essential genes, as defined by a number of metrics (Phi, pLI, RVIS, Missense z-score, EvoTol, LoFtool), use conserved regulatory elements. It is estimated that, with sequencing of 1 million genomes, one every third nucleotide will be observed variant if tolerated.

A. Telenti: A. Employment (full or part-time); Significant; Human Longevity, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Human Longevity, Inc.

E01.3
Increasing the diagnostic yield of genome-wide sequencing for rare diseases

K. M. Boycott

University of Ottawa, Ottawa, ON, Canada

An accurate diagnosis is an integral component of clinical care for patients with rare genetic diseases. Recent advances in sequencing, in particular whole-exome sequencing (WES), are identifying the genetic basis of disease for 25–40% of patients. There are a variety of reasons why up to 75% of patients might be unsolved after WES, including incomplete coverage of the exome and genetic mutations elusive to the technology itself; some of these challenges can be addressed by pursuing additional genomic technologies. However, there are a proportion of these unsolved cases in which the disease-causing variant is in fact within the WES data but for a variety of reasons there is insufficient evidence to support a definitive diagnosis. This is not surprising given that approximately half of

the genes for the estimated 7000 rare diseases remain to be discovered. The wealth of genome sequence data being generated in diagnostic laboratories should accelerate progress in biomedicine - making it possible to integrate genomic and clinical information to reveal the genetic basis of inherited diseases, amongst many other applications. However, we are not organized to seize this extraordinary opportunity — nor are we on a path to do so, highlighting the need for critical large-scale data sharing. Two international consortia, the International Rare Diseases Research Consortium (IRDiRC) and the Global Alliance for Genomics and Health (GA4GH), have recognized rare diseases as a remarkable opportunity for advancement in this realm on a world-wide scale and support initiatives such as the Matchmaker Exchange (www.matchmakerexchange.org) to facilitate case-based matching for discovery. As we come closer to understanding the genetic etiology of all rare diseases, it is likely that we will increasingly rediscover known genes and that the approach to completion of the disease compendium will be asymptotic and much more challenging than predicted.

K.M. Boycott: None.

E01.4
Medical genome sequencing in the 100,000 Genomes Project Rare Disease Programme

R. H. Scott

Genomics England, London, United Kingdom

To bring the predicted benefits of genomics to NHS patients is why the Prime Minister launched the 100,000 Genomes Project in late 2012. The project will sequence 100,000 genomes from around 70,000 people. Participants are NHS patients with a rare disease, plus their families, and patients with cancer.

The Rare Disease Programme has recruited over 20,000 participants and returned interpreted genome results in 3,000 (from 1,500 families) to the recruiting centres. To deliver the programme a novel framework has been established to enable large-scale recruitment, clinical data collection and the development of a semi-automated interpretation pipeline that benefits from the centralisation of sequencing and bioinformatic infrastructure while enabling recruiting clinical and laboratory control over validation and reporting of results and forming a platform for research and innovation. This talk will describe the development of this infrastructure and share insights from our analyses to date.

R.H. Scott: None.

E02
CRISPR/Cas9 genome editing to model disease

E02.1
Functionally assaying thousands of *BRCA1* variants with saturation genome editing

G. M. Findlay, R. Daza, B. K. Martin, A. Leith, M. D. Zhang, L. M. Starita, J. Shendure

University of Washington, Seattle, WA, United States

CRISPR technologies are transforming the way researchers study genome function. The ability to engineer DNA sequence variants in their endogenous genomic context offers great potential for accurately characterizing the effects of variants on both basic biological processes and human disease. However, given the extensive genetic variation observed in clinical sequencing, methods for determining functional consequences of variants should be highly scalable. To address this challenge, we recently developed a genome editing method to interrogate hundreds to thousands of variants at a single locus in a single experiment. This approach, called saturation genome editing, leverages multiplex homology-directed repair of CRISPR/Cas9-derived DNA breaks to introduce a highly diverse set of variants across approximately 100 bp of genomic sequence. With next-generation sequencing, we can measure each allele's effect on phenotypes such as cell growth and transcript abundance. Currently, we are applying saturation genome editing to systematically characterize approximately 4,000 single nucleotide variants within the *BRCA1* gene in human cells. We read out *BRCA1* function in a cell line in which the homologous recombination pathway is essential for viability. Our preliminary results constitute a catalog of functional scores for nearly all single nucleotide variants spanning thirteen high-priority exonic regions that code for the protein's RING and BRCT domains. We show that in addition to nearly all nonsense and canonical splicing variants, a substantial fraction of missense and intronic variants near splice junctions also compromise *BRCA1* function. Furthermore, a small but appreciable fraction of synonymous variants abrogate *BRCA1* function, presumably through effects on splicing efficiency or transcript stability. Ongoing efforts to refine our functional scores and substantiate their clinical utility may be useful for overcoming the challenge of *BRCA1* variants of uncertain significance in genetic testing for hereditary breast and ovarian cancer disease risk. More broadly, by expanding this experimental paradigm for CRISPR/Cas9-mediated variant testing to other clinically

actionable genes we aim to substantially improve the interpretability of genetic testing.

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

E02.2
The CRISPR revolution: engineering structural variants to study disease

D. Lupiáñez[1,2,3], G. Andrey[1]

[1]Max Planck Institute for Molecular Genetics, RG Development and Disease, Berlin, Germany, [2]Institute for Medical and Human Genetics, Charité – Universitätsmedizin, Berlin, Germany, [3]Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité – Universitätsmedizin, Berlin, Germany

Structural variations contribute extensively to the variability of the human genome and are often associated with disease. Their study in murine model systems, however, has been classically burdened by laborious genetic targeting procedures and time-consuming mouse crossing steps. Recently, the CRISPR/Cas technology emerged as a powerful tool to edit genomes in a fast, cost-effective and precise manner. Using this system, chromosomal rearrangements such as deletions, duplication, inversions or translocations can be easily generated, thus facilitating the creation of models of human disease. In this talk, I will discuss the fundaments of this technology, providing exemplary cases that highlight how CRISPR/Cas has revolutionized our current lab methodology and allowed the identification of novel pathomechanisms.

D. Lupiáñez: None. **G. Andrey:** None.

E03 50
Shades of Cancer Genetics

E03.1
Genetic susceptibility to common cancers: what have we learned from large cancer genetic consortia

A. C. Antoniou

Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom

Advances in genomic technologies have enabled more rapid, less expensive genetic sequencing than was possible a few years ago. These technologies allow for the comprehensive genetic profiling for assessing risks to common cancers and include multiplex sequencing panels of several genes and panels of common single nucleotide polymorphisms (SNPs). However, the clinical utility of such multiplex gene and SNP panels depends on having accurate estimates of cancer risks for mutations in the genes included in such panels as well as cancer risk prediction models that consider the multifactorial aetiology to cancer susceptibility. Over the past two decades international consortia, such as the Breast Cancer Linkage Consortium, the Breast and Ovarian Cancer Association Consortia, the Consortium of Investigators of Modifiers of *BRCA1/2* and the International *BRCA1/2* Carrier Cohort Study have enabled us to accurately characterise the cancer risks for rare and common cancer susceptibility genetic variants; to understand how the genetic variants interact with each other on cancer risks; and how genetic variants interact with other lifestyle/hormonal risk factors for the disease. Using breast, ovarian and prostate cancers as examples the presentation will review the key research achievements by the international consortia and how these are helping us to realise a more personalised risk-based cancer prevention and cancer control.

A.C. Antoniou: None.

E03.2
Two decades after BRCA: setting paradigms in personalized cancer care and prevention

K. Offit

New York, NY, United States

No abstract received.

E04
Channelopathies

E04.1
Brain channelopathies

R. Guerrini

Children's Hospital Anna Meyer, University of Florence, Florence, Italy

In the human genome there are more than 400 genes encoding ion channels, i.e. transmembrane proteins regulating ion fluxes across membranes. Dysfunction in ion channels leads to altered membrane excitability most often manifested by paroxysmal disorders. The resulting diseases, cumulatively defined as channelopathies, are manifested as phenotypes ranging from common to very rare disorders, whose severity can be mild, disabling, or life-threatening.

Genetic neurological channelopathies are typically inherited in an autosomal dominant fashion. The resulting paroxysmal disturbances of neurological function can either be the only clinical manifestation or be associated with developmental and intellectual disabilities, with consequent pronounced reproductive disadvantage. Mutations affecting central nervous system expressed sodium and potassium channels most often result in severe epileptic encephalopathies, but may also cause self-limiting benign epilepsies. In spite of the high number of patients and mutations described for some of these conditions, genotype/phenotype correlations are only marginally understood. The study of these disorders has improved our understanding of pathophysiology of several neurological disorders, particularly epilepsy, but also episodic ataxia and hemiplegic migraine. Neuronal channelopathies are individually rare but cumulatively represent a considerable proportion of the practice of pediatric neurology and pose serious challenges for treatment and genetic counselling. Even after a specific ion channel disorder has been diagnosed the therapy remains empirical and symptomatic, often with limited efficacy in most patients. Developing new and more specific therapeutic approaches is a high priority and is proving more difficult that initial discoveries had led to hope for.

R. Guerrini: None.

E04.2
Muscle channelopathies

H. Houlden

UCL Institute of Neurology, Queen Square, London, United Kingdom

Muscle and Neuromuscular Channelopathies

The number of pathogenic mutations causing non-dystrophic myotonias (NDMs) and periodic paralyses in known genes continues to expand. Mutations have also been identified CLCN1, SCN4A, in the ryanodine receptor gene manifesting as an atypical periodic paralysis phenotype and thyrotoxic hypokalaemic periodic paralysis in a novel gene encoding an inwardly rectifying potassium channel, Kir2.6. Work studying molecular mechanisms indicates that 90% of the known mutations causing hypokalaemic periodic paralysis (HypoPP) result in loss of positively charged arginine residues in the S4 segments of either SCN4A or CACNA1S, possibly creating a gating-pore current that may be important in the pathogenesis of HypoPP. Recent studies evaluating clinical features and health status in NDM patients have provided more detailed insights into the significant morbidity associated with these diseases. Ultrasound has been successfully used to demonstrate muscle abnormalities in NDM patients and magnetic resonance spectroscopy studies applied to HypoPP patients suggest that this technique can demonstrate both disease-related and treatment-related changes. Here the clinical syndromes and genetic causes are discussed as well as some of the more recently identified neuromuscular channelopathies.

H. Houlden: None.

E05 Imprinting-related disorders

E05.1
Overview on imprinting related disease

D. J. G. Mackay

University of Southampton, Faculty of Medicine, Southampton, United Kingdom

Genomic imprints in humans are written upon our genomes from the earliest stages of our development, as a permanent memory of our parental origin. The human genome contains approximately 150 imprinted genes, and their expression is distinctively mono-allelic - i.e., from either the paternal or the maternal DNA. Many imprinted gene products regulate growth, from the cellular to the organismal level, and disturbing their exquisite balance of mono-allelic expression leads to imprinting disorders (IDs), with distinctive effects on growth, development, metabolism and behaviour. In this educational session, I will summarise known IDs, their range of genetic and epigenetic causes, and the insights they give us into normal imprinting control. I will explore the challenges of ID diagnosis on both the clinical and epigenetic level, highlighting both the clinical heterogeneity of individual IDs, and the clinical overlap between them. I will describe the recent advances in diagnosis and management of IDs that have been driven by the EU COST network on imprinting disorders, EUCID.net. As examples of this work, both this talk and its companion lecture will describe recent International Clinical Consensus activities in three IDs: Silver-Russell syndrome, Beckwith-Wiedemann syndrome, and Pseudohypoparathyroidism.

D.J.G. Mackay: None.

E05.2
Clinical and molecular overview of Beckwith-Wiedemann and Silver-Russel syndromes

F. Brioude, E. Giabicani, W. Abi Habib, I. Netchine

Universite Pierre et Marie Curie, Assistance Publique Hopitaux de Paris (Hopital Trousseau) and Inserm UMR_S938, Paris, France, Paris, France

Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS) are two imprinting disorders (ID) with foetal growth disturbance. Epigenetic (abnormal methylation at imprinting center regions) or genetic (mutations, duplications, uniparental disomy (UPD)) defects of imprinted genes on chromosome 11 (BWS and SRS), 7 (SRS) and more recently 14 (SRS) have been identified in these two syndromes. In humans, the 11p15 region contains genes which are important for regulation of foetal and postnatal growth. This region includes two imprinted domains: the *IGF2/H19* domain regulated by imprinting center region 1 (ICR1, or *H19/IGF2*:IG-DMR) and the *CDKN1C/KCNQ1OT1* domain regulated by ICR2 (or *KCNQ1OT1*:TSS DMR).

BWS has been described in the 1970s. BWS is an overgrowth syndrome with additional features as macroglossia, abdominal wall defects, hemihyperplasia, organomegaly, neonatal transient neonatal hypoglycemia/hyperinsulinism. Furthermore, BWS is associated with an increased risk of embryonic tumour during early childhood, especially nephroblastomas or hepatoblastomas. ICR1 gain of methylation (GOM), ICR2 loss of methylation (LOM), 11p15 paternal UPD or *CDKN1C* loss-of-function mutations represent the most frequent molecular mechanisms in BWS. A strong correlation between the risk of embryonic tumor and the molecular mechanism has been described, with the most important risk for patients with ICR1 GOM or 11p15 paternal UPD. Tumour screening is mostly based on abdominal ultrasound for children with BWS. However, many tumour screening programs have been proposed, with some of them suggesting different schemes based on the molecular mechanism. Furthermore, some of them add screening of hepatoblastoma with serum alpha fetoprotein.

SRS is a clinical mirror of BWS, and has been described in the 1950s. In 2016, a first international consensus regarding diagnosis and management of SRS has been established. SRS is a clinical diagnosis, for which a clinical scoring system (Netchine-Harbison, NH-CSS) has been validated. NH-CSS includes being short for gestational age, postnatal growth retardation, relative macrocephaly at birth, feeding difficulties/low BMI, protruding forehead and body asymmetry/hemihypoplasia. Later in life, SRS patients are more likely to develop early puberty, overweight and/or metabolic disturbances. About 50% of SRS patients present an ICR1 LOM, and 5–10% present a maternal UPD at chromosome 7. More recently, molecular defects of chromosome 14 (usually linked to Temple syndrome, another ID with growth retardation) have been identified in cohorts of SRS patients (including maternal UPD, deletions or abnormal methylation). Finally mutations of *CDKN1C* (gain-of-function) or *IGF2* (loss-of-function) have been described in rare familial cases of SRS. Management of SRS patients includes (among others) early nutritional support, growth hormone treatment and surveillance of puberty/adrenarche.

F. Brioude: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; IPSEN, SANDOZ. **E. Giabicani:** None. **W. Abi Habib:** None. **I. Netchine:** None.

E06 Bioethics for 'dummies'

E06.1
Gene editing, NIPT

M. C. Cornel

VU University Medical Center, dept Clinical Genetics and Amsterdam Public Health research Institute, Amsterdam, Netherlands

Bioethics for 'dummies': Gene editing, NIPT

Ethics has many definitions. (1) Research ethics. Scientist may think of ethical committees or institutional review boards - long and bothersome procedures needed before the start of a research project. Does the experiment follow research ethics principles as specified in the Declaration of Helsinki?(2) Clarifying the debate. Bioethicist help to understand relationships among life sciences, biotechnology, medicine, politics, law and philosophy. Why do we argue the way we argue. What values are behind controversial issues. For a decision to be made, what are the arguments in favor and against. (3) Ethical practice in profession-led health care. For physicians ethics is an element of professionalism. The CANMEDS competency "professionalism" encompasses ethical practice. For the questions at the beginning of life, gene editing and non-invasive prenatal testing (NIPT), I will focus on the contributions of the Public and Professional Policy Committee (PPPC) of ESHG, to illustrate how recommendations for responsible health care practice were developed, and how the ethical debate was clarified as in the second and third definition of ethics. Ethics for beginners could start with *principles* of ethics. Knoppers and Chadwick in 2005 described emerging trends in ethics, especially in human genetic research, starting from the principles of autonomy, privacy, justice, quality and equity and moving to reciprocity, mutuality, solidarity, citizenry and universality. Autonomy has been a very important element in genetics health care. Especially if reproductive choices are to be made, the women or the couple involved have to decide. Coercion is to be avoided. The PPPC recommendations on NIPT state that it has the potential of helping the practice better achieve its aim of facilitating autonomous reproductive choices. To evaluate this aim, it is not sufficient to

report on the number of participants, the uptake or the prevalence of aneuploidies. Crucial elements for the health care system include information and counseling, education of professionals, accountability to all stakeholders including children born from screened pregnancies and persons living with the conditions targeted in prenatal screening and promotion of equity of access. The ethical principles are often connected. Equity for instance comes in. If women are to make an autonomous choice, this can only be achieved if all women have access, and if, whatever their choice, they and their children will be taken care of. For gene editing the public debate and legislation seems to draw a line when it comes to germline gene editing. The PPPC of ESHG collaborates with The European Society of Human Reproduction and Embryology (ESHRE). Together we are in the process of developing recommendations especially for reproductive gene editing. In many countries germline interventions have been prohibited. What were the arguments behind this legislation, and do these still apply and are they still considered convincing? If a technique can help to avoid serious genetic disorders, in a safe and effective way, would this be a reason to reconsider earlier standpoints? After endorsement of these recommendations, more debate will be needed with stakeholders outside of the genetics/human reproduction community.

M.C. Cornel: None.

E06.2
Balancing public health & biomedical ethics: The case of newborn screening

***Y. Bombard*[1,2]**

[1]*University of Toronto, Toronto, ON, Canada,* [2]*St Michael's Hospital, Toronto, ON, Canada*

Newborn screening (NBS) is considered a public health genomics success story. NBS programs identify serious conditions where early detection and urgent presymptomatic treatment were necessary to avert serious clinical harm. However, three challenges are raising old ethical issues of consent and public health benefits with renewed urgency:

(1) The scope of NBS panels is expanding - around the world - allowing the early detection of dozens of genetic conditions, including conditions without treatment, where treatment does not improve mortality and where evidence of benefit is equivocal.

(2) Further, controversy has emerged concerning the storage of NBS samples and their secondary uses. NBS samples are typically stored for QA purposes, but these dried spots may also be used for other purposes including: research, public health surveillance, and sometimes for identifying disaster victims or in law enforcement.

(3) Finally, there is growing discussion of the potential to use genome sequencing (GS) technologies within NBS programs. However, the possibility of incorporating GS into population-wide NBS programs would lead to a major shift in the scope and scale of information generation, which raises questions about the limits of screening and the moral authority of NBS to continue to operate as a mandatory or as an implied consent program.

Combined, these developments might alter public expectations to participate in universal NBS programs and underscore the need to engage the public on these issues.

This presentation will review these three issues and ethical principles as well as describe research from a national public engagement study on Canadian citizens' views and values on these emerging issues. Results will describe public expectations of expanded NBS panels and their values regarding research with stored samples. Results will also present citizens' personal inclinations, and perceived obligations of others, to participate in NBS using GS. In light of these findings, key ethical and policy considerations as well as future research questions will be discussed.

Y. Bombard: None.

E07 Pharmacogenomics in the clinic

E07.1
Pharmacogenomics Knowledge for Personalized Medicine

***T. Klein*[1], *M. Ritchie*[2]**

[1]*Department of Biomedical Data Science, Stanford, CA, United States,* [2]*Penn State University, Danville, PA, United States*

Pharmacogenomics (PGx) focuses on the use of genomic information to guide drug therapy and is a central component of precision medicine. Despite substantial progress in understanding how genetic variations impact drug efficacy and toxicity, the adoption of pharmacogenomics in clinical practice has been relatively slow. Major challenges in the implementation of pharmacogenomics knowledge include lack of awareness of the available evidence, unsure of how to interpret and use the genetic information, and lack of clear guidance on how to deliver information to the practitioners and patients. A central repository of pharmacogneomic knowledge is critical in addressing all of these challenges. The Pharmacogenomics Knowledgebase (PharmGKB) is a publically available premiere repository

that collects, curates, and disseminates information about the impact of human genetic variation on drug responses. Through our research efforts and collaborations with pharmacogenomics research and clinical communities, we provide a comprehensive catalogue of genes and genetic variations that are most important for drug response phenotypes. I will describe the core content of our knowledgebase and discuss how we use the knowledge to support clinical implementation of PGx. In addition, I will highlight our collaboration with the Clinical Pharmacogenetics Implementation Consortium (CPIC) to develop freely available, peer-reviewed gene/drug practice guidelines for physicians that aids implementation of pharmacogenetic testing and improves the precision of drug selection and dosing. Lastly, I will present the development of The Pharmacogenomics Clinical Annotation Tool (PharmCAT), a software tool that extract all CPIC level-A variants from a genetic dataset (represented as a vcf), interpret the variant alleles, and generate a report that can then be used to inform prescribing decisions. PharmCAT is currently being developed in a collaboration between the PGRN Statistical Analysis Resource (P-STAR), PharmGKB, the Clinical Genome Resource (ClinGen), and CPIC. **References:** M. Whirl-Carrillo, E.M. McDonagh, J. M. Hebert, L. Gong, K. Sangkuhl, C.F. Thorn, R.B. Altman and T.E. Klein. "Pharmacogenomics Knowledge for Personalized Medicine". *Clinical Pharmacology & Therapeutics (2012) 92(4): 414–417.;* M.V. Relling, T.E. Klein. "CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network." *Clin Pharmacol Ther. 2011 Mar;89(3):464–7*

T. Klein: None. **M. Ritchie:** None.

E07.2
Implementation of pharmacogenomics in the clinic

M. Pirmohamed

University of Liverpool, Liverpool, United Kingdom

Pharmacogenetics/genomics has been around for a long time: although this has led to many discoveries, i.e. associations between phenotypes (drug efficacy and safety) and genotypes, translation of these discoveries into clinical practice has generally been poor. Worldwide, there is now greater emphasis on implementation, with many different approaches and areas being investigated including preemptive genotyping. Lack of robust evidence is cited as the main reason for lack of implementation. Conventionally, the randomised controlled trial is regarded as the top of the evidence hierarchy, but very few trials have been undertaken in pharmacogenomics. Undertaking a RCT for a pharmacogenomics phenotype is more complicated than undertaking a conventional RCT - apart from the usual factors such as design, sample size, clinical outcome measures, and follow-up, additional factors such as how patients will be genotyped, how genotype will affect drug dose/choice, whether it will be cost-effective, and how many patients will need to be screened to identify those that fit with the inclusion criteria, all need to be considered. These additional factors inevitably also make it much more expensive to undertake pharmacogenomics-based RCTs - given that many of the drugs being studied for pharmacogenomics are off-patent, it can be difficult to obtain funding to undertake these trials. Therefore, in order to improve the translation of laboratory findings into the clinic, we need to consider different forms of evidence in a more intelligent, rather than purely relying on a hierarchy of evidence. The talk will be illustrated by several case studies to show how different implementation approaches are now being investigated.

M. Pirmohamed: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; EU-PACT Trial European Commission, UK National Institute of Health Research. C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; LGC, MC Diagnostics.

E08
Multi-omics data integration

E08.1
Functional Genomics

P. Beales

London, United Kingdom

No abstract received.

E08.2
Methods of integrating genomics data

M. D. Ritchie

Geisinger Health System, Danville, PA, United States

Modern technology has enabled massive data generation of molecular data types including DNA sequence variation, RNA gene expression, methylation, and protein expression, among others. However, tools and software to work with these data in effective ways are limited. Genome science, in particular, has advanced at a tremendous pace during recent years with dramatic innovations in highthroughput

molecular data generation technology, data collection, and a paradigm shift from single lab science to large, collaborative network/consortia science. These massive datasets provide a rich resource for sophisticated data analysis to identify the underlying patterns that explain or predict complex trait architecture. Our group, and others, have been exploring the use of machine learning strategies to analyze these multiomics datasets. We have specifically focused on meta-dimensional analysis where we integrate multiple data types to identify multivariate models that predict disease states. The goal of this technology is to integrate across multiple datasets as well. In this presentation, I will describe the strategies in the field for integrating genomics data and provide some examples of where these techniques have identified novel association signals.

M.D. Ritchie: None.

E09
Phakomatosis Update

E09.1
Neurofibromatosis Update

D. Evans

Manchester, United Kingdom

Since the discovery of the NF1 gene in 1990 and NF2 in 1993 there has been the promise of gene based therapies. Initial work in NF1 using farnesyl transferase inhibitors was disappointing. However work on NF1 mice suggested that statins may have a role in helping the neuro-cognitive deficit in NF1. Again initial studies were not convincing but larger scale international initiatives are still awaited. More latterly many more target both in NF1 and NF2 have been suggested and early evidence suggested that imatinib was likely to have efficacy in at least a minority of growing plexiform tumours in NF1. This was shown to have only a 17% response rate in a clinical trial. The big breakthrough came nonetheless in NF2 with Bevacizumab. Bevacizumab is a vascular endothelial growth factor inhibitor that has recently been used in the treatment of schwannoma growth associated with NF2 and appears to work in short to medium term tumour shrinkage or control in about 70% of cases with rapidly growing tumours. Bevacizumab was approved for such treatment through the National NF2 service in England in September 2010. Seventy patients have so far been treated through 4 NF2 centres. This includes 10 patients with extracranial schwannomas that have responded to treatment with bevacizumab and 8 ependymomas with both symptomatic improvement, and decrease in tumour size. Bevacizumab treatment appears to have efficacy on rapidly growing schwannomas throughout the nervous system. Just this year MEK inhibitors were shown in a paediatric phase 1 trial to be effective in treating plexiform tumours in NF1 and may have efficacy in other tumour types. Other targets include nilotinib and sorafenib which are in phase 0 studies in Plymouth/Manchester.

E09.2
Tuberous Sclerosis Complex Update

S. Jozwiak[1,2]

[1]*Department of Child Neurology, Warsaw Medical University, Warsaw, Poland,* [2]*The Children's Memorial Health Institute, Warsaw, Poland*

Tuberous Sclerosis Complex (TSC) is one of the most frequent neurocutaneous disorders. It affects 1 in 6,000 in pediatric population. Familial cases comprise about 30% of all TSC patients. Up to 90% of patients suffer from epilepsy and about 50% present with different forms of mental retardation. Diagnosis of TSC may be difficult due to the fact that majority of clinical presentations of the disease develop over time in older children, adolescents and adults. According to the recent recommendations, TSC diagnosis may be established if pathogenic mutation is found in either of *TSC1* or *TSC2* genes, or when two major diagnostic clinical criteria are present in the patient. Recent update on newest diagnostic criteria of TSC will be presented. Usually, cardiac tumors, especially multiple, present a first clinical symptom of TSC and may be disclosed on routine fetal echocardiography performed at about 30th week of pregnancy. Early diagnosis of TSC in young infants may be particularly important as currently available early interventions may change the natural course of this devastating disease. Identification of infants with high risk of epilepsy allows implementation of preventative treatment, which significantly improves their neurodevelopmental outcome. Recent results of preventative studies and the EPISTOP project (www.EPISTOP.eu) will be discussed. Treatment with mTOR inhibitors of TSC-associated renal (angiomyolipomas; AMLs) and brain (subependymal giant cell astrocytomas; SEGAs) tumors, significantly improved long-term prognosis of affected persons. New recommendations for SEGAs treatment will be presented. mTOR inhibitors have been found to be effective also in drug-resistant epilepsy associated with TSC. Also these aspects of management will be covered in the lecture. Acknowledgements. SJ was partly financed by the European Community's 7th FP (FP7/2007–2013; EPISTOP, grant agreement no. 602391) and the Polish Ministerial funds for science (years 2014–2018) for the implementation of international co-financed project.

S. Jozwiak: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Novartis. F. Consultant/Advisory Board; Modest; Novartis, Eisai, UCB.

E10
Whole-genome haplotyping methods for human embryo selection

E10.1
Karyo- and Meio-mapping for human embryo selection

D. Wells

Nuffield, United Kingdom

No abstract received.

E10.2
Haplarithmisis for human embryo selection

J. R. Vermeesch

KU Leuven, Leuven, Belgium

Large scale whole genome and exome sequencing is uncovering a plethora of novel mutations that cause highly penetrant, early-onset, severe, or later-onset life-threatening dominant and recessive disorders. For couples who are known carriers of mutant alleles, preimplantation genetic diagnosis enables the detection of genetic disorders in embryos that have been fertilized in vitro, thereby avoiding their transmission to offspring. Traditional PGD methods require a mutation and family specific work-up. We and others have developed generic methods that can be readily applied for all transmitted genetic disorders. The method reconstructs genome-wide haplotype architectures as well as the copy-number and segregational origin of those haplotypes by employing phased parental genotypes and deciphering WGA-distorted SNP B-allele fractions using a process we coin haplarithmisis. We demonstrate the method can be applied as a generic method for preimplantation genetic diagnosis on single cells biopsied from human embryos enabling to diagnose both disease alleles genome wide, as well as numerical and structural chromosomal anomalies. Moreover, meiotic segregation errors can be distinguished from mitotic ones. In addition to the principles, I will present the results following the first year of clinical implementation. The introduction of genome wide screening of embryo's raised novel ethical questions. The principles guiding embryo selection and prioritization that are applied at our centre according to the chromosomal content and mutational load of the embryos, will also be discussed.

J.R. Vermeesch: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Agilent. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; patents licensed to Agilent.

E11
Strategies to avoid sudden cardiac death

E11.1
Sudden Cardiac Death in the Young

C. Semsarian

Sydney, Australia

Sudden cardiac death (SCD) is a tragic and devastating complication of a number of cardiovascular diseases. The death is most often unexpected and has major implications for the surviving family and the community. While in the older populations, SCD is most frequently caused by underlying coronary artery disease and heart failure, in those aged under 35 years, the causes of SCD commonly include genetic disorders, such as inherited cardiomyopathies and primary arrhythmogenic diseases. [1]In the evaluation of families in which SCD has occurred, the key combination of both clinical screening and targetted genetic testing is the cornerstone of both establishing an underlying diagnosis and in screening at-risk family relatives. Specifically, in cases where no definitive cause is identified at postmortem, i.e. Sudden Unexpected Death (SUD), the "molecular autopsy" has emerged as a key process in the investigation of the cause of death. The combination of clinical and genetic evaluation of families with SCD in the setting of a specialized multidisciplinary clinic provides a platform for early initiation of therapeutic and prevention strategies, with the ultimate goal to reduce sudden death among the young in our communities. [1]Bagnall et al. A Prospective Study of Sudden Cardiac Death among Children and Young Adults. *N Engl J Med* 2016;374:2441–2452.

E11.2
Recommendations for the management of sudden cardiac death

F. Fellmann

Lausanne, Switzerland

In November 2016 the Brocher Foundation hosted an interdisciplinary workshop on *Ethical, legal and practical aspects of post-mortem genetic analysis for sudden cardiac death in young adults*. The workshop was organised in collaboration with the Public and Professional Policy Committee of the ESHG. Twelve experts in (forensic) pathology, cardiology, genetics and law from Europe and Canada presented their views. Together with PPPC members and invited experts they participated in group work to identify common challenges and list recommendations. The workshop focussed on cases of sudden unexpected death in adults below the age of 40, potentially indicative of underlying hereditary cardiac disorders. In such cases post-mortem genetic testing can be relevant for medical reasons as well as for public health purposes. Though (forensic) autopsy procedures are available, these poorly integrate post-mortem genetic testing. Aim of the workshop was to address the lack of coordination between the forensic and medical domain and the various professions and respective regulations by drafting recommendations to improve European guidance.

This presentation will address the ethical, legal, and practical (including economical) challenges of post mortem genetic testing and the use of the results for medical purposes. Autopsy procedures in cases of sudden unexpected death vary per European country or region; the procedures for selecting cases suspected of sudden cardiac death, the number of autopsies performed, and the extent to which the heart is thoroughly examined as part of these autopsies, vary. It needs to be clarified at what stage and by whom the family members are informed and asked for consent to store samples or DNA. It needs to be clarified who is responsible for storing the sample, for what purpose and by whom a genetic test may be performed. Forensic pathologists and/or medical examiners) may not have sufficient training and/or resources (including time) to properly interpret genetic testing results. Further challenges include the transfer of information and samples from the forensic or medico-legal to the medical domain. Insufficient communication between different medical specialties (i.e. pathology, cardiology and genetics), further hinders an adequate information of the relatives of the deceased person.A list of recommendations will be presented to improve communication and attunement between professionals involved in post mortem analysis and genetics at a regional level as well as between different representing bodies and dedicated policymakers to improve adherence to existing guidelines whenever available, while suggesting further guidance in the absence of procedures.

Contributors: C. Basso, Padua, Italy; S. Boers, Utrecht, The Netherlands; P. Charron, Paris, France; A. Clarke, Cardiff, UK; M. Cornel, Amsterdam, The Netherlands; E. Delmarre, Angers, France; A. M. Duguet, Toulouse, France; C. van El, Amsterdam, The Netherlands; F. Fellmann, Switzerland; F. Forzano, London, UK; H. Howard, Uppsala, Sweden; S. Kauferstein, Frankfurt, Germany; K. Michaud, Lausanne, Switzerland; H. Kayserili, Istanbul, Turkey; A. Lucassen, Southampton, UK; A. Mendes, Porto, Portugal; C. Patch, London, UK; D. Radojkovic, Belgrade, Serbia; E. Rial-Sebbag, Toulouse, France; A. Sajantila, Helsinki, Finland; M. Sheppard, London, UK; A. M. Tassé, Canada; S. Temel, Turkey; A. Wilde, Amsterdam, the Netherlands; C. Yakicier, Istanbul,Turkey

E12
The evolution of genetic counseling: Lessons learned from psychotherapy

E12.1
The added value of psychotherapy in the genetic counselling process

R. Moldovan

Babes-Bolyai University, Cluj-Napoca, Romania

Genetic counselling is rooted in many different psychological traditions, ranging from theory-based approaches to evidence-based interventions. However, overarching frameworks that bring together the range of models and perspectives are lacking, and the breadth of research is difficult to translate into effective practice. Research has consistently demonstrated that genetic counselling improves patient knowledge, risk perception and autonomy in decision-making, decreases stigma, emotional distress and generates high satisfaction. Nevertheless, genetic counselling interventions are not traditionally evidence-based while a number of evidence-based psychological interventions are already available. The presentation will focus on how research, practice and training in genetic counselling could take account of the efficacy of evidence-based psychotherapies such as cognitive and behavioural psychotherapy.

R. Moldovan: None.

E12.2
Genetics and Family Dynamics: Navigating the Sometimes Bumpy Road to Effective Communication

S. H. McDaniel

University of Rochester Medical Center, Rochester, NY, United States

Communicating genetic information is challenging because of the complexity and ambiguity of the scientific information that must be transmitted by the clinician, and because the patient hears that information through filters that often include anxiety, idiosyncratic health beliefs, and longstanding family dynamics. This talk will provide specific evidence-based, communication techniques for the clinician to improve patient understanding of complex information. It will also describe tools based on family systems theory to understand and plan for communication with patients and their families in ways that support the genetic information being heard, understood, and acted upon.

S.H. McDaniel: None.

E13
Network Medicine

E13.1
Mining biological networks

N. Pržulj

London, United Kingdom

No abstract received.

E13.2
Cellular Networks and Human diseases

A. Sharma

Boston, MA, United States

No abstract received.

Concurrent Sessions

C01 Personalized Medicine and Pharmacogenomics

C01.1
The role of Next-generation sequencing in tumours in Adolescents and young adults (AYA) with advanced solid tumors participating in phase I trials

T. P. McVeigh, R. Sundar, N. Diamantis, S. Kaye, U. Banerji, J. Lopez, J. de Bono, W. van der Graaf, A. J. George

Royal Marsden NHS foundation trust, Sutton, United Kingdom

Introduction: Adolescents and young adults(AYAs) aged 15–39years at cancer diagnosis are uncommon. Hereditary cancer predisposition syndromes confer significantly increased risks of cancer at unusually young ages. Genetic mutations (somatic and/or germline) represent putative therapeutic targets. The aim of this study is to investigate the clinical and genomic assessment of AYAs with advanced solid tumours managed in a specialist drug development unit (DDU).

Methods: AYAs treated in the DDU at the Royal Marsden Hospital between 2002 and 2016, were identified from departmental databases. Data regarding clinicopathological features, clinical assessments, and germline and tumour genetic testing were retrieved by electronic chart review.

Results: 219 AYA patients were identified. The most common cancer types included sarcoma (42,18%); cervical (27,12%); breast (25; 11%); ovarian (21,10%) and colorectal (21,10%) cancers. Tumour Molecular Characterisation (MC) was performed by next generation sequencing in 45 cases. Mutations were detected most commonly in *TP53* (14,31%); *PIK3CA* (8,18%); *KRAS* (4,9%) and *MET* (4,9%). Twenty-one patients were known to have a cancer predisposition syndrome. Six others were referred to genetics or underwent mainstreamed genetic testing. Using current guidelines, a further 33 would now be eligible for mainstream *BRCA1/2* testing and 5 for *TP53* testing based on their personal history alone. In 108 cases, no family history was taken.

Discussion: A significant proportion of AYAs presenting with advanced tumours will have targetable mutations in the soma or the germline. Thorough assessment of familial risk factors, and inclusion of germline testing in appropriate circumstances can complement tumour testing to help optimize patient management.

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C01.2
Less is More: knockdown of the aberrant HBB $^{IVSI\text{-}110(G>A)}$ mRNA restores HBB expression and enhances gene therapy by gene addition in primary erythroid cells

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Mutations reducing β-globin production and thus causing β-thalassemia are of global clinical importance. β-Thalassemia caused by the *HBB*$^{IVSI-110(G>A)}$ mutation (HGVS name: HBB:c.93-21G > A), which produces an abnormal splice acceptor site, is particularly frequent in many Western countries and causes severe thalassemia major in homozygotes.

Preclinical and clinical studies have highlighted patients with *HBB*$^{IVSI-110(G>A)}$ as difficult to treat with gene therapy by gene addition, suggesting an effect of the mutant locus on normal, endogenous or vector-encoded, β-globin alleles. Towards improved gene-addition treatment of affected patients and supposing that the mutant locus acts in *trans* by aberrant *HBB*$^{IVSI-110(G>A)}$-derived mRNA, we therefore set out to reduce the latter by RNA interference. We recognised, first in a novel humanised murine erythroleukemia model and then in primary CD34^{+}-derived erythroid cells from HBB$^{IVSI-110(G>A)}$-homozygous patients, that specific knock-down of the aberrant *HBB*$^{IVSI-110(G>A)}$ mRNA alone results in extremely significant induction of β-globin production from the mutant locus. In primary cells the resulting β-globin expression and phenotypic correction of erythroid-lineage differentiation is equal to or exceeds that achieved by same-sample control treatment with the clinically successful GLOBE gene-therapy vector. Furthermore, combination of *HBB*$^{IVSI-110(G>A)}$ knockdown with GLOBE results in significant improvement of both disease parameters compared to either treatment alone.

This study establishes aberrant HBB$^{IVSI-110(G>A)}$ mRNA as the main causative agent of disease severity in -*HBB*$^{IVSI-110(G>A)}$ thalassaemia and as a potent target for mutation-specific gene therapy for β-thalassaemia. It moreover puts forward *HBB*$^{IVSI-110(G>A)}$ thalassaemia as a paradigm for the importance of allelic heterogeneity when applying gene therapy by gene addition.

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C01.3
A first genome-wide systems genetics approach identifies risk loci and pathways for candidaemia susceptibility

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Introduction: *Candida albicans* (*C. albicans*) is the most common opportunistic fungal pathogen, causing a bloodstream infection (candidaemia). However, not all at-risk patients develop candidaemia, indicating that genetics influences their susceptibility to the infection.

Materials and Methods: To identify novel susceptibility genes, we have performed the first genome-wide association analysis (GWAS) by using the largest candidaemia cohort to date of European ancestry. Due to the cohort size limitations, we followed a systems genetics approach to profile genetic variants that showed association with known risk factors for candidaemia, such as neutrophil counts, cytokine production and transcriptome. Therefore, we profiled transcriptome response to *C. albicans* in peripheral blood mononuclear cells (PBMCs) of 75 healthy individuals and identified the *Candida*-response expression QTLs (eQTs). *Candida*-induced cytokine profiling in PBMCs from healthy volunteers was also assessed and we mapped cytokine QTLs (cQTLs).

Results: We identified EHD4 locus as the genome-wide significant region to be associated with candidaemia. We further validated the role of EHD4 in regulation of IFNγ, a well-known pathway involved in anti-*Candida* host immune defense, using co-expression data upon *Candida* stimulation. Three genome-wide significant QTLs influencing *Candida*-induced cytokine production (IL6, TNF-a and IL22), and one locus influencing IL17 production induced by *Candida* were identified. In addition, variants with suggestive association ($P < 9.99 \times 10^{-5}$) with candidaemia susceptibility identified to be associated with either neutropenia or cytokine-QTLs.

Conclusions: Integration of transcriptional responses, cQTs and genetic studies in candidaemia patient cohort identified several new susceptibility genes that may represent diagnostic and therapeutic targets in candidaemia.

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C01.4
Whole genome sequencing yields medically significant secondary variants in ~25% of a paediatric cohort

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To pilot paediatric genomic medicine we developed the SickKids Genome Clinic, a research project centered on diagnostic whole genome sequencing (WGS) of children undergoing genetic evaluation. With parents' consent, we also search for predictive secondary variants (PSVs) associated with occult or future disease.

A search of 100 patients' WGS data for candidate PSVs affecting 3000+ disease genes yielded 26 SNVs that met 2015 ACMGG criteria for pathogenic/likely pathogenic classifications. An additional 10 SNVs were deemed returnable PSVs based on more limited evidence of pathogenicity. None of 610 CNVs that overlapped OMIM genes were classified as PSVs. All PSVs were inherited and 9/36 predicted adult onset disease. The majority were rare (MAF < 0.0001) variants that cause dominant Mendelian disorders. The remainder included variants linked to attenuated Mendelian phenotypes, common disease risk variants, an X-linked variant, and a protective variant.

25 children had one PSV, four children had two PSVs and one child had three. Structured family histories performed before result disclosure were negative for disease states predicted by the secondary PSVs. However, following disclosure, positive family histories related to the PSVs were obtained by targeted questioning in more than a third of cases. Reverse phenotyping of children with PSVs and their first-degree relatives uncovered unsuspected disease and demonstrated incomplete penetrance.

Comprehensive genomic searching can yield PSVs in ~25% of children, mostly rare familial mutations associated with childhood disorders. While predictive WGS yields false positives, it uncovers occult disease states and is substantially more sensitive than family history for identifying familial genetic disorders.

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C01.5
Secondary actionable findings identified by whole-exome sequencing from 693 consecutive tests: implications for organization of care and patients?

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Background: With the integration of whole exome sequencing (WES) in medicine practice, there is potential for the reporting of secondary findings unrelated to the indication for prescribing WES but of medical value for patient care. In 2013, the American College of Medical Genetics and Genomics (ACMG) examined the issue of secondary findings in WES/WGS, and introduced recommendations to report medically actionable variants in a minimal set of 56 genes, recently updated to 59.

Methods: In order to evaluate the implications of these recommendations in the organization of care, we conducted a retrospective study to evaluate the frequency of variants in a list of actionable genes in a cohort of 693 probands with multiple congenital anomalies explored by WES, as well as the extra work generated.

Results: We identified 2.4% of patients with pathogenic or likely pathogenic variants in the 59 ACMG actionable genes (3.6% when extended to the Dorschner list). 10% of patients were heretozygous for a variant responsible for the four most frequent autosomal recessive diseases in France. If returned to patients, these results should induce 693 pre-test and 96 post-test genetic consultations, and 31 specialised follow-up and additional family evaluations. Variant interpretation took about 15 min.

Conclusions: This study evaluated consequences in terms of organization of care. Although multiple surveys in various populations have been massively in favour of returning such results, this opportunity should be discussed given the work overload generated, and the absence of large studies evaluating the impact of transmitting such results to affected patients/families.

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C01.6
Genetics-first analysis of high-risk variants for breast, ovarian and prostate cancer in participants of Estonian Genome Center

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The American College of Medical Genetics and Genomics has published a minimum list of 59 genes that should be reported back if found mutated in clinical sequencing. We are adapting these recommendations for genomic analyses in Estonian Genome Center to favour personalized management and early detection. Hereditary breast and ovarian cancer (HBOC) was selected as one of primary candidates for the participant feedback as it has the highest frequency in our dataset, together with familial hypercholesterolemia. By Estonian Gene Research Act, the biobank participants have a right to know about their genetic data. To date, we have identified 34 individuals with 9 known and expected pathogenic variants (3 recurrent variants, 6 singletons) in BRCA1 and BRCA2 genes. 18 individuals were identified from whole genome sequencing data (WGS; $n = 2240$) and additional 16 individuals by using long-range haplotyping. So far, 9 participants have visited Estonian Genome Center for additional validation. 3 of 9 had been diagnosed with breast and prostate cancer; 8 of 9 had a positive family history of HBOC-related malignancies. Based on WGS data from 2240 individuals, the prevalence of BRCA1 and BRCA2 high-risk variants in Estonia is approximately 1/125. Therefore, it will be extremely important to establish a close collaboration with clinicians for a proper follow-up. Giving feedback on high-risk genetic variants to currently healthy individuals may have a large psychosocial impact. This needs to be further studied, together with the individual's compliance to management recommendations. This research was supported by the EU project 2014–2020.4.01.15–0012 and PUT736 personal grant.

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C02
Neurogenetics 1

C02.1
Allele-specific silencing as therapeutic strategy for disorders due to gene duplication: a proof-of-principle in Autosomal Dominant LeukoDystrophy (ADLD)

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Allele-SPecific silencing by RNA Interference (ASP-iRNA) allows to specifically inhibiting the expression of disease-causing alleles with minimal suppression of the corresponding wild-type alleles. This therapeutic strategy has been effectively used to target dominant activating mutations or SNPs *in cis* with aberrantly expanded trinucleotide repeats. We reasoned that ASP-iRNA could be exploited also in genetic diseases due to gene duplication. Here, we demonstrate its efficacy in Autosomal Dominant LeukoDystrophy (ADLD), a fatal disorder associated with CNS demyelination. As ADLD is caused by *LMNB1* gene duplication-mediated overexpression, the paramount choice for ADLD treatment would be a drug capable of restoring physiological levels of *LMNB1* expression without the deleterious side effects of an excessive gene knock-down. ADLD patients have three, equally expressed, *LMNB1* alleles. Hence, we chose to target the non-duplicated allele by ASP-siRNA, exploiting a frequent coding SNP. We designed and screened a siRNA library centred on the SNP

and evaluated siRNAs efficacy and specificity by using a customized dual reporter system. We identified four siRNAs with a high efficacy ($p < 0.0001$) and allele-specificity ($p < 0.001$). These were further tested in four ADLD patient-derived fibroblast lines. Three siRNAs selectively silenced the target allele ($p < 0.0005$) and restored *LMNB1* mRNA level to the wild type. Notably, siRNA treatments restored LMNB1 protein to physiological levels and improved ADLD-specific cellular alterations, corroborating ASP-iRNA therapeutic potential in ADLD. Our work represents a proof-of-principle in the use of ASP-RNAi in genetic disorders with gene overexpression, opening new therapeutic possibilities for all Mendelian and syndromic disorders associated with gene(s) duplication.

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C02.2
CHRNA7 CNVs: shared clinical phenotypes mediated by differing molecular mechanisms

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Chromosome 15q13.3 is an extremely unstable region in the genome with multiple pathogenic recurrent copy number variants (CNVs) observed. Probands with 15q13.3 deletions present with cognitive deficits, epilepsy, and autism spectrum disorder (ASD), while duplication probands have a milder phenotype including borderline cognitive deficits, ASD, and ADHD. Encoding for the α7 nicotinic acetylcholine receptor (nAChR) highly expressed in the brain, *CHRNA7* has been suggested as a candidate gene for these CNVs.

We have utilized patient-derived induced pluripotent stem cells and differentiated neural progenitor cells (NPCs) to determine the molecular phenotypes of 15q13.3 CNVs. In these cells, mRNA levels of genes within 15q13.3 CNVs correlate to their genomic copy number. For *CHRNA7* duplications, we are able to show that the entire *CHRNA7* gene is duplicated, resulting in mRNA overexpression. Importantly, small duplications that include the first exon of *OTUD7A* do not appear to disrupt its expression.

Surprisingly, both *CHRNA7* gains and losses result in decreased α7-specific calcium flux in patient-derived NPCs. While this is puzzling considering the genomic and expression data, it does mirror clinical phenotypes observed in patients. Deletions likely have decreased calcium flux due to haploinsufficiency of *CHRNA7*. Overexpression of α7 subunits from duplications, on the other hand, result in ER stress, likely due to insufficient chaperones required for folding, assembly, and trafficking of nAChRs. Subsequently, fewer functional receptors reach the cell membrane. For both deletions and duplications, the decreased calcium flux results in down-regulation of calcium signaling cascades, which may explain the neurobehavioral phenotypes observed in patients.

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C02.3
17q21.31 duplication causes prominent Tau-related Dementia with Increased *MAPT* expression

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Introduction: Association analyses based on whole-exome sequencing (WES) data have highlighted the genetic contribution of rare single nucleotide variants and indels in Alzheimer Disease (AD). The study of copy-number variations (CNVs) in large case-control series was initially restricted to chip-based analysis focusing on large and frequent CNVs. The development of new algorithms dedicated to CNVs allows the detection from WES data of rare, exon-scale rearrangements.

Methods: To assess the role of rare CNVs in AD, we conducted a case-control study using WES data from 522 early-onset AD cases (onset before 65 years) and 584 controls using CANOES, a software based on read depth comparison.

Results: The most recurrent rearrangement, found in 4 unrelated cases and absent in controls, was a 17q21.31 microduplication, reciprocal to the Koolen-De Vries syndrome microdeletion, overlapping *CRHR1*, *MAPT*, *STH* and *KANSL1*. Interestingly, one was de novo, and one was cosegregating in a large pedigree. Duplication carriers exhibited increased expression of the *MAPT* mRNA; clinical signs, neuroimaging and CSF biomarker profiles were consistent with an AD diagnosis, but amyloid PET imaging was negative. Neuropathological examination confirmed that the *MAPT* duplication causes a complex tauopathy including prominent neurofibrillary tangle pathology, but without Aβ deposits, yet a hallmark of AD.

Conclusion: 17q21.31 duplication is therefore the genetic basis of a novel entity characterized by prominent tauopathy, leading to early-onset dementia with an AD clinical phenotype. This entity could account for a proportion of probable AD cases with negative amyloid PET imaging recently identified in large clinical series.

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C02.4
MCM3AP in recessive axonal neuropathy and mild intellectual disability

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Defects in mRNA export from the nucleus have been linked to various neurodegenerative disorders. We report mutations in the gene *MCM3AP*, encoding the germinal center associated nuclear protein (GANP), in nine affected individuals from five unrelated families. The variants were associated with severe childhood onset axonal (four families) or demyelinating (one family) Charcot-Marie-Tooth neuropathy (CMT). Mild to moderate intellectual disability (ID) was present in six out of nine affected individuals. The affected individuals were either compound heterozygous or homozygous for different *MCM3AP* variants, which were predicted to cause depletion of GANP or affect conserved amino acids with likely importance for its function. Accordingly, fibroblasts of affected individuals from one family demonstrated severe depletion of GANP. GANP has been described to function as an mRNA export factor, and to suppress TDP-43-mediated motor neuron degeneration in flies. Thus our results suggest defective mRNA export from nucleus as a potential pathogenic mechanism of axonal degeneration in these patients. The identification of *MCM3AP* variants in affected individuals from multiple centers establishes it as a disease gene for childhood-onset recessively inherited CMT with ID.

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C02.5
BZRAP1 (RIM-BP1) mutations cause a novel autosomal recessive dystonia syndrome

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RIM-binding proteins (RIMBPs) are large multidomain active zone proteins that bind to calcium channels. In mice, deletion of RIM-BPs disrupts localization of calcium channels at the active zones, thereby impairing the fidelity of synaptic transmission. In humans, *BZRAP1* (*TSPOAP1*) encoding RIMBP1 has not been associated with any Mendelian disorders.

We performed exome sequencing in an Estonian patient with progressive generalized dystonia with onset at 12 years of age. A homozygous stop-gain mutation c.2449_2450delinsTG p.(Gln817*) (RefSeq NM_004758.3) in a *BZRAP1* was identified. Using GeneMatcher, another family originating from India was found with three sibs affected by generalized dystonia that carried a segregating homozygous frameshift mutation in *BZRAP1* (c.538delG p.(Ala180Profs*9)). Importantly, there are no homozygous loss-of-function (LoF) mutations in gnomAD database of 141,352 individuals, supporting the pathogenicity of these mutations.

The shared features between homozygous LoF mutation carriers were onset of dystonia in the second decade, progressive decline in cognitive performance, progressive cerebellar atrophy, and marginally low 5-methyltetrahydrofolate in cerebrospinal fluid. A muscle biopsy indicated a synaptic defect in the Estonian patient. In addition, a patient from Turkey carrying homozygous missense mutation (c.5422G > A p.(Gly1808Ser)) was identified with a significantly milder phenotype, consisting of adult-onset cervical and upper-limb dystonia, without any evidence of cerebellar atrophy.

In conclusion, we describe the novel gene-phenotype association of biallelic mutations in *BZRAP1* gene causing autosomal recessive dystonia syndrome. Functional studies are in progress to characterize the pathogenesis of *BZRAP1*-related disorder.

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C02.6
De novo mutations in regulatory elements cause neurodevelopmental disorders

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De novo mutations in hundreds of different genes collectively cause 25–42% of severe developmental disorders (DD). The cause in the remaining cases is largely unknown. The role of de novo mutations in regulatory elements in severe developmental disorders is largely unexplored. We identified de novo mutations in three classes of putative regulatory elements in almost 8,000 DD patients and find that de novo mutations in highly evolutionarily conserved elements active in the fetal brain are significantly and specifically enriched in neurodevelopmental disorders. We identified a significant two-fold enrichment of recurrently mutated elements. We estimate that, genome-wide, de novo mutations in fetal-brain active elements are likely to be diagnostic for 1–3% of patients without a pathogenic coding variant and that only a small fraction (<1%) of de novo mutations in these elements are pathogenic with high penetrance. Using sequence data from 13,000 unaffected parents, we show that identifying strongly deleterious variants in regulatory elements is not possible using current tools, highlighting the need for improved methods to stratify more or less damaging variation in regulatory elements. Our findings represent a robust estimate of the contribution of de novo mutations in regulatory elements to this genetically heterogeneous set of disorders, and emphasise the importance of combining functional and evolutionary evidence to delineate regulatory causes of genetic disorders.

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C04
Epigenetics and Gene Regulation

C04.1
Polymer physics predicts the effects of structural variants on chromatin architecture

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The 3D folding of the genome and especially the organization of topologically associating domains (TADs) can be disrupted by genomic rearrangements, such as deletions, duplications or inversions, collectively called structural variants (SVs). SVs can originate a re-wiring of enhancer-promoter contacts, gene misexpression and disease. However, the prediction of such effects remains a challenge. We present a polymer physics based approach (PRISMR) to model chromatin folding and predict enhancer-promoter contacts. Our PRISMR algorithm aims to find the minimal number, types and position of binding sites in a String&-Binders (SBS) polymer chain, that best reproduce an input contact matrix of a given chromosomal locus. Using the *EPHA4* locus as a model, the effects of pathogenic SVs were predicted in-silico and compared to capture Hi-C data from mouse limb buds and patient-derived fibroblasts. PRISMR deconvolves the folding complexity of the *EPHA4* locus, and identifies SV-induced alterations of 3D genome organization in homozygous and heterozygous states. PRISMR accurately predicts the specific genomic positions with ectopic contacts that produce extensive rewiring of regulatory interactions, causing disease by gene misexpression. PRISMR can be used to predict interactions in-silico thereby providing a tool for analyzing the disease causing potential of SVs. Furthermore, PRISMR can also be used when affected tissues or equivalent cell types are not available. Thus, polymer modelling by PRISMR emerges as a valid method to facilitate the interpretation and diagnosis of this type of genomic rearrangements.

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C04.2
Changes in chromatin interaction dynamics at the *PITX1* locus cause congenital limb malformations

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Non-coding genomic diversity critically impacts fundamental biological processes (e.g. gene regulation, 3D chromatin folding), and can contribute to human disease. In this study, we systematically investigated the *cis*-regulatory landscape of *PITX1*, a hindlimb specific transcription factor. Mutations and non-coding structural variations at the *PITX1* locus have been shown to associate with a variety of congenital limb defects, including clubfeet, polydactyly, and arm-to-leg transformation. We performed in vivo enhancer reporter assays in transgenic mice and identified several limb enhancer elements at the *Pitx1* locus; surprisingly they all showed both forelimb and hindlimb activity, although *Pitx1* is never expressed in the forelimb. We termed this regulatory region the "*pan*-limb enhancer." Chromosome confirmation capture experiments (Capture Hi-C) in mouse limb buds revealed a tissue-specific chromatin organization at the *Pitx1* locus, which uniquely enables hindlimb-specific contact between the *Pitx1* promoter and the *pan*-limb enhancer. To investigate whether this tissue-specific chromatin folding plays a causal role, we used CRISPR/Cas9 to generate a set of deletions and inversions in the *Pitx1 cis*-regulatory region in mice. Genetic perturbations of this regulated chromosomal conformation led to ectopic forelimb expression of *Pitx1* and an arm-to-leg transformation in mice and in human patients. Our data highlight how non-coding mutations affecting chromatin folding can contribute to congenital disease, and give new insights into *PITX1*-associated limb defects.

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C04.3
Generating large-scale datasets of mutational effects for interpreting regulatory variants

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The use of sequencing approaches for the identification of disease causal mutations is rapidly gaining traction. However, interpretation of the identified variants remains a major challenge. When scaling from exome to genome sequencing, the vast majority of variants fall in non-coding regions. However, we currently have a very limited toolset for their interpretation and almost no training data that can be applied for machine learning strategies.

Recently, we enhanced our massively parallel reporter assays (MPRAs) to directly quantify effects from RNA tag-sequencing read-outs and developed a lentiviral integration system for MPRA (lentiMPRA), achieving replicate correlations of 0.92–0.98 depending on the type of assay (Inoue & Kircher et al. 2016). We believe that the broad cell-type range of lentivirus transduction will permit MPRAs to be conducted in neurons, primary cells or organoids.

In a second effort, we scaled saturation mutagenesis MPRAs to empirically measure effects of >20,000 point mutations in human disease associated *cis*-regulatory elements. More specifically, we aimed to generate variant-specific activity maps for more than 20 clinically relevant promoter (*F9*, *HBB*, *TERT* and others) and enhancer sequences (*SORT1*, *RET* and others). For example, using our map of the *TERT* core promoter, we rediscovered previously identified activating mutations and were able to assign activating effects to variants of unknown significance; comparing across cell-types we further mapped the effects of mutations to an *E2F*-repressor site. Our experiments provide a rich dataset for benchmarking predictive models of variant effects and an unprecedented database for the interpretation of potentially disease-causing regulatory mutations.

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C04.4
Local regulatory networks across two tissues and applications to analyze rare non-coding variants

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Population measurements of gene expression and genetic variation enable the discovery of thousands of expression Quantitative Trait Loci (eQTL), an extensive resource to determine the function of non-coding variants. To describe the effects of eQTL on regulatory elements such as enhancers and promoters, we quantified gene expression (mRNA) and three key histone modifications (H3K4me1, H3K4me3 and H3K27ac) across two cell types, 320 Lymphoblastoid Cell Lines and 80 Fibroblasts densely genotyped European samples. We find that nearby regulatory elements form local chromatin modules (LCM) often comprising multiple sub-compartments and overlapping topologically associating domains (TADs). These modules bring multiple distal regulatory elements in close proximity, vary substantially across cell types and drive co-expression of multiple genes. This regulation is under strong genetic control as ~34,000 chromatin QTLs (cQTLs) affect ~30% of the histone marks and as ~70% of LCMs are associated with QTLs. These LCMs empower association studies of rare variants when whole genome sequencing is available. Using the Geuvadis transcriptomic data we unravel that expression of ~20% of genes is associated with rare non-coding variants in modules, for example. Coordination between regulatory elements located on different chromosomes (i.e. in *trans*) is well supported by Hi-C sequencing data and seem to drive in some cases *trans* eQTL effects. We replicated up to 80% of the strongest inter-chromosomal Hi-C contacts. Overall, this large-scale study integrating gene expression, chromatin activity and genetic variation across two cell types and hundreds of samples provides key insights into the biology underlying gene regulation and eQTLs.

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C04.5
Cis-Regulatory Noncoding Elements, the hidden master weavers of *CDH1* expression: lessons from HDGC patients

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Introduction: Half of Hereditary Diffuse Gastric Cancer (HDGC) families lack genetic diagnosis. We hypothesize that this rare monogenic cancer-syndrome is caused by intronic *cis*-regulatory variants in the major disease-causative gene, *CDH1*/E-cadherin. Supporting our hypothesis, we demonstrated that most *CDH1* coding mutation-negative HDGC families, lacking coding mutations in other genes, display germline *CDH1*-monoallelic downregulation and aberrant E-cadherin expression in tumours. Herein, we aim at disclosing the functional role of *CDH1* intronic cis-regulatory elements (iCREs) and the impact of their disruption in *CDH1*/E-cadherin expression.

Methods: We sequenced (NGS) the full *CDH1* locus of >200 *bonafide* HDGC *CDH1*-negative families. Noncoding variants (NCVs) were bioinformatically prioritized according to their absence from public genome databases and rareness within this cohort. NCVs were integrated with regulatory functional annotation to select iCREs, whose function was evaluated through in vitro and in vivo (zebrafish) reporter assays and CRISPR-Cas9-mediated iCRE deletion.

Results: Twelve potential iCREs were defined within *CDH1*. We found that 2/12 act as enhancers in vitro and in vivo, but its enhancer function was abrogated by random point mutations. Homozygous deletion of iCREs in cell lines led to massive *CDH1* mRNA downregulation and E-cadherin loss of expression, while heterozygous deleted clones showed a strong monoallelic downregulation, recapitulating the phenotype observed in HDGC patients. These

results support our assumption that identified iCREs are true regulatory elements modulating *CDH1*/E-cadherin expression.

Conclusion: High numbers of clinically homogeneous HDGC families and a *locus*-targeted approach allowed identifying new *CDH1* inactivating mechanisms likely causative in HDGC. Support: Portuguese FCT and American NSFC Foundation.

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C04.6
Single Cell Methyl-Seq for Analysis of Epigenomic Diversity in Mammalian Brain

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Epigenomic marks such as cytosine DNA methylation (mC) have highly diverse patterns across brain cell types. Human and mouse brains accumulate high levels of non-CG methylation (mCH) throughout the genome, where > 200,000 regions showing differential CG methylation were identified between three cortical excitatory and inhibitory neuron types. In addition, ~16% of the mouse genome contains differential mC signatures that distinguish neuronal cell types by low coverage single cell WGBS (whole-genome bisulfite sequencing). Extending cell type specific mC analysis to all brain cell types requires unbiased single cell mC profiling, and also enables the study of mC heterogeneity across cells of the same type. To meet the need of large-scale single cell methylome profiling, we developed a single cell methylome workflow using Adaptase™, the technology underlying the Accel-NGS Methyl-Seq Library Preparation Kit. Through more efficient adapter attachment to bisulfite converted ssDNA, libraries with greater complexity are generated and single cell pooling enables high-throughput library preparation. Single cell methylomes were generated from over 4,000 single neuronal nuclei isolated from human and mouse frontal cortex using FACS. We demonstrated robust cell type classification, readily separating excitatory and inhibitory populations and also identified distinct inhibitory cells. The data further allowed an accurate classification of pyramidal neurons in superficial versus inner layers of mouse frontal cortex. The single cell Methyl-Seq method will enable unbiased characterization of brain epigenomic diversity without the need for isolation of specific cell populations.

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C05
Skin and Bones

C05.1
Mutations in three genes encoding proteins involved in hair shaft formation cause uncombable hair syndrome

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Uncombable hair syndrome (UHS), also known as "spun glass hair syndrome", "pili trianguli et canaliculi", or "cheveux incoiffables" is a rare anomaly of the hair shaft which occurs in children and improves with age. UHS is characterized by dry, frizzy, spangly and often fair hair that is resistant to being combed flat. Up to date both simplex and familial UHS cases with autosomal dominant as well as recessive inheritance have been reported. However, none of these cases were linked to a molecular genetic cause. Here, we report the identification of UHS causative mutations located in the three genes *PADI3 (*peptidylarginine deiminase 3*)*, *TGM3 (*transglutaminase 3*)* and *TCHH (*trichohyalin) in a total of eleven children. All of these individuals carry homozygous or compound heterozygous mutations in one of these three genes, indicating an autosomal recessive inheritance pattern in the majority of UHS cases. The two enzymes PADI3 and TGM3, responsible for posttranslational protein modifications, and their target structural protein TCHH, are all involved in hair shaft formation. Elucidation of the molecular outcomes of the disease causing mutations by cell culture experiments and tridimensional protein models demonstrated clear differences in the structural organization and activity of mutant and wild type proteins. Scanning electron microscopy observations revealed morphological alterations in hair coat of *Padi3* knockout mice. All together, these findings elucidate the molecular genetic causes of UHS and shed light on its pathophysiology, and hair physiology in general.

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C05.2
Genotype-phenotype correlation in Jeune thoracic dysplasia/short rib-polydactyly type III: review of 130 cases

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Short rib-polydactyly type III (SRPIII) and asphyxiating thoracic dysplasia (ATD) are ciliopathies ranging from severe fetal form to life-compatible ATD, characterized by narrow thorax and shortened limbs. ATD symptoms may involve respiratory, hepatic and renal dysfunctions and retinal degeneration. To date, mutations in 12 ciliary genes are reported in ATD/SRPIII. To establish genotype-phenotype correlations and delineate long-term evolution of ATD, we recruited 75 fetuses and 55 ATD patients (age range 1–43 years). We performed targeted sequencing of 1221 ciliary genes and further exome if negative. We identified mutations in 84% cases, including 60% in *DYNC2H1* (79/130). Other mutations involved *IFT140 (4.5%), WDR60 (3%), WDR34 (3%), IFT80 (2%), TTC21B (2%), DYNC2LI1 (1%), WDR19 (1%), WDR35 (1%), TCTEX1D2 (1%)*. We also identified mutation in *KIAA0753* in one ATD living case and in *KIF24*, a new ciliary gene, in one fetal ATD. Fetus cases linked to *DYNC2H1* (50/75) mostly presented only skeletal signs. 8/50 (16%) presented few dilated renal tubules and 3/50 (6%) abnormal liver portal spaces. Comparatively, 50% of fetus linked to other genes displayed renal cysts and/or glomerular dysplasia and

33% abnormal liver. Among the 55 alive ATD, 31 had *DYNC2H1* mutations. 9 (16%) patients died of respiratory distress, including 5 with *DYNC2H1* mutations (17%). Abnormal electroretinogenesis equally affected both groups (10%). No case with *DYNC2H1* mutations displayed renal or liver dysfunctions. Conversely 35% patients with mutations in other genes had renal insufficiency and 10% liver fibrosis. Our results suggest that medical follow-up can be modulated depending on the molecular basis.

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C05.3
Phenomics analysis of zebrafish type I collagen mutants reveals a spectrum of skeletal phenotypes mimicking the clinical variability in human brittle bone

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Introduction: The brittle bone disease (or osteogenesis imperfecta, OI) is a rare congenital disorder, caused by defects mainly related to type I collagen, which forms the structural scaffold of the bone extracellular matrix. Clinically, OI is characterized by a broad disease spectrum, ranging from mild forms with minimal fractures, to severely deforming or even lethal forms. The underlying pathogenic mechanisms between the different types of OI, remains one of the most puzzling questions in the field. In this study we illustrate the potential of zebrafish as a tool to better understand and define genotype - phenotype correlations in OI.

Materials and Methods: We conducted a phenomics analysis on a large set of zebrafish mutants representing different forms of OI by mapping and quantifying skeletal parameters.

Results: Our study revealed a remarkably high phenotypic reproducibility of the human disease features between our set of zebrafish mutants and patients with comparable genetic forms of OI. These findings, along with advanced computational analysis of quantitative parameters argued for the presence of similar genetic mechanisms, responsible for influencing the presence and penetrance of disease features, both in zebrafish models and human OI patients.

Conclusions: Bone phenotypes in mice often cause perinatal lethality, making many bone mutants unavailable for the study of later stages. Zebrafish overcomes this challenges. With our study, we demonstrate that zebrafish is able to both genocopy and phenocopy different forms of human OI. We therefore propose zebrafish as a new tool to investigate unknown genetic modifiers and mechanism underlying human OI.

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C05.4
A 10q24.32 duplication causes bilateral femoral hypoplasia through formation of a novel sub-TAD

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Bilateral hypoplasia of the femoral bones is an extremely rare limb malformation with unknown genetic origin. In our study we investigated a sporadic female patient with isolated severe bilateral femoral hypoplasia, in whom arrayCGH revealed a 533 kb duplication at chromosome 10q24.32, which occurred de novo. The rearrangement demonstrated a significant overlap with the split hand/foot malformation type 3 (SHFM3) *locus*, yet included a known

developmental gene, *FGF8*, absent in the SHFM3 causative aberrations.

CRISPR/Cas9 approach was utilized to recapitulate the patient's phenotype in mice. The $Fgf8^{+/dup}$ mutant manifested multiple skeletal abnormalities, including a severe shortening of the humeri and the femoral bones. The second mutant, $Fgf8^{del/dup}$ carried the same duplication on one allele and a corresponding deletion on the other allele, yet manifested the same skeletal defects. Based on the mouse studies we hypothesize that the patient's phenotype resulted specifically from the duplication, which restructured the genes and *cis*-regulatory elements (REs) in the *locus*, and not from the gene dosage effect.

Next, we performed circular chromosome conformation capture (4C) assay to investigate the interactions between relocated genes and REs in patient's fibroblasts. 4C-seq revealed ectopic interaction within the altered region and indicated to the formation of a novel sub-TAD (Topologically Associated Domain) as the underlying pathomechanism of the femoral hypoplasia phenotype.

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C05.5
Cross-mapping analysis identifies 9 modifier loci in Marfan syndrome

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Marfan syndrome (MFS) is a connective tissue disorder with an autosomal dominant inheritance, mainly due to mutation within *FBN1* gene. Several systems are affected, such as ocular (*ectopia lentis*), skeletal (scoliosis) and cardiovascular system (thoracic aortic aneurysm). MFS displays great variability, for age of onset as well as severity of clinical manifestations. Absence of strong genotype-phenotype correlation, extra- and intra-familial variability and the use of quantitative models to estimate heritability suggest the existence of genetic factors underlying phenotypic variability.

We combined various genome-wide approaches and performed a cross-mapping of significant results to identify these genetic modifiers. Within a collection of 1070 clinically well-characterized *FBN1* mutation carriers, we combined 1) an *FBN1* eQTL analysis in 80 fibroblasts of *FBN1* mutation-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 analysis in 98 extreme phenotype samples.

We found 3 genetic factors underlying phenotype variability: 1) new phenotype-genotype correlations, 2) co-occurrence in 5 patients of the *FBN1* disease-initiating mutation with an additional mutation in another vascular disorder gene, and 3) combined effects of frequent alleles with identification of 9 modifier loci (common to at least two different analysis) containing excellent candidate genes, including one in which mutations are known to be involved in familial thoracic aortic aneurysm.

These results confirm that combination of various strategies is efficient to overcome obstacles to identify genetic architectures of clinical variability in rare diseases

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C05.6
Can we predict *PIK3CA*mosaicism genotype from deep phenotyping variables in overgrowth?

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Individuals with *PIK3CA* mutations are currently eligible for international trials of mTOR inhibitors, however many affected individuals present with overlapping features of syndromes previously thought to be clinically distinct, making selection for genotyping difficult. We undertook targeted deep next generation sequencing of 60 known overgrowth genes on tissue samples from a cohort of 110 children with a diagnosis of vascular malformation and/or overgrowth. Multiple logistic regression was used to model *PIK3CA* mutation status on the basis of deep phenotyping variables (clinical, radiological, histopathological and haematological) and clinical outcome measures (pain requiring regular analgesia, thrombotic complications, sclerotherapy, surgery and death). Median age at presentation was 1.2yrs, median follow up 5.5yrs. 42% of patients had truncal/limb overgrowth, 7% macrocephaly, 49% abnormal clotting, and 95% positive mTOR immunohistochemistry. 59% required surgery, 41% sclerotherapy, 21% suffered superficial thrombophlebitis and 2.5% deep vein thrombosis, 30% had significant pain and there were no deaths. 41% had detectable *PIK3CA* mutations, with mutant allele frequency 1.2–28%. No phenotypic variables were statistically predictive of all-genotype *PIK3CA* mosaicism, however, *PIK3CA* hotspot mutations were significantly associated with mixed vascular malformations (OR 15.5, $p = 0.01$), and a requirement for surgery (OR 3.4, $p = 0.039$) which is a proxy measure for severity of overgrowth. Our findings confirm the broad phenotypic spectrum reported with post-zygotic mutations in *PIK3CA*. Thus far we have not identified any phenotypic predictors of genotype. However, our preliminary results suggest exact genotype may help predict clinical outcome.

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C06
ELSI genomics

C06.1
Uncertainty about carrier results from exome sequencing: A randomized controlled trial of disclosure

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Exome sequencing often generates uncertain results. How individuals perceive the uncertainty can affect the utility of the results. Adult participants ($N = 462$) from an NIH clinical cohort study who chose to learn carrier results generated from sequencing were randomized to disclosure by a web-based platform or by a genetic counselor. Half of each group was further randomized to a follow-up counseling session. Participants were on average 63 years old, white, and highly educated. They received a median of 3 heterozygous variants and a median of 0.7 VUS results. A two-way ANOVA was used to evaluate the influence of education mode and counseling on perceptions of practical uncertainty about results at six-months follow-up. The main effect of education mode indicated significantly greater practical uncertainty among participants in the web arm ($p = 0.018$). The interaction effect was also significant, yielding a ratio of $F(1,363) = 4.01$ ($p = 0.046$). Practical uncertainty was significantly correlated with tolerance of uncertainty ($r = 0.169$, $p = .001$) and ambiguity aversion ($r = 0.213$, $p < .001$), demonstrating perceptions consistent with personality characteristics. Our findings demonstrate that receiving results from an online platform can generate higher perceptions of practical uncertainty than receiving results from a counselor, and that follow-up counseling can mitigate the elevated uncertainty. Uncertainty may lead to reduced utility of the results, and may be a limitation of receiving carrier results from a web-based platform without genetic counseling. This study was funded by the intramural research program of the National Human Genome Research Institute, National Institutes of Health.

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C06.2
Recommendations for the reporting of results from diagnostic next generation sequencing

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Introduction: Although next generation sequencing technologies (NGS) are well-embedded in the clinical setting for identification of genetic causes of disease, most current recommendations by professional bodies do not provide detailed guidance about whether variants of uncertain significance (VUS) and unsolicited findings should be reported by laboratory personnel to clinicians.
Methods: A working group was formed comprising a panel of experts (clinical geneticists, laboratory specialists, ethicists, researchers and lawyers) from Europe, Canada and Australia. The working group considered the current guidelines, their own experiences and data from a recent qualitative study exploring laboratories' reporting practices in these countries, to develop recommendations for clinical laboratory scientists and policy makers regarding the reporting of VUS and USF from diagnostic NGS.
Results: The working group recommends that in general, a targeted approach to analysis of data from NGS should be considered. VUS that are identified in known genes, which are related to the clinical question but with insufficient evidence of pathogenicity, and also variants identified in candidate genes, should be reported to clinicians. Unsolicited findings that are relevant to the health of adult patients should be reported, provided that informed consent has been obtained for such reporting from patients prior to sequencing. A patient's choice not to know unsolicited findings should be respected. In children, unsolicited findings that are a) relevant to their health during childhood or adolescence, and b) medically actionable, should be reported, even without the consent of the parents
Conclusions: These recommendations will help guide laboratory practices in Europe and internationally.

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C06.3
Legal framework for genomic data sharing in view of the new EU General Data Protection Regulation

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Genomic data contain sensitive health and non-health related information about the individuals and their family members. Therefore, adopting adequate privacy safeguards is paramount when processing genomic data for research or clinical purposes. One of the major legal instruments for personal data protection in the EU is the new General Data Protection Regulation (GDPR), which has entered into force in May 2016 and repealed the Directive 95/46/EC, with an ultimate goal of enhancing effectiveness and harmonization of personal data protection in the EU. This paper explores the major provisions of the new Regulation with regard to processing genetic data, and assesses the influence of such provisions on reinforcing the legal safeguards when sharing genomic data for research purposes. The new Regulation attempts to elucidate the scope of personal data, by recognizing pseudonymised data as personal (identifiable) data, and including genetic data in the catalogue of special categories of data (sensitive data). Moreover, a set of new rules is laid out in the Regulation for processing personal data under the scientific research exemption. For instance, further use of personal data for scientific research purposes, without obtaining additional consent will be allowed, if the specific conditions are met. The new Regulation has already fueled concerns among various stakeholders, owing to the challenges that may emerge when implementing the Regulation across the countries. Notably, the provided definition for pseudonymised data has been criticized, because it leaves too much room for interpretations, and it might undermine the harmonization of the data protection across the countries.

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C06.4
Recontact about clinically significant variant reclassifications in cardiogenetics; patient experiences

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Research question: What are patient perceptions on and experiences with our current recontacting practice?
Introduction: Technical advances and increasingly broader diagnostic genetic testing have highlighted the importance of recontact for variant reclassifications. It is ethically desirable to inform patients about reclassifications, especially those of clinical significance. Information on best practices regarding recontacting is limited. Due to an adaptation of the diagnostic guidelines in our centre, 62 patients who previously underwent genetic testing for cardiomyopathy were recontacted by an extensive letter from their counselor about the reclassification of 'their' variant. We explored how patients experienced recontact, their understanding of the information, and what actions they took.
Method: Semi-structured interviews in 8/25 eligible cardiomyopathy patients who were recontacted about a Likely Pathogenic to VUS or Pathogenic reclassification.
Results: Patients were positive and felt reassured about being informed about the reclassification and the advancing knowledge. Understanding of the information about the reclassification was incomplete and led to misinterpretations, e.g. on perceived changed risks for family members, hampering family communication. None of the patients took initiative to discuss the reclassification with medical professionals, although encouraged to do so. Trust in genetics/their counselor was not affected negatively. Recontact led to expectations about updates of test results in the future.
Conclusions: Preliminary findings suggest that patients are positive about recontacting. Information on reclassification does not impair trust in genetics/their counselor. To ensure better understanding though, information must be adapted to patient's needs and patients should feel encouraged to contact their counselor.

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C06.5
Evaluation of the 100,000 Genomes Project Consent Process and Participant Materials

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Introduction: The 100,000 Genomes Project is an English National Health Service Transformational Project focusing on patients with rare diseases (RD) and cancer. The evaluation included 10 pre-defined topic areas, selected from feedback raised during initial recruitment.
Methods: We undertook a cross-sectional, formative process evaluation using mixed methods (exit postcards, focus groups and on-line survey). Working with three stakeholder groups, consented participants (exit postcards), patient & public (focus groups) and active recruiting staff (on-line survey), we evaluated the consent experience in multiple geographical sites and from contrasting perspectives.
Results: All 13 Genomic Medicine Centres participated. Respondents included 174/1,196 participants (14.6%), 58/161 recruiters (36%), and five focus groups (34 members). For participants, 98% described their consent experience as good or excellent, only 66% read in detail the participant information sheet (PIS), and 81% of consents took more than 30 min (RD). Fifty percent of recruiters said participants often or very often presented with specific needs e.g. needing extra explanation, low levels of literacy, high anxiety levels or visual impairment. Views differed on the amount of information and length of the PIS, with most participants stating it was 'about right', but focus group members and recruiting staff felt it would benefit from revision.
Conclusions: Recommendations made, 8 'things to maintain' and 15 'changes to consider'. These were adopted and following research ethics committee approval went live in February 2017.

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C06.6
Recontacting in clinical practice: results from an investigation of the perspectives of patients and healthcare professionals in the United Kingdom

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As the interpretation of genomic data evolves, the question of whether former patients should be recontacted when updated information is available becomes more pressing. There is no professional consensus about whether and how recontacting should happen. There is also limited empirical evidence concerning the perspectives of healthcare professionals (HCPs) in genetic and 'mainstream' specialties, and patients.

We discuss the findings of a multi-site project conducted in the UK and with international collaborators. The project investigated socio-ethical and legal issues related to recontacting and gathered empirical evidence on: current clinical practices in the UK via a survey of 20/24 genetics centres; expectations of patients and HCPs via interviews ($n = 71$) and questionnaires ($n = 130$). Our data show that HCPs sometimes recontact, however there is no uniform system for how and when. HCPs and patients tended to view recontacting as desirable, but were unsure about whether formalized recontacting systems should be implemented. Both groups expressed concerns about the feasibility of recontacting within the current constraints of the National Health Service, and a lack of clarity about roles and responsibilities. Some patients' preferences (e.g. to receive regular updates from HCPs) corresponded to a model of follow-up rather than recontacting.

We make several recommendations to guide future policy and professional debate. These include clarifying: differences between recontact and follow- up; which methodologies and infrastructures might be required and with whom responsibilities lie; how genome data should be shared; any differences between professional, legal, or ethical duties/responsibilities to recontact, and the implications of these differences.

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C07
Novel genomics technologies

C07.1
Mosaic mutation detection using single molecule molecular inversion probes (smMIPs) for autoinflammatory disorders

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Systemic autoinflammatory diseases (SAID) affect patients that suffer from an uncontrolled innate immune system leading to recurrent inflammation. Due to the variety of symptoms, diagnosis of patients is difficult. This can result in a delayed treatment and irreversible organ damage as a consequence. In several patients with a phenotype consistent with an autosomal dominant form of SAID, mosaicism for a somatic mutation has been detected. Only a small percentage of mosaicism is necessary to generate even severe forms of SAID. Currently many laboratories use Next Generation Sequencing (NGS) to detect mutations in SAID genes. However, with the standard coverage, filtering and analysis settings many patients with low frequency somatic mosaics will be missed. NGS deep sequencing could solve this, although it is not able to discriminate mosaic mutations with a low allele frequency from PCR or sequencing artefacts. We designed and validated a sensitive deep sequencing assay using single molecule molecular inversion probes (smMIPs) for SAID gene mutation detection. Our results show the accurate detection of variant allele frequencies as low as 1%. Moreover, we can

distinguish heterozygous mutations from higher levels of mutational mosaicism. Additionally, we detect differences in variant allele frequencies of a patient when investigating mutational allele frequencies at different time points. These findings make smMIPs a very promising assay to study the development and progress of mutational mosaicism in time. Moreover it is a flexible, time- and cost effective assay to use in a diagnostic setting to prevent misdiagnosing of SAID patients with somatic mosaic mutations.

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C07.2
Ultra-sensitive detection of mosaic mutations in blood DNA of healthy individuals provides new insights into age-related clonal hematopoiesis

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Clonal hematopoiesis (CH) results from somatic mutations in hematopoietic stem cells, which grant an advantage to mutant cells and drive their clonal expansion. The acquisition of CH-driver mutations (CHDMs) occurs with normal aging and these mutations are reportedly detectable in blood in over 10% of individuals over 65. We present a targeted re-sequencing assay combining high throughput with ultra-high sensitivity based on single-molecule molecular inversion probes (smMIPs). Using smMIPs, we screened for CHDMs in over 100 loci in 2,007 blood DNA samples from healthy donors between 20 and 69 years of age and with no previous diagnosis of cancer. Loci screened included 40 known drivers of CH and 64 novel candidate loci. We identified a total of 225 somatic mutations throughout our cohort, of which 217 were in known CH-driver genes, such as *DNMT3A, JAK2, GNAS, TET2* and *ASXL1*, including 196 substitutions and 21 indels. Our assay allowed for the detection of mutations with variant allele frequencies < 0.1%. This improved sensitivity led to the identification of CHDMs in over 20% of individuals between the age of 60 and 69 and in close to 3% of individuals between 20 and 29 years of age, suggesting that CHDMs are more prevalent in all age groups than previously reported. Our findings support the occurrence of CH due to CHDMs as a widespread phenomenon associated with ageing, suggesting that clonal evolution of cells harboring somatic mutations is a universal mechanism which occurs at all ages in healthy humans.

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C07.3
Quantifying the role of paralogous genes in tissue selective hereditary diseases

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We aim to tackle a long standing enigma in human genetics: what limits the clinical manifestation of hereditary diseases to certain tissues or cell types, while their causal genes are expressed across the human body? Previously, we developed a novel interactome analysis to tackle this question for over 300 hereditary diseases. We showed that causal genes tend to have elevated transcript levels in their disease-manifesting tissue. Moreover, causal genes were significantly more involved in protein interactions that were unique to the disease-manifesting tissue, thus pointing to potential disease mechanisms. Here we investigate the role of causal genes paralogs. It has been shown across organisms that paralogs can compensate for the loss of each other. Here we hypothesize that, specifically in the disease-manifesting tissue, this compensation may become insufficient, and thus disease phenotypes emerge. Notably, this hypothesis has been demonstrated previously in the context of few specific diseases, but was never assessed at large-scale. We analyzed 152 tissue-specific hereditary diseases that manifest specifically in one of seven tissues. To test whether insufficient compensation occurs specifically in the disease tissue, we used hundreds of RNA sequencing data covering over 40 different human tissues. We found strong evidence for insufficient compensation in the disease tissues for most hereditary diseases. Moreover, in 20% of the diseases, insufficient compensation arose only due to the paralog being significantly under-expressed in the disease tissue. Our results shed a new light on this long-standing enigma, and may point to new directions in treating tissue-specific hereditary diseases.

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C07.4
Mapping and phasing of structural variation in patient genomes using nanopore sequencing

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Large capital investments are needed for second generation sequencing equipment, which has led to the concentration of human genome sequencing efforts in specialized sequencing centers. An interesting alternative for human genome sequencing is the MinION nanopore sequencer, a small and low-cost device that can generate long sequence reads in real-time. Here, we demonstrate sequencing of the genomes of two patients with congenital abnormalities using the MinION at 11x and 16x mean coverage, respectively. We developed a bioinformatic pipeline - NanoSV - to efficiently map genomic structural variants (SVs) from the nanopore data. Using NanoSV, we readily detected all de novo rearrangements involving multiple chromosomes and comprising complex chromothripsis events. Genome-wide surveillance of SVs, revealed 1,090 (27,4%) novel variants that were missed in short-read data of the same sample, the majority of which are short variations <200bp in size. Nanopore reads enabled efficient phasing of genetic variations, allowing the construction of genome-wide maps of phased SVs. Finally, we show that all de novo chromothripsis breakpoints occurred on paternal chromosomes and we used this information to resolve the long-range structure of the chromothripsis. This work demonstrates the value of portable and low-cost sequencing devices for human genome sequencing in future life sciences research and clinical diagnostics.

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C07.5
Enrichment of unamplified DNA and long-read SMRT Sequencing to unlock repeat expansion disorders

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Nucleotide repeat expansions are a major cause of neurological and neuromuscular disease in humans, however, the nature of these genomic regions makes characterizing them extremely challenging. Accurate DNA sequencing of repeat expansions using short-read sequencing technologies is difficult, as short-read technologies often cannot read through regions of low sequence complexity. Additionally, these short reads do not span the entire region of interest and therefore sequence assembly is required. Lastly, most target enrichment methods are reliant upon amplification which adds the additional caveat of PCR bias.

We have developed a novel, amplification-free enrichment technique that employs the CRISPR/Cas9 system for specific targeting of individual human genes. This method, in conjunction with PacBio's long reads and uniform coverage, enables sequencing of complex genomic regions that cannot be investigated with other technologies. Using human genomic DNA samples and this strategy, we have successfully targeted the loci of Huntington's Disease (HTT; CAG repeat), Fragile X (FMR1; CGG repeat), ALS (C9orf72; GGGGCC repeat), and Spinocerebellar ataxia type 10 (SCA10; variable ATTCT repeat) for examination. With this data, we demonstrate the ability to isolate hundreds of individual on-target molecules in a single SMRT Cell and accurately sequence through long repeat stretches, regardless of the extreme GC-content. The method is compatible with multiplexing of multiple targets and multiple samples in a single reaction. This technique also captures native DNA molecules for sequencing, allowing for the possibility of direct detection and characterization of epigenetic signatures.

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C07.6
CLIP-Cap: Combined Long-Insert Paired-End and Capture sequencing, a novel method for the analysis of complex genomic aberrations

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CLIP-Cap is a method that allows to completely resolve complex chromosome rearrangements in the human genome using standard laboratory equipment and an efficient and multiplexable workflow.

CLIP-Cap is based on creating a paired-end sequencing library where the inserts of genomic DNA are of variable length in the kbp-range (i.e. 2–8 kbp as opposed to 400–800 bp in standard paired-end sequencing), also known as mate-pairs, and then to carry out semi-targeted capture from that paired-end library with oligomers representing one of the chromosomes involved in the structural genome aberration at hand. This is then followed by limited (i.e. benchtop or multiplexed) 'next-generation' DNA sequencing and data analysis with standard software tools (for paired-end mapping, split-read analysis and read-depth analysis, respectively).

We resolved the three main Philadelphia Chromosome aberrations, in three different cancer cell lines (Kasumi-4, which contains a three-way translocation, SUP-B15 and K562). And we furthermore resolved the complex rearrangements in four cases of developmental disorder that all involved chromosome 22, but also other chromosomes (9, 10 or 11) or multiple different aberrations (e.g. deletions and isodicentric translocations) present in a mosaic fashion.

Currently there is no single approach to resolve typical complex chromosomal rearrangements that are a common hallmark of cancer and also of developmental disorders. We have tested CLIP-Cap on seven different cases of genomic rearrangements to demonstrate that it is sufficient to resolve a wide range of scenarios, the only requirement at the outset being knowledge of one of the chromosomes involved in the aberration.

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C08 Neuromuscular Disorders

C08.1
Biallelic mutations in the myopalladin gene, MYPN are associated with childhood-onset, slowly progressive nemaline myopathy

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Nemaline myopathy (NM) is a common form of congenital nondystrophic skeletal muscle disease characterized by muscular weakness of proximal dominance, hypotonia, and respiratory insufficiency, but typically without cardiac dysfunction. Wide variation in severity has been reported. Intranuclear rod myopathy is a subtype of NM in which rod-like bodies are seen in the nucleus, often expressing a severe phenotype. Although ten mutant genes are currently known to be associated with NM, only *ACTA1* is associated with intranuclear rod myopathy. In addition, the genetic cause remains unclear in approximately 25–30% of individuals with NM. We performed whole exome sequencing on individuals with histologically confirmed but genetically unsolved NM. Our study included individuals of milder NM with later onset age, and identified biallelic loss-of-function mutations in the myopalladin gene (*MYPN*) in four families. Encoded MYPN is a sarcomeric protein exclusively localized in striated muscle in humans. Individuals in all four of these families with identified *MYPN* mutations had relatively mild NM with childhood- to adult-onset, slowly progressive muscle weakness. Walking difficulties were recognized around their forties. Decreased respiratory function, cardiac involvement, and intranuclear rods in biopsied muscle were observed in two individuals. MYPN was localized at the Z-line in control skeletal muscles, but was absent from affected individuals. Homozygous knock-in mice with a nonsense mutation in *Mypn* showed Z-streaming and nemaline-like bodies adjacent to a disorganized Z-line on electron microscopy, recapitulating the disease. *MYPN* screening should be considered in individuals with mild NM, especially when cardiac problems or intranuclear rods are present.

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C08.2
Neurocalcin delta as a novel protective modifier for spinal muscular atrophy: A full story from gene identification to therapy

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Autosomal recessive spinal muscular atrophy (SMA) with an incidence of 1 in 6,000 people and a carrier frequency of 1:35 is the most frequent cause of infant lethality. Recently, the first SMA therapy based on antisense oligonucleotides (ASOs) elevating SMN protein levels, namely SPINRAZA, has been FDA-approved. SMN is crucial for all cells but particularly for motoneurons (MN) and neuromuscular junctions (NMJ). In the most severe type I - accounting for 60% of SMA-affected individuals - the elevated SMN level may be still insufficient to restore MN function lifelong.

Using a combined strategy of linkage and transcriptome analysis, we identified neurocalcin delta (NCALD) as a novel SMA protective modifier. Low NCALD expression was found in five asymptomatic *SMN1*-deleted individuals in comparison to type III SMA affected individuals, all carrying four *SMN2* copies. We demonstrate that low SMN level reduces Ca^{2+}-influx and impairs endocytosis. NCALD binds clathrin Ca^{2+}-dependently and acts as a Ca^{2+}-dependent negative regulator of endocytosis. Indeed, NCALD inhibition restores impaired endocytosis in SMA.

Using three different SMA models, *C. elegans*, zebrafish, and mouse, we prove that NCALD downregulation ameliorates SMA disease pathologies at MN and NMJ level and improves motoric abilities. Most importantly, a combinatorial therapy using low dose of SMN-ASOs and 50% NCALD reduction restored survival, MN and NMJ function and motoric abilities in a severe SMA mouse model. A similar strategy may cure and not only ameliorate SMA in the future.

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C08.3
Dissecting the causal mechanism of X-linked dystonia-parkinsonism by integrating genome and transcriptome assembly

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Introduction: X-linked Dystonia Parkinsonism (XDP) is a neurodegenerative disorder endemic to the Philippines that was linked to a founder haplotype over two decades ago.
Materials and Methods: We constructed a high-resolution population-specific genetic map of XDP from de novo genome and transcriptome assembly using short-read and multiple long-read and linked-read technologies. We characterized transcriptomes from fibroblasts among 46 subjects (probands, carriers, and unaffected family members) as well as induced pluripotent stem cell derived neural stem cells (NSCs), and induced neurons from a subset of individuals.
Results: These analyses identified 17 alleles shared among all XDP probands, as well as three independent recombination events that narrowed the putative causal region to a genomic segment including *TAF1*. Differential gene expression and de novo transcriptome assembly in NSCs revealed novel alternative splicing and partial transcription of intronic sequence as a consequence of retrotransposition of a disease-specific sine-VNTR-Alu (SVA) into the XDP founder haplotype; remarkably, both the aberrant splicing and reduced *TAF1* expression signatures in XDP probands were rescued following CRISPR/Cas9 excision of the SVA. We also identified a highly interconnected co-expression network that have been previously implicated in neurodevelopmental disorders.
Conclusions: Collectively, the integration of XDP genome and transcriptome assembly suggest a unique pathogenic mechanism of XDP involving aberrant splicing and transcription as a consequence of SVA retrotransposition, as well as perturbations of pathways of relevance to dystonia, parkinsonism, and neurodevelopment. These studies further suggest a potential role for emerging technologies in reference-free discovery of novel sequences and pathogenic mechanisms.

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C08.4
Application of exome sequencing technologies to 1,000 patients affected by limb-girdle weakness of unknown origin

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Introduction: Rare diseases collectively affect 8% of the general population, eliciting a significant global health and economic burden. Muscular dystrophies are a heterogeneous group of rare genetic disorders that are characterised by progressive skeletal muscle wasting and weakness, and can directly precipitate premature mortality. Here, we apply targeted whole exome sequencing (WES) to the largest ever cohort of patients with unexplained proximal muscle weakness. We aim to decipher disease aetiology, enhance diagnostic pathways and heighten the awareness of neuromuscular disorders - particularly limb-girdle muscular dystrophies (LGMDs).

Materials and Methods: 1,000 European participants presented with limb-girdle weakness and/or elevated creatine kinase activity. WES was performed using Illumina exome capture (38 Mb target) and a Picard-based pipeline. The variant call set was uploaded onto *seqr* and 169 candidate genes examined.

Results: Suspected pathogenic variants were identified in 49% of participants across 73 genes. LGMD2A (*CAPN3*) was the most common disease accounting for 15% of solved cases. Patients with extremely rare diseases including LGMD2Z (*POGLUT1*) were identified in our cohort, while a LGMD2J (*TTN*) founder mutation was detected in a Serbian sub-population. Genotype-phenotype correlations and inheritance patterns were expanded for many diseases. Crucially, we diagnosed nineteen patients with treatable conditions including CMS5 (*COLQ*), CMS10 (*DOK7*), glutaric acidemia IIC (*ETFDH*), Pompe (*GAA*), CMS12 (*GFPT1*) and CMS11 (*RAPSN*).

Conclusions: We have facilitated the integration of next-generation sequencing technologies into healthcare. This study has significantly enhanced the capacity of standard clinical work-ups and has pioneered an accessible pathway to expedite future diagnoses.

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C08.5
Autosomal recessive myopathy associated with cataracts caused by mutations in the gene INPP5K, in inositol phosphatase

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INPP5K encodes the type II inositol phosphatate 5-phoaphatase K, a muscle enriched inositol phosphatase interacting with the BiP chaperone and negatively regulating assembly of the actin cytoskeleton. Here, we report on recessive missense mutations and one in-frame deletion affecting the functionality of the INPP5K protein in six families with congenital muscular dystrophy further complicated by bilateral early childhood cataracts and intellectual disability or learning difficulties. Notably, mutations of another binding partner of BiP called SIL1 have been linked to a similar phenotype. Muscle biopsy specimen of INPP5K-patients reveal clear features of muscle dystrophy and moreover indicate that the mutant protein is still expressed in the diseased tissue. Pathogenicity of loss of functional INPP5K was confirmed in zebrafish via injection of respective morpholinos. Knock-down of protein expression resulted in disturbed architecture of skeletal muscle, narrowed eyes and cranial nerve abnormalities 48 h post

injection. Moreover, label-free global proteome profiling utilizing patient-derived fibroblasts was carried out. The obtained results are in line with the known cellular functions of INPP5K and provide further insights into the molecular basis of the phenotype by showing vulnerability of proteins affected in phenotypically similar disorders, thus biochemically linking a group of rare disease. Identification of INPP5K mutations along with the results of our zebrafish studies and proteomic findings put INPP5K on the growing list of complicated myopathic phenotypes und in addition build a molecular bridge between some subtypes of those.

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C08.6
Homozygous variants in *LMOD1* and *MYLK* cause Megacystis Microcolon Intestinal Hypoperistalsis Syndrome by disruption of smooth muscle contractility

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Introduction: Megacystis Microcolon Intestinal Hypoperistalsis Syndrome (MMIHS) is a rare congenital disorder characterized by severe dilation of the bladder and intestinal obstruction. To date, two genes are known to be involved in MMIHS pathogenesis, *ACTG2* and *MYH11*, but in some patients the genetic etiology of this disease is still unknown. **Material and Methods:** Homozygosity mapping and whole exome sequencing were performed in four MMIHS patients derived from three consanguineous families for which no variant in *ACTG2* and *MYH11* was identified. Pathogenicity of the variants identified was determined by a series of expression studies, in vitro and in vivo assays. **Results:** Homozygous variants in two different genes where identified in the four patients analyzed. In one family a nonsense variant in the Leimodin-1 gene (*LMOD1*) was found, while the other two families carried two different homozygous variants in the Myosin light chain kinase gene (MYLK). Expression studies showed that the variants in *LMOD1* and *MYLK* affected protein expression, and in vitro data confirmed pathogenicity of these variants due to impairment of smooth muscle contractility. Moreover, *Lmod1* knock out mice generated using CRISPR-Cas9 genome editing, and homozygous mutant mice for Mylk previously described[1], showed pathology consistent with MMIHS.

Conclusions: Our results present *LMOD1* and *MYLK* as two new disease causing genes for the recessive form of MMIHS, confirming that MMIHS is a myopathy with multiple patterns of inheritance caused by disruption of the smooth muscle contractile apparatus.

[1]He et al., 2008;135(2):610-20.

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C09 Molecular Mechanisms of Disease

C09.1
X chromosome inactivation in human single cells

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One X chromosome is randomly inactivated in each female cell (XCI). However, some genes on the silenced X chromosome escape from XCI and are expressed from both X chromosome alleles. To date, the majority of X inactivation studies were performed in populations of cells (bulk) from organs and tissues. The ability to study the single cell transcriptome (scRNA-seq) now provides an unprecedented opportunity to revisit X-inactivation. We have studied 902 single cell fibroblasts from five female individuals and

performed scRNA-seq in order to investigate XCI at single cell resolution. To this aim we developed a computational and statistical framework integrating single cell transcriptome and whole genome sequencing to robustly eliminate confounding artifacts and identify genes which escape X inactivation. We identified 19 genes as escapees (14 known and 5 novel genes significantly escaping X inactivation). Unexpectedly, among cells coming from the same individual, we observed that each gene exhibited a variable propensity to escape XCI. Through the calculation of an Inactivation Score as the mean of the allelic expression profiles of the escapees per cell, we discovered some cells being "inactive", i.e. exclusively expressing the escaping genes from the active allele and others being "escapers", i.e. expressing the escapee from both alleles. A possible mechanism to explain this cellular heterogeneity is the single cell variability of *XIST* transcription in concert with *LINEs* expression, which we revealed being both positively correlated with the Inactivation Score. These results provide evidence of an unexpected cellular heterogeneity of the mechanism of X-inactivation.

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C09.2
Morbidity risk of chromosomal breakpoints in topological domains enriched in non-exonic conserved elements

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Introduction: The human genome is organised into regulatory regions termed topological associating domains (TADs). Disruption of TADs by balanced chromosomal rearrangements (BCRs) e.g. translocations and inversions, can be deleterious due to long range position effect (LRPE) caused by displacement of regulatory elements. Most BCRs rearrange TADs without phenotypic consequences, so definition of TADs at high risk for LRPE is needed. Several known LRPE loci overlap with clusters of evolutionarily conserved non-exonic elements (CNEs). Here we identify the most CNE-enriched TADs (CNE-TADs) and examine the effects of chromosomal breakpoints within these in affected and unaffected BCR carriers.

Materials and Methods: All human TADs were ranked by enrichment of CNEs to define CNE-TADs. We mate-pair sequenced two-way BCRs from 87 affected and 117 healthy carriers. Together with breakpoints from ~200 published BCRs, and 120 known BCRs associated with LRPE, we analysed a total of ~900 breakpoints in the context of CNE-TADs.

Results: We define ~400 CNE-TADs covering ~16% of the genome. Notably, boundaries of CNE clusters correlate with TAD boundaries, highlighting the conservation of CNE-TADs as regulatory units. CNE-TADs are enriched for key developmental genes and they overlap with 23 out of 32 (70%) known LRPE-associated loci. Breakpoints truncating CNE-TADs are more frequent among affected versus healthy BCR carriers ($p = 0.003$), and even more so are intergenic breakpoints within CNE-TADs ($p = 0.0015$).

Conclusions: CNE-TADs are high risk regions for LRPE, and their disruption by balanced genomic rearrangements are associated with an increased morbidity risk.

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C09.3
Whole genome characterization of array defined clustered CNVs reveals two distinct complex rearrangement subclasses generated through either non-homologous repair or template switching

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Introduction: Clustered copy number variants (CNVs) as detected by chromosomal microarray are often reported as germline chromothripsis. However, such cases might need further investigations by massive parallel whole genome sequencing (WGS) in order to properly define the underlying complex rearrangement.

Material and Methods: 22 carriers of clustered CNVs, previously referred to the Departments of Clinical Genetics at the Karolinska University Hospital (Stockholm, Sweden) or Kennedy Center, Rigshospitalet (Copenhagen, Denmark) for a clinical chromosome microarray due to congenital developmental disorders, were sequenced using either Paired-End or Mate Pair libraries. To utilize the WGS data for structural variant analysis, a WGS caller (TIDDIT), a pipeline (FindSV) and a program to visualize the rearrangement end-products were developed.

Results: By combining read depth and discordant read pair analysis 154 junctions were characterized (range 4–26; median = 5) and an overall connectivity picture is given in 21 cases. These rearrangements were sub-classified depending on the patterns observed:

(1) Cases with clustered deletions only (e.g. del-nml-del-nml-del) often had additional hidden structural rearrangements, such as insertions and inversions, that may be the result of multiple simultaneous double-strand DNA breaks followed by non-homologous repair typical to chromothripsis.

(2) Cases with only duplications (e.g. dup-nml-dup) or combinations of deletions and duplications (e.g. del-nml-dup-del-nml-dup-nml-del), demonstrated a pattern of inversions, deletions and duplications more consistent with serial template switching during DNA replication suggesting chromoanasynthesis.

Conclusion: Multiple copy number changes clustered on a single chromosome may arise through both chromothripsis and chromoanasynthesis.

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C09.4
Biallelic mutations of Prune-1 are causing PEHO-like syndrome with microcephaly and neurodevelopmental impairment

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Introduction: The correct development of the brain is strictly regulated by the correct balance between symmetric and asymmetric division of neuronal progenitor cells (NPGs). Alterations in NPGs proliferation, migration and differentiation result in neurodevelopmental disorders with intellectual disabilities and brain malformations, including tubulinopathies.

Material and Methods: Using cell-proliferation/migration (XCELLinge),biochemical-assays, immunofluorescence, microtubules(MT)spin-down/polymerization, we examined the functional consequences of Prune1 mutations.

Results: Prune1 biallelic-mutations were found in several individuals in extended families with tubulinopathies: Microcephaly(MIM#251200) and PEHO-syndrome (MIM#260565) (Zollo et al, Brain.2017.https://doi.org/10.1093/brain/awx014). The mutations identified in Prune1 (D30N-R297W-D106N) show enhancement of its enzymatic exopolyphosphatase (PPase/PPX) activity. These "neomorphic-mutations" enhance the catalytic ability of Prune1 to hydrolyze tetrapolyphosphates(P4) producing inorganic-polyphosphates(PPi-Pi), that mediate signal transmission in mammalian brain. We further found wild-type and mutated Prune1 colocalize with Microtubules(MT) to mitotic-spindle via binding to a/b-Tubulin. Our genotype/phenotype correlation studies demonstrate that these Prune1-mutants caused a delay in MT-polymerization in-vitro, affecting nucleation-phase in patients-derived-fibroblasts. These alterations result in mitotic-defects (micronuclei/lagging-chromosomes) and impaired cell-proliferation/migration. Moreover, we found NDPK-A/-B (D'Angelo et al,2004) colocalize with mitotic-spindle and negatively affect in-vitro MT-polymerization. These findings suggest a role for Prune1/NDPK-protein-complex in MT-dynamics during cell division. Furthermore,two small molecules are indicating action on impairment of Prune1 augmented PPase/PPX-activity in-vitro.

Conclusions: We link the clinical feature of these neurodevelopmental-disorders to PPX-iperactivity of Prune1 mutants. Our data establish biochemical-activity of Prune1 is necessary to assure a correct cell division, especially during brain/cerebellum development. These results would benefit future therapeutic applications with small molecules impairing PPase/PPX activity. Our results in-vitro are encouraging.

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C09.5

A human developmental syndrome caused by germline mutation to a histone H4 gene highlights the importance of H4K91 in DNA damage response and cell cycle control

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Chromatin is a repeat of nucleosomes, consisting of the DNA fiber wrapped around histone octamers. Its covalent modifications, whether affecting DNA or histone N-terminal tails, govern cellular processes such as DNA replication, transcription and repair. Mutations on genes belonging to histone tail-modifying complexes have been reported to cause developmental disorders or carry an oncogenic effect. Recently, mutations directly affecting the histone tail were shown to play a role in tumorigenesis. Very little is known, however, about modifications affecting the histone core.

Here we show that mono-allelic missense mutations affecting Lysine 91 in the histone H4 core (H4K91) cause a human syndrome of growth delay, microcephaly, intellectual disability, characteristic facial features and foot ray anomalies. We report three patients with a missense mutation at H4K91 and overlapping phenotypes.

The human genome contains fifteen histone H4 genes, all differing at the nucleotide level but coding for an invariant H4 protein. RNA sequencing analysis of patient cells showed that ± 8% of H4 cDNA molecules carried the mutated allele. We observed differentially expressed histone genes and cell-cycle related genes compared to controls, suggesting an effect on processes such as DNA replication or cell cycle progression.

Expression of the H4 variants in zebrafish embryos recapitulated the anomalies seen in individuals carrying the H4 mutations. We link the H4 mutations to genomic instability, resulting in increased apoptosis and cell cycle progression anomalies during early development. Our findings imply an important role for ubiquitination of H4K91 in genomic stability during embryonic development.

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C09.6
The ciliopathy protein *Talpid3/KIAA0586* plays a role upstream of Rab8 activation in outer segment formation and maintenance in zebrafish retinal photoreceptors

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Ciliopathies are a group of human disorders caused by dysfunction of primary cilia, ubiquitous microtubule-based organelles involved in signal transduction. Cilia are anchored inside the cell through basal bodies, modified centrioles also acting as microtubule organization centers. Photoreceptors (PR) are sensory neurons, whose primary cilium has evolved into a highly specialized compartment called the outer segment (OS) responsible for sensing incoming light. Thus, retinal degeneration is frequent in ciliopathies.

Mutations in the novel ciliopathy gene *KIAA0586*/Talpid3, cause Joubert syndrome and lethal ciliopathies. To elucidate the function of Talpid3 in photoreceptors, we studied zebrafish *ta3* mutants which undergo progressive retinal degeneration. The majority of *ta3*-/- PR do not form OS due to a defect in the initial basal body docking steps required for ciliogenesis. These PRs also display intracellular accumulation of the photopigment opsin and progressive loss of cell polarity. In a small subset of PRs, persistence of maternally deposited Ta3 rescues this phenotype and allows formation of initially normal-looking OS. However, progressive abnormalities in cell shape and OS maintenance in these PRs indicate that Ta3 plays additional roles beyond basal body docking in ciliary-directed trafficking or cytoskeletal maintenance. We further show that a constitutively active form of the small GTPase Rab8 rescues the OS formation defect but not the subsequent cell shape defect. Together, our results indicate that Ta3 plays a role upstream of Rab8a activation in the initial steps of ciliogenesis and additional roles in ciliary function and cell shape maintenance which are independent of Rab8.

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C10
GWAS: Resolving Missing Causality

C10.1
Systems Genetics and Transcriptome analysis on Circulating Proteins

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Proteins circulating in blood are often measured as biomarkers for various diseases, including immune diseases, cancers and cardiovascular diseases (CVD). There is considerable evidence that human genetic variation influences gene expression. The effect of genetic variants on serum level of circulating proteins is still largely unknown. We performed a systems genetics analysis to integrate genetics, transcriptome and circulating proteins in 1,294 individuals from a Dutch population cohort (LifeLines-DEEP), for whom we had data on genome-wide genotype, transcriptome by RNAseq and serum levels of 92 CVD-relevant proteins, as well as over 200 various clinic parameters. At FDR 0.05, we identified 125 novel cis-pQTLs. The strongest cis-effects were observed for IL-6R ($P = 3.3 \times 10–310$), SHPS1 ($P = 2.2 \times 10–307$) and IL-17RA ($P = 3.1 \times 10–184$). Thirty novel trans-pQTLs have suggested pleiotropic effects of KLKB1, ABO and PLAUR loci that trans-affected 10, 7 and 3 proteins respectively. Over 70% of pQTLs effects cannot be explained by expression level in blood. These proteins were enriched for "leakage proteins"

from other tissues. These proteins have shown associations not only to metabolic traits but also to electrocardiography parameters of heart function, suggesting their promise in disease diagnosis. It was further confirmed by mendelian randomization approach that 48 proteins were associated with genetic risk scores (GRS) of blood lipids, coronary artery disease, metabolites and blood cell counts. Most associations remained significant after controlling cis-pQTL effect. Our study highlights that plasma proteomics is under strong genetic control, which should be taken into account when using them as potential biomarkers for complex diseases.

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C10.2
The deCODE replication server, a resource for the replication of published genotype-phenotype associations

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Introduction: Since 2005, the number of genome-wide association (GWAS) studies reporting associations of sequence variants with disease and quantitative traits has grown fast. Publications of validation of finding have not always kept pace. By focusing on the Icelandic population deCODE genetics contributed early and often to the field of GWAS and after 20 years of data collection, information on the most common disease and traits is available for a large fraction of the population.

Materials and Methods: We have created a resource that access matching genotype-phenotype associations of published GWASs and rare variant associations in the Icelandic population using sequence variants identified through whole-genome sequencing of over 30 thousand Icelanders and imputed into 300 thousand chip-typed individuals and their close relatives.

Results: Using sequence variants for which statistical power can be estimated, we quantify the extent to which published GWAS associations replicate across and within different phenotypes and ancestry groups. We show that the rational in choosing genome wide significance thresholds drastically affects replication outcomes. Finally, scrutinizing association studies for Alzheimer's disease for the full spectrum of allele frequencies we demonstrate the usefulness of independent replication in investigating rare variant effects.

Conclusions: This resource provides a powerful tool for assessing published GWAS associations and to examine the proposed role of common as well as rare variants on disease risk identified by GWAS or other approaches.

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C10.3
Assessing the causal role of body mass index on cardiovascular health in young adults: a Mendelian randomization and recall-by-genotype analysis

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Introduction: The association between body mass index (BMI) and cardiovascular health may be explained by reverse causation, confounding or bias, rather than

causality. Mendelian randomization (MR) studies in mid-to-late life imply causality but whether this applies to younger ages is unclear. Using complementary Mendelian randomization (MR) and recall-by-genotype (RbG) methodologies, we estimated the causal effect of BMI on cardiovascular health in European individuals aged 17–21 from the Avon Longitudinal Study of Parents and Children
Methods: For MR analyses, a genetic risk score (GRS) comprising 97 genetic variants was used as an instrument to test the causal effect of BMI on cardiovascular phenotypes measured at 17 (N = 7924). An independent sample of participants from the same cohort participated in a RbG study at 21, which allowed additional detailed cardiovascular phenotyping (N = 418; 191/227 from the tails of a GRS predicting variation in BMI).
Results: In both MR and RbG analyses, results suggest that increased BMI causes higher blood pressure and left ventricular mass (indexed to height2.7, LVMI) (e.g. change in LVMI per kg/m2 using MR: 1.09g/m2.7; 95% CI: 0.63, 1.54; P = 3.32 × 10-06), plus increasing stroke volume (estimate per 3.55kg/m2: 1.49ml/m2.04; 95% CI: 0.62, 2.35; P = 0.001) and cardiac output (estimate per 3.55kg/m2: 0.11l/min/m1.83; 95% CI: 0.03, 0.19; P = 0.01) in RbG analyses. Neither analysis suggested a causal role for BMI on heart rate.
Conclusions: Complementary causal methodologies showed greater BMI causing poorer cardiovascular health, even in young adults.Grants: BHF (RG/10/004/28240, PG/06/145 and CS/15/6/31468); UK MRC, UoB and Wellcome Trust (MC_UU_12013/1–9, 102215/2/13/2, 096989/Z11/Z, 086676/7/08/Z and MR/M009351/1).

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C10.4
Fine-mapping analysis of 158 breast cancer risk loci from OncoArray data

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Introduction: One hundred and fifty-eight breast cancer risk loci have been discovered through genome-wide association studies (GWAS). For most, the mechanisms underlying these associations remain unknown. To explore them in more detail, we combined association data for all 158 known susceptibility regions with in-silico genomic feature annotation.
Material and Methods: Genotypes for 660,053 variants across these regions were determined using the custom arrays iCOGS and Oncoarray, or estimated by imputation, in 88,937 Controls and 84,642 Cases of European ancestry. We determined, using stepwise multinomial logistic regression, the signals associated with ER-positive (ER +) or ER-negative breast cancer risk, and the credible sets of candidate variants driving each signal. We also determined whether these variants overlapped with transcription factor (TF) binding sites, histone marks or DNase hypersensitivity sites, specifically in breast tissue.
Results: Across the 158 confirmed loci, we identified 202 independent association signals and the credible candidate variants within each one. For ER + disease, we found significant enrichment of binding sites for 21 different TFs, including FOXA1, GATA3, and ESR1; as well as histone marks H3K27ac and H3K4me1 coincident with the positions of the candidate variants.
Conclusions: Our results suggest significant overlap of credible causal variants with active gene regulatory elements and binding sites for specific TFs. Further analysis will elucidate the roles of these TFs in breast cancer development. Funding: EC [MSCA-IF-2014-EF-656144], CR-UK [C1287/A10118, C1287/A16563], Genome Canada, NCI [U19 CA148065, X01HG007492].

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C10.5
Prospects of fine-mapping causal genetic variants using summary statistics from genome-wide association studies

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Identifying the causal genetic variants from a much larger set of highly correlated ones is an important next step towards translating results from Genome-Wide Association Studies (GWAS) into therapeutic targets. Public availability of GWAS summary statistics from large international consortia has generated exciting new opportunities to carry out such fine-mapping studies without access to the original data. This is a promising approach to utilize the increasing GWAS sample sizes while avoiding privacy concerns and logistics of sharing individual-level genotype data. Although all fine-mapping methods using summary statistics require information about the Linkage Disequilibrium (LD) between variants, so far, it has not been verified whether LD information from publicly available reference genotype panels could replace the original genotype data in these analyses. We show that one should not rely only on the reference genotypes from the 1000 Genomes Project due to its small sample size for any one population, whereas a reference panel of 1,000 individuals from the study population is typically enough for cohort size of up until 10,000 individuals. More generally we show, both theoretically and empirically, that the size of the reference panel needs to scale with the GWAS sample size, which has important consequences for large GWAS meta-analyses and biobank studies. We conclude by providing software tools and practices for sharing LD information to efficiently exploit summary statistics in biomedical research. Our results are based on Finnish population cohorts, UK Biobank data and detailed fine-mapping of the APOE region discovering a novel variant associated with LDL-C.

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C10.6
Genome-Wide Inferred Statistics (GWIS) for Homeostatic Model Assessment of β-cell function and Insulin Resistance

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Introduction: Genome wide association studies (GWAS) of Homeostatic Model Assessment of β-cell function and Insulin Resistance (HOMA-B/-IR) require Fasting Glucose (FG) and Fasting Insulin (FI) to be measured in the same individual.

Materials and Methods: We implemented the Genome Wide Inferred Statistics (Nieuwboer et al. 2016. GWIS for Functions of Multiple Phenotypes. AJHG) approach to carry out a GWAS of HOMA-B/-IR based on the summary statistics for both traits, thereby increasing power over traditional HOMA-B/-IR GWAS. We used partially overlapping GWASs of FI ($N = 64{,}090$) and FG ($N = 88{,}320$) from recent MAGIC meta-analyses and did GWIS tests for HOMA-B/-IR in up to 75,240 non-diabetic individuals of European descent. Next, we used LD Score regression to evaluate genetic relations with related traits.

Results: We detected one novel HOMA-B (*FOXA2*) and three novel HOMA-IR (*LYPLAL1, PER4, PPP1R3B*) loci, and confirmed one HOMA-IR (*GCKR)* and eight HOMA-B (*ADCY5, DGKB, GCK, SLC30A8, GLIS3, TCF7L2, ARAP1, MTNR1B*) loci previously associated with type 2 diabetes (T2D). Blood triglycerides, body mass index, waist-to-hip ratio, waist and hip circumferences show positive genetic correlation ranging from $r_g = 0.35$ to $r_g = 0.70$ ($p < 10^{-4}$) with both HOMAs. Genetic correlation with high-density lipoprotein cholesterol is inverse ($r_g = -0.36$ and $r_g = -0.53$, respectively for HOMA-B/-IR, $p < 10^{-4}$). Additionally, HOMA-IR correlates with T2D ($r_g = 0.56$, $p < 10^{-9}$).

Conclusions: GWIS can aid GWAS of traits calculated from other phenotypes using mathematical formulae, especially if some subjects or cohorts have incomplete measures. Characterization of genetic loci within HOMA-B/-IR GWIS provides mechanistic clues about heterogeneous processes contributing to T2D pathophysiology and glycaemic traits variability in non-diabetics.

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C11
Sensory disorders

C11.1
FDXR mutations cause sensorial neuropathies, a new mitochondrial Fe-S disease

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Hearing loss and retinis pigmentosa have mostly genetic origins, some of them being related to sensorial neuronal defects. Here, we report eight subjects from four independent families presenting with auditory neuropathy and optic atrophy. Whole-exome sequencing revealed biallelic mutations in the *FDXR* gene in affected subjects of each family. *FDRX* encodes the mitochondrial ferredoxin reductase, the sole human ferredoxin reductase which is implicated in the biosynthesis of iron-sulfur clusters (ISC) and in the heme formation. ISC proteins are involved in enzymatic catalysis and gene expression, DNA replication and repair. We observed deregulated iron homeostasis in FDXR mutant fibroblasts and indirect evidence of mitochondrial iron overload. Functional complementation in a yeast strain deleted for *ARH1*, the human *FDXR* counterpart, established the pathogenicity of these mutations. These data emphasize the wide clinical heterogeneity of mitochondrial disorders related to ISC synthesis.

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C11.2
A homozygous variant in mitochondrial RNase P subunit PRORP is associated with Perrault Syndrome characterised by hearing loss and primary ovarian insufficiency

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Perrault syndrome is a rare autosomal recessive condition characterised by sensorineural hearing loss in both sexes and primary ovarian insufficiency in 46 XX, females. It is genetically heterogeneous with biallelic variants in five genes identified to date (*HSD17B4, HARS2, LARS2, CLPP* and *C10orf2*). We describe a consanguineous family with three affected individuals homozygous for a novel missense variant c.1454C > T; p.(Ala485Val) in *KIAA0391* encoding proteinaceous RNase P (PRORP). PRORP is the metallonuclease subunit of the mitochondrial RNase P complex,

responsible for the 5'-end processing of mitochondrial precursor tRNAs. In enzyme activity assays, RNase P complexes containing the PRORP disease variant produced ~35–45% less 5'-processed tRNA than wild type PRORP. Consistently, the accumulation of unprocessed polycistronic mitochondrial transcripts was observed in patient fibroblasts, leading to an observable loss of steady-state levels of mitochondrial oxidative phosphorylation components. Rescue experiments demonstrated that expression of wild type PRORP in patient cells partially recovered tRNA processing. Immunocytochemistry analyses of mouse inner ear sensory epithelium showed high levels of PRORP in the efferent synapses and nerve fibres of the auditory hair cells which highlights a possible pathology for the sensorineural hearing loss observed in affected individuals. We have identified a novel Perrault syndrome gene and with the identification of this variant, defects in all three subunits of mitochondrial RNase P have now been found to cause mitochondrial dysfunction, each with distinct clinical presentations.

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C11.3
Rare genetic variants in MEPE cause congenital facial paresis with stapes fixation, and are associated with otosclerosis

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Whole exome sequencing in a family with Hereditary Congenital Facial Paresis (HCFP) associated with mixed hearing loss and stapes fixation resulted in the identification of a pathogenic heterozygous frameshift variant c.1273delC (p.Gln425Lysfs*38) in *MEPE*. *MEPE* encodes a matrix extracellular phosphoglycoprotein and plays an inhibitory role in bone mineralization. Because of the stapes fixation it was hypothesized that *MEPE* might be important in the pathophysiology of otosclerosis. *MEPE* was screened for variations in 91 patients with familial otosclerosis, resulting in the identification of an additional heterozygous frameshift variant, c.199_202delGAAA (p.Lys70Ilefs*26) in two unrelated families. Subsequently, *MEPE* was screened in 336 otosclerosis patients, which lead to identification of the p.Lys70Ilefs*26 variant in three additional patients and p.Ser206Ilefs*3 in one patient. Analysis of a single amplicon including the p.Lys70Ilefs*26 variant was performed in 1065 unrelated otosclerosis patients and 1308 controls. The p.Lys70Ilefs*26 frameshift variant was present in 5 patients and none of the controls, resulting in an overall presence of the variant in 8 patients ($p = 0.003$, Fisher's exact test). Two additional rare variants (c.184G > T; p.Glu62* and c.229G > A; p.Ala77Thr) were identified in cases and not in controls (overall $p = 0.0020$, Fisher's exact test). These results pinpoint that MEPE might be a key player in temporal bone and middle ear mineralization implicating its importance in the pathogenesis of otosclerosis. Currently, a

follow-up study is being performed investigating the contribution of rare variants in the complete gene in a population of approximately 3400 otosclerosis patients and ethnically matched controls.

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C11.4
Naturally-occuring exon-skipping allows bypassing complete *CEP290* loss-of-function in individuals with unusually mild retinal disease

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Introduction: CEP290 is pivotal for the assembly/maintenance of primary and motile cilia in a wide range of cell systems. Biallelic *CEP290* mutations cause a spectrum of devastating ciliopathies ranging from monosymptomatic Leber congenital amaurosis (LCA10) to multi-visceral and sometimes embryo-lethal syndromes (Meckel Syndrome, MKS). LCA10 manifests invariably as a congenital and dramatically severe cone-dominant disease with visual function reduced to light perception. Using targeted-NGS, we identified homozygosity and compound heterozygosity for *CEP290* truncating mutations (p.Ile556Phefs*17 and p.Lys170*/p.Glu1364*) in two unrelated individuals having a rod-dominant disease with preserved cone function (visual acuity from 3/10 to 6/10). The present study aimed at understanding the molecular bases of these observations.

Material and Methods: mRNA, protein and ciliation analyses were performed in patient, LCA, MKS and control fibroblasts.

Results: mRNA analysis evidenced the presence of *CEP290* isoforms lacking exons encompassing the premature termination codons (PTC) in the two individuals but not in other cell lines. Protein analysis detected a CEP290 protein around 290 KDa, which like the wildtype counterpart, could be detected in a 670 KDa protein complex (BNPage), supporting the view that exon-skipping allowed bypassing PTC. In sharp contrast with MKS and LCA fibroblasts, which exhibited severe to mild cilia defects, the fibroblasts from the two individuals had apparently unaltered ciliation ability, as determined by comparison to control cells.

Conclusions: Here, we report naturally occurring exon-skipping and protein synthesis in two individuals having an unusually mild retinal phenotype despite biallelic PTC mutations, providing strong support for *CEP290* splice switching oligonucleotide-mediated therapy.

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C11.5
Hidden genetic variation in Stargardt disease: novel copy number variations, cis-regulatory and deep-intronic splice variants of the ABCA4 locus

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Purpose: Stargardt disease (STGD1) is hallmarked by a large proportion of patients with single coding variants in the disease gene ABCA4, suggestive for hidden genetic variation in non-coding regions. We aimed to assess the contribution of copy number variations (CNVs) and non-coding sequence variations in STGD1 patients.

Methods: A total of 116 monoallelic STGD1 patients underwent targeted resequencing of the whole ABCA4 gene. Candidate splice variants were tested by mini-gene assays while putative cis-regulatory variants were investigated by ex vivo electroporation in mouse retinas. 4C-seq was performed on human retinal cells, using an anchor in

the ABCA4 promoter region. Customized arrayCGH (arrEYE) was used for CNV analysis of 5 patients.

Results: Mini-gene assays were performed for a subset of 12 intronic variants, confirming a splice effect for 3 variants. The cis-regulatory effect of 3 promoter and 2 deep-intronic variants were tested in mouse retinal explants, revealing a significant effect on regulation for some of them. A chromatin interaction map of the ABCA4 region was generated by 4C-seq in human adult retinal cells. CNV analysis revealed a novel ABCA4 deletion (ex 40–50) and a duplication (ex 2–6) in 2 patients without candidate non-coding variants.

Conclusions: Resequencing of the whole ABCA4 locus uncovered novel deep-intronic splice variants and cis-acting regulatory variants, representing the first report of non-coding regulatory ABCA4 variants. In addition, a retinal chromatin interaction dataset for the ABCA4 locus was generated. Finally, apart from a novel deletion, the first duplication was identified in ABCA4, expanding the CNV spectrum of ABCA4.

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C11.6
New diagnostic biomarkers for peroxisomal biogenesis disorders revealed by untargeted metabolomics profiling include significant reduction of sphingomyelin, bile acid alterations, and unique long chain fatty acid elevations

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Peroxisomal biogenesis disorders in the Zellweger spectrum (PBD-ZSD) encompass a range of multisystem diseases with prominent neurological, hepatic, renal, and bone features, comprising a broad clinical spectrum, with phenotypes ranging from severe, presenting with intractable seizures, neuronal migration defects and neonatal hypotonia, to milder forms with mild-moderate developmental delay, retinopathy, adrenal insufficiency, and hearing loss. We ascertained a cohort of 18 individuals with mild to moderate PBD-ZSD with mutations in *PEX1* who presented with hearing loss, developmental delay, and varied observations of microcephaly, retinopathy, and movement disorders. Analysis of plasma samples included quantitative peroxisomal biochemical diagnostics in parallel with untargeted small molecule metabolomic profiling with detection of > 650 named molecules. Metabolomic profiling show anticipated elevations in pipecolic acid, long chain fatty acids, and several bile acids, as well as reduced plasmalogens, with concordance between quantitative and untargeted measurements. Metabolomics analyses also revealed unanticipated significant reductions in sphingomyelins in every patient sample. These perturbations in the plasma metabolomic profiles are unique, specific, and not previously seen in >700 other samples analyzed as normal controls or for other indications. Reduced sphingomyelin was one of the strongest effects observed in these samples and detected only by the untargeted metabolomic profiling process. As the clinical presentation of mild PBD-ZSD broadens and as sequencing identifies novel genetic variants, the need for assessment of peroxisomal function increases. Untargeted metabolomic screening identifies several specific biomarkers that taken together allow for effective detection of these mild cases of PBD-ZSD, ending the diagnostic odysseys for many of these patients.

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C12
Engaging Patients in Genomics

C12.1
SEQUAPRE: Preferences and representations from patients and parents with regard to the use of Next-Generation Sequencing technologies in medical genetics. The case of development anomalies

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P. Ancet[8], A. Lapointe[9], P. Morin[9], P. Edery[4], M. Rossi[4], D. Sanlaville[4], S. Bejean[3,2], E. Cretin[1,2], L. Faivre[7,2]

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Background: Next-Generation Sequencing (NGS) of genome has revolutionized diagnostic odyssey in development diseases. In order to optimize information given to patients to obtain consent to undergo this examination, it is necessary to ask them about their preferences and representations with regard to the arrival of these new technologies in healthcare.

Methods: A mixed methodology was implemented to answer these questions in two centers of expertise for rare diseases in France. The study involved teams from human and social sciences as well as patient organizations. The objectives were: 1/ to estimate and analyze the preferences of parents of patients with development anomalies, who are potential candidates for NGS, concerning the nature and the announcement of the results (quantitative study before the NGS); 2/ Describe, analyze and understand, following the use of NGS for diagnostic purposes, the experiences, expectations and reactions of families with regard to their diagnostic trajectory and the announcement of the results (qualitative study).

Results: 528 parents completed questionnaires for the quantitative analysis. The respondents were strongly in favor of the communication of uncertain (particularly the most likely uncertain results) and unsolicited results, and for the reanalysis of examinations, mainly in an automatic way. 47 interviews with parents were conducted. Whatever the result of the NGS, it was considered a step and not an end in itself. Even when a diagnosis is made, the trajectory continues and a feeling of powerlessness may persist.

Conclusions: NGS thus represents a transition from a diagnostic odyssey to a step in care trajectory.

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C12.2
Children with a rare chromosome disorder. How have UK families' experiences of diagnosis and counselling changed over the ten year period 2003 - 2013?

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ABSTRACT

Background: The UK 2014 Strategy for Rare Diseases (SRD) recommends involving those affected by such diseases in order to improve diagnosis, intervention, and coordination of care in clinical genetics services[1]. Patient Reported Outcome Measures (PROMS) for genetics services are still in their infancy[2]. UK families' experiences of RCD diagnosis and counselling have been analysed over a ten year period (2003 - 2013) leading up to the launch of the national SRD.

Methods: Two surveys were undertaken ten years apart (2003 and 2013) by Unique (http://www.rarechromo.co.uk/html/History.asp). An identical questionnaire investigated seven stages of the clinical genetics service pathway including: pre-testing process; testing and communication of test result; referral to genetics expert; conduct of genetics consultation; RCD information provided; follow-up genetics counselling; sign-posting to peer support; plus the quality of the overall service. Comparison of responses at different time-points revealed trends and changes over time, and helped identify areas for improvement.

Results: 583 UK families' responses in 2003 were compared to 575 in 2013. Respondents were mainly mothers (reducing from 92.3% to 85.9% in 2013); mean age was similar (42.3 years and 43.0 years in 2013). Most families had only one child with a RCD (rising from 86.1% to 92.3%). Families' ratings of overall service received were not particularly high, and have not improved significantly over time. Key areas for improvement are identifiable.

Conclusions: The findings reported will hopefully enable the experiences of families of children with RCDs to be integrated more effectively into national strategies and policies for rare diseases.

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C12.3
BRCA1/BRCA2 population screening in Ashkenazi Jews: Long term impact

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Background: Ashkenazi Jews (AJ) population screening for common (2.5%) BRCA1/BRCA2 mutations could identify all carriers, including many (approximately half) lacking suggestive family history. Towards implementation, we examined the long-term impact of BRCA screening.
Methods: Unaffected AJs, age ≥ 25 years were either self-referred (SR) or recruiter-enrolled (RE), received pre-test written information and self-reported family history (FH). Post-testing, non-carriers with significant FH and carriers received in-person genetic counseling. Psychosocial outcomes and health behaviors were assessed quantitatively one week, 6 months and 2 years post-testing
Results: We report the 2 year follow-up of 1771 participants, including 32 carriers.
Psychosocial outcomes: RE and SR participants had similar rates of satisfaction (94%), and endorsement of population screening (90%), and similarly low stress (IES score = 4.1). Knowledge scores were higher in SR vs. RE (7.5 vs. 6.9/10, $P < 0.001$). Among carriers, 94% expressed satisfaction and 92% endorsed population screening. Stress was higher but declined over time (IES score 13.9 vs. 19.9 at 6 months (NS)). Knowledge was greater than in non-carriers (8.7 vs. 7.15/10, $p < 0.001$).
Health behaviour: All 25 women carriers had breast surveillance, 3/25 (12%) underwent risk-reducing bilateral mastectomy (similar to published rates in Israeli carriers). 15/16 (94%) carriers age > 40 underwent Risk-reducing salpingo-oophorectomy (RRSO). Among non-carriers, mammography screening rates did not decline compared to pretest rates, and even increased in non-carriers > 50 with non-suggestive FH ($p = 0.003$).
Conclusions: Long-term, BRCA screening is highly acceptable. Non-carriers do not demonstrate false reassurance, whereas carriers universally adopted increased surveillance with the vast majority undergoing age-appropriate RRSO. Funded by BCRF.

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C12.4
The European Gen-Equip project to create accessible resources for genetics education in primary care: an account of the process, the challenges and the successes

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The Gen-Equip project was undertaken to provide accessible genetics education to primary care health professionals in Europe. With advancing genomic medicine, all health professionals require knowledge and skills to detect patients at genetic risk, to provide them with appropriate healthcare. However, obtaining access to current genetics education, appropriate to their setting and language, is challenging. Partners from six European countries have worked collaboratively to produce nine online modules based on patient cases relevant for primary care. The process has involved: 1) Writing materials and agreeing content, 2) Production of videos and other resources to supplement text, 3) Uploading to a multi-media platform, 4) Translation and adaptation of modules to suit specific requirements of each partner country. Educational evaluation is crucial. Learners can undertake pre and post-module quizzes: we have now collected over 200 sets of pre and post-module data. Statistical analysis indicates that there are highly significant differences in knowledge as a result of taking a module. For example, mean pre-test score for the breast/ovarian cancer module was 77.6% (standard error = 2.97), compared with the post-test score of 90.4% (standard error = 2.69) and this represented a highly significant difference in the means ($t(33) = -5.87$, $p = 0.000$). We present the full results of the module evaluations, the process of preparing and disseminating these materials, and how we have addressed the challenges of involving multiple partners from different professions and countries. The online

case-based approach seems effective and the Gen-Equip experience has transferable lessons for further genetics education programmes in Europe.

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C12.5
UK investigation of the experiences and information preferences of patients with an increased risk of bowel cancer; Family Web Study survey results informing website content

S. M. A. Goodman, H. Skirton, R. Jones

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Relatives of people diagnosed with an increased risk of bowel cancer may also share a high lifetime risk of this cancer and it is important that patients share information about the implications of their diagnoses. However, evidence indicates that less than half of at-risk relatives access genetic testing or screening colonoscopy: this is sometimes due to a lack of understanding of personal risk.

The aim of this cross-sectional survey was to investigate experiences and preferences for receiving information about the familial diagnosis and risk. Participants (n = 238) at risk of colorectal cancer were recruited either online via charity websites or by post via genetics and colorectal clinics in six hospitals. Quantitative data were analysed using descriptive statistics with additional qualitative analysis of free text responses. Results indicate that only a third (79/238) received all or most of the information they wanted when they learnt of their risk. Most (148/238) indicated they would like information in other formats; via email, websites or social media, but also through follow-up appointments (102/238). Issues of particular interest were: healthy lifestyle (111/238) genetic testing (108/238) and talking to children (73/238).

These data are being used in the Family Web study to improve the dissemination of information within families via a website (www.familyweb.org.uk) designed so relatives can share personal information securely online. This survey has informed web site development. Survey responses endorse the need to improve existing information and support to this population, but these results may also be applicable to other genetic conditions.

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C12.6
Genomics Education at Scale

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Health Education England's Genomics Education Programme (GEP) has the mandate to ensure all staff working within the National Health Service (NHS) are prepared for the integration of genomic medicine into clinical practice. To educate and inform at this scale the GEP is utilising different learning technologies. In 2016, the GEP launched a three-week Massive Open Online Course (MOOC) on whole genome sequencing via the FutureLearn platform. Over three iterations, the course has registered 15,000 learners, representing a range of professions within the NHS. This MOOC is a synchronous learning activity, with course educators and mentors facilitating the education experience in real time. Crucially, the platform allows participants to interact and learn from each other's experiences. This interprofessional learning environment, as well as the presence of course mentors (clinical scientists in the NHS) was shown via course evaluation to be extremely effective. We have also used social media as a tool to educate and inform the largest NHS professional group, nurses and midwives. Using the WeCommunities platform, the GEP has facilitated a series of twitter conversations, with experts driving the discussion by posting key questions. Our data demonstrates that the power of this platform is its reach, and therefore its potential to inform and educate on a large scale: each conversation reached five million people. As well as proving to be successful learning and awareness raising tools, these platforms have also provided valuable insight into education and training gaps and highlighted areas good practice within genomic medicine in the NHS.

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C13
Innovative Variant Interpretation

C13.1
Novel diagnostic guidelines for prediction of variant spliceogenicity derived from a set of 311 combined in silico and in vitro studies: an international collaborative effort

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Variant interpretation is the key issue in molecular diagnosis. Splice variants exemplify this issue as each nucleotide variant can be deleterious *via* disruption, creation of splice site consensus sequences. Consequently, reliable *in silico* prediction of variant spliceogenicity would be a major improvement. Thanks to a collaborative effort from the French UGG splice network and the ENIGMA consortium, a set of 311 *BRCA1* and *BRCA2* variants studied at the mRNA level and occurring in 5' and 3' consensus regions (defined as the 9 and 23 bases surrounding the exon/intron junction, respectively) was collected and used to optimize previous prediction guidelines. Among these 311 variants, 147 (including 40 novel ones), were used to train a new prediction protocol named SPiCE (Splicing Prediction in Consensus Elements). Briefly, SPiCE first uses *in silico* splice predictions from Splice Site Finder and MaxEntScan. After that probability of splicing alteration and optimal decision thresholds are calculated by logistic regression. Following training, SPiCE was evaluated on a set of consecutive publications gathering 164 *BRCA* variants with their corresponding transcript analysis. We met an unprecedented sensitivity of 99.2% (134 out of 135) and specificity of 93.7% (30 out of 32). In other words, impact on splicing was correctly predicted for 161 out of 164 variants (98%). We therefore propose the SPiCE protocol as the new guideline for prediction of variant spliceogenicity. It could be easily implemented in any diagnostic laboratory as a routine decision making tool to help geneticists to face the deluge of variants in the nextgen era.

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C13.2
PEDIA study Phase 2: Prioritizing Exomes of unsolved patients with Image Analysis

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Combining molecular data with phenotype information has become the key bioinformatics strategy in interpreting exomes of patients with rare Mendelian disorders. With this approach, the correct mutation can be ranked first place in roughly half of patients with known dysmorphic syndromes and a disease-causing sequence variant in the coding part of the genome. As the high information content of dysmorphic human faces is only incompletely describable by the terminology of the human phenotype ontology, we aimed to analyze the gain in performance by including automated image analysis. We used facial recognition technology from FDNA to detect dysmorphic features in frontal photographs of patients, and derived similarity scores for the comparison of the gestalt to all known syndromes. In a multicenter effort with currently 15 participating institutions, we built a cohort of more than 400 meticulously studied and molecularly confirmed monogenic syndromic cases. The inclusion of pattern recognition for the human face in the prioritization process increased the ratio of exome cases with the top-ranked disease-causing mutation by more than thirty percent. In the clinical routine, where only a limited number of candidate mutations is evaluated, this also translates into a higher diagnostic yield. Interestingly, we were also able to delineate classifiers for gene-specific phenotypes of recently identified disease genes and we are therefore advocating to define case groups of yet undiagnosed patients by computer-assisted image analysis.

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C13.3
Genetic diagnosis of Mendelian disorders via RNA sequencing

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Across a large variety of Mendelian disorders, ~50–75% of patients do not receive a genetic diagnosis by whole exome sequencing indicative of underlying disease-causing variants in non-coding regions. In contrast, whole genome sequencing facilitates the discovery of all genetic variants, but their sizeable number, coupled with a poor understanding of the non-coding genome, makes their prioritization challenging. Here, we demonstrate the power of transcriptome sequencing to provide a confirmed genetic diagnosis for 10% (5 of 48) of undiagnosed mitochondrial disease patients and identify strong candidate genes for patients remaining without diagnosis. We found a median of 1 aberrantly expressed gene, 5 aberrant splicing events, and 6 mono-allelically expressed rare variants in patient-derived fibroblasts and established disease-causing roles for each kind. Private exons often arose from sites that are weakly spliced in other individuals, providing an important clue for future variant prioritization. One such intronic exon-creating variant was found in three unrelated families in the complex I assembly factor TIMMDC1, which we consequently established as a novel disease-associated gene. In

conclusion, our study expands the diagnostic tools for detecting non-exonic variants of Mendelian disorders and provides examples of intronic loss-of-function variants with pathological relevance.

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C13.4
Mutation spectrum of *NOD2* reveals recessive inheritance as a main driver of Early Onset Crohn's Disease

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Introduction: Inflammatory bowel disease (IBD), encompassing Crohn's Disease (CD) and Ulcerative Colitis (UC), results in chronic and destructive inflammation of the gastrointestinal tract in genetically susceptible individuals. IBD is typically diagnosed in the 3rd decade of life; however pediatric-onset IBD, diagnosed before age 18, represents ~25% of all diagnoses and is generally more severe. *NOD2* was the first and to date most replicated gene associated with adult CD; its role in pediatric IBD remains not well understood.

Methods: We performed WES in a cohort of 1183 probands with pediatric IBD (<18y) and their available parents and siblings. Trio-based analysis was executed on 492 complete trios for initial discovery, and replicated our findings in the remaining probands.

Results: In total, we identified 92 probands in our cohort with recessive inheritance of *NOD2* alleles, carrying homozygous or compound heterozygous combinations of rare and low-frequency CD-risk alleles or novel variants. We investigated the contribution of recessive inheritance of *NOD2* in IBD patients from the DiscovEHR study and confirmed that recessive inheritance of *NOD2* rare and low-frequency variants explained ~7% of cases.

Conclusions: In sum, ~8% of the probands in our pediatric-onset IBD cohort conform to a recessive inheritance mode of *NOD2* rare and low frequency deleterious variants. This was similarly confirmed in an independent IBD cohort with several cases having been diagnosed with CD at an earlier than average age. Our findings implicate *NOD2* as a Mendelian disease gene for a subset of early-onset CD, molecularly defined by recessive inheritance of *NOD2* variants.

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C13.5
Podocytes differentiated from urine renal precursor as a tool for Alport syndrome diagnosis and for assessing therapeutic strategies based on patient-derived cells

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Alport syndrome is a genetic disorder caused by mutations in collagen IV genes, leading to ultrastructural lesions of the glomerular basement membrane up to end-stage renal disease. COL4 chains expression is restricted to kidney, eye and ear. Podocytes, key component of the glomerular structure, are the only cells able to produce the three COLIV alpha chains and thus, they are key-players in ATS pathogenesis. However, podocytes-targeted therapeutic strategies, have been hampered by the difficulty of isolating them by non-invasive methods and transcripts-based diagnostic approaches have been complicated by the inaccessibility of other cell types expressing COL4 chains. For the first time, we have recently demonstrated that it is possible to isolate and differentiate renal precursors from urine of Alport syndrome patients and healthy carriers, providing an easily available cell system closer to podocytes' physiological conditions. RT-PCR analysis revealed *COL4A3*, *COL4A4* and *COL4A5* expression associated with Podoplanin expression, marker involved in shaping podocytes membrane. RNA studies on patients-derived podocytes harboring intronic variants of uncertain significance in *COLIV* genes led to identify aberrant splicing patterns leading to premature stop codons and truncated proteins. Our data highlight that urine-derived podocytes' precursors can be used as tool to establish the pathogenic role of uncertain variants, thus allowing us to provide the patient with a molecular diagnosis and with a recurrence risk for the siblings. Furthermore, the established system opens up the possibility of testing personalized gene therapy-based approaches on disease-relevant cells taking advantage of a CRiSPR/Cas9 technology, in combination with the easily-deliverable AAV9 system.

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C13.6
Machine learning models for the characterization of genes associated with adult brain diseases

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Over the past 5 years there has been a massive growth in genetic testing and this has had a huge and arguably disproportionate impact on our understanding of adult neurological disorders. In parallel there has been an equally impressive growth in the availability of omics data. Given this critical mass of data we ask whether we can identify the key features of a gene relevant to adult brain disease. We apply Machine Learning (ML) on DNA, RNA and protein features of disease genes defined by DisGeNET and expert curation to achieve this. Regarding DNA, we include as a predictor ExAC pLI (probability of being intolerant to Loss of Function). Regarding RNA data, we consider specificity of gene expression in 42 tissues as detailed in GTExV6, and the overall and specific connectivity patterns of the genes as measured by WGCNA-based co-expression networks. We use data from HEXEvent to account for variability in transcript structure. Using this approach we demonstrate that gene connectivity rather than gene expression is a more useful classifier. Furthermore, this approach highlights the importance of gene expression within adipose tissue (over brain tissue) for Alzheimer's disease (AD), in keeping with evidence linking adiposity and AD risk. A p-value of 5.79e-10 for the classifier accuracy being better than the non-information ratio suggests the utility of this approach. Thus, we conclude that by using ML we can efficiently generate novel insights into the location and processes driving disease in genetic forms of adult neurological disorders.

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C14
Population Genetics and Ancient DNA

C14.1
Extremely rare variants reveal patterns of germline mutation rate heterogeneity in humans

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Precise estimates of the single-nucleotide mutation rate and its variability are essential to the study of human genome evolution and genetic diseases. However, estimates using common variants are biased by selection and biased gene conversion while analyzing de novo variants provides insufficient observations to consider sequence context. Here we use ~36 million singleton variants observed in 3,716 whole-genome sequences to characterize the heterogeneity of germline mutation rates across the genome. These singletons arose very recently in the population, and are thus largely unaffected by confounding evolutionary factors. We show that nucleotide context is the strongest predictor of mutability, with mutation rates varying by >650-fold depending on the identity of three bases upstream or downstream of the mutated site. Histone modifications, replication timing, recombination rate, and other local genomic features further modify mutability; magnitude and direction of this modification varies with the sequence context. We evaluate the estimated models in an independent dataset of ~46,000 de novo mutations and show that singleton-based estimates provide a more accurate prediction of the mutation patterns than estimates based on common variants used in previous approaches. Incorporating the effects of genomic features further improves the prediction. Finally we demonstrate how highly mutable 7 base pair motives can help identify new mechanisms of germ-line mutation. The effects of sequence contexts, genomic features, and their interactions we present capture the most refined portrait to date of the germline mutation patterns in humans.

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C14.2
Clustered de novo mutations with large intra-mutational distance contribute to the maternal age effect

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Clustered mutations are series of point mutations occurring in close proximity. Clustering of mutations has been observed in humans in germline, somatic and cancer cells. Evidence suggests that these mutation clusters mostly arise from single mutational events, giving rise to point mutations with reciprocal distances of up to 20kb. However, the exact mutational mechanism of germline mutation clusters remains elusive.

Here, we collected data on clustered de novo mutations by sequencing whole genomes of 1,291 parent-offspring trios. We identified 1,796 clustered de novo mutations (cDNMs) and determined the parent-of-origin for 660 cDNMs (37%) to investigate the differences between male and female germline cDNMs.

We show that mutation clusters containing C-G substitutions prefer specific mutation orders, suggesting that the underlying mutational influences are oriented according to the DNA backbone. Unlike unclustered DNM, we do not find a paternal age effect for clustered DNM. However, specifically maternal DNM clusters with inter-mutational distances above 1kb are correlated with maternal age, accounting for a significant proportion of the maternal age effect of all DNMs. Interestingly, these clusters are enriched on chromosomes 8, 9 and 16 in known maternal accelerated regions.

In conclusion, our study sheds light on the potential mechanisms underlying these events and reveals the significant contribution of cDNMs to the maternal age effect.

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C14.3
Admixture mapping identifies Inuit ancestry loci associated with metabolic traits in the Greenlandic population

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Background: Greenland is inhabited by a small, historically isolated Inuit population, which only in the last few centuries admixed with Europeans. Indigenous Inuit Greenlanders have adapted to harsh Arctic conditions, including low temperatures and restricted access to plant-based food. We aim to make use of recent admixture in the Greenlandic population to investigate ancestry specific genetic associations with metabolism.
Materials and Methods: We genotyped 1570 Greenlanders and inferred locus-specific (local) ancestry. Using linear mixed models to control for the population structure, we performed admixture mapping with 232 serum metabolites quantified by NMR spectroscopy.
Results: We found three loci where local ancestry associated with changed levels of one or more metabolites. The first is located on 11q13.2, where Inuit ancestry was associated with several metabolites including decreased degree of fatty acid unsaturation ($p = 8.5e-38$). At the second locus, located on 8q21.3, Inuit ancestry was associated with an increased ratio of ω-3 fatty acids to all fatty acids ($p = 1.1e-6$). Finally, at 12q13.13, Inuit ancestry was associated with increased levels of lactate ($p = 1.1e-6$).
Conclusions: Our findings indicate that Inuit ancestry at specific regions in the genome is associated with the regulation of metabolism in the Greenlandic population, demonstrate that admixture mapping can localize ancestry-specific functional variants, and illustrate the power of genetic studies in small, historically isolated populations.
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C14.4
Farming in Estonia was introduced by Early Bronze Age migrants from the Steppe

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The shift from hunting-gathering to farming and animal husbandry happens relatively late in Northeast Europe and the extent to which it involved genetic ancestry change is still poorly understood.

To shed more light on the genetic changes during the shift to farming based economies in Estonia, we extracted and sequenced aDNA from skeletal remains uncovered in the context of Mesolithic Narva Culture (MNC) (7,200–5,900 yr BP), and Neolithic Comb Ceramic Culture (CCC) (5,900–3,800 yr BP) and Corded Ware Culture (CWC) (4,800–4,000 yr BP) from Estonia. We compared these autosomal as well as mtDNA, X and Y chromosome data to sequence and genotype data from modern and ancient populations of Europe, West Asia and Siberia to make inferences about the extent of continuity and genetic change during the end of the Stone Age in Estonia.

We find that Estonian hunter-gatherers of Comb Ceramic Culture are closest to Eastern hunter-gatherers. The Estonian first farmers of Corded Ware Culture show high similarity in their autosomes with Steppe Belt Late Neolithic/Bronze Age individuals, Caucasus hunter-gatherers and Iranian farmers while their X chromosomes are most closely related with the European Early Farmers of Anatolian descent.

These findings suggest that the shift to intensive cultivation and animal husbandry in Estonia presents a unique case of a sex-specific process triggered by the arrival of new people and culture, whose ancestry can be traced back to the Steppe rather than to Anatolia.

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C14.5
Complex spatio-temporal distribution and genogeographic affinity of mitochondrial DNA haplogroups in 24,216 Danes

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Mitochondrial DNA (mtDNA) haplogroups (hgs) are evolutionarily conserved sets of mtDNA SNPs. Associations of hgs with geographical origin, disease and physiological characteristics have been reported, but have frequently not been reproducible. We assessed, using 418 mtDNA SNPs on the PsychChip (Illumina), the spatio-temporal distribution of mtDNA hgs in DNA isolated from 24,642 geographically un-biased dried blood spots (DBS), collected from 1981 to 2005 through the Danish National Neonatal Screening program. Geno-geographic affinity was established with ADMIXTURE using a reference of 100K + autosomal SNPs in 2,248 individuals from nine populations. The hg distribution was typically Northern European, and hgs were highly variable based on median-joining analysis, suggesting multiple founder events. Considerable heterogeneity and variation in autosomal geno-geographic affinity (ancestry background) was observed. Thus, individuals with hg H exhibited 95 %, and U hgs 38.2 % – 92.5 %, Danish ancestry. Significant clines between geographical regions and rural and metropolitan populations were found. Over 25 years, macro-hg L increased from 0.2 % to 1.2 % ($p = 1.1$*E-10), and M from 1 % to 2.4 % ($p = 3.7$*E-8). Hg U increased among the R macro-hg from 14.1 % to 16.5 % ($p = 1.9$*E-3). Geno-geographic affinity, geographical skewedness, and sub-hg distribution suggested that the L, M and U increases are due to immigration. The complex spatio-temporal dynamics and geno-geographic heterogeneity of mtDNA in the Danish population reflect repeated migratory events and, in later years, net immigration. Such complexity may explain the often contradictory and population-specific reports of mito-genomic association with disease.

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C14.6
From lost empires to modern cities with ancient GPS

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Recent studies have demonstrated that geographical origin can be accurately inferred from genomic data and prompted us to embark on the unresolved question of inferring the geographical origin of skeletal finds, thus far assumed to be synonymous with their burial site. Whereas geographical inference based on anatomical or morphological information is highly complex and error-prone, particularly when the remains are physically damaged or fragmented, using ancient DNA for localization entails different challenges due to the lack of intermediate samples over space or time, the small number of SNPs, and their spurious nature. We developed the ancient Geographic Population Structure (aGPS), an admixture-based method that uses the relationship between admixture and geography to predict the geographical locations of samples. aGPS implements a genetic clustering approach and uses a dynamic reference panel based on the chronology of the sample. Applied to a genomic dataset of over 300 ancient Eurasians and Near-Easterners (Pleistocene - Late Iron Age), aGPS localized ~50% of the samples within 0–200km from their burial site, ~32% within 200–1,000km, and the remaining within 1,000–3,175km, with an overall average accuracy of 525km. We have also identified "biodiversity centers", which correspond with ancient Empires that drew immigrants from other countries, and the spatio-temporal structure of the corresponding migration fluxes. Our results confirm the massive Yamnaya migration from the steppe to Central Europe during the Late Neolithic. Our findings allow addressing long standing questions in history concerning the identity of the Old World residents.

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C15
Reproductive Genetics

C15.1
Aging oocytes accelerate regional sequence diversity in humans and African apes

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Introduction: The accumulation of germline de novo mutations (DNMs) is a primary requisite for evolution. Despite this, there is considerable uncertainty about the parent-of-origin effects on DNM accumulation and the mutational mechanisms inducing them.

Material and Methods: We whole-genome sequenced 1,548 Icelanders (~35X coverage), their parents, and for a subset of 225, at least one child.

Results: We found 108,778 DNMs, whereof we identified parental origin of 42,961 DNMs. We estimated a paternal and maternal age effect of 1.51 and 0.37 DNM per year of the age of the parents, respectively. There was a considerable difference in the relative contribution of maternal CpG > TpG (0.26% decrease per year) and C > G (0.33% increase per year) DNMs with increasing maternal age. Strikingly, the maternal age at conception affects the regional distribution of DNMs especially for C > G DNMs, resulting in a 50 fold greater C > G mutation rate in a 20 megabase region of chromosome 8p. We found that age-related gene conversions from mothers mostly occur within the regions enriched with maternal C > G DNMs, providing a causal link between double strand break repair and the accumulation of DNMs with maternal age. This age-related regional influx of maternal C > G DNMs is reflected in rare and common C > G mutations and pairwise divergence between African great ape species.

Conclusions: Our result showcase the interaction of parental age effect on the location, type and rate of mutations; and how these sex differences have shaped generation of sequence diversity throughout human history and African great ape divergence.

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C15.2
Interactome between embryo trophectoderm cells and endometrial epithelial and stromal cells: novel insights into implantation process in human

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Human embryo implantation is a complex process that requires dialogue between the receptive lineage of uterus i.e. endometrium and blastocyst stage embryo. As it is ethically and technically impossible to study human embryo implantation in vivo, the molecular processes during the implantation process are still not well known. Protein-protein interaction (PPI) networks were constructed using transcriptome data on upregulated genes from receptive endometrial epithelium or stromal cells and polar-trophectodermal cells. The detected mRNAs were converted into corresponding proteins, the subset of proteins localized in cell membrane and surface were extracted, and PPI networks were created based on interactions in STRING 10.0 database. Endometrial epithelial-trophectodermal cell network contains 157 and endometrial stromal-trophectodermal network 138 interacting cell surface proteins. Biological processes related to cell attachment and proliferation were detected in both blastocyst-epithelial and blastocyst-stromal cell-specific PPI

networks. The largest super-cluster in stromal-specific cell network was related to the positive regulation of kinase activity and extracellular matrix organization, while in epithelial-specific cell network blood coagulation and cell junction assembly clusters were detected. The hyaluronan metabolism pathway, known to be important in embryo implantation, was uniquely enriched in epithelial cell network, underlining the cell type specific nature of human embryo implantation process. This is the first comprehensive study of molecular networks between blastocyst and specific cell types of receptive human endometrium, providing new understanding about the molecular processes that lead to successful embryo implantation, thus allowing to predict the best timing for embryo transfer in in vitro fertilization cycles. Funding: grants IUT34-16; EU48695; EU324509.

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C15.3
Diagnostic value of non-invasive prenatal testing (NIPT) using genomic imbalance profiling (GIPseq)

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Non-invasive prenatal testing (NIPT) enables risk estimation for common fetal autosomal aneuploidies with high sensitivity and specificity. Using clinical analysis of over 20.000 pregnancies, we show that NIPT by in-house optimized genomic imbalance profiling (GIPseq) increases the sensitivity for detection of fetal trisomy 21, 18 and 13 to 100%, 97,2% and 100% respectively without reducing the specificity, which exceeds 99,9% for each of these trisomies. Furthermore, NIPT by GIPseq offers the advantage of detecting other genomic imbalances that are clinically relevant for fetal or maternal health. These findings include (i) other aneuploidies (0.5%), with the highest incidence for trisomy 7, 16 and 22 respectively, (ii) fetal or maternal segmental imbalances (15 and 13 cases respectively) and (iii) maternal cancer (7 cases). We show that, although uncommon aneuploidies mostly exist as confined placental mosaicism with different grades of mosaicism depending on the sampled region, they pose a risk for intra-uterine growth retardation and uniparental disomy (UPD), as illustrated in a fetus with mosaic trisomy 15 and a UPD15 cell line. We also demonstrate that placental chromosomal imbalances can pass the developmental barrier, resulting in complex fetal segmental anomalies with severe clinical implications. In addition, the detected maternal and fetal segmental imbalances triggered expert ultrasound follow-up and, in some cases, had implications on future reproductive choice. Genome-wide NIPT also enables presymptomatic detection of maternal tumors. Altogether, we demonstrate that genome-wide NIPT analysis improves pregnancy management without introducing unnecessary invasive testing.

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C15.4
Clinical implementation of non-invasive prenatal diagnosis (NIPD) for single gene disorders

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Introduction: We have developed and implemented a method for NIPD of multiple single gene disorders (SGD), including spinal muscular atrophy (SMA), Duchenne and Becker muscular dystrophies (DMD/BMD), cystic fibrosis (CF) and congenital adrenal hyperplasia (CAH). We launched a diagnostic service for SMA and DMD/BMD in September 2016.

Materials and Methods: The test involves targeted enrichment of thousands of SNPs across multiple genomic regions and massively parallel sequencing (Illumina MiSeq) of cfDNA followed by relative haplotype dosage (RHDO) analysis. Maternal, paternal and proband genomic DNA samples are tested alongside cfDNA for haplotype phasing and to measure fetal fraction. Our method can test 2–3 patients on a single MiSeq run, thus increasing the multiplexing capacity and decreasing testing costs for clinical laboratories.

Results: We have received referrals from across the world, including 8 pregnancies at risk of SMA, 1 pregnancy at risk of DMD and 1 pregnancy at risk of BMD. Samples have ranged in gestational age from 8 to 13 weeks, with an average turnaround time for results of 12 calendar days.

Overall, we have reported 3 normal, 5 unaffected carrier and 2 affected pregnancies.

Conclusions: We have encountered a number of scenarios, including consanguinity, 2 + 0 SMA carrier parents and recombination events. We have shown that NIPD by RHDO is feasible in a clinical setting, increasing accessibility to many more couples with a pregnancy at risk of a SGD. Validation is still ongoing for CF and CAH, with similarly promising results so far.

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C15.5
Exome sequencing of 406 parental/fetal trios with structural abnormalities revealed by ultrasound in the UK Prenatal Assessment of Genomes and Exomes (PAGE) project

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PAGE aims to apply whole exome sequencing (WES) to 1000 trios recruited in the UK-NHS over 3 years to identify pathogenic variation underlying heterogeneous fetal structural abnormalities detected by ultrasound scan (USS).

Trio WES is conducted after resolution of pregnancy if conventional testing (QF-PCR, chromosomal microarray or targeted single/panel gene testing) fails to establish a definitive diagnosis. Genetic variants are triaged via a stringent filtering pipeline established for the UK Deciphering Developmental Disorders (DDD) project and potentially pathogenic variants are assessed and classified by a UK-wide multidisciplinary clinical review panel (CRP), technically validated in NHS accredited labs and reported back to Clinical Genetics units and families where appropriate.

Thus far from 259 trios reviewed by the CRP, 16 likely diagnoses have been revealed, giving a diagnostic yield of ~6%. Diagnostic yield varies by phenotypic class, with multisystem phenotypes showing the highest (~16%). Diagnoses include de novo mutations in known dominant developmental disorder genes (n = 9), biparentally inherited homozygous/compound heterozygous variants (n = 6) and maternal UPD chromosome 15. The majority of variants are SNVs/indels which would escape targeted detection by conventional testing. When compared to a null model based on triplet mutation rate, an excess of de novo mutation is observed, more pronounced in known dominant genes (such as KMT2D). Further analysis is predicted to identify new gene and mechanistic associations underlying observed phenotypes as more samples are processed. PAGE aims to catalyse responsible adoption of WES and potentially WGS in routine diagnostics in the prenatal setting.

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C15.6
Evaluation of an expanded carrier screening offer in a non commercial setting

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Since May 2016, expanded carrier screening for 50 severe recessive disorders is available in a non-commercial hospital setting in Amsterdam, to facilitate informed reproductive decision-making. The screening aims at couples without a priori increased risk (no family history). Couples can apply for counseling via www.dragerschapstest.nl, or hospital referral. Pre- and posttest counseling is provided by genetic professionals at the outpatient clinic. External geneticists can send in blood samples as well. Outcome and impact of testing was evaluated.

Methods: A capture-based next generation sequencing strategy is used. Only pathogenic variants are reported (individual reports). CNV analyses is included. Reimbursement is possible for couples with high-risk indication (HRI) based on ancestry/consanguinity. Pre- and posttest questionnaires were completed including reasons for testing, knowledge, psychological impact, and satisfaction. Partners could opt for parallel or sequential testing.
Results: In the first nine months, 39 couples (25 with HRI) and 6 individuals (4 with HRI) visited the outpatient clinic. One couple refrained from testing. Sixteen couples choose parallel testing. Eventually, eighty individuals were tested, including 5 partners sequentially tested after positive tested carriers, and 15 additional external requests. Carrier status of one (n = 21 persons), two (n = 3), three (n = 1) or four (n = 1) mutation(s) was identified. Fifty-four (68%) individuals tested negative. No carrier couples were found.
Conclusions: Preliminary analyses shows that about one third of tested individuals were carriers. Although the test was designed for couples with no a priori risk, more than half reported HRI. One-year outcome will be presented at ESHG Conference, including results from questionnaires.

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C16
Intellectual Disability

C16.1
Mutations in epigenetic regulation genes are a major cause of overgrowth with intellectual disability

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To explore the genetic architecture of overgrowth syndromes and human growth control we performed experimental and bioinformatic analyses of 710 individuals with overgrowth (height and/or head circumference ≥ + 2SD) and intellectual disability (OGID). We identified a causal mutation in one of 14 genes in 50% (353/710). This includes *HIST1H1E*, encoding histone H1.4, which has not been associated with a developmental disorder previously. The pathogenic *HIST1H1E* mutations are predicted to result in a product that is less effective in neutralising negatively-charged linker DNA because it has a reduced net charge, and in DNA binding and protein-protein interactions because key residues are truncated. Functional network analyses demonstrated that epigenetic regulation is a prominent biological process dysregulated in individuals with OGID. Mutations in six epigenetic regulation genes, *NSD1*, *EZH2*, *DNMT3A*, *CHD8*, *HIST1H1E* and *EED*, accounted for 44% of individuals (311/710). There was significant overlap between the 14 OGID genes and 611 genes in regions identified in GWAS to be associated with height ($P = 6.84 \times 10^{-8}$), suggesting common variation impacting OGID gene function influences height at a population level. Increased cellular growth is a hallmark of cancer and there was striking overlap between the OGID genes and 260 somatically mutated cancer driver genes ($P = 1.75 \times 10^{-14}$). However, the mutation spectra of genes involved in OGID and cancer differ, suggesting complex genotype-phenotype relationships. These data reveal insights into the genetic control of human growth and demonstrate that exome sequencing in OGID has a high (50%) diagnostic yield, and could be have utility as a first-line test in OGID. Funder, Wellcome:100210/Z/12/Z.

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C16.2
Mutations in *EBF3* disturb transcriptional profiles and cause intellectual disability, ataxia, and facial dysmorphism

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From a GeneMatcher-enabled international collaboration, we identified ten individuals affected by intellectual disability, speech delay, ataxia, and facial dysmorphism and carrying a deleterious *EBF3* variant detected by whole-exome sequencing. One 9-bp duplication and one splice-site, five missense, and two nonsense variants in *EBF3* were found; the mutations occurred de novo in eight individuals, and the missense variant c.625C > T (p.Arg209Trp) was inherited by two affected siblings from their healthy mother, who is mosaic. EBF3 belongs to the early B cell factor family (also known as Olf, COE, or O/E) and is a transcription factor involved in neuronal differentiation and maturation. Structural assessment predicted that the five amino acid substitutions have damaging effects on DNA binding of EBF3. Transient expression of EBF3 mutant proteins in HEK293T cells revealed mislocalization of all but one mutant in the cytoplasm, as well as nuclear localization. By transactivation assays, all EBF3 mutants showed significantly reduced or no ability to activate transcription of the reporter gene *CDKN1A*, and in situ subcellular fractionation experiments demonstrated that EBF3 mutant proteins were less tightly associated with chromatin. Finally, in RNA-seq and ChIP-seq experiments, EBF3 acted as a transcriptional regulator, and mutant EBF3 had reduced genome-wide DNAbinding and gene-regulatory activity. Our findings demonstrate that variants disrupting *EBF3*-mediated transcriptional regulation cause intellectual disability and developmental delay and are present in ~0.1% of individuals with unexplained neurodevelopmental disorders.

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C16.3
Recurrent de novo missense mutations in small GTPase gene *RAB11B* cause severe intellectual disability and a distinctive brain phenotype

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The Rab GTPase family comprises approximately 70 GTP-binding proteins that function in vesicle formation, transport and fusion. Interaction with downstream proteins is only present in their GTP-bound, active state. Here, we report six patients with two recurrent de novo missense mutations in RAB11B (p.(Val22Met)/four patients; p.(Ala68Thr)/two patients). An overlapping neurodevelopmental (ND) phenotype, including severe intellectual disability with absent speech, epilepsy, and spasticity was observed in all patients. Additionally, visual problems, musculoskeletal abnormalities and microcephaly were present in the majority. Re-evaluation of brain MRI images of four patients, showed a shared distinct brain phenotype, consisting of severely decreased white matter volume, thinned corpus callosum, hypoplasia of the cerebellar vermis, optic nerve hypoplasia and mild ventriculomegaly. To study the functional effect of the identified *RAB11B* variants, and to compare this with known inactive GDP- and active GTP-bound RAB11B mutants (p.(Ser25Asn) and p.(Gln70Leu), respectively), we modeled the variants on the three-dimensional protein structure and performed subcellular localization studies. We found that both variants altered the binding pocket of GTP/GDP and resulted in disturbed cytosolic and Golgi localization in transfected hTERT-RPE1 cells. Interestingly, these observations were comparable to the known GDP-bound inactive mutant, suggesting that the *RAB11B* mutations in patients resulted in a predominantly inactive state of the protein. In line with these findings, we observed that the patient mutations altered protein-protein interactions with canonical RAB11B binding partners. In conclusion, we report two recurrent dominant mutations in *RAB11B* leading to a ND syndrome, likely caused by impaired GTP binding and subsequently, altered protein-protein interactions.

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C16.4
A syndromic neurodevelopmental disorder is caused by de novo disruption of the proteasome regulatory subunit PSMD12

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Introduction: Degradation of proteins by the ubiquitin-proteasome system is an essential biological process in the development of eukaryotic organisms and the maintenance of physiological homeostasis. Dysregulation of this mechanism leads to numerous human neurodevelopmental and/or neurodegenerative disorders. Two independent teams recently highlighted the same candidate gene for intellectual disability within the ubiquitin-proteasome system.

Materials and Methods: Additional anomalies of the candidate gene were sought in data from 50,000 exomes and 59,000 chromosomal microarray analyses, thanks to an international collaboration between six research centers specialized in neurodevelopmental disorders. To determine the role of the candidate gene in the disorder, a zebrafish model was generated and in vitro ubiquitination assays were performed from normal and affected individuals' cells.

Results: We identified six de novo genomic deletion CNVs and four de novo point mutations involving *PSMD12*, encoding non-ATPase subunit RPN5 of the 19S regulator of the 26S proteasome complex, in unrelated individuals with intellectual disability, congenital malformations, ophthalmologic anomalies, and subtle dysmorphic facial features. We observed a reduced RPN5 protein level and an accumulation of ubiquitinated proteins without any impairment of proteasome catalytic activity. Our *psmd12* loss-of-function zebrafish CRISPR/cas9 model exhibited microcephaly, as well as renal and craniofacial anomalies.

Conclusions: Conclusions: our data support the importance of RPN5 to human biology and disease directly implicating *PSMD12* haploinsufficiency, and aberrant functioning of the scaffolding subunit of the proteasome, in impaired neurodevelopment. These enable the definition of a neurodevelopmental disorder due to *PSMD12* variants, expanding the phenotypic spectrum of ubiquitin-proteasome dependent disorders.

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C16.5
Biallelic variants of *UBA5* reveal that disruption of the UFM1 cascade can result in early-onset encephalopathy

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Via whole-exome sequencing, we identified rare autosomal-recessive variants in *UBA5* in five children from four unrelated families affected with a similar pattern of severe intellectual deficiency, microcephaly, movement disorders, and/or early-onset intractable epilepsy. *UBA5* encodes the E1-activating enzyme of ubiquitin-fold modifier 1 (UFM1), a recently identified ubiquitin-like protein. Biochemical studies of mutant UBA5 proteins and studies in fibroblasts from affected individuals revealed that UBA5 mutations impair the process of ufmylation, resulting in an abnormal endoplasmic reticulum structure. In Caenorhabditis elegans, knockout of *uba-5* and of human orthologous genes in the UFM1 cascade alter cholinergic, but not glutamatergic, neurotransmission. In addition, *uba5* silencing in zebrafish decreased motility while inducing abnormal movements suggestive of seizures. These clinical, biochemical, and experimental findings support our finding of UBA5 mutations as a pathophysiological cause for early-onset encephalopathies due to abnormal protein ufmylation.

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C16.6
Is Rett syndrome treatable? In vitro restoration of neuronal microtubule dynamics and preclinical studies

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Introduction: Rett syndrome (RTT) is predominantly caused by Methyl-CpG-binding protein 2 (*MECP2*) mutations. Development of targeted therapeutics has been hampered by an imprecise understanding of the pathophysiology of RTT. We and others have demonstrated that neuronal microtubule dynamics are disrupted in RTT, in association with reduced α-tubulin acetylation and increased histone deacetylase 6 (HDAC6). We hypothesized that pharmacological inhibition of HDAC6 could restore microtubule stability and function, potentially ameliorating the RTT phenotype.

Materials and Methods: We tested this hypothesis in a number of in vitro studies and in the $Mecp2^{T158A}$ mouse model of RTT. Acetylated tubulin and HDAC6 levels were measured in MeCP2-deficient cells. Microtubule stability was measured in RTT patient fibroblasts and the trafficking speed of mitochondria was measured in $Mecp2^{T158A}$ cultured cortical neurons. Further, we tested if a highly specific HDAC6 inhibitor could ameliorate the impaired motor and behavioural phenotype of the $Mecp2^{T158A}$ mice.

Results: We found reduced acetylated tubulin and increased HDAC6 expression in both patient and $Mecp2^{T158A}$ mouse brain, a reduction in mitochondrial velocity RTT mouse cortical neurons, increased microtubule instability in patient fibroblasts, and that HDAC6 inhibition restores tubulin acetylation levels and improves microtubule stability. Preliminary studies with our HDAC6 inhibitor revealed that treated $Mecp2^{T158A}$ mice show improvement in their impaired motor, behavioural and respiratory phenotype.

Conclusions: Together, these early studies suggest that pharmacological inhibition of HDAC6 potentially provides a novel therapeutic option for RTT, restoring neuronal trafficking deficits, and may stabilize the neurological phenotype associated with the disorder in our animal model of RTT.

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C17
Hereditary Cancer

C17.1
The contribution of rare variants, polygenic risk, and novel candidate genes to the hereditary risk of

breast cancer in a large cohort of Breast Cancer families

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Identifying the missing hereditary factors underlying the familial risk of breast cancer could have a major and immediate impact on managing the breast cancer risk for these families.

Methods: We identified candidate breast cancer predisposition genes through whole exome sequencing of BRCAx families, and sequenced, up to 1325 genes, along with 76 common variants associated with breast cancer, in index cases from 5,900 BRCAx families and 5,600 cancer free women (ethnically matched on PCA).

Results: The role of recently described (*PALB2*) or suspected (*MRE11A*) moderately penetrant genes was confirmed. Conversely, the size of the cohort means that the absence of enrichment for LoF mutations provides strong evidence against other reported breast cancer genes (*BRIP1*, *RINT1*, *RECQL*). For further moderate risk variants (in *CHEK2*, *ATM*, *BRCA2*) we observed significant risk modification based on the polygenic risk score (PRS - calculated from the common variant data), with the risk restricted to the co-occurrence of the rare variant and high PRS. Novel candidate genes were identified based on LoF mutations, including *NTHL1* (38 cases versus 15 controls, OR 2.5 p = 0.002): a member of the base excision repair (BER) pathway. We analysed data from additional genes in the BER pathway, along with somatic sequencing, tumour mutation profiling and familial segregation to examine this association.

Conclusions: Our data shows that the effect of rare variation in established and novel breast cancer genes, along with consideration of the background polygenic risk, together explains a substantial component of the heritable risk of breast cancer in our cohort.

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C17.2
Assessing risk of familial breast cancer: effectiveness of current UK guidelines

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Introduction: Breast cancer risk is a common indication for referral to clinical genetics. UK National Institute of Health and Care Excellence (NICE) guidelines use family history to stratify by 10yr risk of breast cancer from age 40. Patients are divided into low (LR, 10-year risk <3%), moderate (MR, 3–8%) and high risk (HR, >8%). Women at increased risk are offered screening at or prior to age 40.

Methods: Family history data was obtained for all unaffected women with a family history of breast cancer aged <50, referred to Tayside clinical genetics from 2000–2010. Patients were risk stratified de novo by NICE criteria, identifying patients who subsequently developed breast cancer.

Results: 1,409 women had 15,414 patient-years of follow up. 30 invasive breast cancers developed, 13 in MR and 13 in HR women. Kaplan-Meier analysis demonstrated no significant difference in breast cancer rate between LR and MR women from ages 40–49 (Log rank $p = 0.431$). There was a significant difference from 40–49yrs between LR and HR women ($p = 0.036$), but not on exclusion of *BRCA* mutation carriers ($p = 0.136$). NICE absolute 10yr risk thresholds from 40–49 were not met in any risk group (LR = 0.82%, MR = 1.68%, HR = 3.56%).

Conclusions: Screening prior to age 50 in those without a *BRCA* mutation may be unnecessary. NICE family history criteria do not identify women with the suggested 10yr risk values. There is a need for further evaluation of the benefits of early screening, and criteria for identifying women at increased risk of breast cancer may need to be improved.

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C17.3
The optimal cancer genetics testing tool? - Diagnostic whole genome sequencing in research participants with multiple primary tumours

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Whole genome sequencing (WGS) offers a variety of potential benefits in diagnostic settings such as improved coverage of coding regions and the opportunity to detect structural variation. In the clinical cancer genetics context, application of WGS has thus far been limited and assessments of performance are not abundant in the literature. Here we present diagnostic interpretation of WGS data from the Multiple Primary Tumours arm of the NIHR BioResource-Rare Disease study (recruitment criteria ≥2 primaries <60 years or ≥ 3 primaries <70 years and no identified molecular diagnosis), to which 720 individuals have been recruited. This cohort is expected to be enriched for tumour predisposing variants but is unselected for tumour type, arguably representing the kind of patient group that agnostic diagnostic testing might be applied to in a "mainstreaming" model of genetic testing. Variants were called using Illumina Isaac (SNVs and indels), Canvas (CNVs) and Manta (CNVs, translocations and inversions). They were extracted for analysis if occurring in a gene included in a comprehensive list (n = 133) of cancer predisposition genes (CPGs) and annotated with a sequence ontology term reflecting potential alteration in function. Variants where then assessed with stringent criteria based on quality, predicted biological consequence and literature. The detection rate of clinically relevant variants is presented and the added value of WGS compared with other techniques is assessed. The majority of samples in the study had concurrent sequencing of 94 CPGs using the Illumina TruSight Cancer panel, allowing identification of SNVs and indels missed by WGS.

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C17.4
A somatic mutational signature in different tumor types associated with biallelic germline *NTHL1* mutations

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We previously found that biallelic germline mutations affecting the base excision repair gene *NTHL1* predispose to the development of adenomatous polyposis and colorectal cancer (CRC). However, the clinical characteristics associated with biallelic germline *NTHL1* mutations suggest a broader tumor spectrum that includes multiple extracolonic malignancies. Characterization of the somatic mutation profile in multiple colorectal carcinomas derived from individuals with biallelic germline *NTHL1* mutations revealed a bias towards C:G > T:A (C > T) transitions. The aim of this study was to genetically characterize colorectal and extracolonic tumors in order to delineate the somatic mutational signature caused by NTHL1 deficiency to provide further insight in the associated tumor spectrum.

Whole-exome sequencing was performed on six tumors from four different tissues (colon ($n = 3$), thyroid-gland, urothelium, and tonsil) derived from four individuals with biallelic *NTHL1* mutations. Mutational signatures were identified using MutationalPatterns (https://doi.org/10.1101/071761).

We analyzed the somatic mutation profiles of three CRCs from three newly identified NTHL1-deficient families. These revealed a strong bias towards C > T mutations at non-CpG sites, which is clearly distinct from the CpG > TpG mutations commonly encountered in sporadic CRC. This mutation profile fits a unique mutational signature that comprises 40–70 mutations in the three extracolonic tumors, but appeared to be extremely rare in the mutational profiles from The Cancer Genome Atlas (https://cancergenome.nih.gov).

Our results demonstrate that NTHL1 deficiency is associated with a unique mutational signature, and confirms the broad tumor spectrum found in individuals with biallelic germline *NTHL1* mutations. This finding provides an interesting strategy to correlate tumors to this novel NTHL1-associated tumor syndrome.

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C17.5
Genotoxic chemotherapies and X-rays are responsible for the development of multiple primary tumours in patients with Li-Fraumeni syndrome

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Introduction: Li-Fraumeni syndrome (LFS), one of the most severe predispositions to cancer, is due to *TP53* germline mutations, and is characterized by the development of early-onset and multiple primary cancers (MPC). We recently reported in LFS patients a rate of MPC above 40% including secondary tumours in radiation fields.

Materials and Methods: We first adapted the genotoxicity assay, previously developed in our laboratory and based on the p53-transcriptional response to DNA damage in human lymphocytes, to mouse cells. Then, *Trp53* KO-/-, *wt/-* and *wt/wt* mice were exposed to X-rays or to various anti-cancer drugs. The control group was NaCl treated. Tumour development was monthly monitored using whole-body MRI.

Results: The genotoxicity assay performed on murine cells exposed in vitro or in vivo confirmed that all classical chemotherapies, except spindle poisons, are genotoxic. X-rays and Etoposide (a topoisomerase inhibitor) were shown to accelerate tumour development in *Trp53-/-* and *wt/-* mice ($OR = 6.46$, p-value $= 1.24.10^{-3}$ and $OR = 3.91$, p-value $= 5.76.10^{-4}$, respectively), unlike Docetaxel (a spindle poison), which was devoid of effect.

Conclusions: This study provides evidence that genotoxic chemotherapies contribute to MPC development in LFS patients. Therefore, in germline *TP53* mutation carriers, radiotherapy should be avoided, surgical treatment prioritized, and non genotoxic treatments should be considered in the future to reduce the risk of MPC. As we validated in vivo the results obtained using the genotoxicity assay, this assay will be helpful to discriminate drugs with and without genotoxic risk for LFS patients.

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C17.6
Raising the age limit for routine MMR testing in colorectal cancer from 50 to 70 years improves recognition of new Lynch syndrome families

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Background: To improve Lynch syndrome (LS) recognition, in the Netherlands, the age-limit for routine mismatch repair (MMR) deficiency testing in colorectal cancer (CRC) by the pathologist was raised from 50 to 70 years. Referral for genetic counseling is advised for patients with MMR deficient CRC without *MLH1* hypermethylation.

Methods: The Dutch Pathology Registry (PALGA) was used to evaluate MMR testing in 14 pathology laboratories. Patients referred to two regional genetic centers were coupled to PALGA data to evaluate referral rates. Pathology laboratories received feedback on percentage of correctly tested CRCs.

Results: From January 15th to October 2016, in 1499 of 1951 (77%) CRCs before age 70 MMR test results were available. Of 68 patients with MMR test results suggestive for LS, 30 (44%) were referred for genetic counseling. In eight of 18 (44%) patients with complete diagnostic workup, germline LS mutations were identified. Six of these new LS patients (75%) represent previously unknown LS

families. Five (83%) had CRC between age 50 and 70, and did not comply with former referral criteria on young age at diagnosis or family history.

Conclusions: Raising the age-limit for routine MMR testing in CRC from 50 to 70 years improves recognition of new LS families, of which the majority would not have been identified by criteria based on young age at diagnosis or family history.

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C18
Internal organs

C18.1
Mutations in the leukemia inhibitory factor receptor (*LIFR*) gene and *Lifr* deficiency cause urinary tract malformations

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Congenital anomalies of the kidneys and urinary tract (CAKUT) are the most common cause of chronic kidney disease in children. As most CAKUT cases are genetically unexplained, we aimed to identify new CAKUT causing genes. Using whole-exome sequencing and trio-based de novo analysis, we identified a novel heterozygous de novo frameshift variant in the leukemia inhibitory factor receptor (*LIFR*) gene causing instability of the mRNA in a patient presenting with bilateral CAKUT and requiring kidney transplantation at one year of age. *LIFR* encodes a transmembrane receptor utilized by IL-6 family cytokines, mainly by the leukemia inhibitory factor (LIF). Mutational analysis of 121 further patients with severe CAKUT yielded two rare heterozygous *LIFR* missense variants predicted to be pathogenic in three patients. LIFR mutants showed decreased half-life and cell membrane localization resulting in reduced LIF-stimulated STAT3 phosphorylation. *LIFR* showed high expression in human fetal kidney and the human ureter, and was also expressed in the developing murine urogenital system. *Lifr* knockout mice displayed urinary tract malformations including hydronephrosis, hydroureter, ureter ectopia, and, consistently, reduced ureteral lumen and muscular hypertrophy, similar to the phenotypes observed in patients carrying *LIFR* variants. Additionally, a form of cryptorchidism was detected in all $Lifr^{-/-}$ mice and the patient carrying the *LIFR* frameshift mutation. Altogether, we demonstrate heterozygous novel or rare *LIFR* mutations in 3.3% of CAKUT patients, and provide evidence that *Lifr* deficiency and deactivating *LIFR* mutations cause highly similar anomalies of the urogenital tract in mice and humans (supported by Else Kröner-Fresenius-Stiftung grant no. 2014_A234).

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C18.2
The microbiome of inflammatory bowel disease and irritable bowel syndrome - a case-control study of 1792 individuals

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Irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) are two of the most common gastrointestinal (GI) disorders, affecting respectively 7–21% and 0.3%–0.5% of the global population. Next to host-genetics, microbiome plays an important role in disease pathology. Here, we present the largest gut microbiome case-control analysis in both IBD and IBS to date, using metagenomic shotgun sequences of stool samples from 1025 Healthy Controls 355 IBD and 412 IBS patients. Taxonomy was determined for bacteria, viruses and micro-eukaryotes. Bacterial pathways were determined using HUMAnN2. In addition, bacterial strain diversity and growth rates were inferred from the sequencing data. In the case-control analyses, correcting for 25 previously identified microbiome-modifying factors, we observed 157 differentially abundant species associated with CD, 87 species associated with UC and 125 species for IBS. We observed an increased strain level diversity for Eschericia coli and Bacteroides vulgatus and several species with differential growth rate dynamics in patients compared to healthy controls. Prediction models for differentiating between IBS and IBD based on microbiome data show a best predictive value up to 94.6%. In addition, we previously described alterations of the gut microbiota of healthy individuals with a high genetic risk for IBD. In this study, we present a high-resolution characterization of changes in the microbiome, and its functional implications, in patients with IBD or IBS, which can be used in the disease prediction.

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C18.3
Whole-genome sequencing identifies associations of sequence variants with clinically relevant urinary disease markers

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Introduction: Chemical analysis of urine with dipstick measurements allows for the simultaneous testing of a wide range of traits typically assessed in clinical practice.

Materials and Methods: We identified 35.5 million sequence variants through whole-genome sequencing of 15,220 Icelanders and imputed them into up to 143,900 Icelanders with urinary dipstick measurements. These variants enabled us to perform a genome-wide association study to search for sequence variants influencing the urinary markers of metabolic disease and kidney function.

Results: We tested all variants for association with the presence of glucose, ketones, blood and protein in urine and detected associations at several loci for each trait. These included four loci that are located in or near genes with a function that matches the associating urinary trait (SLC5A2, OXCT1, LRP2 and COL4A3). We detected common and rare independent variants, coding in or close to SLC5A2, associating with glucosuria. This gene encodes Sodium Glucose Transporter 2, a protein targeted pharmacologically to treat diabetes. We observed two proteinuria associations, a novel signal at LRP2 and a previously reported urinary albumin excretion signal at CUBN. Interestingly LRP2 and CUBN encode cubilin and megalin, which interact and together mediate proximal tubule protein uptake. We identified a rare Iceland-specific 2.5 Kb exonic deletion in COL4A3 that associates with hematuria (OR = 9.4). This deletion is present in 1/700 Icelanders and mutations in COL4A3 cause autosomal dominant familial benign hematuria.

Conclusions: The identification of these associations provide insight into the biology underlying urinary disease markers, potential disease mechanisms, and treatment.

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Mikaelsdottir: A. Employment (full or part-time); Significant; deCODE genetics/Amgen, Inc.. **G.I. Eyjolfsson:** None. **B.O. Jensson:** A. Employment (full or part-time); Significant; deCODE genetics/Amgen, Inc. **U. Thorsteinsdottir:** A. Employment (full or part-time); Significant; deCODE genetics/Amgen, Inc. **D.F. Gudbjartsson:** A. Employment (full or part-time); Significant; deCODE genetics/Amgen, Inc. **P. Sulem:** A. Employment (full or part-time); Significant; deCODE genetics/Amgen, Inc. **K. Stefansson:** A. Employment (full or part-time); Significant; deCODE genetics/Amgen, Inc..

C18.4
Genome wide association study identifies two novel loci associated with female stress and urgency urinary incontinence

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Introduction: Stress and urgency incontinence are heritable, but no risk loci have been identified. We undertook a GWAS, using three European cohorts, followed by replication in six further studies, with supplementary transcriptomic analyses using human bladder biopsies.

Materials and Methods: Genotyping in discovery cohorts (n = 8,979) was conducted using Illumina arrays. Replication genotyping used competitive PCR (n = 4,069). Biopsies from women with urgency or stress incontinence were run on Affymetrix-U133 arrays.

Results: Discovery analyses identified five genome-wide significant loci. Two loci replicated: rs138724718 ($p = 3.39 \times 10^{-09}$) and rs34998271 ($p = 1.70 \times 10^{-09}$). In analysis of differential expression, the top-ranked process (GO:0003012, $p = 7.5 \times 10^{-10}$), includes *CHRM3* (fold difference:4.23, p = 0.0007), which is the main drug target for urgency incontinence, *SULF2* (fold difference:1.52, p = 0.005) in the top locus from the discovery phase, and *EDN1* (fold difference:−1.60, p = 0.09) in the top locus from the replication phase.

Conclusions: We identified two genetic variants strongly associated with urinary incontinence. The first, rs138724718, is situated near *MARCO*, with a role in host defense. The second, rs34998271, is situated near *EDN1* a potent constrictor of smooth muscle, which was differentially expressed in bladder. This work highlights the myogenic and urotheliogenic mechanisms for incontinence, and suggests the potential of endothelin modulating drugs for urgency incontinence

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C18.5
A *PMM2* promoter mutation causing congenital polycystic kidney and hyperinsulinemic hypoglycemia

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Hyperinsulinemic hypoglycemia and congenital polycystic kidney disease are rare, genetically heterogeneous disorders. The co-occurrence of hyperinsulinemic hypoglycemia and enlarged polycystic kidneys in 18 children from 12 unrelated families suggested a shared cause. Autozygosity analysis of SNP-chip genotype data in a consanguineous family with 3 affected members revealed a

	GRCh37 Position	SNP	Effect Allele	Other Allele	MAF	Phenotype	Discovery Cohorts (n = 8,997)	Replication Cohorts (n = 4,069)	Overall				
							OR	95% CI	p	OR	95% CI	p	p
20	46424160	rs139329202	c	g	0.01	UUI	8.50	4.12–17.55	8.07×10^{-9}	1.35	0.82–2.21	0.238	2.38×10^{-05}
6	1430664	rs146033157	a	t	0.03	UUI	0.33	0.23–0.48	1.73×10^{-8}	0.97	0.35–2.71	0.960	n/a
14	55489229	rs146757102	a	g	0.05	UUI	0.45	0.34–0.60	1.95×10^{-8}	1.13	0.87–1.45	0.360	5.12×10^{-03}
7	141328145	rs78851245	t	c	0.02	Any UI	3.22	2.13–4.86	2.92×10^{-8}	1.46	1.00–21.3	0.051	2.11×10^{-07}
7	34354797	rs78878767	a	c	0.01	UUI	4.26	2.56–7.10	3.04×10^{-8}	0.86	0.51–1.43	0.556	2.11×10^{-07}
3	55473083	rs13059018	c	g	0.07	SUI	0.70	0.61–0.81	1.01×10^{-7}	1.14	1.00–1.29	0.054	1.40×10^{-06}
12	11049362	rs201363123	ag	a	0.06	Any UI	0.65	0.56–0.76	1.03×10^{-7}	1.14	1.00–1.29	0.053	5.43×10^{-05}
1	154881110	rs1218596	t	c	0.06	Any UI	0.64	0.55–0.75	1.04×10^{-7}	0.95	0.75–1.20	0.681	4.49×10^{-06}
11	39642765	rs10768519	a	c	0.26	UUI	0.80	0.74–0.87	2.26×10^{-7}	1.00	0.85–1.17	0.954	1.40×10^{-04}
9	105517298	rs72738866	t	c	0.26	SUI	0.79	0.71–0.87	2.55×10^{-7}	1.01	0.89–1.14	0.895	5.97×10^{-05}
2	119587824	rs138724718	a	g	0.02	SUI	1.85	1.47–2.35	2.89×10^{-7}	1.73	1.20–2.48	0.003	3.39×10^{-09}
6	12533066	rs34998271	a	g	0.05	UUI	1.70	1.40–2.07	4.97×10^{-7}	1.55	1.20–2.01	0.0008	1.70×10^{-09}

homozygous 2.5Mb region on chromosome 16p13.2. Whole genome multipoint parametric linkage analysis in this and 4 other informative families confirmed and refined this to a single significant locus of 2.3 Mb that includes 14 annotated genes (combined LOD score 6.5). Exome or genome sequencing of the coding regions of these 14 genes failed to identify bi-allelic mutations but a *PMM2* promoter variant (c.-167G > T) was found in all patients. This variant was either homozygous or *in trans* with *PMM2* coding variants, including mutations previously reported in patients with congenital disorder of glycosylation type 1a. Typical features of systemic autosomal recessive PMM2 glycosylation disease were absent and the diagnostic test of transferrin isoelectric focusing was normal. In vitro studies in patient cells revealed decreased transcription activity of the mutant promoter. Electrophoretic mobility shift assay demonstrated impaired binding of the transcription factor ZNF143. In silico analysis reveals the importance of ZNF143 for the structural confirmation of a chromatin loop including *PMM2* to enable tissue-specific transcription. In conclusion we report a previously undescribed rare disease characterized by the combination of hyperinsulinemic hypoglycemia and congenital polycystic kidney disease. The novel promoter variant appears to exert a critical tissue-specific effect on *PMM2* transcription, leading to an organ-specific phenotype.

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C18.6
GREB1L and *ROBO1* -Two novel genes associated with renal agenesis

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Variants in families with renal agenesis

Family	NGS findings	Prediction Polyphen Mutation Taster Sift Provean CADD	MAF 1000Genomes ExAC 2000DK
1	***ROBO1* c.526C > T p. (Pro176Ser)**	-probably damaging -disease causing -damaging -deleterious -PHRED 24.7	- - -
1	***ROBO1* c.4823C > G p. (Ser1608*)**	-NA -disease causing -NA -NA -PHRED 50	- - -
2	***GREB1L* c.5608 + 1del p.?**	-NA -disease causing -NA -NA -PHRED 25.8	- - -
3	***GREB1L* c.5378T > G p. (Leu1793Arg)**	-probably damaging -disease causing -damaging -deleterious -PHRED 31	- - -
4	***GREB1L* c.371G > T p. (Gly124Val)**	-probably damaging -disease causing -damaging -deleterious -PHRED 29.2	- - -
5	***ROBO1* c.3685G > T p (Glu1229*)**	-NA -disease causing -NA -NA -PHRED 44	- - -
5	***ROBO1* c.4823C > G p. (Ser1608*)**	-NA -disease causing -NA -NA -PHRED 50	- - -

Introduction: Renal agenesis is the extreme example of congenital anomalies of the kidney and urinary tract.We identified two novel genes associated with renal agenesis, and provide functional validation in zebrafish for one of these, *GREB1L.*

Methods: Fetuses with bilateral kidney agenesis were screened using a targeted kidney-gene panel. In cases where candidate variants were not identified, whole-exome sequencing was performed. Genes uncovered by this analysis were added to our targeted panel, and placed in a pipeline for functional validation in zebrafish.

Results: Whole-exome sequencing identified *GREB1L* and *ROBO1* variants in families with renal agenesis. We added *GREB1L* and *ROBO1* to our targeted kidney-gene panel and identified additional variants in both genes (Table 1). *SLIT* and *ROBO* genes are involved in cell guidance. Although variants in *ROBO2* or *SLIT2* have previously been associated with kidney anomalies, this is the first example of a renal agenesis phenotype associated with *ROBO* variants. *GREB1L* encodes a co-activator of steroid hormone/retinoic acid receptors but has not previously been associated with kidney anomalies. Analysis of a zebrafish *greb1l* mutant revealed severe defects in the pronephros, the proper specification of which is required for downstream kidney development.

Conclusions: We report identification of multiple *ROBO* and *GREB1L* variants in renal agenesis cases, implicating two pathways (cell guidance and steroid hormone/retinoic

acid signaling) not previously known to be associated with human renal agenesis.

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C19
Diagnostic variant interpretation and quality control

C19.1
The spectrum of sequence and copy-number variants in 80,000 patients: Implications for test development and validation

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Introduction: Many medically important genes are located in technically challenging regions of the genome. Moreover, complex but highly medically relevant classes of mutation are well-known. The overall impact of these facts on diagnostic yield and on appropriate technologies for routine clinical genome/exome sequencing has not yet been thoroughly described.

Methods: We examined over 80,000 patients clinically tested for physician-specified genes underlying a hereditary cancer, cardiovascular, neurological or pediatric condition. Sensitive methods using NGS, long read sequencing, MLPA, and arrays were used to detect and confirm a broad spectrum of variants.

Results: Of 12,489 pathogenic, potentially actionable findings, approximately 10% belong to a technically challenging class not easily addressed by short-read NGS methods. Approximately 3% are CNVs affecting only a single exon, 2% are either large indels or complex variants, and 5% were in low-complexity, highly conserved or extreme-GC regions. This general observation was consistent across clinical areas, although specifics varied. Very large triplet repeat expansions and cytogenetic abnormalities are not included and would increase this total.

Discussion: Technically challenging variants are a substantial fraction of findings in routine clinical testing. However, published validation studies often omit these variants, and benign SNPs dominate many sensitivity calculations. This may, in part, be due to difficulty obtaining positive controls. We have thus developed synthetic controls containing a diverse set of challenging mutations in commonly tested genes. These have been tested in multiple laboratories using different protocols and will be available to the ESHG community by the time of the meeting.

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C19.2
ClinGen Sequence Variant Interpretation Work Group recommendations for ACMG-AMP guideline specification

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In 2015, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) published a guideline for variant interpretation that provides an evidence-based framework to classify variants (PMID:25741868). The guideline defines 28 criteria that address types of variant evidence. The committee recognized that the guideline was a starting framework that would evolve over time. ClinGen's Sequence Variant Interpretation (SVI) Working Group aims to standardize application of the ACMG-AMP guidelines by providing recommendations to adapting the guidelines and converting qualitative criteria to quantitative, where applicable. To date, SVI has refined the "stand-alone" benign allele frequency criterion (BA1) for additional clarity, worked with ClinGen Clinical Domain WGs

(CDWG) to harmonize approaches to calculating gene-specific allele frequency thresholds, provided nomenclature recommendations for documenting criteria strength modifications, and developed a draft proposal for quantifying segregation data. Additionally, a subgroup of SVI is focused on computational and predictive evidence, working to provide additional guidance on the use of computation tools sequence variant interpretation. SVI also reviews and approves gene/disease specifications to the guidelines made by CDWGs and will review proposals from CDWGs to develop new evidence categories. These harmonization approaches by the SVI will facilitate transparency and consistency in application of the ACMG-AMP guidelines and variant classifications across different CDWGs and laboratories. The SVI has also begun work on a longer term goal of developing a general Bayesian framework, to better quantify and integrate different types of evidence in an overall assessment of pathogenicity. Funded by NIH-NHGRI U41HG006834.

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C19.3
ClinGen: The Clinical Genome Resource

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Introduction: The Clinical Genome Resource (ClinGen) is focused on defining the clinical relevance of genes and variants through sharing genomic information, developing and applying standards, deploying tools to store and evaluate evidence, and creating a knowledgebase to make this information freely available.

Materials and Methods: ClinGen utilizes genomic data, health data and curated knowledge submitted by laboratories, clinicians, and patients. Interpreted variants are shared through the ClinVar database. Standardized evidence frameworks and curation interfaces enable evaluation of gene-disease associations, gene dosage sensitivity, variant pathogenicity and clinical actionability.

Results: To date, 642 submitters from 59 countries have submitted 384,623 interpreted variants to ClinVar. Reassessment of variants with interpretation differences resolved 72% and 87% of differences in two studies. Ten expert groups are curating variants using gene-specific modified ACMG-AMP criteria. Over 1,200 genes have been reviewed using one or more ClinGen curation processes, including: 45 gene-disease associations for clinical validity, 66 gene-condition pairs for clinical actionability and 1,237 genes for dosage sensitivity. Results are available on clinicalgenome.org.

Conclusions: ClinGen's curated information on genes, diseases, variants and clinical actions is publicly available. This information is useful in a variety of clinical and research applications, including building evidence-based clinical genetic testing panels, determining the effects of deletions or duplications identified on cytogenomic microarray, resolving discrepancies in variant interpretation, and developing actionability frameworks for deciding when to return results to patients.

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C19.4
High-resolution variant filtering empowers clinical interpretation and provides insights into variant penetrance and population-specificity

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Allele frequency (AF) is a key discriminator between pathogenic variants and benign bystanders, but the thresholds that should be used to classify variants as benign are often unclear, given that Mendelian diseases may also be present in the population. The recent Genome Aggregation Database (gnomAD) comprises variation from 138,633 individuals, providing unprecedented power to characterise rare variants.

We have developed a rigorous statistical framework to evaluate whether an observed AF is compatible with pathogenicity. Our approach considers disease prevalence, genetic/allelic heterogeneity and variant penetrance, to determine whether a variant frequency is compatible with disease causation, whilst taking into account sampling variance.

We evaluated our approach using high quality reference variants from 7,855 clinically classified cardiomyopathy cases, with gnomAD as a population reference dataset. Compared to a conventional 0.1% AF filter, our more stringent filter reduced the number of 'Variants of Uncertain Significance' by 10% (69 variants), while preserving 375/376 'Pathogenic' variants. Additionally, of 28 ClinVar 'pathogenic' variants that failed our stringent filter: none had robust evidence of disease-causation.

In addition to permitting the safe and appropriate use of much more stringent AF thresholds, our approach facilitates investigation of disease architecture. Using a diverse dataset of >1,500 cardiomyopathy cases we can accurately quantify disease penetrance and identify disease-causing variants that are specific to a single ethnic population.

To aid variant interpretation, we have released pre-computed frequency annotations for all variants in the gnomAD dataset. Additionally, to facilitate investigation of disease architecture and variant penetrance, we have created an online tool: cardiodb.org/allelefrequencyapp/.

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C19.5
External Quality Assessment of Clinical Genetics: from pilot assessment to full EQA scheme

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Quality assessment has long been associated with laboratory, but not clinical, services. To address this gap, in 2012 the ESHG Genetic Services Quality Committee explored the needs for a European Quality Assessment (EQA) scheme for clinical genetics and counselling. European national societies of human genetics were surveyed and a need for EQA for clinical genetics was expressed. CEQAS was chosen as the EQA provider and three pilot EQAs have now been completed and evaluated. Each year, the working group prepares four case scenarios in the fields of cardiogenetics, oncogenetics, monogenetics and dysmorphology. Each scenario started with a referral letter and consisted of multiple stages, to reflect an episode of clinical care. At each stage further information was given and a number of questions presented. For each question, consensus answers were agreed by independent experts including two clinical geneticists and a patient organisation. In 2014, 2015 and 2016, a total of 15, 29 and 42 genetic centres from 11, 16 and 21 countries respectively participated in the pilot EQA. All answers were reviewed by two clinical geneticists. Based on the experiences with the three pilot EQAs, a guideline for the development of suitable case scenarios was written, including how questions and criteria should be formulated. The answers provided by the centres highlighted differences in clinical genetics practice across Europe. The EQA for clinical genetics proved to be highly educational. The working group will continue to improve the process for the benefit of the Clinical Genetics community.

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C19.6
Assessing clinical consistency among inconsistent variant classifications

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It is essential for diagnostic genetics laboratories to interpret and report sequence variants consistently. Much effort is being directed towards achieving consistency through the introduction of standardized and objective classification guidelines (eg ACMG standards and guidelines 2015). Once a variant has been classified, it is potentially more important that clinicians are consistent in whether they use that variant to manage their patients.

To assess and improve consistency between laboratories the UK National External Quality Assessment Service (UK NEQAS) for Molecular Genetics has been assessing annually how laboratories classify and interpret variants since 2012. Laboratories are given typical referral scenarios and asked to classify and interpret a number of variants using the standard 5 point pathogenicity scale.

In a pilot scheme in 2016 twelve clinical geneticists were asked to classify 7 variants that 44 laboratories had assessed previously, and to state whether they would use the variants to manage patients. Only one variant was classed uniformly by all laboratories and clinicians (class 1, benign); four variants were classed as either 4 or 5, or 1 or 2, respectively; two variants were classed into one of three classes, (1–3 or 3–5). Despite similar inconsistencies in classification by clinicians and scientists, reassuringly there was good consensus among the clinicians regarding use of the variants to manage patients.

This assessment scheme is being expanded via a virtual training and competency tool (G-TACT) to help large numbers of scientists and clinicians learn and improve consistency in this important skill of classifying and interpreting genomic variants.

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C20
Molecular syndromology

C20.1
Mutations in the cadherin-catenin complex in Blepharo-Cheilo-Dontic Syndrome

A. Kievit[1], F. Tessadori[2,3], J. Douben[1], I. Jordens[2], M. Maurice[2], A. Hoogeboom[1], R. Hennekam[4], S. Nampoothiri[5], H. Kayserili[6], M. Castori[7], M. Whiteford[8], C. Motter[9], C. Melver[9], M. Cunningham[10], A. Hing[10], N. Kokitsu-Nakata[11], S. Vendramini-Pittoli[11], A. Richieri-Costa[11], A. Baas[2], C. Beugem[12], K. Duran[2], M. Massink[2], P. Derksen[13], W. F. J. van IJcken[14], L. van Unen[14], F. Santos-Simarro[15], P. Lapunzina[15], V. L. Gil-da Silva Lopes[16], E. Lustosa-Mendes[16], M. Krall[17], A. Slavotinek[17], V. Martinez-Glez[15], J. Bakkers[3], K. L. I. van Gassen[2], A. de Klein[1], M. J. van den Boogaard[2], G. van Haaften[2]

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Blepharocheilodontic syndrome (BCDS) consists of lagophthalmia, ectropion of the lower eyelids, distichiasis of the upper eyelids, euryblepharon, cleft lip/palate and dental anomalies and has an autosomal dominant inheritance with variable expression. We identified heterozygous mutations in two genes of the cadherin-catenin complex, *CDH1*, encoding E-cadherin, and *CTNND1*, encoding p120-catenin delta1 in 15 of 17 BCDS index patients. *CDH1* plays an essential role in epithelial cell adherence; *CTNND1* binds to *CDH1* and controls the stability of the complex. Functional experiments in zebrafish and human cells showed that the *CDH1* mutations impair the cell-adhesion function of the cadherin-catenin complex in a dominant-negative manner. Mutations in *CDH1* have been linked to familial hereditary diffuse gastric cancer and invasive lobular breast cancer, however no cases of gastric or breast cancer have been reported in our BCDS cases. Functional experiments reported here indicated the BCDS mutations comprise a distinct class of *CDH1* mutations. Together, we discovered the genetic cause of BCDS enabling DNA diagnostics and counseling, in addition we describe a novel class of dominant negative CDH1 mutations.

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C20.2
Heterozygous loss-of-function *ACTB* mutations result in a novel developmental syndrome

S. Cuvertino[1], H. Stuart[1,2], K. E. Chandler[2], N. A. Roberts[1], R. Armstrong[3], L. Bernardini[4], S. Bhaskar[2], B. Callewaert[5], J. Clayton-Smith[1,2], C. H. Davalillo[6], C. Deshpande[7], K. Devriendt[8], M. C. Digilio[9], A. Dixit[10], M. Edwards[11], J. M. Friedman[12], S. Joss[13], B. Kerr[2], A. K. Lampe[14], R. McGowan[13], M. D. Medt[8], J. O'Sullivan[2], S. Odent[15], M. J. Parker[16], C. Pebrel-Richard[17], F. Petit[18], Z. Stark[19], S. Tinschert[20], P. Vasudevan[21], O. Villa[6], &. M. White[19,22], F. Zahir[12,23], The DDD study, R. Lennon[1], A. S. Woolf[1], S. Banka[1,2]

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Introduction: *ACTB*, encodes β-actin an abundant cytoskeletal house-keeping protein. Homozygous *Actb* mice are embryonic lethal and, in humans, postulated gain-of-function missense mutations cause Baraitser-Winter syndrome (BRWS), characterised by intellectual disability, agyria/pachygyria, coloboma, sensorineural deafness and characteristic facial features. To date, the effects of loss-of-function *ACTB* mutations are unknown.

Methods and Results: We describe heterozygous *ACTB* deletions (gross or intragenic) or truncating mutations in 33 individuals with growth retardation, developmental delay, intellectual disability, internal organ malformations (affecting heart, kidneys, spine and palate amongst others), minor anomalies (e.g. nail hypoplasia and overlapping toes), variable systemic manifestations and a recognisable facial gestalt (interrupted eyebrows, dense eyelashes, wide nose, wide mouth and a prominent chin) that is distinct from individuals with BRWS. Strikingly, this spectrum overlaps with that of several chromatin remodelling disorders.

In developing mice, β-actin was enriched in tissues correlating with the human disease. *ACTB* mRNA levels in patient-derived lymphoblastic lines and fibroblasts were approximately 50% versus control cells. Patient-derived fibroblasts and *ACTB* siRNA knockdown in wild-type fibroblasts showed altered cell shape and migration, consistent with known roles of cytoplasmic β-actin. Notably, a decrease in β-actin protein levels was detected in nuclei of these cells along with altered expression of cell cycle genes correlating with a decrease in cell numbers.

Conclusions: Loss-of-function *ACTB* mutations cause a novel developmental syndrome. Our linked developmental and cell biology studies suggest that a critically reduced level of this protein alters cell morphology, migration and gene expression to the detriment of brain, heart and kidney development.

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C20.3
Variants in the degron motif of *AFF3* cause a multisytem disorder with skeletal dysplasia and severe neurologic involvement

N. Voisin[1], R. E. Schnur[2,3], S. Douzgou[4,5], A. J. Tanaka[6], C. F. Rustad[7], S. M. Hiatt[8], E. Del Giudice[9], A. Mikhaleva[1], The DDD study[10], B. Yalcin[1], D. Donnai[4,5], N. Brunetti-Pierri[9,11], A. Reymond[1], W. K. Chung[6]

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The ALF transcription factor paralogs, *AFF1-AFF4* are components of the transcriptional super elongation complex that regulates expression of genes involved in neurogenesis and development. We identified five individuals with de novo missense variants in the AFF3 degron motif, a signal for protein degradation. They present with a recognizable pattern of anomalies including microcephaly, global developmental delay, intellectual disability, brain atrophy, seizures, dysmorphic features, horseshoe kidney, a mesomelic form of skeletal dysplasia resembling

Nievergelt/Savarirayan type and other skeletal features, only partially overlapping the *AFF4* variants-associated CHOPS syndrome. Consistent with a causative role of *AFF3* variants in this syndrome, a previously reported individual with a microdeletion encompassing *AFF3* exhibited overlapping clinical features. Murine models lacking the orthologous gene display brain and skeletal anomalies, and kidney defects.

The three described CHOPS individuals carry de novo variants in the degron of AFF4 that altered the binding to the SIAH E3-ubiquitin ligase and its stability. These individuals and two additional probands with de novo *AFF4* variants we identified allow to define that seizures, failure to thrive and a distinctive skeletal dysplasia are specific to individuals with *AFF3* variants and absent in CHOPS. In conclusion, although proteins encoded by *AFF2*, *AFF3* and *AFF4* were reported to be partially redundant, the phenotypes associated with *AFF3* and *AFF4* variants are clinically distinct and show only minimal phenotypic overlap. While CHOPS syndrome is due to an accumulation of AFF4 protein, functional analyses are warranted and underway to understand the mechanism of *AFF3*-associated syndrome that may explain phenotype differences.

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C20.4
KIAA1109 variants are associated with a severe disorder of brain development and arthrogryposis

L. Gueneau[1], R. Fish[2], H. Shamseddin[3], N. Voisin[1], F. Tran Mau-Them[4], E. Preiksaitiene[5], G. Monroe[6], F. Allias[7], Q. Ambosaidi[8], L. Ambrozaityte[5], L. Cimbalistiene[5], J. Delafontaine[9], N. Guex[9], M. Hashem[3], W. Kurdi[8], T. Pippucci[10], S. Pradervand[9], B. Roechert[11], P. Van Hasselt[6], M. Wiederkehr[1], C. Wright[12], DDD Study, I. Xenarios[9], G. Van Haaften[6], C. Shaw-Smith[12], E. Schindewolf[13], M. Neerman-Arbez[2], J. Chelly[4], V. Kucinskas[5], F. Alkuraya[3], A. Reymond[1]

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Whole exome sequencing of 10 individuals from 9 unrelated families with overlapping clinical manifestations identified loss-of-function (LoF) and missense variants in *KIAA1109* allowing description of a new autosomal recessive multi-system syndrome. Shared phenotypic features representing the cardinal characteristics of this syndrome combine brain atrophy, hydrocephalus and encephalic Dandy-Walker malformation with clubfoot and arthrogryposis. Severe cases were incompatible with life, whereas those with milder missense variants presented with severe global developmental delay, syndactyly of 2nd and 3rd toes and severe muscle hypotonia resulting in incapacity for patients to stand without support. Histology of the brain of two affected fetuses revealed defects of cerebellar cortical lamination. The 5005 amino acid KIAA1109 is evolutionarily conserved and interacts with proteins previously associated with intellectual disability and regulation of cell division such as CTNNB1, PPP2R4 and BUB3. Consistent with a causative role for *KIAA1109* loss-of-function and hypomorphic variants in this new syndrome, knockdowns of the zebrafish orthologous gene with morpholinos resulted in embryos with hydrocephaly, abnormally curved notochords, and dysmorphic overall body shape. Similarly, published knockouts of the *Mus musculus* and *Drosophila* orthologs of *KIAA1109* resulted in lethality or severe neurological defects reminiscent of proband features.

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C20.5
Heterozygous *BMP2* mutations leading to haploinsufficiency cause a recognisable human syndrome comprising short stature, palatal anomalies, congenital heart disease and skeletal malformations

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Bone morphogenetic protein 2 (*BMP2*) on chromosome 20p12 belongs to a gene superfamily encoding TGF-beta signalling peptides involved in bone and cartilage biology. Heterozygous chromosome 20p12 deletions are variably associated with cleft palate, short stature and developmental delay. We report individuals with short stature, a recognizable gestalt, skeletal anomalies and congenital heart disease but normal intellect with heterozygous mutations in *BMP2*. Craniofacial features include cleft palate, flat midface, short, upturned nose, long philtrum and low-set ears. Skeletal features include 11-pairs of ribs, clinodactyly and pectus deformity. Congenital heart disease includes transposition of great arteries and Ebstein anomaly. In affected sisters with a *BMP2* splice site mutation we demonstrate abnormal exon 2 splicing and paternal mosaicism; in an unrelated individual we identified a de novo frameshift mutation. A heterozygous chromosome 20p12 deletion involving *BMP2* was identified in another individual and his father with a similar phenotype. The craniofacial and skeletal phenotype of individuals with intragenic *BMP2* mutations is similar to the deletion phenotype, suggesting that haploinsufficiency of BMP2 is the primary phenotypic determinant. *Bmp2*-null mice die from defects including heart malformation in early gestation while mice with heterozygous loss of *Bmp2* appear normal. We present analyses of secreted BMP2 peptide in affected and control cell lines as well as the effects of *bmp2* knockdown on zebrafish craniofacial/skeletal cartilages and cardiac development. Our data suggest an elevated sensitivity to reduced BMP2 levels in human development and demonstrate involvement of *BMP2* mutations in a recognizable human syndrome comprising craniofacial, skeletal and cardiac malformations.

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C20.6
Reverse phenotyping of whole-genome sequencing data from patients with 22q11.2 deletions identifies an extensive catalog of broader phenotypic variability and benign variation in pathogenic disease genes

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The 22q11.2 deletion syndrome is the most common chromosomal deletion syndrome in humans with an incidence of 1 in 2–4000 live births. Individuals with the syndrome most often have a classically associated 1.5–3 Mb deletion and extremely variable clinical presentations. The

hemizygous nature of the region offers the opportunity to evaluate mutations in relation to known recessive disorders.

We have evaluated the remaining allele from 382 whole-genome sequenced patients. A total of 90% were identified to have the typical 3Mb deletion, 6% the 1.5Mb deletion, and the remainder a variety of more atypical deletion sizes. We identified 8,093 total variant positions, of which 117 are predicted to be rare (frequency $\leqslant$5%) and protein damaging across a total of 185 subjects. Reverse phenotyping is now being performed to associate this genetic variation with expected phenotypes. Findings in these subjects may provide evidence for broader phenotypic variability in the previously described conditions and/or new features that were not previously recognized to be associated prior to the identification of mutations on the other allele. Alternatively, variation that appears damaging may be demonstrated to actually be benign. For example, four patients (3 variants) have SCARF2 mutations, but do not clinically present signs of Van den Ende-Gupta syndrome. It has become evident that mapping the variation in the remaining alleles of genomic deletion disorders provides a rich annotation of more detailed pathogenic variation, but also benign variation.

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C21
Cardiovascular disorders

C21.1
Inactivation of *KLHL24* is associated with hypertrophic cardiomyopathy and abnormal glycogen storage in heart and skeletal muscle

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Hypertrophic Cardiomyopathy (HCM) is a common autosomal dominant genetic disease associated with sudden death and progressive heart failure. Pathogenic variants of genes associated with metabolic disorders may also cause HCM, but usually with recessive inheritance, or maternal inheritance in the case of mitochondrial DNA mutations. Here we report seven young adults with hypertrophic cardiomyopathy from two consanguineous families. Two individuals died suddenly and one woman had a cardiac transplant at age 26 due to heart failure. Endomyocardial biopsy in two individuals from one family showed storage of polyglucosan, and these two individuals also showed abnormal glycogen storage and other structural abnormalities in skeletal muscle biopsy but no muscle weakness. Intermediate filaments composed of desmin were accumulated in both heart and skeletal muscle. Whole-exome sequencing of affected individuals in both families identified homozygous potentially deleterious variants in the Kelch-like family member 24 gene, *KLHL24*. One variant was a nonsense mutation, c.1048G > T, p.Glu350*, and the other a missense mutation, c.917G > A, p.Arg306His. Kelch-like proteins have been identified as adaptors for the recruitment of substrates for Cul3-based E3-ubiquitin ligases and are important for protein turnover. *KLHL24* is highly expressed in striated muscle. In zebrafish, the homolog gene klhl24a is expressed in the heart. Down-regulation by antisense morpholino resulted in defective heart development visible at 2 days post fertilization, leading finally to death at around day 5, thus demonstrating the importance of klhl24 for cardiac development and function. These findings support the pathogenicity of *KLHL24* mutations, and suggest that *KLHL24* is a new cardiomyopathy-associated gene.

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C21.2
Epigallocatechin-3-gallate prevents cardiac hypertrophy in a Williams-Beuren syndrome mouse model

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Introduction: The hallmark feature of Williams-Beuren syndrome (WBS) is a generalized arteriopathy secondary to elastin deficiency that may lead to serious cardiovascular complications. A mouse model with complete deletion (CD) of the orthologous interval deleted in WBS recapitulates most cardiovascular features, increased oxidative stress, and heart hypertrophy as adults. Epigallocatechin-3-gallate (EGCG) is a flavonoid widely studied due to its safety and remarkable antioxidant effects that include radical-scavenger functions.

Materials and Methods: CD mice were fed with EGCG diluted in the drinking water. Cultured cardiomyocytes were also treated with EGCG in the media. We then compared treated and untreated CD mice and wild-type animals and cells for several features: 1) heart weight and cardiomyocyte's area by histology; 2) oxidative stress levels in cardiac tissue by quantification of dihydroethidium staining; and 3) gene and protein expression (oxidative stress pathways) by RT-PCR and immunocytochemistry.

Results: Treated CD mice did not show the cardiac hypertrophy present in untreated CD animals, mainly due to normalization of cardiomyocytes size. A slight increase in oxidative stress levels that did not change with treatment was observed in CD tissue. Treated animals showed a normalized expression levels of *Nqo1* (NADPH-dehydrogenase quinone), decreased in untreated CD mice. The nuclear proportion of NRF2 (direct regulator of *Nqo1*) was also recovered by treatment in cultured CD cardiomyocytes.

Conclusions: EGCG prevents cardiac hypertrophy in CD mice by normalizing alterations in NRF2 pathway. Thus, EGCG is a potential therapeutic agent for preventing cardiovascular complications in WBS individuals.

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C21.3
Generalized compound heterozygosity analysis highlights associated loci for coronary artery disease in genetic and exome data

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Background: The active search for genetic factors contributing to coronary artery disease(CAD) has seen major progress from (*hypothesis-free*) genome-wide association studies(GWAS) of common SNPs. However for CAD, a complex diseases, GWAS's only explain a small portion of phenotypic variance. A new proposed approach is to look at the generalized form of compound double heterozygosity (GCDH) to detect the genetic associations caused by compound heterozygosity(CH) and thereby aid in explaining some of the 'missing heritability'. Our study aimed to use the GCDH test to discover genetic loci associated with CH of CAD in European ancestry genetic and exome data.

Methods: Our genetic sample are based on the German Myocardial Infarction Family Study V(GerMIFS V) population-based study cohort which consists of 1,261 families(Men = 64.52%) whereby all cases($N_{cases} = 2{,}459$; $N_{controls} = 1{,}611$) had myocardial infarction. Our exome samples are based on the German CAD North and South Studies(Ncase|Ncontrol for North:4,464|2,886 and South:5,255|2,921). The CollapsABEL software tool was used, to conduct a genome-wide association scan – PLINK2 was called internally - and subsequently a GCDH test was performed.

Results: For chr1, we found 2 genetic loci to be significantly associated ($\leq 1.25 \times 10^{-10}$) with CH in CAD. Interestingly, of these, one SNP was already identified using the "traditional" single-SNP based method while the other SNP was only found by using GCDH. Remaining autosomal chromosomes and exome data are currently being analysed.

Conclusions: Based on the analysis of one chromosomone, GCDH test was able to reveal a locus that was associated

with CH in CAD which could have otherwise been 'missed' using single-SNP based method.

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C21.4
Copy number variants account for at least 2% of non-syndromic cardiomyopathies

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Copy-number variants (CNVs) are generally underascertained in routine diagnostics, although their involvement in heritable diseases is widely appreciated. CNV calling from next-generation sequencing data suffers from low precision - producing uncertain findings that have to be tested by orthogonal techniques - while short deletions and duplications involving one or two exons can remain undetected. Recently, clinically-significant CNVs have been found in less than 1% of cardiomyopathy patients, questioning the worth of CNV calling in these conditions.

We have developed a method for reproducible CNV calling from targeted next-generation sequencing data and implemented it into routine variant detection and assessment in our laboratory. Using a panel of 169 genes implicated in inherited cardiac conditions, we have validated the CNV-calling method with known deletions and duplications and by confirming new CNV predictions with digital PCR and MLPA. We have compared our method to three widely used CNV-calling tools and found a superior precision in calling short intragenic deletions and duplications.

We have examined the burden of CNVs in 882 healthy volunteers and 1681 cardiac disease patients and observed a significant enrichment of rare CNVs ($OR = 3.5$, $p = 0.001$) in the patient cohorts. In contrast to published reports, we find clinically-relevant CNVs in more than 2% of cardiomyopathy patients. We present likely-pathogenic rare CNVs in non-syndromic heart disease demonstrating the significant contribution of these variants to heritable cardiac conditions.

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C21.5
A major beneficial effect of angiotensin II receptor blockade for preventing spontaneous aortic rupture in a new mouse model of vascular Ehlers Danlos syndrome

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Vascular Ehlers-Danlos syndrome (vEDS) is a rare disorder caused by genetic defects at the COL3A1 gene which codes for the pro-α1 chain of collagen type III, a fibrillar collagen arranged as an homotrimer. The prognosis is worsened by unpredictable arterial ruptures occurring in young adulthood. Two thirds of the disease-causing variants correspond to missense substitutions at glycine residues of the mature triple helix, acting in a dominant negative manner. We report here the characterization of the first knock-in mouse model of the pathology. Heterozygous mice $col3a1^{+/G183R}$ were viable, had a 10% reduction in body weight without significant changes in blood pressure (BP). Their main striking feature was a 60% mortality at 24 weeks of age (0% in controls), mainly observed in males and caused by aortic rupture which was not preceded by aortic dilatation but with reduced arterial wall thickness. Electronic microscopy showed collagen fibers less dense and heterogeneous as well as altered morphology of adventitial fibroblasts with dilated endoplasmic reticulum suggesting retention of abnormal collagen. Compared to placebo, survival rate was not modified by propranolol, but considerably improved by losartan (10% mortality at 24 weeks) that markedly decreased BP -25 mmHg). Stopping losartan was associated with aortic rupture and mortality in the following weeks. The use of amlodipine which also reduced BP, albeit to a less extent (-10 mmHg), was conversely associated with an increased mortality. In this vEDS mouse model, we demonstrate the benefits of BP

reduction and angiotensin I receptor blockade. This therapeutic strategy should be tested in humans.

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C21.6
Patterns of co-occurrence of congenital heart defects follows distinct patterns

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Introduction: Congenital heart defects (CHD) affect almost 1% of the population and the number of adults with CHD is increasing. In families where CHD has occurred previously, estimates of recurrence risk and the type of recurring heart defect are important for counseling and clinical decision making, but the recurrence patterns of CHD in families are poorly understood.

Materials and Methods: We investigated the co-occurrences of congenital heart defects in 1,163 CHD families, comprising 10,278 individuals, of which 3,080 had a clinically confirmed CHD diagnosis. We calculated rates of concordance and discordance for 41 types of CHD and odds ratios for each of 1,640 pairs of discordant lesions observed between affected family members.

Results: We observed a high variability in the rates of concordance and discordance and we were able to identify 178 pairs of malformations that co-occurred significantly more or less often than expected in families. The data show that distinct groups of cardiac malformations co-occur in families, suggesting influence from underlying developmental mechanisms. Analysis of human and mouse susceptibility genes showed that they were shared in 19% and 20% of pairs of co-occurring discordant malformations, respectively, but none of malformations that rarely co-occur, suggesting that a significant proportion of co-occurring lesions in families is caused by overlapping susceptibility genes.

Conclusions: Our data show that familial CHD follow specific patterns of recurrence and suggest that part of co-occurrence of malformations in familial CHD may be caused by shared susceptibility genes.

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C22
Systems Genetics

C22.1
Four glycaemic trait trans-ethnic genome-wide association meta-analyses using densely imputed genetic data in up to 281,416 non-diabetic individuals

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Introduction: Large-scale glycaemic trait genome-wide association studies (GWAS) have identified more than 120 loci in Europeans. We aimed to dissect the trans-ethnic (71% European, 13% East Asian, 7% Hispanic, 6% African-American and 3% South Asian) genetic architecture of fasting glucose (FG), fasting insulin (FI), glycated haemoglobin (HbA1c) and 2-hour glucose (2hG), in up to 281,416 non-diabetic individuals from 144 studies.

Materials and Methods: We inverse normally transformed all phenotypes, adjusted FG/FI/2hG for body mass index (BMI) and tested for association the 1000 Genomes Project-imputed (Mar,2012) GWAS, assuming additive genetic

effects. Ethnic-specific fixed-effects meta-analyses were combined using MANTRA, allowing for heterogeneity in allelic effects between ethnicities and assuming $\log_{10}$ Bayes factor ($\log_{10}$BF) ≥ 6 for genome-wide significance.

Results: We detected 102 signals for FG (53 novel), 62 for FI (43 novel), 130 $HbA1_c$ for (60 novel) and 21 for 2hG (10 novel). These included loci at *NFX1* ($\log_{10}BF = 12.42$) and *ZBTB38* ($\log_{10}BF = 11.79$) genes for FG; waist-to-hip ratio/BMI-associated *BCL2* ($\log_{10}BF = 11.66$) for FI, *LRRC16A* ($\log_{10}BF = 18.60$) involved in platelet count and volume for HbA1c, and human cerebrospinal fluid monoamine metabolite level-associated *CLEC14A* ($\log_{10}BF = 9.68$) for 2hG not previously known to be involved in diabetes. Cluster analysis classified ~70% of HbA1c signals as influencing erythrocytic and ~30% glycaemic pathways. Enrichment analyses with GARFIELD and DEPICT highlighted blood, hemic and immune system signatures for HbA1c; pancreas for HbA1c/FG; adipose, fat tissues, adrenal glands and cortex for FI.

Conclusions: Novel clues about genetic architecture of glucose and insulin level regulation from this trans-ethnic analysis provide new hypotheses about their biology.

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C22.2
Expression insights into the human miRNA-mRNA interactome

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Introduction: miRNAs play a key role in the regulation of gene expression, while a majority of miRNA-mRNA interactions remain unidentified. The recent development of a high-throughput CLASH (crosslinking, ligation and sequencing of hybrids) technique for discerning miRNA-mRNA interactions allowed an experimental analysis of the human miRNA-mRNA interactome.

Materials and Methods: The direct miRNA-mRNA interaction data were gained from the experimental CLASH data. Expression levels for mRNAs and miRNAs were retrieved from FANTOM5 and GEO.

Results: Expression analysis of miRNA revealed two interesting groups: "specific" regulators expressed at high levels while forming only a few interactions with cognate mRNAs and "promiscuous" regulators expressed at low levels each forming more than 100 interactions with mRNAs. In normal cells, these "promiscuous" miRNAs are kept under tight transcriptional control that may be relaxed in pathophysiological states, thus forming an attractive pool of potential biomarkers, while the "specific" regulators may become candidates for therapeutic modulation.

Conclusions: Both of these miRNA groups are recognized as biomarkers associated with adverse prognostic features in cancer and other severe pathologies. Only about 1% of mRNAs are actively engaged in miRNA interactions. Furthermore, we identified several coding mRNAs with a substantial sponge effect, including AGO1, which function may reflect the competition and resultant coevolution of mRNAs and miRNAs.

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C22.3
Deconvolution of whole blood eQTLs into rare immune-subpopulations uncovers key players of immune mediated diseases

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Many genetic variants have been associated to immune mediated diseases, yet their molecular consequences are unclear. Expression quantitative trait loci (eQTL) studies revealed that the majority of these variants have regulatory effects and that these eQTLs are often cell-type specific (CTs). To study these effects, each subpopulation should be sorted and transcriptionally profiled, but this is often impossible due to resource constraints. In our study, we have identified these CTs eQTL effects by employing a deconvolution approach that does not require expression profiles from purified cells. We used 89 samples with whole blood (WB) RNA-seq data and quantifications of 73 different blood subpopulations using FACS. From these subpopulations, 22 could be inferred using WB RNA-seq ($r >= 0.5$ predicted against measured). Next, we imputed these subpopulations in an independent cohort ($N = 2,176$) and subsequently tested how 29,750 previously identified *cis*-eQTL are influenced by these cell-types.

To validate our approach we show that our identified CTs eQTLs have significantly bigger eQTL effect sizes in purified cell, such as CD4 and CD8 T cells. We also observed that the expression of genes in our detected CTs

eQTL is significantly higher in their relevant cell types, when compared to other cell subpopulations.

Finally, we overlapped the genetic risk factors of immune-related diseases and observed and enrichment of CTs eQTLs from immune cell subpopulations. In conclusion, our proposed method detects CTs eQTLs effects without transcriptionally profiling purified cell subpopulations, aiding in the characterization of the downstream effects of auto-immune genetic risk factors.

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C22.4
Adipose cis-eQTL variants at enhancer-promoter interaction circuits regulate obesity genes

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Introduction: Obesity has a high heritability, but its polygenic nature and environmental factors have made it difficult to uncover underlying biological mechanisms.
Materials and Methods: To identify genetic variants influencing obesogenic gene expression in adipose tissue, we combined expression quantitative trait loci (eQTLs) with chromatin interaction data. We detected *cis* eQTLs (+/−500 kb from the TSS, FDR < 1%) using RNA-sequence data from 793 subcutaneous adipose biopsies from the Finnish METSIM cohort, and then detected distal enhancers interacting with promoters by generating promoter capture Hi-C data in primary human white adipocytes.
Results: We identified 10,667 adipose *cis*-eQTL variants residing inside distal enhancers that loop to physically interact with the promoter of the eQTL and enhancer target gene (permutation $p < 0.001$). To focus on genes related to obesity, we correlated the expression of the target genes with body mass index (BMI). Of 1,312 genes with looping *cis*-eQTLs, 54 were correlated with BMI (genome-wide corrected $p < 3.197 \times 10^{-6}$). Of these, *MAP2K5* and *IL20RB*, are regulated by looping *cis*-eQTLs that are LD proxies ($r^2 > 0.80$) for BMI GWAS SNPs, suggesting them as the underlying genes at these wide BMI GWAS loci. By performing gene-gene expression correlations separately in the lean (BMI < 25, n = 118) and obese group (BMI > 30, n = 118), we discovered that correlation coefficients for 34 of the 54 genes show significant differences between lean and obese individuals (permutation $p < 0.01$), implying that obesity disrupts normal correlation structure in adipose transcriptomes among these BMI-correlated genes.
Conclusions: Our results uncover variants in regulatory enhancer-promoter interaction circuits influencing adipose gene expression underlying obesity.

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C22.5
Trans-eQTL analysis in 25,000 individuals reveals clear differences between diseases in the types and number of causally involved biological pathways

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Understanding the role of genetic risk factors identified through genome-wide association studies (GWAS) in complex disease remains difficult. For many diseases, hundreds of mostly non-coding variants contribute to disease risk through largely unknown molecular pathways. To identify these disease pathways, the eQTLGen consortium is integrating genetic and gene expression data from whole blood of >25,000 unrelated individuals, by conducting expression quantitative trait locus (eQTL) meta-analysis. We performed local *cis*- eQTL mapping for 7 million genetic variants and distal *trans*-eQTL mapping for >10,000 known genetic risk factors for disease. Finally, by using GWAS summary statistics for >1,000 (disease) phenotypes we calculated polygenic risk scores for each individual and correlated these to gene expression levels.

In an intermediate analysis ($N = \sim 18{,}000$), we identified 36,730 significant *trans*-eQTLs, representing almost 20% of all tested GWAS Catalog SNPs. *Trans*-acting SNPs are 5.4 times as likely to also affect the expression of a *cis*-gene. Integrating the effects from single SNPs and combined disease risk allows for comparison of the number and the nature of molecular pathways involved in several complex diseases. For example, inflammatory bowel disease SNPs affect various pathways (such as the type 1 and type 2 interferon response and B-cell receptor signalling), while the combined risk SNPs for HDL cholesterol specifically modulate the expression level of genes known to cause familial hypercholesterolemia. In addition, preliminary

analyses suggest that the large number of blood *trans*-eQTLs can also be used to infer relevant associations in other tissues, and to assess which genes are likely causal for disease.

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C22.6
Men with LOY and cells without the Y chromosome - transcriptomes and functional effects studied in 6000 single cells by RNA sequencing using the 10X Chromium platform

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The life expectancy of men is shorter compared to women but the underlying mechanism(s) are not well understood. Our recent discoveries regarding pathogenic effects from mosaic loss of chromosome Y (LOY) in blood might help explain this difference in longevity (*Nat. Genet.* 2014 PMID:24777449, *Science* 2015 PMID:25477213, *AJHG* 2016 PMID: 27231129).

Here we present the results of single cell RNAsequencing (scRNAseq) of more than 6000 peripheral blood mononuclear cells (PBMCs) using blood from a 93 year old man, utilizing the 10X Genomics chromium system. Using t-Distributed Stochastic Neighbor Embedding together with clustering techniques we could determine cell types and the fraction of LOY for each cell population.

We also studied the level of LOY in six FACS sorted cell fractions from the same man using the Illumina Infinium QC Array−24 kit and found a strong concordance in LOY fractions in the different cell types. Furthermore, we could identify genes showing LOY specific expression within cell-types.

A goal of the project is to describe the functional consequences of LOY in blood that could explain associations with increased risk for disease processes in other organs, such as non-hematological cancers and Alzheimer's disease in aging men. Our results show that scRNAseq methods can be used to identify LOY in PBMCs. Furthermore, we provide proof-of-concept that our approach will be useful for studying the impact of LOY at both a cellular and transcriptional level.

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C23
Neurogenetics 2

C23.1
Complex cis-interaction is responsible for the craniofacial and neuroanatomical defects of the 4p16.1 copy number variant

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Copy number variants (CNVs) are frequent lesions involved in both rare and complex disorders. We have shown previously how the use of structural surrogate phenotypes in zebrafish embryos can identify the major genes responsible for CNV-associated phenotypes. Here we dissected functionally the five genes present in a CNV on 4p16.1. Existing cases from both Decipher and AChro-Puce databases indicated that the 4p16.1 deletion was associated with micrognathia (small jaw) and microcephaly whereas the duplication was associated with an abnormal facial shape and macrocephaly. We thus sought to determine the contribution of these genes to brain and face development. To mimic the duplication, we expressed each of the five human transcripts in zebrafish embryos. We found discrete drivers for the two major anatomical features tested. Overexpression of either *SLC2A9* and *ZNF518B* were sufficient to induce macrocephaly. However, scoring for the possible drivers of the craniofacial defect neither gene induced appreciable pathology. In contrast, expression of *CLNK* and *WDR1* led to macrognatia and abnormal U-shaped Meckel's cartilage respectively. Finally, we asked whether the same transcripts might be relevant to the deletion by inducing deletions in each of *WDR1* and *CLNK* orthologs by CRISPR/Cas9; in contrast to the duplication experiment, only the loss of *Wdr1* led to micrognatia. Taken together

these data suggest that the craniofacial and neuroanatomical phenotypes aredue to the dose imbalance of several genes present in the 4p16.1 following a cis-interaction complex model rather than the effect of a major gene driver.Labex Starting Grant, NIH R01 MH106826

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C23.2
Foxp1 is essential for sex-specific murine neonatal ultrasonic vocalization

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Autism and speech and language deficits are predominantly found in boys, however the causative mechanisms for this sex bias are unknown. Human *FOXP1* is associated with autism, intellectual disability and speech and language deficits. Its closely related family member *FOXP2* is involved in speech and language disorder and Foxp2 deficient mice have demonstrated an absence of ultrasonic vocalization (USV). Since Foxp1 and Foxp2 form heterodimers for transcriptional regulation, we investigated USV in neonatal brain-specific *Foxp1 KO* mice. *Foxp1 KO* pups had strongly reduced USV and lacked the sex-specific call rate from WT pups, indicating that Foxp1 is essential for normal USV. As expression differences of Foxp1 or Foxp2 could explain the sex-dimorphic vocalization in WT animals, we quantified both proteins in the striatum and cortex at P7.5 and detected a sex-specific expression of Foxp2 in the striatum. We further analyzed *Foxp1* and *Foxp2* expression in the striatum and cortex of CD1 mice at different embryonic and postnatal stages and observed sex differences in both genes at E17.5 and P7.5.

Sex hormones, especially androgens are known to play a crucial role in the sexual differentiation of vocalizations in many vertebrates. We show that Foxp1 and the androgen receptor are co-expressed in striatal medium spiny neurons and that brain-specific androgen receptor KO mice exhibit reduced *Foxp1* expression in the striatum at E17.5 and P7.5 and an increased *Foxp2* level in the cortex at P7.5. Thus, androgens may contribute to sex-specific differences in *Foxp1* and *Foxp2* expression and USV.

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C23.3
Dominant mutations in DCC cause isolated agenesis of the corpus callosum with sex specific penetrance

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Agenesis of the corpus callosum (ACC) is the most frequent viable brain malformation in humans, usually detected during the prenatal period by ultrasound. It is a widely heterogeneous condition that can be associated with a normal or subnormal cognitive development when isolated, or with variable degrees of intellectual disability (ID). Although mutations in many genes cause syndromic ACC with ID, genes accounting for isolated ACC remain largely unknown. The netrin receptor Dcc (Deleted in Colorectal Cancer) plays a critical role in formation of corpus callosum in mice by guiding callosal axons at the midline. *DCC* mutations have previously been associated with mirror movements (MM) in humans but not ACC. We used a combination of genetic approaches to investigate the cause of isolated ACC in 4 multigenerational families and 70 sporadic cases. We demonstrate that *DCC* mutations leading to premature termination codons or altering amino

acids preferentially located in the netrin binding domain cause dominant isolated ACC, with or without MM and incomplete penetrance. In individuals with ACC and/or MM, mutations were associated with failure of corticospinal axons to cross at the pyramidal decussation. Interestingly, individuals with truncating *DCC* mutations showed sex-biased ACC or MM phenotypic expression, possibly related to enhanced *DCC* expression by testosterone during brain development. *DCC* mutations therefore cause a variable phenotype ranging from MM to isolated ACC with a favorable cognitive development in humans and testosterone could act as a phenotypic modifier.

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C23.4
WD40-repeat 47 is essential for brain development via microtubule-mediated processes and autophagy

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The family of WD40-repeat (WDR) proteins is one of the largest in eukaryotes but little is known about their function in brain development. Among 26 WDR genes assessed, we found seven displaying a major impact in neuronal morphology when inactivated in mice. Remarkably, all seven genes showed corpus callosum defects, from thicker (*Atg16l1*, *Coro1c*, *Dmxl2* and *Herc1*), thinner (*Kif21b* and *Wdr89*) to absent (*Wdr47*), revealing a common implication of WDR genes in brain connectivity. We focused on the poorly studied WDR47 protein sharing structural homology with LIS1, which causes lissencephaly. Mice lacking *Wdr47* showed lethality, extensive fibre defects, microcephaly and sensory-motor gating abnormalities reminiscent of a patient harbouring mutation in *WDR47*. We demonstrated that WDR47 indeed shares functional characteristics with LIS1, as it participates in key microtubule-mediated processes. Interestingly, WDR47 specific CTLH domain was associated with functions in autophagy for the first time in mammals. Silencing WDR47 in hypothalamic GT1–7 neuronal cells and yeast models independently recapitulated these findings, demonstrating highly conserved mechanisms. Finally, our data identified two WDR47-interacting partners: SCG10 and Reelin. Taken together, these results provide a starting point for studying the implication of WDR proteins in neuronal regulation of microtubules and autophagy, bringing a new insight to the biology of corpus callosum.

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C23.5
ATPase-deficient ATAD3A alters mitochondrial dynamics in hereditary spastic paraplegia

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ATAD3A is a mitochondrial inner membrane AAA ATPase with an unknown precise function. Membrane AAA ATPases form hexameric rings, which are catalytically dependent on the co-operation of the subunits. If ATP hydrolysis is blocked in one subunit the entire protein is rendered nonfunctional. ATAD3A was only recently associated with inherited human disease when de novo mutations were reported in isolated patients presenting with a neurological syndrome. On the contrary, we identified dominant inheritance of mutant *ATAD3A*, leading to hereditary spastic paraplegia (HSP), a disorder of the upper motor neurons. The mutation p.G355D in our patients affects the Walker A motif, which is responsible for ATP binding in the AAA module of ATAD3A. We show that the

recombinant mutant ATAD3A protein has a strong dominant-negative effect on the wild type enzyme. We further observed that the patient fibroblasts with the ATPase-deficient ATAD3A have elongated mitochondria and substantially increased lysosome mass. These alterations were verified to associate with upregulated basal autophagy through mTOR inactivation, resembling starvation. We also derived motor neurons through differentiation of patient-specific induced pluripotent stem cells, and observed alterations in mitochondrial network dynamics in the neurons, particularly presenting as elongated mitochondria. We currently focus on transcriptome profiling of the ATAD3A-deficient motor neurons using single cell RNA sequencing.

We present the first dominantly inherited ATAD3A mutation associated with loss of ATPase activity, inducing mitochondrial elongation. This finding adds ATAD3A to the group of mitochondrial inner membrane AAA proteins associated with spasticity.

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C23.6
Mutation of ribosomal RNA-processing protein 7 homolog A (*RRP7A*) cause autosomal recessive microcephaly with intellectual disability

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Introduction: Autosomal recessive microcephaly is a rare neurodevelopmental disorder characterized by small brain and intellectual disability. The disorder is usually associated with aberrant neuronal progenitor cell proliferation, caused by mutation of genes encoding centrosomal proteins.

Materials and Methods: Genome-Wide SNP Array 6.0 and whole exome sequencing (WES) was used to identify regions of homozygosity and rare mutations. Human fetal brain sections were used for expression analysis. Patient's and control fibroblasts and RPE cells were used for functional studies. Zebrafish and P19.CL6 cells were used as model systems.

Results: We identified a rare homozygous missense mutation in the gene encoding ribosomal RNA-processing protein 7 homolog A (RRP7A). Immunostaining showed that RRP7A is highly expressed in radial glia cells and motile cilia of the human developing brain, and zebrafish *rrp7a* mutants display impaired craniofacial development, small heads and eyes, cell proliferation defects and increased apoptosis. Further, P19.CL6 *Rrp7a* mutant stem cell clones show defects in neuronal differentiation. At the cellular level, RRP7A localizes to nucleoli, primary cilia, centrosomes as well as spindle pole bodies during all stages of mitosis in fibroblasts and RPE cells. The missense mutation in patient-derived primary skin fibroblasts causes ribosome biogenesis defects detected by impaired rRNA processing as well as cell cycle defects with slower proliferation rate compared to wild-type cells. Similarly, RPE cells subjected to RNAi-mediated depletion of RRP7A display overly long primary cilia, which are associated with defects in cell cycle entrance.

Conclusions: Impairment of ribosomal RNA processing is a novel cause of autosomal recessive microcephaly.

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