Human Genetics Goes Somatic
Dear colleagues,

On behalf of the BeSHG Board and the Local Organising Committee, we cordially welcome you to the 17th Belgian Society of Human Genetics (BeSHG) Meeting in Louvain-la-Neuve.
On behalf of all Board members
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Human Genetics Goes Somatic

Aula Magna, Louvain-la-Neuve

08.30 – 09.45  Registration and Coffee  Hall
09.45 – 10.00  Welcome and Introduction: Paul Coucke, president BeSHG  Theatre

10.00 – 11.40  Morning session  Theatre

10.00  Inherited predisposition to hematopoietic malignancies
Lucy Godley

10.50  Revertant mosaicism in human skin disease
Marcel Jonkman

11.45 – 12.45  Parallel Session IA: Selected Oral Presentations  Theatre

Florence Arts
PDGFRB mutations cause infantile myofibromatosis
Romain Péanne

ATP6AP2-CDG: Identification using whole exome sequencing of a new subtype of v-ATPase assembly defects causing liver disease
Miriam Bauwens

Hidden genetic variation in Stargardt disease: novel copy number variations, cis-regulatory and deep-intronic splice variants within the ABCA4 locus
Bieke Decaesteker

SOX11 acts as part of the MYCN-WEE1 regulatory protein complex implicated in neuroblastoma
11.45 – 12.45  **Parallel Session IB: Selected Oral Presentations**

*Foyer Royal*

**Chairs:** Lut Van Laer & Anabelle Decottignies

**Kris Vleminkx**

*Modeling human hereditary cancer syndromes using CRISPR/Cas9 mediated genome editing in Xenopus tropicalis*

**Siebe Loontiens**

*Scrutinizing PHF6 regulatory networks using zebrafish*

**Gretl Hendrickx**

*Conditional mouse models support the role of SLC39A14 (ZIP14) in Hyperostosis Cranialis Interna and in bone homeostasis*

**Olga Ťuiko**

*In vitro procedures exacerbate chromosome instability in cleavage-stage embryos*

12.45 – 14.00  **Lunch, Poster viewing, Exhibition & Company Visits**  

**Hall**

14.00 – 14.40  **Annual General Assembly BeSHG**  

**Theatre**

14.40 – 15.55  **Parallel Session IIA: Selected Oral Presentations**  

*Theatre*

**Chairs:** Sonia Van Dooren & Pascal Brouillard

**Hilde Brems for Multiplicom**

*Somatic Mosaicism in Genodermatoses*

**Simon Ardui**

*Detecting AGG interruptions in male and female FMR1 premutation carriers by single-molecule sequencing*

**Koen Theunis**

*Cost effective DNA copy number profiling of single cells without upfront whole-genome amplification*
Annelies Dheedene

*Detection of copy number variation using shallow whole genome sequencing (CNVseq) as a cost-effective alternative to genomic microarrays*

Antonio Colaprico

*TCGAbiolinksGUI: A graphical user interface to analyze cancer molecular and clinical data*

**14.40 – 15.55**  **Parallel Session IIB: Selected Oral Presentations**  **Foyer Royal**

**Chairs:** Damien Lederer & Anne De Leener

**Geert Mortier**

*Evidence that bi-allelic mutations in NPR3 result in a peculiar phenotype with tall stature, arachnodactyly, long hallucs and multiple extra epiphyses in hands and feet*

**Josephina Meester**

*Loss-of-function mutations in the X-linked gene BGN cause a severe syndromic form of thoracic aortic aneurysms and dissections*

**Bert Callewaert**

*Mutations in ATP6V1E1 or ATP6V1A cause autosomal recessive cutis laxa*

**Nele Cosemans**

*The clinical relevance of intragenic NRXN1 deletions*

**Danya Vears**

*Which next generation sequencing results are reported to clinicians? A qualitative study*

**15.55 – 16.15**  **Coffee break, Poster session and Exhibition**  **Hall**
16.15 – 18.15  Afternoon session  
Chairs:  Paul Coucke & Mikka Vikkula

16.15  Uncovering genetic interactions in haploid human cells  
Vincent Blomen

17.05  Interrogating the architecture of cancer genomes  
Peter Campbell

17.55 – 18.15  Prizes and Closing remarks: Mikka Vikkula, Nisha Limaye  
Theatre

18.15 – 19.15  Reception  
Hall

19.15 – 00.00  Walking dinner and party  
Foyer du Lac
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Invited Speakers
Inherited predisposition to hematopoietic malignancies

Lucy A. Godley

The University of Chicago

Familial predisposition to hematopoietic malignancies is likely more common than generally appreciated. Currently, we know most about predisposition syndromes that confer risk for myeloid malignancies. These can be divided into several classes: those for which individuals have an increased risk only for myeloid malignancies (e.g., germline \textit{CEBPA} and \textit{DDX41} mutations as well as genomic duplications of 14q32.2); those that confer thrombocytopenia and abnormalities of platelet aggregation (e.g., germline \textit{RUNX1}, \textit{ANKRD26}, and \textit{ETV6} mutations); those that confer risk for systemic diseases in addition to myeloid malignancies (e.g., germline \textit{GATA2} and \textit{SRP72} mutations); and the inherited bone marrow failure syndromes (e.g., dyskeratosis congenita and Fanconi anemia). Familial predisposition to lymphoid malignancies has also been described and is an area of great possibility for discovery.

Accurate diagnosis of the germline basis for disease in patients presenting with hematopoietic malignancies is critical for their clinical management, especially when considering allogeneic stem cell transplantation using a related donor. At The University of Chicago, we have established a comprehensive approach to patient evaluation, which includes documentation of a complete family and bleeding history, consultation with a certified genetic counselor familiar with inherited hematopoietic predisposition syndromes, and testing using DNA derived from cultured skin fibroblasts to ensure testing of germline material. We have developed a robust clinical testing pipeline that includes next-generation sequencing to identify point mutations as well as array-based methods to assess for genomic rearrangements. In our experience, we are able to identify a germline mutation in about 20% of individuals/families presenting with familial clustering of myeloid malignancies.

Patients who test negative for the known predisposition syndromes are offered participation in research to allow discovery of new syndromes. Thus, the vast majority of cases present an opportunity to identify new syndromes/predisposition alleles. Although widespread application of next-generation sequencing makes screening relatively straightforward, the relative rarity of families with germline mutations means that new gene discovery is likely to require the cooperative effort of groups throughout the world. Moreover, affected individuals should be encouraged to participate in translational research studies to help elucidate answers to remaining questions in the field, including: What percentage of MDS/AML cases actually have a familial/genetic basis? How many germline predisposition syndromes exist? What are the associated clinical findings in these syndromes? What are the phenotypic similarities among syndromes? How do mutations in predisposition syndromes lead to myeloid malignancies? What is the genetic progression from germline mutation to overt malignancy? Addressing these questions in the coming years will provide great insight into the contribution of germline genetics to myeloid malignancies.
Revertant mosaicism in skin disease

Marcel F. Jonkman

University of Groningen

A revertant is a former mutant that has regained, partly or completely, the wild-type phenotype and/or genotype. Revertant mosaicism (RM) is a special form of somatic mosaicism that refers to the co-existence of cells carrying disease-causing mutations with cells in which the inherited mutation is genetically corrected by a spontaneous post-zygotic event. RM in skin was first reported by us in 1997 in the genetic disorder epidermolysis bullosa (EB), which is characterized by lifelong fragile skin that easily forms blisters and erosions. In a patient with generalized atrophic benign EB, caused by compound heterozygosity at the COL17A1 locus, we found several patchy areas of healthy skin and provided molecular proof that the keratinocytes in the clinically unaffected skin were corrected by a gene conversion event, and consequently produced normal type XVII collagen.

Mutations in as many as 19 genes can result in EB. Five of these genes have shown to revert: KRT14 encoding keratin 14 in EB simplex, LAMB3 encoding the β3 chain of laminin-332, and COL17A1 encoding type XVII collagen in junctional EB, COL7A1 encoding type VII collagen in dystrophic EB, and FERMT1 encoding kindlin-1 in Kindler syndrome. RM was also found in other heritable skin diseases: dyskeratosis congenita, and in ichthyosis in confetti (ichthyosis variegata) induced by increased homologous recombination of KRT10. Similar examples of “natural gene therapy” by RM have been described in Bloom syndrome, leukocyte adherence deficiency type 1, Wiskott-Aldrich syndrome, and RAG1-deficient severe combined immunodeficiency.

This “natural gene therapy” phenomenon manifests as normal appearing skin areas surrounded by affected skin. Although initially thought to be rare, RM is now considered relatively common in genetic skin diseases. To address the issues relevant to RM, we will discuss the following questions: 1) What are the repair mechanisms in RM? 2) When does revertant mutations occur? 3) What is the incidence of RM in heritable skin diseases, and what role plays clonal expansion of the fittest cell? 4) How is revertant skin recognized? The answers to these questions allow us to acquire knowledge on these reverted cells, the mechanisms of RM, and utility of the reverted cells to the advantage of the patient.

The revertant skin could potentially be used to treat the patient's own affected skin. Revertant skin cells can be used for transplantation by means of: 1) own skin biopsies, 2) cell suspension, 3) cultured epithelial cell sheet, 4) induced pluripotent stem cells reprogrammed as epithelial sheet for skin grafting or as haemopoietic stem cells for infusion. Transplantation of revertant skin biopsies has already successfully been performed in a patient with EB.
Exploring genetic interactions in haploid human cells

Vincent A. Blomen and Thijn R. Brummelkamp

Netherlands Cancer Institute

Gene-associated phenotypes often depend on the activity of other genes resulting in genetic interactions. In model organisms, the large-scale generation of combinations of mutations has resulted in the identification of genetic interactions and provided insights into their contribution to complex phenotypes. Here we use haploid human cells combined with genome-wide mutagenesis to address to study genetic interactions. We have made a synthetic lethality network using query genes acting in the secretory pathway and observe that human genes frequently engage in genetic interactions. Beyond cell lethality, we search for genes, which upon loss deregulate cellular processes such as signaling pathways. Genetic modifier approaches in those mutants reveals back-up mechanisms or genetic networks contributing to the phenotype of interest. This provides a much-needed window into the robust genetic networks governing human cells.
Interrogating the architecture of cancer

Peter Campbell

Wellcome Trust Sanger Institute

Cancer is driven by mutation. Using massively parallel sequencing technology, we can now sequence the entire genome of cancer samples, allowing the generation of comprehensive catalogues of somatic mutations of all classes. Bespoke algorithms have been developed to identify somatically acquired point mutations, copy number changes and genomic rearrangements, which require extensive validation by confirmatory testing. The findings from our first handful of genomes illustrate the potential for next-generation sequencing to provide unprecedented insights into mutational processes, cellular repair pathways and gene networks associated with cancer development. I will also review possible applications of these technologies in a diagnostic and clinical setting, and the potential routes for translation.
Selected Oral Presentations
01: PDGFRB mutations cause infantile myofibromatosis

Florence A. Arts¹, Raf Sciot¹, Bénédicte Brichard¹, Marleen Renard¹, Laura A. Noël¹, Amélie I. Velghe¹, Christine Galant¹, Maria Debiec-Rychter⁶, An Van Damme¹, Pascal Brouillard¹, Miikka Vikkula¹, Raphaël Helaers⁷, Nisha Limaye⁷, Hélène A. Poirel⁸ & Jean-Baptiste Demoulin¹

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Infantile myofibromatosis, one of the most prevalent benign tumors of soft tissue of childhood, is characterized by the presence of nodules in the skin, subcutaneous soft tissues, bones or viscera. Multifocal nodules with visceral lesions are associated with a poor prognosis. Recently, germline and somatic heterozygous mutations in the platelet-derived growth factor receptor β (PDGFRB) have been associated with the familial form of infantile myofibromatosis and two cases of overgrowth syndrome. These mutations had not been characterized.

The first aim of this project was to characterize these new PDGFRB mutants functionally. We expressed three PDGFRB mutants associated with familial infantile myofibromatosis (R561C, P660T and N666K) in Ba/F3 cells. In the absence of ligand, the R561C and N666K mutants activated pathways that are activated by the wild-type receptor only in response to PDGF. The P660T mutant showed no difference with the wild-type receptor, suggesting that it might represent a polymorphic variant unrelated to the disease. Moreover, both activated mutants, namely R561C and N666K, were able to transform NIH3T3 and Ba/F3 cells. The mutant identified in an overgrowth syndrome, P584R, was also constitutively active. In the second part of this project, we sequenced PDGFRB in 16 patients with sporadic myofibromatosis or solitary myofibroma, which are much more frequent than familial cases, by targeted deep sequencing (Ion Torrent technology). Gain-of-function mutations in the coding sequence of PDGFRB were identified in seven patients, six of whom had the sporadic multicentric form of the disease. Mutations were located in the transmembrane, juxtamembrane and kinase domains of the receptor. Finally, we tested the sensitivity of the mutants to tyrosine kinase inhibitors. Most mutated receptors were inhibited by imatinib, suggesting a treatment for severe myofibromatosis. One mutant was resistant to imatinib but remained sensitive to dasatinib and ponatinib.

In conclusion, the PDGFRB mutations previously identified in familial infantile myofibromatosis and overgrowth syndrome activate the receptor in the absence of ligand. In addition, we identified gain-of-function PDGFRB mutations in 75% (6/8) of multifocal infantile myofibromatosis cases, shedding light on the mechanism of disease development, which is reminiscent of multifocal venous malformations induced by TIE2 mutations. Our results provide a genetic test to facilitate diagnosis, and preclinical data for development of molecular therapies.
02: ATP6AP2-CDG: identification using whole exome sequencing of a new subtype of \(v\)-ATPase assembly defects causing liver disease

Romain Péanne\(^1\), Magda Cannata Serio\(^2\), Maria Rujano\(^2\), Ganna Panasyuk\(^3\), Matias Simons\(^2\) & Gert Matthijs\(^1\)

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\(^2\) Imagine Institute - Paris Descartes University, Paris, France
\(^3\) Institut Necker-Enfants Malades, INSERM - Paris, France

Background: Congenital Disorders of Glycosylation (CDG) are a rapidly growing and heterogeneous group of rare, genetic diseases of metabolism, caused by defects in the glycosylation of glycoproteins and glycolipids. Next Generation Sequencing (NGS) - including Whole exome sequencing (WES) - held the promise to quicken the identification of additional, novel genetic defects, particularly in extremely heterogeneous diseases like CDG.

Objectives: We aim to solve the genetic defect in patients with CDG-II (disorders of Golgi homeostasis or vesicular trafficking) using a combined approach of clinical phenotyping and NGS, and to decipher the (patho)physiology of the involved pathway(s) thanks the use of cellular and animal models.

Main approach: Using whole exome sequencing, we identified 3 individuals from two unrelated families with hemizygous mutations in the X-linked ATP6AP2 gene. The two index cases were from a cohort of unexplained CDG patients.

Results: WES revealed two hemizygous variants in ATP6AP2 in three CDG-II patients. All patients presented a partially overlapping clinical phenotype, including borderline IQ, immunodeficiency, skin laxity/cutis laxa and liver involvement. The first mutation (c.293T>C – p.Leu98Ser) is a missense variant identified in a male Portuguese patient. This was in contrast to the SNP c.268C>G in the same gene, which was transmitted by the mother to all sons. The missense variant is situated at a conserved site in the extracellular domain of ATP6AP2 and was scored as a disease-causing variant using Polyphe-2, SIFT and Mutation Taster. An additional mutation in ATP6AP2 (p.Arg71His) was found in a German family. Arg71His scored weaker than Leu98Ser with SIFT and Polyphen-2. Yet, the phenotypes were for the most part stronger. Taken together, those findings uncover a novel ATP6AP2-dependent glycosylation disorder featured hypogammaglobulinemia, liver defects, serum lipid abnormalities and cognitive impairment. ATP6AP2 is one of the two accessory subunits (together with ATP6AP1) of the multi-subunit \(V\)-ATPase complex. This complex acidifies intracellular organelles, thereby controlling a number of events in the secretory and endocytic pathway, such as proteolytic processing, protein degradation and autophagy.

We next tested the effect of the mutations on Drosophila development in a rescue assay. We focused on L98S, because unlike R71H it is conserved between humans and flies and could, thus, be introduced in a rescue construct. Consistent with the cell culture findings, the L98S mutation caused impaired protein stability and led to developmental defects and impaired lipid metabolism when introduced into Drosophila. Moreover, a murine model carrying a floxed allele of ATP6AP2 was used to evidence the link between defective lipophagy
and liver damage. Compared to control GFP-Cre animals, the conditional inactivation of ATP6AP2 in the liver of Adeno-Cre injected animals led to impaired autophagy flux and presented similar disease features of ATP6AP2-CDG patients.

Conclusion: To summarize, we show that the identified mutations in ATP6AP2 using WES cause a syndromic liver disease, presenting as a glycosylation disorder. ATP6AP2 deficiency is characterized by a similar organ manifestation as in mutations of other assembly factors of the v-ATPase, particular of ATP6AP1. Using Drosophila and mice, we also provide evidence that impaired autophagic and lysosomal degradation is a major determinant of the cellular pathology of ATP6AP2-CDG.
O3: Hidden genetic variation in Stargardt disease: novel copy number variations, cis-regulatory and deep-intronic splice variants within the ABCA4 locus

Miriam Bauwens1, Riccardo Sangermano2, Timothy Cherry3, Caroline Van Cauwenbergh1, José Luis Gómez-Skarmeta4, Nicole Weisschuë5, Susanne Kohl1, Bart P. Leroy6, Frans P. Cremers2 & Elfride De Baere1

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5 Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tuebingen, Tuebingen, Germany.
6 Dept of Ophthalmology, Ghent University Hospital & Ghent University, Gent, Belgium.

Purpose: A large proportion of Stargardt disease (STGD1) patients have single coding variants in the disease gene ABCA4, suggestive of hidden genetic variations in non-coding regions. We aimed to assess the contribution of copy number variations (CNVs) and non-coding sequence variations in monoallelic STGD1 patients. Moreover, we attempted to gain more insight into the cis-regulatory landscape of ABCA4.

Methods: A total of 116 monoallelic STGD1 patients underwent targeted resequencing of a conserved block of synteny encompassing the ABCA4 gene (HaloPlex enrichment). Filtering of variants was based on criteria such as publicly available minor allele frequencies and frequency in the total cohort of sequenced patients. Altered splice predictions, presence in cis-regulatory regions of ABCA4 and RegulomeDB data served as a secondary filter. 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 2 of the promoter variants. A chromatin interaction map of the ABCA4 region was generated by 4C-seq in human adult retinal cells. 11 putative cis-regulatory variants are being tested by luciferase assays in Y79 cells and nine putative splice variants are being assessed by mini-gene assays. CNV analysis revealed a novel ABCA4 deletion (ex 40-50) and a duplication (ex 2-6) in 2/5 patients without candidate non-coding variants.

Conclusions: Resequencing of the whole ABCA4 locus uncovered novel deep intronic splice variants and cis-acting regulatory variants in unsolved cases of STGD1, representing the first report of non-coding regulatory ABCA4 variants. In addition, a chromatin interaction dataset for the ABCA4 locus was generated in retinal cells. Finally, apart from a novel deletion, the first duplication was identified in ABCA4, expanding the CNV spectrum which represents a very small fraction of the known mutational load in ABCA4.
04: SOX11 acts as part of the MYCN-WEE1 regulatory protein complex implicated in neuroblastoma

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Neuroblastoma (NB) is an aggressive lethal pediatric cancer of the developing sympatho-adrenergic nervous system and characterised by a low mutation burden while exhibiting recurrent DNA copy number alterations including chromosome 2p gain and MYCN amplification. Rare amplifications have previously allowed to identify additional oncogenes implicated in NB such as ALK and LIN28B. Here we report on four NB tumours and two NB cell lines with high level focal gain containing the SRY-related HMG-box transcription factor 11 (SOX11) as only protein-coding gene in the smallest region of overlap. The high expression levels of SOX11 in NB and the developing sympathetic nervous system, and high correlation of SOX11 mRNA and protein levels with survival outcome, suggested a role for SOX11 as oncogene and prompted us to further investigate its role in MYCN driven NB formation. SOX11 knock down in NB cell lines showed reduced colony formation capacity and G1-S cell cycle arrest. We observed increased SOX11 levels upon MYCN induction in NB cell lines, in MYCN overexpressing mouse neuroblastomas compared to normal ganglia as well as a high correlation of SOX11 and MYCN mRNA and protein levels in cell lines and tumors. As these data could indicate direct MYCN regulation of SOX11, we performed MYCN ChiPseq and indeed confirmed binding of MYCN to the SOX11 promotor. Next, we demonstrated MYCN and SOX11 cooperative binding to common targets using SOX11 and H3K27ac ChiP-sequencing. First, we showed localisation of more than half (62%) of the SOX11 binding sites at enhancers. Moreover, 19% of SOX11 targets contained an E-Box motif, typically known to be targeted by MYCN, and we showed overlap of 30% of SOX11 and MYCN binding targets. In a further step to unravel SOX11 function in NB cells, we analysed SOX11 binding partners using IP-MS and identified 9 robust interaction partners in multiple NB cell lines. These included MYCN which was confirmed by reciprocal co-IP, as well as WEE1, a tyrosine kinase critically implicated in G2-M checkpoint control for which multiple potent and specific small molecule inhibitors are available. Remarkably, upon testing of the AZD1775 WEE1 inhibitor in 10 NB cell lines, we observed the strongest effects in cells with the highest SOX11 protein levels indicating that SOX11 expression levels can serve as predictive biomarker for AZD1775 treatment response.

In conclusion we identified high-level focal gain of SOX11 in NB tumors and cell lines and demonstrated the oncogenic role of SOX11 in MYCN driven neuroblastoma with therapeutic opportunities through interaction with WEE1.
05: Modeling human hereditary cancer syndromes using CRISPR/Cas9 mediated genome editing in Xenopus tropicalis

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CRISPR/Cas9 mediated genome editing is creating unique and unmatched opportunities in several research fields, including cancer research. For the first time it is now possible to create functional gene knockouts in a number of model organisms. We have recently generated the first genetic tumor models in the organism Xenopus tropicalis. Because of the external development of the Xenopus embryo and its diploid genome, gene targeting experiments using CRISPR/Cas9 are extremely efficient and cheap in this organism. Interestingly, by mosaic targeting of the tumor suppressor gene APC we generated tadpoles that rapidly (< 1.5 months) and efficiently (>90%) developed neoplasia characteristic for Familial Adenomatous Polyposis (Van Nieuwenhuysen et al., Oncosience 2015). Similarly, using CRISPR/Cas9 techniques we found that RB1/RBL1 double mosaic mutant tadpoles rapidly develop retinoblastoma (Naert et al. Sci. Rep. 2016). Preliminary work also indicates the possibility of modeling Neurofibromatosis Type 2 by mosaic targeting of the NF2 gene. The rapid kinetics of tumor development in the tadpoles and froglets pave the way for use as pre-clinical models. Additionally, these tumor models provide unique possibilities for fast identification of novel drug targets by multiplexed CRISPR/Cas9 gRNA injections (tumor suppressor gene + candidate therapeutic target gene). We believe that our models offer a unique experimental platform that can be easily plugged into the research lines of several groups active in the field. We will present our first promising results with multiplexed gene targeting in desmoid tumors.
06: Scrutinizing PHF6 regulatory networks using zebrafish

Siebe Loontiens¹, Kaat Durinck¹, Inge van de Walle², Finola Moore³, Suzanne Vanhauwaert¹, Els Janssens¹, Pieter Rondou¹, Givani Dewyn¹, Aline Eggermont¹, Lisa Depestel¹, Christophe Van Neste¹, David Langenau³, Pieter Van Vlierberghe¹, Tom Taghon² & Frank Speleman¹

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In T-ALL, various oncogenic driver events and cooperative mutations have been identified in the past decade that contribute to malignant T-cell transformation. One of the major T-ALL subtypes is characterized by ectopic expression of the TLX1 transcription factor and primary TLX1⁺ cases often harbor inactivating mutations in the gene PHF6. So far, the functional role of PHF6 in normal and malignant hematopoietic lineage development is currently unknown. In this study we aimed to unravel the role of PHF6 in normal T-cell development and in TLX1 driven T-ALL in human hematopoietic precursors cells using the well-established OP9 in vitro co-culture system and zebrafish as an in vivo model.

In vitro differentiation of human CD34⁺ hematopoietic progenitors was evaluated upon stable PHF6 knockdown. We observed an acceleration in CD4⁺CD8⁺ double positive T-cell stage transition upon PHF6 downregulation. In addition, PHF6 depletion favoured TCR⁻⁺T-cell development at the expense of TCR⁻⁻ T-cell differentiation. Notably, these observed phenotypic effects mimicked those induced by reduced Notch1 signaling.

We show that combined PHF6 knockdown and pharmacological Notch inhibition act synergistically. This additive interaction could further be supported at the level of gene expression. We were able to validate these observations in vivo by the use of a TALEN based PHF6 knock out zebrafish line (Moore et al., Plos One, 2012). Upon phf6 knockdown, accelerated T-cell maturation was seen and Notch inactivation by GSI treatment accelerated this developmental effect, in line with the acquired in vitro data.

Using the same TALEN knock out line, we were able to model the TLX1-PHF6 T-ALL cases in zebrafish. We crossed PHF6⁻⁻ zebrafish with rag2-TLX1-GFP zebrafish and are currently screening their offspring for T-ALL development. At the age of 10 months, already 1/20 fish show a full-blown T-ALL. Further screening of these zebrafish is ongoing at the moment. To validate our model, the obtained T-ALL’s will then be analysed on genome, transcriptome and epigenome level.

In conclusion, our work reveals fundamental novel insights into the role of PHF6 in normal T-cell development and this study provides for the first time an in vivo model to scrutinize the role of PHF6 in TLX1 driven T-ALL, aiming to provide novel therapeutic opportunities to treat TLX1⁺ leukemia.
07: Conditional Mouse Models Support the Role of SLC39A14 (ZIP14) in Hyperostosis Cranialis Interna and in Bone Homeostasis

Gretl Hendrickx¹, Vere M. Borra¹, Ellen Steenackers¹, Timur A. Yorgan², Christophe Hermans³, Evelyne Boudin¹, Jérôme J. Waterval⁴, Ineke D. Jansen⁵, Tolunay B. Aydemir⁶, Niels Kam⁷, Geert J. Behets⁸, Patrick C. D’Haese⁹, Vincent Everts¹, Martin Lammens¹, Geert Mortier¹, Robert J. Cousins⁶, Thorsten Schinke², Robert J. Stokroos⁹, Johannes J. Manni⁹ & Wim Van Hul¹

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Hyperostosis Cranialis Interna (HCI) is a rare bone disorder characterized by intracranial hyperostosis and osteosclerosis of the skull base and calvaria, whereas the remainder of the skeleton is unaffected. This results in symptoms caused by entrapment of cranial nerves due to progressive bone overgrowth. We previously localized the disease-causing gene for HCI on chromosome 8p21. With whole-exome sequencing we now detected a mutation (p.L441R) in the SLC39A14 (ZIP14) gene, encoding a zinc transporter. The p.L441R mutation results in trafficking defects of ZIP14 towards the plasma membrane and accumulates intracellular zinc in vitro. Immunohistochemistry indicates expression of ZIP14 in osteoblasts and osteoclasts. We therefore generated two conditional knock-in mouse models, overexpressing p.L438R Zip14 in osteoblasts and osteoclasts respectively. Remarkably, the calvariae of these mice were unaffected, whereas the rest of the skeleton was affected by p.L438R Zip14. Both knock-in models, and mainly the osteoblast-specific knock-in mice, exhibit an increased cortical thickness due to an increased endosteal bone formation, which is the underlying cause of intracranial osteosclerosis in patients with HCI. Trabecular bone volume was differentially modulated by p.L438R Zip14, i.e. decreased (osteoblast knock-in) or unaffected (osteoclast knock-in). Moreover, p.L441R ZIP14 significantly increased cAMP-CREB signaling, known for its anabolic effects on osteoblasts. NFAT signaling was also significantly increased, which has been linked to the production of chemoattractants (TNF-?, IL-6) by osteoblasts to attract osteoclast progenitors and hence increase osteoclast numbers, as was seen in our osteoblast-specific knock-in mice. Collectively, we postulate that actions of mutant ZIP14 are similar to that of estrogen and IGF-1 and opposite to PTH, resulting in the calvarial phenotype of HCI patients and the skeletal phenotype of our conditional knock-in mice. We therefore identified the disease-causing gene for HCI and introduce ZIP14 as a novel gene in the field of bone homeostasis.
In vitro procedures exacerbate chromosome instability in cleavage-stage embryos

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Embryo chromosomal aneuploidy is a major factor contributing to embryo implantation failure and spontaneous miscarriage, and may explain the relatively low success rate of in vitro fertilization (IVF) procedures. Because only 30% of human conceptions result in live birth and chromosomal abnormalities are detected in at least half of all the spontaneous miscarriages, it is speculated that the post-zygotic events leading to CIN in IVF embryos may also occur in natural conceptions. Due to ethical and legal constraints associated with human embryo research, naturally conceived human cleavage-stage embryos are not accessible and chromosome instability (CIN) in human IVF and in vivo embryos cannot be compared directly. To compare chromosome stability in vitro versus in vivo cleavage-stage we used to bovine embryos, an adequate model for mimicking early human embryogenesis. We used five donor animals to analyse (1) in vivo embryos, (2) in vitro embryos produced after oocyte retrieval via ovum pick up with ovarian stimulation (OPU-IVF), and (3) in vitro produced embryos from in vitro-matured oocytes without ovarian stimulation (IVM-IVF). We applied an advanced genome analysis method for haplotyping and copy number profiling of single cells to investigate the influence of ART treatments on the rate and nature of CIN during early embryo development.

The study revealed that although CIN is also present in vivo embryos, in vitro techniques influence embryo development and survival. Genomic stability of single blastomeres in both in vitro-cultured embryo cohorts was severely compromised (P<0.0001), and the frequency of whole-chromosome and segmental aberrations was increased in embryos produced in vitro, compared to embryos derived in vivo. Only 18.8% of in vivo-derived embryos carried at least one blastomere with chromosomal abnormalities, compared to 69.2% of OPU-IVF (P<0.01) and 84.6% of IVM-IVF embryos (P<0.001). Our data represents a starting point for characterizing the differences in genome dynamics of early embryogenesis that can be influenced by in vitro manipulations of oocytes and early embryo. It also highlights the importance of understanding in vivo regulation of mammalian oocyte maturation and subsequent embryonic development to refine assisted reproduction in human. Therefore, in the absence of human data, it is of paramount importance to propose ART only to couples, who have a medical indication for IVF treatment.
O9: Detecting AGG interruptions in male and female FMR1 premutation carriers by single-molecule sequencing

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The FMR1 gene contains an unstable CGG repeat in its 5’ untranslated region. Premutation alleles range between 55 and 200 repeat units and confer a risk for developing fragile X-associated tremor/ataxia syndrome or fragile X-associated primary ovarian insufficiency. Furthermore, the premutation allele often expands to a full mutation during female germline transmission giving rise to the fragile X syndrome. The risk for a premutation to expand depends mainly on the number of CGG units and the presence of AGG interruptions in the CGG repeat: large CGG repeats with few AGG triplets interrupting the CGG repeat have the biggest risk to expand into a full mutations.

In spite of its importance, AGG measurement is not yet a standard feature of FMR1 diagnostic work-up because the detection of AGG interruptions is hampered by technical difficulties. Those difficulties are mainly encountered in females because the FMR1 CGG repeat on each X-chromosome camouflages each other’s repeat structure. By using single-molecule sequencing developed by Pacific Biosciences, reads derived from different alleles can be separated based on differences in repeat size and interruption pattern. A novel workflow was developed and validated on 7 males and 34 females. This workflow enables not only the determination of the repeat size, but also of the complete repeat sequence including AGG interruptions in male and female alleles with repeats ranging from 45 to 100 CGG units.

This method facilitates research and diagnostic analysis of the FMR1 repeat expansion. We now implement this method diagnostically.
**O10: Cost effective DNA copy number profiling of single cells without upfront whole-genome amplification**

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Single-cell sequencing is a powerful tool to investigate the biology of cellular heterogeneity and is revolutionizing different fields of life sciences. To sequence a single cell, whole-genome amplification (WGA) and subsequent library preparation is currently still the standard. While there are many approaches to achieve this, most are time consuming and expensive. We and others have previously shown that the detection of copy number aberrations (CNAs) can be performed by sparse sequencing of single-cell sequencing libraries. Thus, a large part of the cost for profiling CNAs in single cells is due to expensive WGA and the preparation of sequencing libraries. To enable studies that require sequencing of hundreds to thousands of single cells, we developed a novel cost and time effective method, called SC-NxtSeq. In SC-NxtSeq we omit the upfront whole-genome amplification step and immediately prepare sequencing libraries from single-cell lysates. We evaluated the robustness of our method by comparing SC-NxtSeq to conventional single-cell sequencing approaches using 7 different WGA technologies. The accuracy of the copy number profiles following SC-NxtSeq was at least as good as the other methods and often even better at more than an order of magnitude lower cost. Finally, to demonstrate that SC-NxtSeq can be used for primary tissue, we applied it on single cells biopsied from a human breast tumor. In conclusion, SC-NxtSeq is an accessible, low-cost and highly accurate method for CNA profiling of single-cell genomes.
011: Detection of copy number variation using shallow whole genome sequencing (CNVseq) as a cost-effective alternative to genomic microarrays.

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Genomic Copy Number Variations (CNVs) are an important cause of several human genetic disorders. Conventional karyotyping has been the gold standard for many years, but has been replaced by genomic microarrays, enabling a higher throughput and more important, a much higher resolution. More recently, next generation sequencing has been introduced in genetic diagnostics by means of targeted resequencing such as (clinical) exome sequencing. Despite the big advantage of whole exome sequencing (WES) to interrogate patient genomes at the nucleotide level to identify disease-associated single nucleotide variants, additional testing is still warranted to unequivocally detect, or rule out, CNVs. Although it has been proven that microarrays perform well for detecting submicroscopic CNVs, throughput is still rather limited, consumable cost is high, and resolution is fixed depending on the probe density of the microarray used. We therefore investigated whether genomic microarrays can be replaced by shallow whole genome sequencing for the detection of sub-microscopic copy number variants in a diagnostic setting.

First, a statistical model was built to predict the theoretical amount of sequence reads necessary for a certain resolution. Subsequently, this model was evaluated by shallow whole genome sequencing at several read depths and bin sizes for several patient samples with clinically relevant genomic aberrations. The DNA was isolated from blood, prenatal samples and tumour specimen. We defined the number of reads necessary to achieve the same resolution as with commonly used microarrays and a thorough comparison between genomic microarrays and CNVseq was made. Moreover, we show that CNVseq results in a higher dynamic range and is more sensitive for the detection of low-grade mosaicism. Several clinical samples were run through our pipeline with mosaic CNVs of variable sizes to validate the detection potential of mosaic aberrations.

Since library preparation is completely automatable and many samples can be pooled on one sequencing run, the hands-on time diminishes. Together with the plummeting cost of sequencing, this results in a more cost-effective workflow and a higher throughput. Furthermore, resolution is merely dependent on sequencing depth and is not limited to the probes present on the array, hence CNVseq enables more flexibility compared to genomic microarrays.
012: TCGAbiolinksGUI: A graphical user interface to analyze cancer molecular and clinical data.

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The National Cancer Institute’s (NCI’s) Genomic Data Commons (GDC), a data sharing platform that promotes precision medicine in oncology, provides a rich resource of molecular and clinical data of more than 14,000 tumor patients across 38 different cancer types and subtypes. The platform includes data from The Cancer Genome Atlas (TCGA) and Therapeutically Applicable Research to Generate Effective Treatments (TARGET). The data, which is publicly available, have been utilized by Researchers to make novel discoveries and/or validate important findings. To enhance these findings, several important bioinformatics tools to harness genomics cancer data were developed, many of them belonging to the Bioconductor project ((Gentleman et al. 2004)). Among those tools is our tool TCGAbiolinks ((Colaprico et al. 2016)), which was developed in order to facilitate the analysis of TCGA data by incorporating the query, download and processing steps within the Bioconductor project ((Gentleman et al. 2004)). This tool allows users to integrate TCGA data with Bioconductor packages thus harnessing a wealth of statistical methodologies for biologically derived data. In addition, it provides integrative methodologies to perform several important downstream analyses, such as DNA methylation and Gene expression integration. Although TCGAbiolinks is a suitable package for most data analysts, its target audience is users with a strong knowledge and familiarity with R. To enable user access to the methodologies offered in TCGAbiolinks (specifically biologists with limited knowledge of R/programming), we developed a new package which takes in all the important features of TCGAbiolinks and offers a graphics user interface (GUI). Here we present TCGAbiolinksGUI an R/Bioconductor package which uses the R web application framework shiny ((Chang et al. 2016)) to provide a GUI to process, query, download, and perform integrative analyses of TCGA data.

The user interface of TCGAbiolinksGUI has been divided into three main types of menus. The first type defines the acquisition of GDC data (search, download and processing). The second defines the analysis steps which were subdivided according to the molecular data types (e.g. DNA methylation, Gene Expression, Mutation) as well as clinical data types (e.g. survival). And the third one is dedicated to harnessing integrative analyses. We designed the GUI to control parameters of the analysis and visualization functions and to export the results from the analysis as a csv spreadsheet. Moreover, we provide a guided tutorial for users via a vignette document detailing each steps and menu functions.
TCGAbiolinksGUI was used for training activities during our last course ‘Mining and analysis of genomic and epigenomic data (TCGA) using R’


It is possible to have an overview looking at this tutorial:
http://www.ulb.ac.be/di/map/colsen/biopark/day2/Biopark_TCGA_day2_tutorialGUI.pdf

TCGAbiolinks is an R Bioconductor package as a collaborative’s project with several universities dealing with GDC’s data.


TCGAbiolinks is in the top 5% of Bioconductor’s packages downloaded since 2015
http://bioconductor.org/packages/stats/bioc/TCGAbiolinks/

TCGAbiolinks was recently used for section (4) mRNA Expression and (5) DNA methylation profiling in last TCGA’s marker paper published in early 2016.

https://tcga-data.nci.nih.gov/docs/publications/

See citation in supplementary informations.
O13: Evidence that bi-allelic mutations in NPR3 result in a peculiar phenotype with tall stature, arachnodactyly, long hallucus and multiple extra epiphyses in hands and feet

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Studies in mice and humans have shown that natriuretic peptides and their corresponding receptors control endochondral ossification and linear bone growth. Reciprocal translocations resulting in an increased production of the C-type natriuretic peptide (CNP) and heterozygous activating mutations in the natriuretic peptide receptor 2 (NPR2) have been reported in individuals with tall stature. In contrast, bi-allelic inactivating mutations in NPR2 have been identified in patients with severe short stature (acromesomelic dysplasia Maroteaux type) while heterozygous inactivating mutations in the same gene are found in patients with an idiopathic short stature.

With this paper we provide strong evidence that bi-allelic hypomorphic mutations in the clearance receptor NPR3 can also result in enhanced growth. Compound heterozygosity for a missense (NM_001204375.1, c.442T>C; p.Ser148Pro) and a nonsense mutation (NM_001204375.1, c.1524delC, p.Tyr508*) in NPR3 was identified by whole exome sequencing in two affected sibs born to non-consanguineous, healthy parents of Dutch origin. Both the parents and the unaffected younger sib are heterozygous carriers.

Using mRNA isolated from whole blood, we demonstrated that the nonsense mutation resulted in nonsense mediated mRNA decay while localization studies revealed that the NPR3 receptor with the p.Ser148Pro substitution was no longer incorporated into the plasma membrane. These data suggest that both mutations have a loss-of-function effect on the protein. The phenotype observed in both affected boys was characterized by tall stature (Marfanoid habitus), generalized joint hyperlaxity with hypotonia, mild pectus excavatum, long fingers (arachnodactyly) and markedly long hallucus. Furthermore, more recently aorta dilatation (z-score +2.09) was also reported in the oldest patient. In addition, hand radiographs of both patients revealed the presence of extra (pseudo)epiphyses in first metacarpals and all proximal and middle phalanges. Extra (pseudo)epiphyses were also visible in the proximal phalanges of both feet. This remarkable radiographic finding is retrospectively also visible in the family with an activating mutation of NPR2 reported by Miura K et al. (PLoS ONE 2012). It suggests that enhanced growth due to defects in either the NPR2 or NPR3 controlled signaling pathways may be partially mediated through the creation of extra growth plates in tubular bones.
014: Loss-of-function mutations in the X-linked gene BGN cause a severe syndromic form of thoracic aortic aneurysms and dissections

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Thoracic aortic aneurysm and dissection (TAAD) is typically inherited in an autosomal dominant manner but rare X-linked families have been described. So far, FLNA is the only X-linked gene associated with a syndromic form of TAAD, namely the periventricular nodular heterotopia type of Ehlers-Danlos syndrome. However, FLNA only explains a small number of the X-linked TAAD families.

We performed targeted resequencing of nearly 500 extracellular matrix and TGFβ related genes in a cohort of 11 Marfan-like probands without known causal mutation. We identified two patients with loss-of-function mutations in BGN, encoding the extracellular matrix small leucine-rich proteoglycan biglycan. Subsequent Sanger sequencing of BGN in 400 male and 200 female TAAD-patients, negative for known TAAD genes, identified a splice site mutation in a male proband and suggested a deletion in two other male probands. The latter were confirmed by micro-array analysis. The clinical phenotype is characterized by early onset aortic aneurysm (as young as age 1) and dissection (as young as age 15). Other recurrent findings include hypertelorism, pectus deformity, joint hypermobility, contractures and mild skeletal dysplasia. Histological stainings of the patients’ aortic wall revealed a low to normal collagen content, while elastin fibers appeared normal. An increase in TGF-beta signaling was observed via an increase in nuclear pSMAD2. Biglycan deficiency in male BALB/cA mice leads to sudden death from aortic rupture, indicating that biglycan is both structurally and functionally essential for the integrity of the aortic wall.

These results confirm that BGN gene defects in human cause an X-linked syndromic form of severe TAAD.
015: Mutations in ATP6V1E1 or ATP6V1A Cause Autosomal Recessive Cutis Laxa

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Defects of the V-type proton (H+) ATPase (V-ATPase) impair acidification and intracellular trafficking of membrane-enclosed compartments including secretory granules, endosomes and lysosomes. Whole-exome sequencing in five families with mild to severe cutis laxa, dysmorphic facial features and cardiopulmonary involvement identified biallelic missense mutations in ATP6V1E1 and ATP6V1A. These genes encode the E1 and A subunits of the V1 domain of the heteromultimeric V-ATPase complex, respectively. Structural modeling indicated that all substitutions affect critical residues and inter- or intrasubunit interactions. Furthermore, complexome profiling, a method combining blue native gel electrophoresis and liquid chromatography tandem mass spectrometry, showed that they disturb either the assembly or the stability of the V-ATPase complex. Protein glycosylation was variably affected. Abnormal vesicular trafficking was evidenced by delayed retrograde transport after Brefeldin A treatment and abnormal swelling and fragmentation of the Golgi apparatus. In addition to reduced and fragmented elastic fibers, the histopathological hallmark of cutis laxa, transmission electron microscopy of the dermis also showed pronounced changes in the structure and organization of the collagen fibers. Our findings expand the clinical and molecular spectrum of metabolic cutis laxa syndromes and further link defective extracellular matrix assembly to defects in protein processing and cellular trafficking caused by genetic defects in the V-ATPase complex.

Also on behalf of the other contributors:

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016: The clinical relevance of intragenic NRXN1 deletions

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Neurexins (NRXNs) are cell-adhesion molecules concentrated at the synapses. They are involved in synapse formation, differentiation and function, which makes them important in brain development and function. Rare intragenic copy number variants (CNVs) affecting NRXN1 have been associated with neuropsychiatric disorders, such as developmental delay, language disorders, autism spectrum disorder, epilepsy, schizophrenia and aspecific dysmorphic features. Understanding the factors that contribute to variable expressivity and penetrance is a key goal for clinical genetics. Although there is evidence that the intragenic position of the variant may be an important factor (e.g. exonic versus intronic variants, 3' versus 5' variants or alpha versus beta isoform affecting variants), no guidelines are available to assess the clinical relevance of an intragenic CNV in a particular patient or family. Well described patient cohorts are relatively small (less than 30 patients). Moreover, combining genotype-phenotype data from different studies and case reports is hampered by differences in ascertainment, by selective reporting (e.g. single case reports describing mainly exonic deletions) and by the lack of phenotypic data in large scale CNV studies. In addition, the exact CNV breakpoints have rarely been determined which makes the delineation of the variant dependent on the local resolution of the array platform. Despite these limitations, we analysed data of 707 individuals, including 170 controls, with an intragenic NRXN1 variant to derive guidelines for the clinical interpretation of intragenic NRXN1 deletions. In addition, we will apply the new knowledge in the genetic counselling of 46 patients with intragenic NRXN1 deletions that have been identified in Leuven.

The study cohort was assembled through data extraction from 74 literature reports and consists of 385 probands and 152 relatives from clinical cohorts or case reports and 170 individuals from control cohorts. All individuals in the study cohort are characterised by clinical data, molecular data (position and size of the variant) and family relationships. The phenotypes of all probands and relatives have been reviewed by a clinical geneticist and all cases were assigned to a particular phenotype category according to the major presenting clinical diagnosis. All reports providing data from control cohorts were extensively reviewed and individuals were assigned to either the group of ‘screened’ or ‘unscreened’ controls. The described literature cohort consists mostly of heterozygous deletions (668), hence the following analyses will focus hereupon.

First, the prevalence of NRXN1 deletions was estimated to be 0.0032 (99% confidence interval: 0.0027 – 0.0038), 1/300, based on 39 unscreened control cohorts (233 deletions in 71,796 individuals). Second, analysis of the intronic deletions in controls revealed the presence of an apparently common ~65 kb deletion within intron 5 that had also been described in patients. Furthermore, the distributions of intronic NRXN1 deletions in controls and reported probands were very comparable, with de novo intronic deletions occurring very rarely in probands (only in 2 out of 64 intronic deletions with inheritance information). Finally, analysis of the exonic deletions in controls revealed only 18 out of 162 control deletions included in our dataset were exonic and, with the exception of one variant, all these exonic deletions in controls affected one or more of the 5' exons...
(exons 1 to 5). Following the latter observation, the 70 probands reported with deletions affecting 3’ exons (exon 6 until exon 24) were studied in more detail. For 35 of these 3’ exonic deletions, inheritance information was available showing 18 de novo, 13 maternally inherited and 4 paternally inherited cases. Moreover, the 3’ deletions for which segregation information was available (17) were de novo in 7 probands with sporadic neurodevelopmental disorder and inherited from a parent with cognitive problems or a neuropsychiatric disorder in 6 probands.

In conclusion, the results of the analysis provide additional support for a pathogenic effect of exonic NRXN1 deletions. However, unlike the conclusion from a recent report by Lowther et al. (2016), these results support the importance of 3’ exons in the expression of neurodevelopmental disorders and provide evidence for a higher penetrance of 3’ deletions as compared to 5’ deletions. The relationship between these findings and the particular phenotypic characteristics, such as intellectual disability, epilepsy and autism, is currently being studied in the literature cohort. Guidelines for counselling will further be applied and illustrated in the Leuven patient cohort.
017: Which next generation sequencing results are reported to clinicians? A qualitative study

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Next-generation sequencing (NGS) technologies can identify causative mutations in individuals who would have previously remained undiagnosed, influencing their treatment and providing important genetic counselling information to their families. However, they also have the potential to identify additional genomic information that is unrelated to their illness, such as variants of uncertain significance (VUS) and mutations in genes causing phenotypes extraneous to the clinical question (incidental findings, or IFs). Recommendations by professional bodies outlining which findings should be reported by laboratories to the clinician requesting the test are inconsistent. Little is known about which variants laboratories report to genetic health professionals and how they make decisions about this.

In-depth interviews were conducted with 27 personnel from 24 laboratories in Europe (12), Canada (5) and Australasia (7) to explore reporting practices for diagnostic NGS. Although the laboratory personnel interviewed were generally comfortable with the reporting practices within their laboratory, they discussed a range of challenges their teams experience. Participants highlighted decisions regarding which VUS to report as challenging and reporting practices often differed between laboratories depending on the perception of its relevance to the clinical question. There was considerable variation in the filtering strategies that participants described. While some laboratories use quite stringent filtering in order to limit the number of IFs that are identified, others are less strict, allowing identification of these variants.

Differences were also seen in the reporting of IFs between laboratories with some limiting reporting to variants which relate to the phenotype of the patient and others reporting IFs which may or may not be medically actionable. Determining whether to report incidental findings involved detailed discussions by committees usually composed of molecular and clinical geneticists and ethicists, to consider the potential benefit of returning incidental findings to the patient or their family. The participants highlighted the importance of close relationships with the referring genetic health professional in order to both assess the pathogenicity of variants and also determine which IFs are relevant to report.

Our study highlights that laboratories are still grappling with decisions about which variants to report to genetic health professionals. These findings will assist laboratories to learn from each other’s experiences, to enhance the relationships between laboratory personnel and clinicians, and also to refine reporting guidelines.
Poster Presentations
**P1: Strategies and clinical outcome of Preimplantation Genetic Diagnosis for Polycystic kidney disease.**

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In this study, preimplantation genetic diagnosis (PGD) data for polycystic kidney disease (PKD) from 2005 until 2015 are reported. Fifteen single-cell clinical tests for PKD based on STR (short tandem repeat) marker polymerase chain reaction (PCR), with or without a specific mutation were developed and were applied for 73 cycles in 35 couples (25 for polycystic kidney disease 1 (PKD1), 2 for polycystic kidney disease 2 (PKD2) and 8 for autosomal recessive polycystic kidney disease (ARPKD)). In total, 450 embryos were biopsied and a diagnosis was obtained for 95.1% of the analysed embryos of which 174 were genetically transferable. Transfer of 68 embryos in 48 fresh cycles resulted in an implantation rate of 35.3% and a delivery rate of 39.6% per embryo transfer with 17 singleton and 2 twin deliveries. Transfer of 23 cryopreserved embryos in 21 FET (frozen-thawed embryo transfer) cycles resulted in an implantation rate of 43.5% and a delivery rate of 42.9% per FET with 9 singleton deliveries. The cumulative delivery rate was 62.9% per couple after five treatment cycles.
P2: A search for digenic inheritance in primary microcephaly using patients exome data and zebrafish mutants

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Disorders of brain development carry a heavy burden of intellectual deficit, autism, or epilepsy. Primary microcephaly (PM) is a fascinating disorder of brain development where the brain is too small since birth, which serves as a model disease for the study of brain growth and of neuronal organization of the cerebral cortex in humans. Many causal genes have been identified, but the mechanism(s) of PM remain unclear. Specifically, while many PM genes are expressed at the centrosome in experimental conditions, some are expressed in other cellular compartments, and it is not clear whether such expression data truly reflect distinct pathways. Here, we test for digenic inheritance of selected pairs of genes known to cause PM, because digenic inheritance constitutes evidence for functional interaction in a common pathway. We use two independent, holistic, in vivo approaches: 1) zebrafish modeling of digenic inheritance by knock-out of 3 genes in zebrafish lines which will be crossed to produce double heterozygous knock outs, for genes of the same, or of two distinct pathways implicated in PM by current models; 2) analyzing exomes from PM patients in search of new PM genes or cases of digenic inheritance, and develop a bioinformatic tool to identify digenic inheritance in a predefined subset of 68 genes known to cause PM, using sequencing data of whole exomes from PM patients, compared to exomes of non PM-patients. We performed Mutation burden tests and observed a significant excess of variants in a predefined subset of 68 PMs-related genes in cases in comparison to controls. When performing the same experiment with different subsets of control (non neural) genes, we observed no significant differences between cases and controls. Our project should identify new causes of PM, give direct in vivo evidence for one or for distinct pathways in PM, by-passing possible limitations of in vitro approaches, and produce a bioinformatics predictor of digenic inheritance in PM.
P3: Selective single base deletion in BCL11A gene is associated with severe oral, speech and manual dyspraxia

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We report the case of a 7-year-old Caucasian male presenting moderate intellectual disability and a speech disorder with severe childhood apraxia of speech characterized by oral motor dyspraxia and impaired phonological processing, excessive drooling, and manual dyspraxia.

Since genetic factors are important contributors to language and learning development, we first performed a 180K CGH-array (Agilent) which returned normal results. However, by exome sequencing, we detected a heterozygous cytosine deletion in the fourth exon of BCL11A, a gene recently demonstrated as being involved in cognition, and language development (Dias et al. 2016). This single base deletion causes a frameshift leading to a premature stop codon, and occurred de novo; two elements in favor of its pathogenicity. The depth sequencing of parent’s lymphocyte DNA failed to revealed a somatic mosaicism.

Together with a previously described patient with a 200kb 2p15p16.1 deletion limited to the entire BCLA11 gene displaying similar phenotype, we characterize in depth how BCL11A is involved in clinical aspects of language development, and oral praxis.
P4: Do we need to screen Smith-Magenis Syndrome patients for Renal Tumors?

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Smith-Magenis syndrome (SMS) is a contiguous-gene disorder most commonly caused by a deletion of chromosome 17p11.2. It is characterized by intellectual disability, behavioral abnormalities, characteristic facial features and variable organ malformations. Renal anomalies have been reported in 15-35%, but kidney tumors have never been observed in association with SMS. We report a 57 year-old man with SMS who presents bilateral renal tumors. This is most likely related to haploinsufficiency of FLCN gene, located in the deleted region, and a known tumor suppressor gene. Haploinsufficiency of FLCN causes Birt-Hogg-Dubé syndrome (BHDS), characterized by pulmonary cysts, renal and skin tumors. The present observation suggests that the follow-up of patients with SMS should also focus on possible manifestations of BHDS.
PS: Troyer Syndrome in three Moroccan siblings with a novel nonsense mutation in the SPG20 gene

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Troyer syndrome is an autosomal recessive form of complicated hereditary spastic paraplegia, characterized by progressive lower extremity spasticity and weakness, dysarthria, distal amyotrophy, developmental delay, short stature, and subtle skeletal abnormalities. It is caused by deleterious mutations in the SPG20 gene, encoding spartin. Until now, 6 unrelated families with a genetically confirmed diagnosis have been reported. Here we report the clinical findings in three brothers of a consanguineous Moroccan family, with spastic paraplegia, short stature, motor and cognitive delay and severe intellectual disability. Targeted exon capture and sequencing showed a homozygous nonsense mutation in the SPG20 gene, c.1369C>T (p.Arg457*), in the three affected boys.
P6: Five new cases with FATCO syndrome

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Fibular aplasia, tibial campomelia and oligosyndactyly (also termed FATCO syndrome) is a descriptive term for a syndrome with thus far unknown aetiology. Fifteen cases have been reported in the literature. We report the variable clinical findings of 5 patients with a probable diagnosis of FATCO syndrome. As previously reported, all cases we observed were sporadic, suggesting a dominant de novo mutation. We will discuss the clinical presentation and differential diagnosis.
P7: AZFb deletions compatible with sperm production?!

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Background: Deletions of the long arm of the Y chromosome are a well-known cause of male infertility. The detection of a Yq microdeletion is of diagnostic and prognostic use: only AZFc deletions are compatible with sperm production (in ~70% of patients). Since 2014 new EMQN guidelines have been endorsed for the detection and interpretation of Yq microdeletions. An important revision is the inclusion of the extension analysis, i.e. in case of the detection of a microdeletion, extra markers should be analysed.

Aim: In a routine setting, we test for the presence of Yq microdeletions according to the new guidelines. We have a special interest in the AZFb region.

Methods: The standard multiplex PCR reaction is performed in duplex, as instructed by Simoni et al., 2004 and Krausz et al., 2014. When a deletion is detected, we continue with the suggested extension analysis (Krausz et al., 2014). In case an AZFb deletion is detected, markers sY105, sY1224, sY1192 and sY153 (from proximal to distal) are tested. Markers sY1224 and sY1192 are expected to be absent in case of an AZFb deletion.

Results: We have detected two patients with residual sperm production, where the first Yq microdeletion test shows the absence of markers sY127 and sY134, located in the AZFb region. For the first patient, extension analysis showed that markers sY105 and sY1224 are absent, while markers sY1192 and sY153 are present. For the second patient, only sY1224 was shown to be absent.

Conclusion: We have detected two patients, were the 'old' Yq microdeletion test would classify the patients as having a 'complete AZFb' deletion. However, the extension analysis showed an atypical pattern. Presumably, a re-organisation of the AZFb region has occurred, resulting in a partial absence of genes in this region. Overall, these results show the importance of performing the extension analysis for patients with a Yq microdeletion, especially in case of an AZFb deletion. For patients with an atypical AZFb deletion, testicular sperm extraction might still be an option.
P8: Novel PIGN mutations in a patient with multiple congenital anomalies hypotonia seizures syndrome

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Introduction: Mutations in the phosphatidylinositol-glycan biosynthesis class N (PIGN) gene are causing defects in the glycosylphosphatidylinositol (GPI) anchor biosynthesis pathway. Problems in this pathway are associated with multiple autosomal recessive disorders, often involving the central nervous system. Mutations in PIGN are causing 'multiple congenital anomalies hypotonia seizures syndrome 1' (MACHS1).

Materials and Methods: Exome sequencing was performed in an 12-year old child from non-consanguineous parents from Belgian/Peruvian origin. The girl presented with early onset hypotonia and developmental delay. She had dysmorphic features including a flat nasal bridge and prominent nose. Over the years she remained severely delayed and hypotonic with choreo-athetotic movements and generalized epilepsy, responsive to treatment. ERG was abnormal. The MRI of the brain showed progressive cortical atrophy.

Results: Exome sequencing showed the presence of biallelic variants, c.1158delG, p.Leu386Phefs*17 and c.T956G, p.Val319Gly in the PIGN gene (RefSeq: NM_012327.5) as Sanger sequencing analysis of the parents showed the independent segregation of the two observed alterations.

Conclusions: Exome sequencing showed the presence of two novel changes in the PIGN gene causing MACHS1. The c.T956G alteration is predicted to be pathogenic (SIFT score 0 and PolyPhen score 0.986); while c.1158delG change is completely disrupting the protein. Consequently, MACHS1 in this patient has been confirmed.
P9: First the gene, then the syndrome: reverse syndromology illustrated by a child with a BCOR mutation.

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Introduction: Mutations in the BCL6 Corepressor (BCOR) gene, located on Xp11.4, are linked to OculoFacioCardioDental syndrome (OFCD, MIM 300166). It affects both male and female patients and ophthalmological symptoms are often predominant.

Materials and Methods: Exome sequencing was performed in a boy of Caucasian, non-consanguineous and healthy parents. He has one younger healthy sib. Intrauterine growth retardation was observed from 32 weeks of gestation on and he was born at 40 weeks with a weight of 2485 g and length of 46 cm. Severe pulmonary hypertension made ECMO necessary in the neonatal period. The child has delayed psychomotor development and attends a special school. At the age of 4 years, he still needs tube feeding because of severe feeding difficulties. Cardiac ultrasound shows a persistent ductus arteriosus and ophthalmological examination reveals mild microcornea. Facial gestalt is characterized by small palpebral fissures, synophris, bilateral epicanthic folds, small ears, thin upper lip and widely-spaced small teeth. Pro- and supination are limited due to radio-ulnar synostosis. Hypospadias and cryptorchidism are present.

Results and conclusions: Exome sequencing showed the presence of a hemizygous variant, c.5000C>T, p.Ser1667Leu in the BCOR gene (RefSeq: NM_001123385.1). This alteration of a highly conserved amino acid is predicted to be pathogenic (SIFT score 0.01, MutationTaster score 1, PolyPhen score 1.000). This change has not been detected in dbSNP, and ExAC shows a very low population frequency (2.3x10⁻⁵). The observed clinical features seen in this patient correspond very well with the phenotype for alterations in the BCOR gene as reported in the literature.
P10: In-house developed 15-gene sequencing panel for acute myeloid leukemia and myelodysplasia allows robust detection of genetic defects including FLT3-ITD and CEBPA mutations

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The mutational landscapes in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) reveal distinct molecular subgroups that provide insight into the pathophysiology of AML/MDS and aid in the disease classification and prognostic stratification. In daily routine practice, these clinically-relevant genetic defects are characterized using different molecular techniques. To achieve a uniform and cost-effective workflow, we developed and validated an in-house PCR-based enrichment strategy followed by massive parallel sequencing on a MiSeq benchtop sequencer for mutation detection in AML and MDS. Our AML-MDS panel covers 15 genes with proven diagnostic, prognostic or therapeutic significance in a high proportion (mutation frequency >5%) of well-described AML-MDS patient populations. Single nucleotide substitutions as well as insertions and deletions are reliably detected down to 5% variant allele frequency (VAF) with a minimal coverage of 300x for each region of interest. By enrichment of CEBPA amplicons in each patient-specific pool, a high coverage (>300x) of the entire CEBPA gene was achieved in all samples, in contrast to what can be obtained with commonly used commercial kits. To a similar extent, optimization of our datamining workflow enabled robust detection of FLT3 internal tandem duplications (ITDs) ranging from 21 bp to 189 bp with a VAF <5%. Comparison of the NGS data of 30 diagnostic AML or MDS samples to the results obtained by standard techniques showed 100% concordance. Inter-run and intra-run imprecision values (CV%) of the VAF were, on average, 3.5 and 4.5%, respectively. In conclusion, we designed and validated a 15-gene NGS panel for mutation detection in AML-MDS, showing 100% concordance for all mutational targets including FLT3-ITD and CEBPA mutations. Furthermore, we successfully implemented the in-house cost-effective assay for mutation detection in routine diagnostics. Of importance, our in-house designed NGS workflow allows a flexible and rapid adaptation of the NGS panel by adding new clinical-relevant gene targets and can serve as a model for analysis of other cancer types and acquired malignancies.
P11: Cohort study of Kabuki syndrome, phenotype comparison of KMT2D and KDM6A mutated patients.

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Kabuki syndrome was first described in 1981. The five cardinal features of the syndrome are a characteristic face, skeletal anomalies, unusual dermatoglyphic patterns, mild to moderate intellectual deficiency and postnatal growth retardation. In 2010, KMT2D (previously MLL2) mutations were found in 9 patients with clinical diagnosis of Kabuki syndrome by exome sequencing. Since the discovery of KMT2D, 9 cohorts of patients with Kabuki syndrome were published and a KMT2D mutation was found in 34-76% of patients. In 2012, genic and exonic deletions of KDM6A were found in three patients. A role for KDM6A in Kabuki syndrome was then confirmed by four studies.

Since 2012, we have collected 318 patients with a clinical suspicion of Kabuki syndrome. We found a mutation in KMT2D in 87 of them for a diagnostic yield of 34%. Clinical data were available for 54 patients and confirmed the 5 cardinal features of Kabuki syndrome. Feeding difficulties were present in 75% of infants but BMI study showed that they tend to be overweight after 8 years old. Palate abnormalities, hearing loss, cardiac malformations and recurrent infections were common. A phenotypic association study showed that cleft palate and cardiac defect are often associated and are part of a more severe phenotype.

Including our cohort, 444 mutations in KMT2D were described. Truncating and splice mutations are scattered along the gene and missense mutations occur recurrently in few hotspots in or outside protein functional domains.

In our cohort, we found 14 mutations in KDM6A (4%). In total, 28 patients were reported with a mutation in KDM6A in our study and in the literature. Clinically, boys are more severely affected but some girls present with a severe phenotype. Typically, patients with KDM6A mutations present with dysmorphism, developmental delay, short stature, microcephaly, feeding difficulties, hypotonia, hypertaxity, hirsutism and congenital heart defect. The face is less typical and large superior incisors were seen in some patients. Chronic hyperinsulinism was reported in 4 patients. Even though not statistically significant, some features were more frequent in patients with KDM6A mutations, such as growth retardation, microcephaly and hirsutism. On the other hand, cleft palate and uro-genital malformations were more frequent in individuals with KMT2D mutations.

Moreover, with an encephalopathy gene panel, we detected 3 de novo mutations, 1 missense in KMT2D and 2 frameshift in KDM6A. Interestingly, the missense mutation in KMT2D occurred outside the amino acids typically substituted in KS. Another splice mutation was detected in KMT2D. In those 4 patients, KS was not diagnosed before gene panel analysis and reverse dysmorphology did not lead to a diagnosis of KS.
In conclusion, this study described an extended phenotypic spectrum for KMT2D and KDM6A mutations, from typical KS to isolated encephalopathy. Mild differences in KMT2D and KDM6A phenotype could reflect differences in the developmental role of those genes.
P12: Targeted next-generation sequencing using BRCA Tumor MASTR Plus Dx is an accurate and precise diagnostic method for BRCA1 and BRCA2 mutation detection in formalin-fixed paraffin-embedded tissue-derived DNA

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Targeted next-generation sequencing (NGS) has tremendous potential in clinical diagnostics as it allows oncogenetic profiling to steer targeted therapy. Inhibitors of poly-(ADP-ribose) polymerase (PARPi) have emerged as a new class of targeted anti-cancer drugs, specifically for tumors showing homologous recombination repair deficiency, including BRCA1- and BRCA2- mutated ovarian and breast cancers.

This multicentre study evaluated the performance of BRCA Tumor MASTR Plus Dx (Multiplicom, Belgium) to routinely diagnose somatic and germline BRCA mutations in formalin-fixed paraffin-embedded (FFPE) tumor tissue-derived DNA.

Three genetic centres performed the BRCA Tumor MASTR Plus Dx to detect single nucleotide variants (SNV) and small indels in the BRCA genes at a variant allele frequency as low as 5%. The sample population comprised 54 FFPE-derived DNA extracts from 51 clinical and 3 reference samples. DNA extracts were subjected to quality control using Multiplicom’s QC plex. The clinical samples were characterized using an independent targeted NGS method and Integrative Genomics Viewer (IGV) analysis of the mapped raw reads. Data analysis was performed with the SeqNext module version 4.1.2 (JSI Medical Systems) or the Sophia DDM platform (ILL1MR1SS_BRCA_Tumorv2). The target region was defined as the BRCA1 and BRCA2 coding regions +/- 2 bp.

BRCA Tumor MASTR Plus Dx showed a uniformity of amplification of 93.9%, i.e. the percentage of amplicons with at least 0.2x the mean amplicon coverage, and a target specificity of 99.1%. The limit of detection (LOD) proved to be as low as 1%. The diagnostic accuracy was 99.99% [95% CI ? 99.98%] (100% sensitivity [95% CI ? 99.02%] and 99.99% specificity [95% CI ? 99.98%]) using JSI SeqNext data analysis software and 100% [95% CI ? 99.99%] using the Sophia DDM pipeline. Both repeatability and reproducibility were 99.99% [95% CI ? 99.98%] using SeqNext and 100% using the Sophia DDM pipeline [95% CI ? 99.99%]. Regardless of the data analysis approach, lot equivalence was 100% [95% CI ? 99.99%].

This multicentre study demonstrated that BRCA Tumor MASTR Plus can be routinely applied as an accurate and precise method with a low LOD. The assay can be used as a companion diagnostic to direct patients carrying somatic or germline BRCA1 or BRCA2 mutations to PARPi therapy. An additional performance evaluation study for BRCA Tumor MASTR Plus and Multiplicom’s MASTR Reporter data analysis software is currently ongoing and will be available soon.
P13: Homozygosity mapping-guided exome sequencing in LCA patients of consanguineous origin reveals mutations in known genes and a novel candidate gene.

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Purpose: Leber congenital amaurosis (LCA) is the most severe autosomal recessive inherited retinal dystrophy (IRD) accounting for 5% of childhood blindness. To date, 70% of LCA cases can be explained by mutations in one of the 23 known LCA genes. Here we aimed to identify the underlying genetic cause of LCA in 15 Saudi-Arabian families with reported or suspected consanguinity.

Methods: A total of 20 probands, ranging between 2-9 years old, were clinically diagnosed with LCA or early-onset IRD. The genetic workup consisted of homozygosity mapping followed by targeted next generation sequencing (NGS) or Sanger sequencing, combined or not with whole exome sequencing (WES). Co-segregation could be demonstrated for all identified mutations.

Results: Overall, we identified 13 putative pathogenic homozygous mutations in 10 known IRD genes in 14/15 (93.3%) of the studied families, six of which are novel. Eight of these genes were known LCA genes: CRB1 (3/15, 20%), RPGRIP1 (2/15, 13.3%), SPATA7 (2/15, 13.3 %) and CABP4, CEP290, GUCY2D, MERTK, RDH12 (1/15, 6.7% for the latter five) respectively. Specifically, the recurrent RPGRIP1 mutation c.1007delA p.(Glu370Asnfs*5) is a reported potential founder mutation in the Saudi population (Khan et al. 2014). Moreover, mutations were found in ATF6 and ALMS1 (1/15, 6.7% for each), known to be implicated in autosomal recessive achromatopsia and in Alström syndrome, respectively. In two affected sibs with LCA and autism, we identified a novel nonsense mutation c.3283C>T p.(Arg1095*) in the Regulating Synaptic Membrane Exocytosis 2 (RIMS2) gene (NM_001100117.2), located in the largest autozygous region of 25 Mb. RIMS2 was found to be expressed in human retina and in cerebral cortex, which might be consistent with the phenotype observed in the sibship. A mutation in a paralog RIMS1 was previously reported in autosomal dominant cone-rod dystrophy (CORD7; MIM 603649), implicating a protein with a synaptic function in retinal disease.

Conclusions: We identified 14 putative pathogenic mutations in all studied LCA families, demonstrating the power of autozygome-guided WES in a genetically heterogeneous consanguineous IRD cohort. Apart from mutations in known IRD genes (14/15; 93.3%), we uncovered RIMS2 as a potential novel LCA gene.
P14: Bi-allelic variants in COL3A1 encoding the ligand to GPR56 are associated with cobblestone-like cortical malformation, white matter changes and cerebellar cysts

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Background: Cobblestone lissencephaly is a malformation of cortical development, associated with an impaired neuronal migration and cortical lamination beyond the pial membrane during the formation of the neocortex. Collagens are one of the major constituents of this pial membrane. Type III procollagen, the chains of which are encoded by COL3A1, is the ligand of the G protein-coupled receptor 56 (GPR56), also known as Adhesion G protein-coupled Receptor G1 (ADGRG1). Bi-allelic mutations in GPR56 give rise to cobblestone-like malformation, white matter changes and cerebellar dysplasia. This report shows that bi-allelic mutations in COL3A1 are associated with a similar phenotype.

Methods: Exome analysis was performed in a family consisting of two affected and two non-affected siblings. Brain imaging studies of this family and of two previously reported individuals with bi-allelic mutations in COL3A1 were reviewed. Functional assays were performed on dermal fibroblasts.

Results: Exome analysis revealed a novel homozygous variant c.145C>G (p.Pro49Ala) in exon 2 of COL3A1. Brain MRI in the affected siblings as well as in the two previously reported individuals with bi-allelic COL3A1 mutations showed a brain phenotype similar to that associated with mutations in GPR56. Functional analysis of dermal fibroblasts showed a significant upregulation of COL3A1 transcript in our patients.

Conclusion: This report shows that homozygous or compound heterozygous mutations in COL3A1 are associated with cobblestone-like malformation in all three families reported to date. The variability of the phenotype across patients suggests that genetic alterations in distinct domains of type III procollagen can lead to different outcomes. The presence of cobblestone-like malformation in patients with bi-allelic COL3A1 mutations emphasizes the critical role of the type III collagen-GPR56 axis and the pial membrane in the regulation of brain development and cortical lamination.
P15: Small molecule inhibitors in the treatment of venous malformation.

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Venous malformations are localized developmental defects of the vasculature associated with significant clinical morbidity. Current treatments are limited to surgery and sclerotherapy. We previously discovered that somatic activating mutations in TEK, encoding the endothelial cell tyrosine kinase receptor TIE2, and PIK3CA, encoding the p110α catalytic subunit of PI3K, cause 60% and 20% of sporadic VMs, respectively. Mutations in both proteins activate the PI3K/AKT pathway, resulting in the inactivation of FOXO1 and dysregulation of its transcriptional targets, extracellular matrix and mural cell layer irregularities, as well as abnormal morphology, increased survival and invasiveness of mutant endothelial cells. The mTOR inhibitor rapamycin acts on this pathway, and was able to control lesion growth in a mouse xenograft model of VM, as well as symptoms such as pain, bleeding, and coagulopathy in six patients with severe disease. This provided a proof-of-concept of the utility of molecular inhibitors in the treatment of intractable VM. Rapamycin has immunosuppressive activity and side-effects; we have therefore begun to screen other small molecular inhibitors. We find that the PIK3CA inhibitor BYL719 and a novel TIE2 switch-control inhibitor are both effective at restoring all VM-associated in vitro phenotypes, while the AKT inhibitor MK2206 and rapamycin restore many but not all phenotypes.
P16: Implication of syndromic cleft genes in non syndromic forms : towards translational phenotypes ?

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Cleft lip with or without cleft palate (CL/P) and cleft palate (CP) are the most common craniofacial birth defects with an approximate incidence of ~1/700. In 30% of cases, clefts are seen in syndromic forms (associated with other congenital anomalies) likely caused by genetic factors. Non syndromic forms (NS) are believed to be caused by a combination of genetic and environmental factors. Several genome-wide association studies (GWAS) have proposed a few loci in NSCL/P.

A few genes, such as IRF6, have been implicated in both syndromic and non-syndromic forms of CL/P. Moreover, there are families with NSCL/P following Mendelian transmission with low penetrance and variable expressivity. These two points suggest that a strategy of studying genes or pathways associated with syndromic forms as the cause of NS clefts could be productive.

To this end we decided to perform whole exome sequencing (WES) on patients with NS cleft, after having ruled out IRF6 mutations and cytogenetic anomalies. Patients from 12 families with CP, 10 families with CL/P, 2 families with velopharyngeal insufficiency, and 6 sporadic CP cases were selected.

Several likely causative variants were identified in five families: in GHRL3 (2 families), in TP63 (1 family), in LRP6 (1 family) and in TBX1 (1 family). Clinical reassessment confirmed the isolated occurrence of cleft with variable expressivity in affected patients, as well as low penetrance, given the number of unaffected carriers.

CL/P. It raises the question of an important part of the “missing heritability” of NSCL/P possibly being explained by modest-to-medium penetrant variants in such genes.

Our study illustrates the involvement of genes known to be mutated in syndromic clefts, in non syndromic.
Segregation and tumor phenotype analyses are helpful to filter out rare germline variants in high-risk BRCA 1/2 mutation-negative breast cancer families

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Background
Breast cancer is the most frequent malignant disease among women. Approximately 10% of the cases are considered hereditary, following an autosomal dominant inheritance pattern. Among these, about 30% are attributed to germline defects in the tumor suppressor genes BRCA1 and BRCA2. Several other breast cancer predisposition genes of high, moderate or low penetrance have been identified. Altogether, it is however considered that only 40% of the inherited risk of breast cancer can be explained. Whole-exome sequencing (WES) of germline DNA generates a huge amount of variants that need to be prioritized in order to decipher disease-risk alleles. We used WES to identify the patterns of genetic variation in BRCA 1/2-negative high-risk familial breast cancer patients. Several filtering steps were applied to reduce the number of variants.

Methods
Germline exomes of 26 breast cancer index patients with a very high probability of developing a new breast cancer or of carrying a high-penetrance mutation, as calculated by BOADICEA, were sequenced on a 5500 SOLiD or an Illumina HiSeq system. Variants were called within a panel of 236 genes already associated to cancer or to DNA repair. Variants with minor allele frequency (MAF) above 1.5% in ExAC, GoNL and 1000 Genomes were discarded. Variants had to be considered pathogenic by at least two out of the six prediction programs used. Candidate variants were validated with Sanger sequencing. Familial validation through co-segregation with the disease in one or two other family members was done. Tumor phenotype (tumor histology, expression level of hormonal receptors and HER2 receptor) was defined for all patients.

Results
Quality and gene-panel filtering could narrow the list of variants to 28,773 from the 2,050,698 identified. Of these, 83 were kept after MAF filtering, alignment validation and confirmation by Sanger sequencing. Co-segregation was revealed for 29 of these variants (35%). In the subgroup of 13 families with similar tumor phenotype within the family, 22 variants out of the 40 cosegregated (55%). In contrast, significantly less variants cosegregated in the subgroup of 13 families with diverging tumor phenotypes within the family: 7 variants out of the 43 (16%, p=0.000218).

Conclusion
In these high-risk BRCA 1/2 mutation-negative breast cancer patients, we found a large number of rare germline variants in genes linked to breast cancer or DNA repair despite stringent filtering. Segregation analysis helped to further narrow the list of potentially predisposing mutations. However, tumor phenotype has to be taken into account, as significantly less segregation was observed if the breast cancer of the relative had a different phenotype.
**P18: Mosaic Somatic KRAS mutation is responsible for Oculo-cerebro-cutaneous syndrome (Delleman-Oorthuys syndrome): a seventh patient report**

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**Background**

Oculo-cerebro-cutaneous syndrome (Delleman-Oorthuys syndrome) represents a multisystemic condition with skin (aplasia cutis congenita, lipomatosis on subcutaneous regions located from any subcutaneous regions), ophthalmic (choristome and epibulbar cyst), intra medullary and brain lesions (Moog 2005). Non ossifying fibromas may develop during the first decade on jaw and throughout life on long bones. Follow-up should guarantee precise diagnosis and prompt management. DNA extracted from femoral fibroma compared to blood DNA, applying whole genome shotgun sequencing, allowed to select variants in 2 genes and KRAS was selected since found in other tissues and absent in unaffected relatives (Peacock 2015). These findings were confirmed in the second available report so far on 4 patients (Boppudi S. 2016): this last underlines absence of germline mutation, various degrees of mosaicism and hot spot mutations at codon 13,19,146. Expressivity is highly variable, prognosis factors are not defined so far.

**Patient Report and Result**

We report the natural history of girl secund child born from unrelated healthy parents. From birth were noted: aplasia cutis on left parieto-occipital region, epibulbar dermoid kyst of left conjonctiva and a subcutaneous mass on nuchal region. At the age of 18 months, milestones were normal, she has no seizure and no behavior problem. Ophthalmic lesion as lipoma's and aplasia cutis are not creating morbidity so far. Standard array CGH was normal. Ultrasound at the posterior cervical mass identified lipomatous tissue. Extracted DNA from lipoma and targeted KRAS gene sequencing identified presence of the c.38G>A (p.Gly13Asp) in 47% of these cells. This mutation is one of the 3 (hotspot) reported so far.

**Conclusion**

Present patient is the 7th patient with OCCS identified carrier of somatic mutation in KRAS gene. Clinical diagnosis together with histology and molecular studies enabled appropriate genetic counselling. Precise gain-of-function effect of the missense change in the RAS pathway related to phenotype and indication for mTOR inhibitor therapy remain to be evaluate.

**References**

P19: KIF1B and NF1 are the most frequently mutated genes in paraganglioma and pheochromocytoma tumours from a Belgian multicentric cohort

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Background and objectives: Pheochromocytomas/paragangliomas (PPGLs) are rare chromaffin-cell tumours arising from the adrenal medulla or extra-adrenal paraganglia, respectively. Forty percent of PPGLs are explained by germline mutations in known susceptibility genes. Recently, somatic point mutations were identified in an additional 30% of PPGLs, using Sanger sequencing, mainly in the NF1, VHL, RET, and HRAS genes. However, recently identified susceptibility genes, such as FH and KIF1B, were not screened. Our aim was to look for the prevalence and nature of somatic mutations in patients from a Belgian multicentric PPGL cohort using deep-targeted sequencing.

Design and method: Tumours and their corresponding blood samples were available for 74 PPGL patients (47 pheochromocytomas and 27 paragangliomas). Targeted Next Generation Sequencing was performed using the Ion Torrent system (Life Technologies®) with a panel including 17 susceptibility genes. Variants predicted as damaging by at least 5 programs, or present in COSMIC and predicted by at least 3 programs were considered to be mutations.

Results: Somatic mutations were identified in 40 patients (54%), 6 of which had a germline mutation (5 SDHD and 1 SDHB). The average number of mutations per patient was 7.9 ± 19.4 (range: 1-119). Mutations were more frequent in genes involved in Cluster 2 than in Cluster 1 (58% vs 42%, p-value= 0.003). The most frequently mutated genes were: NF1 (20.8%), KIF1B (20.4%) and RET (11%). Notably, RET mutations were more frequent in paragangliomas than in pheochromocytomas (20% vs. 5%, p<0.008).

Conclusions: Somatic mutations were found in 54% of patients. The high prevalence of somatic mutations in NF1 is in accordance with previous studies, while the similarly elevated prevalence of mutations in KIF1B is a novel finding. The second main result is the high proportion of RET somatic mutations in head and neck paragangliomas compared to pheochromocytomas. Finally, we identified a minority of tumours with a large number of somatic mutations, which may reflect perturbations in DNA damage repair. Whether such tumours are more prone to dissemination and recurrence needs to be demonstrated in prospective studies.
P20: Determining the Deletion, Repeat Composition, and Pathogenic Hemizygous Variants in ~400 Whole-Genome Sequenced Patients with the 22q11.2 Deletion Syndrome

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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. The clinical presentation of the syndrome is extremely variable, but the underlying reason for this variation remains unknown. Individuals with the syndrome most often have a classically associated 3 MB or 1.5 MB deletion as a result of meiotic non-allelic homologous recombination events between flanking low copy repeats. We have addressed three possible variation types that could underlie the phenotypic variability: 1) deletion size, 2) low copy repeat composition, or 3) SNPs in the remaining allele.

To investigate these three possibilities Illumina whole-genome sequencing was performed on ~400 patients with the 22q11.2 deletion syndrome. First we evaluated the structure of the region with the hypothesis that variation in deletion breakpoints or low-copy repeat composition can alter gene structure and/or regulation. Sequencing depth was used to identify 90% of the patients have the typical 3 MB deletion, 6% the 1.5 MB deletion, and the remainder having a variety of more atypical deletion sizes. To determine low copy repeat composition the reference genome was decomposed into repeat subunits and reads aligned to these subunit families. Normalized coverage was determined compared to 60 whole-genome sequenced individuals without the 22q11.2 deletion syndrome. Initial results identify deletion breakpoints down to subunit resolution and demonstrate inter-individual variability in low-copy repeat composition. Finally, hemizygous variants were called to investigate the hypothesis that the deletion unmasks pathogenic recessive variants on the remaining allele leading to specific phenotypes. Filtering for rare protein-altering variants with predicted damaging effects identified 117 SNP positions across 185 patients. Reverse phenotyping is now being performed to associate this genetic variation with specific phenotypes. This will lead to an increased understanding of the genetic components that contribute to variable phenotypes in the 22q11.2 deletion syndrome.
P21: CRISPR/Cas9-mediated genome editing in naive human embryonic stem cells followed by neural differentiation: an optimized workflow

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Human embryonic stem cells (hESCs) are derived from early blastocyst embryos and are characterized by their ability for self-renewal and pluripotency, which makes hESCs an attractive model system to study (candidate) genes involved in neural differentiation. Two phases of pluripotency can be defined: naive and primed. Since naive hESCs have some advantages like single cell passaging by trypsin digest and single cell cloning, the naive state might be more preferable for efficient genome-editing.

We set up a pipeline to generate knockouts for coding and noncoding genes in the naive hESCs using the double nicking CRISPR/Cas9 system. It relies on two single guide RNAs to direct site-specific chromosome breakage mediated by the Cas9 nickase, resulting in deletions or insertions at the target region. To subsequently test whether the altered genes play a role in neural differentiation, the genomically engineered cells are differentiated towards neural progenitor cells.

As a proof of concept, CRISPR/Cas9 assays were designed to target the conserved region of the already functionally validated long noncoding RNA TUNA, which plays a role in pluripotency and neural differentiation of ESCs. These CRISPRs show an editing efficiency of 40% at the on-target site, while no disruptions were noted at the in silico predicted off-target sites. After monoclonal isolation, 5-6% of the clones survived and both homozygous as compound heterozygous TUNA genome-edited colonies were obtained. Both showed altered expression of the downstream targets in the hESCs. Currently we are evaluating the expression levels of specific neural markers (e.g. nestin & PAX6) during neural differentiation of respectively genome-edited as well as wild-type hESCs.
P22: Highlander: variant filtering made easier

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The field of human genetics is being revolutionized by exome and genome sequencing. A massive amount of data is being produced at ever-increasing rates. Targeted exome sequencing can be completed in a few days using NGS, allowing for new variant discovery in a matter of weeks. The technology generates considerable numbers of false positives, and the differentiation of sequencing errors from true mutations is not a straightforward task. Moreover, the identification of changes-of-interest from amongst tens of thousands of variants requires annotation drawn from various sources, as well as advanced filtering capabilities. We have developed Highlander, a Java software coupled to a local database, in order to centralize all variant data and annotations from the lab, and to provide powerful filtering tools that are easily accessible to the biologist. Data can be generated by any NGS machine (such as Illumina's HiSeq or MiSeq, or Life Technologies' Solid or Ion Torrent) and most variant callers (such as Broad Institute's GATK). Variant calls are annotated using DBNSFP (providing predictions from 6 different programs, splicing predictions, prioritization scores from CADD and VEST, and MAF from 1000G and ESP ), ExAC, GoNL and SnpEff, subsequently imported into the database. The database is used to compute global statistics, allowing for the discrimination of variants based on their representation in the database. The Highlander GUI easily allows for complex queries to this database, using shortcuts for certain standard criteria, such as “sample-specific variants”, “variants common to specific samples” or “combined-heterozygous genes”. Users can browse through query results using sorting, masking and highlighting of information. Highlander also gives access to useful additional tools, including visualization of the alignment, an algorithm that checks all available alignments for allele-calls at specific positions, and a module to explore the ‘variant burden’ gene by gene. Highlander is Open-Source and is available at http://sites.uclouvain.be/highlander/.
P23: Genetic causes of endothelial neoplasms

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Our previous work identified numerous genes involved in vascular malformations and cancers. These allowed better stratification and management of the patients. However, numerous morbid vascular pathologies remain unexplained. In this project, we focus on 3 aggressive proliferative endothelial neoplasms. Gorham-Stout disease is characterized by proliferation of endothelial-lined vessels within bones, resulting in their progressive destruction. Maffucci syndrome presents vascular lesions called spindle-cell hemangioma, low-grade vascular tumors associated with multiple enchondromas, which often degenerate into chondrosarcoma. Kaposiform hemangioendothelioma is an aggressive vascular endothelial neoplasm occurring in infancy. Our objectives are to identify their genetic causes, to understand how these induce pathogenicity, and to develop models in which to test therapeutic agents.

As oncogenic mutations have already been identified in various vascular lesions, a similar scenario is very likely for these invasive endothelial neoplasms. We will first focus on a panel of cancer-related genes sequenced at high coverage. If no causative mutation is found, paired tissue and blood samples will undergo whole exome sequencing to identify somatic mutations in other genes. The third step would be to sequence the entire genome. In this case, the coverage will be lower, but it allows to search for mutations in regulatory regions (promoters) and to detect copy-number variations. Once identified, mutations usually need further validation. Depending on what is already known about the genes and the types of mutations, we will plan in vitro experiments to assess the pathogenic effect in endothelial cells. Furthermore, to validate in vivo the “oncogenic” potential of the candidate mutations, the endothelial cells designed to express them will be injected in mice, using matrigel-based implantation system developed by collaborators. These will be invaluable to test potential therapies.
P24: Genes for primary lymphedema explain 40% of familial primary lymphedema

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Primary lymphedema is the most frequent type of lymphatic anomaly, characterized by swelling of tissues, mostly on the extremities, due to bad development or function of the lymphatic system. The expression of lymphedema is highly variable by the age of appearance, location, severity and by association with other features. Mutations in 24 different genes have been identified and their responsibility has been demonstrated in isolated and syndromic forms of primary lymphedema. However, their respective prevalence is still unknown.

In this study, the exons of 15 of the known genes (CCBE1, FLT4, FOXC2, GATA2, GJA1, GJC2, IKBKG, ITGA9, KIF11, PTPN11, PTPN14, RAF1, RASA1, SOX18, and VEGFC) were screened by targeted next generation sequencing (PGM, Ion Torrent) in a cohort of 542 index patients. Data analysis was performed with the in-house developed Highlander software. Filtering criteria of variants were applied as follows: pass the GATK filters; same change present in less than 50 patients; comprised between -2 before an exon and +5 after the exon; reported less than 10 times in 1000 Genomes; less than 5 times in gnoNL; frequency ?0.0014 in ExAC; impact damaging by at least 3 programs of prediction; and checked in silico by IGV.

We found 175 interesting variants that we considered for 68% as disease-causing mutations, the other 32% remaining variants of uncertain significance (VUS). Taking into consideration the mutations only, the genetic cause of primary lymphedema is explained for around 40% of patients with a familial history and 18% of sporadic or with unknown origin cases. Globally it explained 21.5% of the cases. The most frequently mutated gene was VEGFR3 explaining the lymphedema for 9.2% of patients, followed by KIF11 (5.0%) and FOXC2 (3.1%), the rest of genes explained not more than 2% of the cohort each. No mutation was found in GJA1, IKBKG, and PTPN14. This allowed us to have a global view on the involvement of each of these 15 genes in primary lymphedema, but also to observe the types of mutations encountered in a large cohort of patients.

Interestingly, all the mutated genes/proteins seem to act in a common functional pathway involving VEGFR3 signaling. This underscores the important role this pathway plays in lymphatic development and function, and suggests that the unknown genes may also have a role in the same pathway.
P25: Improving CRISPR/Cas-mediated Homology-Directed Repair efficiencies in zebrafish

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The recent emerge of site-directed genome editing technologies such as CRISPR-Cas9 is of great interest for research in medicine. It has been shown extensively that the technology can be used to generate knock-out disease models with high efficiencies, enabling the study of gene function. However, to generate more disease-relevant models, the introduction of specific base pair alterations (knock-in) is desired, since numerous diseases are caused by specific point mutations, leading to amino acid substitutions or splicing defects. Also in zebrafish, CRISPR-Cas9 has been successfully applied to generate knock-out models. However, results from a limited number of small scale studies, showed that the efficiency of CRISPR-Cas9 mediated knock-in approaches to introduce point mutations, is relatively low. These approaches are based on CRISPR-Cas9 stimulated homology-directed repair (HDR), using either single-stranded oligodeoxynucleotide (ssODN), double stranded DNA (dsDNA) or plasmid templates. In this work, we assessed the suitability of different types of ssODN templates to introduce specific point mutations at multiple sgRNA target sites in the zebrafish genome by means of CRISPR-Cas9 stimulated homology-directed repair (HDR). First, we tested if there is a difference in mutagenesis efficiency when using sense or antisense ssODNs and we evaluated the influence of homology arm length and symmetry of ssODNs on mutagenesis efficiency. Next, we assessed the potential of several chemical compounds to increase HDR-based knock-in efficiency in zebrafish, either by blocking the non-homologous end joining (NHEJ) or by stimulating the HDR pathway.
P26: Next generation sequencing shows a high frequency of mutations in patients with therapy-related myeloid neoplasms

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Background
Therapy-related myeloid neoplasms (t-MN) include acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) complicating previous cytotoxic treatment and/or radiotherapy for malignant or non-malignant disorders. Frequently patients are classified according to previous therapy or cytogenetic abnormalities: recurrent unbalanced translocations (e.g. -5/-7, del5q/del7q, +8), recurrent balanced chromosomal abnormalities (e.g. KMT2A (MLL) or RUNX1 rearrangement, PML/RARA) or normal karyotype. Besides these factors, reduced response to chemotherapy and higher incidence of TP53 mutations in comparison with de novo MDS/AML have been described. Our objective was to evaluate gene mutations by next generation sequencing (NGS) in a subgroup of t-MN patients with previous history of a solid tumor.

Methods
We performed a retrospective study of medical records of patients with suspected therapy-related MN. Of patients with t-MN a small subgroup (n=33; t-MDS=14; t-AML=16; t-MDS/MPN=2; t-CML=1) with a history of a solid tumor (breast (n=22), rectal (n=4), ovarian (n=2), prostate cancer (n=2), seminoma with a second carcinoma (prostate (n=1) and thyroid (n=1)) and spinocellular carcinoma of the tongue (n=1)) was selected for NGS. Stored gDNA from diagnostic samples was used. The genomic library was prepared using 50 ng of unfragmented gDNA and the TruSight Myeloid sequencing panel targeting 54 genes (full gene or hotspot) from Illumina. Samples were sequenced on a MiSeq sequencing platform.

Results
Sixty-nine mutations in 26 genes were found in 27 of 33 samples (82%; mean number of mutations=2; range=[1-6]). Mutations were detected in the following genes: TET2 (n=9), TP53 (n=8), FLT3 (n=6), DNMT3A (n=5), ASXL1, NPM1 and U2AF1 (n=4), BCOR, BCORL1, EZH2 and RUNX1 (n=3), IDH2 and SRSF2 (both n=2), BRAF, CUX1, GATA2, IKZF1, JAK2, KDM6A, KRAS, NRAS, PHF6, SETBP1, SF3B1, STAG2 and WT1 (all n=1). Seven out of 33 (21%) t-MN patients had a TP53 mutation, all were associated with a poor outcome: they had a survival range between 3 months and 1 year (except for one patient that was recently diagnosed). These patients had a history of breast or ovarian cancer, presented with a higher percentage of blasts in peripheral blood (75%) and had a very complex karyotype (except for one patient with t(6;9)(P2₃;Q34)). Furthermore, in 5 patients 2 different mutations were found in the same gene (3 in TET2, 1 in RUNX1, 1 in TP53). Mutations in TET2, TP53,
DNMT3A, NPM1, BCOR, EZH2 and IDH2 were found in both t-MDS and t-AML. Interestingly, we found one BRAF mutation in a t-MDS patient.

Conclusion
Our study revealed a variety of mutations in 82% of the patients with a t-MN after cytotoxic treatment for a solid tumor. Based on our results, we confirm the previously reported higher frequency of TP53 mutations in t-MN, which is associated with a very complex karyotype and poor outcome.
Non-compaction cardiomyopathy (NCCM) is a rare genetic congenital disease, resulting from abnormal embryonic myocardial development. Anatomically, patients present with prominent ventricular trabeculations and deep intertrabecular recesses. The clinical manifestations can vary from asymptomatic to ventricular arrhythmias, heart failure and systemic thrombo-embolism. While NCCM mostly occurs as an isolated condition, association with other cardiovascular manifestations, such as Ebstein Anomaly has been reported. Ebstein anomaly (EA), is a congenital heart defect characterized by downward displacement of the tricuspid valve. In EA patients, the posterior and septal leaflets are adherent to the underlying myocardium, which leads to partial atrialization of the right ventricle and, hence, diminished ventricular size and function.

We pursued the genetic disease cause in an autosomal dominant NCCM-EA family consisting of three patients with isolated left ventricle non-compaction cardiomyopathy (LV-NCCM) and two patients with the combined LV-NCCM/EA-phenotype. Whole exome sequencing on two most distant affected family members, followed by shared variants analysis, revealed a missense mutation (p.Leu113Val) in TPM1. TPM1 encodes for alpha-tropomyosin, which is involved in myocardial contraction as well as in stabilization of non-muscle cytoskeletal actin filaments. Pathogenicity of p.Leu113Val is supported by: (i) its absence in the Exome Aggregation Consortium, Exome Variant Server and 1000 Genomes databases; (ii) its co-segregation with disease in the family; (iii) high evolutionary conservation of p.Leu113 (up to Zebrafish); (iv) MutationTaster, SIFT and PolyPhen2 pathogenic predictions; and (v) the fact that it has already been reported in an unrelated primary dilated cardiomyopathy case.

So far, NCCM-EA has predominantly been linked to mutations in MYH7, encoding the cardiac sarcomere protein alpha-myosin heavy chain 7. Recently, one sporadic NCCM-EA case with a de novo TPM1 mutation has been described. We here report the first NCCM-EA family segregating an inherited TPM1 mutation, further establishing the association between EA predisposition and TPM1-related NCCM. Consequently, we recommend genetic testing for both MYH7 and TPM1 in patients and/or families in which NCCM and EA coincide.
P28: Mosaic KRAS mutation in a oculoectodermal syndrome: a case report

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Oculoectodermal syndrome (OES) is a rare neurocutaneous syndrome characterised by congenital scalp lesions and ocular dermoids although other manifestations have been described. A 16-months-old boy was referred to our genetic consultation for a suspicion of Goldenhar syndrome. When we showed him at 16 months, examination showed two area of alopecia on the left of the scalp. The left eye shows a small nodule on the upper eyelid, an upper palpebral coloboma, an unilateral ptosis and a temporal limbal dermoid that overruns the cornea.

There was no facial asymmetry. His ears were normal. We did not objective hypomelanotic or hyperpigmentation macule. He showed a hypertrophic temporoparietal suture. Growth parameters and neurologic examination were normal. The different ultrasounds, x-ray column and the audiometric tests were normal. At the visit, a diagnostic of OES was suspected. At 17 months, he had a resection of the upper eyelid nodule. To identify the genetic etiology of this syndrome, we performed a screening of KRAS mutation in the skin tissue by high debit sequencing. We funded a c.437C>T (p.(Ala146Val)) in the KRAS gene, with 10 % of abnormal cell lines. The somatic screening did not find the mutation on the leucocytes.

The RAS-MAPK pathway regulates cellular processes including DNA synthesis, cell growth and differentiation. Somatic mosaicism for KRAS alterations leads to several forms of neoplasia, and also to several other phenotypes including OES or encephalocraniocutaneous lipomatosis (ECCL). The aim of this case report is to expand the mutational and the clinical spectrum of OES, and the and the features of our patient will be compared with the few cases described in the literature.
P29: Molecular and Genetic Determinants of Infantile Hemangioma Pathogenesis

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Infantile hemangioma (IH) is a benign vascular tumor affecting 10-12% of newborns. It can arise anywhere on the body, especially on the head and neck. The life cycle of IH begins with a rapidly proliferative phase lasting six to twelve months, followed by spontaneous involution, leaving behind a fibrotic-fatty deposit. Current treatments include surgical removal and administration of beta-adrenergic receptor blockers, especially propranolol. The pathogenic cause of IH is unknown. IH is predominantly sporadic; rare familial cases are however known, suggesting a genetic component.

We are using two complementary approaches to identify genes that contribute to IH pathogenesis. (1) We are carrying out Whole Exome Sequencing (WES) on DNA from multiple affected members from families with rare, inherited IH, to identify genetic variants that co-segregate with disease. (2) In order to determine if somatic mutations cause sporadic IH, we are carrying out targeted candidate-gene deep-sequencing on snap-frozen tumor samples and paired blood-DNA. We are also performing WES on DNAs extracted from different cell-types (endothelial cells, pericytes, stem cells) isolated from tumors. Sequences are analyzed on an in-house pipeline for variant detection, followed by extensive variant annotation, filtering, and visualization (« Highlander », Dr. R. Helaers, submitted).

We have so far WESed 21 patients and 5 unaffected individuals from 8 families. In addition, targeted deep sequencing was performed on 4 snap-frozen tumor samples and paired blood-DNA, and WES was carried out on isolated cells from 3 tumors along with paired blood-DNA. Bioinformatic analyses have so far implicated some candidate genes, known to play a role in endothelial cell proliferation and vascular development. All of the candidate genes we identify using the approaches described above will be screened in expanded series of sporadic IH samples (of which we have >300), using custom-panels for targeted sequencing on the Ion Torrent Personal Genome Machine (PGM). Functional analyses will then be carried out on the most promising genetic variants implicated.
P30: Arterial Tortuosity Syndrome: Clinical and Molecular Findings in 36 Newly Identified Families

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Background
Arterial tortuosity syndrome (ATS, MIM#208050) is a rare autosomal recessive connective tissue disorder, characterized by widespread vascular involvement with mainly elongation and tortuosity of the large and middle-sized arteries. ATS is caused by mutations in the SLC2A10 gene.

Aim
To characterize 36 newly identified families including 44 individuals affected with ATS.

Results
All patients harbor biallelic SLC2A10 mutations of which ten are novel. Sixteen families demonstrate consanguinity. Ages range from 9 months to 30 years (mean 11.1 years, median 9 years). Triggers to the diagnosis are cardiovascular manifestations (aortic coarctation and cardiac murmurs) in about half of the patients, aspecific connective tissue findings (hernias, stretchy skin) in 25%, pulmonary manifestations (neonatal respiratory distress or dyspnea in infancy) in about 15%, and a familial history in the remaining 10%. Half of the patients show typical craniofacial features. Recorded prenatal manifestations in four patients include intra-uterine growth retardation, oligohydramnios and aortic tortuosity. All patients have arterial tortuosity, mainly affecting the pulmonary and cerebrovascular circulation. Half of the patients have pulmonary artery stenoses. Stenoses locate at the isthmus aortae (5), abdominal aorta (3) and renal circulation (1). Two patients had a history of a cerebrovascular accident, and four infants have been treated for aggressive aneurysm formation of the aortic root. So far, no arterial dissections or ruptures are observed. Six patients had severe infant respiratory distress syndrome. Gastrointestinal manifestations include sliding hernias and pyloric stenosis. All patients show skin abnormalities varying from a velvety texture with some hyperextensibility to frank cutis laxa. Skin histology shows severe elastic fiber fragmentation, but increased collagen deposition. Transmission electron microscopy shows clear elastic fiber abnormalities with a patchy and irregular deposition of elastin that is poorly connected to the microfibrillar bundles, mainly at the periphery of the elastic fiber. The collagen fibers are irregularly packed and show varying diameters.

Conclusion
Our study confirms the less severe natural history as previously suggested, but respiratory distress, aggressive aneurysm formation, and cerebrovascular accidents may occur. Moreover, the occurrence of stenoses warrants
caution for the use of angiotensin converting enzyme inhibitors or angiotensin II receptor blockers. Skin biopsies show specific elastic fiber and collagen anomalies not seen in any related cutis laxa syndromes.

On behalf of all referring physicians:
N. Canham, Saint Mark’s Hospital London, UK; J.M. Chopin, Hôpital Calmette, Lille; M. Dasouki, University of Kansas, USA; K. Devriendt, Catholic University of Leuven, Belgium; H. Dietz, John Hopkins University School of Medicine, USA; B. Fischer, Charité-Universitätsmedizin Berlin, Germany; A. Gezdirici, Kanuni Sultan Suleyman Training and Research Hospital, Turkey; F. Giuliano, Université de Versailles St-Quentin en Yvelines, France; M. Guitart, Corporación Sanitaria Parc Taulí, Spain; M.Z. Haider, Kuwait University, Kuwait; X. Jeunemaitre, Université Paris Descartes, France; U. Kornak, Charité-Universitätsmedizin Berlin; B. Loeys, Antwerp University Hospital, Belgium; S. Lyonnet, Hôpital Universitaire Necker-Enfants Malades, France; F. Malfait, Ghent University Hospital, Belgium; H. Michael, Leeds Teaching Hospital NHS Trust, UK; S. Mohammed, Guy’s Hospital, London; S. Nampoothiri, Amrita Institute of Medical Sciences and Research Center, India; K. Pichler, University of Innsbruck, Austria; M. Ramos, Complejo Hospitalario de Navarra, Spain; M. Rossi, Hôpital Femme-Mère-Enfant Lyon, France; M Salih, Security Forces Hospital, Kingdom of Saudi Arabia; M.Z. Seid Ahmed, Security Forces Hospital, Kingdom of Saudi Arabia; E. Steichen-Gersdorf, Medical University of Innsbruck, Austria; L. Van Maldergem, Hôpital Saint Jacques, France.
P31: Phen?, an interface for the collection of phenotypic data and prioritization of variants

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5 HUDERF - BKF
6 ULB CHG - IB² - BrIGHTcore - IRIBHM

With genetic disorders, and neurodevelopmental disorders in particular, assigning a pathogenic nature to a given genetic variant is extremely challenging. Accurately collected phenotypic and genomic data tremendously increases the chances to tackle this obstacle. The objective of Phen? is primarily to allow for efficient prospective collection of a patient’s features, using the Human Phenotype Ontology, a unified vocabulary now used by several centers around the world. Such a patient profile can easily be shared with other centers and compared, within the MatchMaker Exchange initiative for example. Matches could lead to a higher rate of diagnosis. Secondly, Phen? implements a variants prioritization pipeline to propose a ranked list of pathogenic variants, based on the phenotype of the patient. We performed a benchmark, using a group of patients with neurodevelopmental disorders, the ADReSSE project which displays a 32% diagnosis rate, in order to highlight the prioritization tool’s performance. Other key functions of Phen? are to communicate with the lab system, to offer an assisted process to prospectively collect the phenotype, and to search, filter and analyze the data, which is secured by authentication of the user and by the encryption of the communications. As a conclusion, the collection of the data in this structured form would allow for a more efficient understanding of rare genetic disorders and a better rate of diagnosis. Also, this approach is a first step towards an extended integrator of genome, phenome and epigenome data.
P32: pBRIT: Gene Prioritization by Correlating Functional and Phenotypic Annotations Through Integrative Data Fusion

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Identification of top candidate genes from a pool of large data sets is computationally challenging. Existing prioritization tools typically utilize fusion of different annotation sources. However, these tools have important shortcomings. First, outdated annotation sources lead to annotation errors propagating to downstream analysis. Second, data fusion approaches generally fail to address sparsity and dependencies in annotation sources. Finally, high-throughput scalability is limited.

We propose pBRIT an advanced tool for candidate gene prioritization using Bayesian Ridge Regression and Information-Theoretic model. It is a fast and adaptive tool integrating ten different annotation sources (Pubmed, GO, HPO, Pathway, Interactions, Disease Ontologies, GAD, HuGe, BLAST, Mouse Ontologies) into the prioritization approach. Our hypothesis states that genes involved in similar disease types share similar “functional and phenotype” characteristics that can be used in disease gene identification.

pBRIT is based on an Information-Theoretic approach that models feature dependencies and sparsity of annotation sources for effective feature mining. Bayesian regression is applied to a training set, consisting of known disease genes, to learn a linear mapping between functional and phenotype annotations. Based on this mapping, candidate genes are ranked according to their relatedness to the training genes.

We compared the performance of pBRIT with 7 different existing methods on their corresponding benchmark datasets. With each benchmark validation set we tried to explore the various aspects of prioritization. This includes the effect of early vs intermediate vs late data integration. Additionally, we validated our method against 32 disease genes published in top medical journals after construction of pBRIT data sources. We achieved a maximum AUC score ranging from 0.92 to 0.96 among all the available benchmark dataset and an AUC score of 0.82 for the recently published genes. These results indicate good performance of pBRIT with high sensitivity and specificity. pBRIT does not show a strong bias towards well studied genes and can predict new genes with minimal existing information. Additionally, pBRIT is fast and scalable to handle thousands of samples. pBRIT is publicly available for academic usage at http://biomina.be/apps/pBRIT.
P33: Type III collagen is important for type I collagen fibrillogenesis and for dermal and cardiovascular development.

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Type III collagen, a major fibrillar collagen consisting of three identical ?1(III)-chains, is expressed in early embryos, throughout embryogenesis and in a wide variety of adult tissues. Mutations in the COL3A1 gene cause vascular Ehlers-Danlos Syndrome (vEDS), a severe, life-threatening disorder, characterized by thin, fragile skin and propensity to arterial and intestinal rupture. Most mutations substitute a crucial glycine residue, which is pivotal for correct folding of the collagen triple helical domain. However, the mechanisms by which mutant type III collagen causes dermal and vascular fragility are not well understood.

To study the role of type III collagen in development and disease, we generated a Col3a1 transgenic mouse model using a BAC transgenic approach. The Col3a1Tg-G182S mice overexpress the Col3a1 transgene harbouring a typical glycine substitution (p.(Gly182Ser)) in the ?1(III)-procollagen triple helical domain, whereas the Col3a1Tg-WT mice overexpress WT Col3a1. Col3a1Tg-G182S mice present a phenotype resembling human vEDS, including dermal and vascular fragility, thin, translucent skin, and wound healing problems. The adventitia of Col3a1Tg-G182S mice is significantly thinner and smooth muscle cells make less connection with elastic fibers and show more intracellular space. Ultrastructural analysis reveals loose packaging of collagen fibrils in the ECM of Col3a1Tg-G182S mice. Although no difference in posttranslational modifications has been detected, and the thermal stability of mutant type III collagen is only slightly shifted, we show that collagen fibrils in Col3a1Tg-G182S mice are severely malformed. Furthermore, skin fibroblasts of Col3a1Tg-G182S mice are not able to form a proper cellular matrix, and type III collagen affects the formation of type I collagen fibrils in vitro. No abnormalities have been observed in the Col3a1Tg-WT control mouse model, indicating that the observed phenotype in Col3a1Tg-G182S mice is due to the Gly substitution, and not merely caused by type III collagen overexpression.

Together, our findings underscore a key role for type III collagen in collagen fibrillogenesis in skin and arterial tissue. This novel transgenic mouse model, overexpressing mutant Col3a1, provides an important new tool for pathogenesis exploration and advanced understanding of the role of abnormal type III collagen in collagen fibrillogenesis and vEDS in general.
P34: Rare modifier variants alter the severity of cardiovascular disease in Pseudoxanthoma Elasticum

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Introduction: Pseudoxanthoma elasticum (PXE), an autosomal recessive ectopic mineralization disorder caused by ABCC6 and ENPP1 mutations, is characterized by skin, ocular and cardiovascular symptoms. Due to striking phenotypic variability, modifier genes are intensively researched to improve counseling. We evaluated the collective influence of multiple rare variants on cardiovascular disease severity in PXE.

Methods: Mixed effects of rare missense/nonsense variants were assessed by Whole Exome Sequencing in 12 PXE patients with an extreme cardiovascular phenotype (based on clinical presentation and vascular calcium scoring). Statistical analysis (SKAT-O and C-alpha testing) was performed to identify new modifier genes for the cardiovascular PXE phenotype and enrichment analysis was used to evaluate pathway and gene ontology features for these modifiers (Reactome Database).

Results: Respectively 16 (SKAT-O) and 57 (C-alpha) genes were identified as significant modifiers of the cardiovascular disease in PXE. Top significant genes could be stratified in 3 groups - calcium homeostasis (OTOP2, HCAR3), association with vascular disease (TOR2A, NLRP1) and induction of apoptosis (AHNAK2, BRWD1) - while enriched pathways involved FGFR1 signaling (FLG) and gamma-carboxylation (GGCX, VKORC1), both associated with mineralization.

Conclusion: This study explored for the first time the cumulative effect of rare variants on the severity of cardiovascular disease in PXE, leading to a panel of candidate modifier genes. Hypothesis-free analysis revealed the most significant genes and enriched pathways to be already involved in vascular disease or mineralization, both hallmarks of PXE. This panel will aid in risk stratification and genetic counseling of PXE patients.
P35: GGCX-associated phenotypes: an overview in search of genotype-phenotype correlations

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Gamma-carboxylation is an essential process in the activation of vitamin K-dependent proteins which are important in numerous biological processes, such as blood clotting, inflammation, bone formation and cell proliferation. This posttranslational modification process is executed by the GGCX (gamma-glutamyl carboxylase) enzyme. Mutations in the gene encoding GGCX have been linked to multiple distinct phenotypes, affecting the heart, skin, eyes, blood clotting and bone metabolism.

As genotype-phenotype correlations were never described, literature was systematically reviewed in search of patients with at least 1 GGCX mutation with a phenotypic description, resulting in a case series of 47 patients. Though this number was too low for statistically valid correlations – a frequent problem in orphan diseases – we demonstrate the importance of mutations in the HTTM (horizontally transferred transmembrane) domain for at least the cardiac and bone phenotype, as all of the patients had at least 1 mutation in this domain, whereas multiple patients without cardiac or osseous manifestations had no mutations in the HTTM domain. Further, age was identified as the most important determinant of the development of PXE-like skin symptoms and to a lesser extent ophthalmological manifestations. Finally, distinct parts of the HTTM domain seem to have a specific role in the development of skin symptoms and not of VKCFD1 (Vitamin K-dependent coagulation factor deficiency-1; OMIM#277450).

Based on our results, patients in whom GGCX mutations are identified should be informed during genetic counseling about the possibility of skin lesions appearing in the course of their disease, taking into account that these lesions may be subtle at onset. In all, a detailed ophthalmological evaluation should be performed and adequate follow-up should be organized, even though the complication rate of the retinopathy in this case series is rather low. Because of its association with reduced bone mass, a bone densitometry should be offered to all patients harboring at least one mutation in the HTTM domain of GGCX. In conclusion, this systematic review suggests that there indeed may be genotype-phenotype correlations for GGCX-related phenotypes, which can guide patient counseling and management.
Introduction: Congenital contractural arachnodactyly (CCA) is an autosomal dominant connective tissue disorder manifesting joint contractures, arachnodactyly, crumpled ears, and scoliosis as main features. Many of the features improve with age. Its rarity and substantial overlap with other conditions including Bethlem myopathy, Marfan syndrome and distal arthrogryposes make the diagnosis challenging, though important for clinical management. CCA is caused by mutations in FBN2.

Aim and methods: We performed a comprehensive clinical and molecular assessment in a large cohort of CCA patients to delineate clinical diagnostic criteria and guide molecular analyses for FBN2. FBN2 analysis using either Sanger Sequencing or PCR-based next-generation sequencing was performed in 122 clinically well-characterized probands. We collected data on 10 main clinical characteristics, and determined the sensitivity, specificity, and positive and negative predictive value to find an FBN2 mutation for each feature, in order to establish a weighted clinical scoring system on 20 points.

Results: Fifty-five probands harbored an FBN2 mutation (mutation uptake rate 45%). All but 2 mutations were located in the neonatal region (exons 22-36) with half of them altering or producing cysteines in the cbEGF-
like domains. Seventeen patients had a large intragenic deletion, or a splice site mutations predicted to result in exon deletions.

Logistic regression analysis revealed a significantly higher clinical score in FBN2+ versus FBN2- patients (mean resp. 11.6 vs 7.7 ; p < 0,001). ROC curve analysis revealed that a clinical score of 11 or more yields a sensitivity of 54.5% and a specificity of 90.5% to find an FBN2 mutation. A score of 6 or less is unlikely to be associated with an FBN2 mutation, unless in adult patients. In addition, review of cardiovascular features revealed non- or slowly progressive aortic root dilatation in five FBN2+ patients and six FBN2- patients. Though aortic root dilatation is a non-discriminative feature, its occurrence in CCA does warrant echocardiographic follow-up.

Conclusion: Our clinical criteria for CCA, based upon a clinical score on 20 points, are helpful to guide molecular analyses. Especially in patients with a score between 7 and 11, FBN2 analysis is warranted. As some patients with a score above 11 do not harbor an FBN2 defect, molecular heterogeneity is likely.
P37: A two-generation family with Myhre syndrome identified through whole exome sequencing

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Myhre syndrome (MYHRS) (MIM 139210) is a rare autosomal dominant disorder characterized by mental retardation, dysmorphic facial features, muscular pseudohypertrophy, a thickened skin and skeletal anomalies (mainly short stature, broad ribs, flattened vertebrae, and brachydactyly). Other features, such as congenital heart defects (CHD) and laryngeal complications have been reported. MYHRS is caused by recurrent missense mutations in SMAD4 (MIM 600993). SMAD4 encodes the common SMAD protein required for most transcriptional responses to transforming growth factor-beta (TGFB) and bone morphogenic proteins (BMP) signaling. Perturbation of these pathways are known to result in multiple developmental defects, often with connective tissue involvement.

We report on a two-generation family with MYHRS identified through whole exome sequencing (WES). In the meantime fifty years old proband had history of a CHD (either a patent ductus arteriosus or aortic coarctation), vertebral segmentation defects causing severe thoracic scoliosis, and a progressive tracheal stenosis requiring tracheostomy. She had repeated miscarriages. Her first live-born child, eleven years old at last evaluation, was diagnosed with a muscular ventricular septum defect, coarctatio aortae for which coarctectomy was performed, hemivertebrae T5-7, and a hypoplastic twelfth rib on the right side. The second life born, a boy aged six at last evaluation, was diagnosed with tetralogy of Fallot, a butterfly vertebra at level T6, and a plexus cyst on echography of the brain. All three patients showed similar craniofacial dysmorphic features, with small low set ears, short palpebral fissures, and a prominent chin. All had bilateral clinodactyly of the second toe and a rather firm skin that was not thickened or stiff. The proband and her daughter had a limited range of motion of the shoulders.

Despite karyotyping, array comparative genome hybridization, JAG1 analysis to exclude Alagille syndrome, and RASopathy associated genes (PTPN11, SOS1, RAF1, KRAS, BRAF, MEK1, MEK2 and HRAS), the cause remained elusive.

In a next step, WES in the proband revealed the presence of a heterozygous c.1486C>T missense mutation in exon 12 of SMAD4 (NM_005359), which has previously been reported as a pathogenic mutation for MYHRS. It results in an Arginine to Cysteine substitution at position 496, which is a highly conserved residue in the MH2
domain involved in transcriptional activation. The mutation was confirmed with Sanger sequencing and was also present in both children.

In conclusion, we report the first familial occurrence of MYHRS in a two-generation family and report on the variability of the clinical hallmarks including the thick skin, short stature and pseudomuscular build, all being absent in this family. The laryngeal stenosis in combination with heart and skeletal defects should trigger the diagnosis in milder cases.
P38: Value of gene panel targeted next generation sequencing in the diagnostic of genetics causes of isolated congenital diaphragmatic hernia

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Congenital Diaphragmatic Hernia (CDH) is a rare condition occurring as an isolated defect in 50% of cases. CDH is thought to have a strong genetic component. However, its genetics is still poorly understood and is highly challenging in diagnostic setting, especially for isolated cases. Previously, a whole exome sequencing study performed on a large cohort of isolated and complex CDH, has shown a significant enrichment of rare variants in known mouse and human CDH causing genes. These variants were present in 30% of the cohort. ZFPM2 gene has been the most commonly affected and accounted for 5% of CDH cases. The objectives of our study are to evaluate the diagnostic yield of gene panel targeted next generation sequencing for isolated CDH cases and fine map the mutational burden in these cases.

Exons, splice-sites and UTRs of a total of 146 genes were captured in 89 fetuses and patients with apparently isolated CDH. The gene panel included known CDH genes in mice and humans, candidate genes in CDH associated CNVs and a selection of candidate genes that are known to directly interact with CDH causing genes.

Variants were filtered and interpreted based on the American College of Medical Genetics guidelines. This filtering resulted in 280 variants in 92 genes, of which 98% are missense. Only 4 patients did not have any rare variants. Fifteen patients had a single rare variant and for the remaining patients, variants number varied from 2 to 8. We identified 2 pathogenic variants in NR2F2 and ZFPM2 in one patient each; and 3 likely pathogenic variants in GATA4, ZFPM2, SEMA3A in one, three and one patient respectively. In addition 3 likely pathogenic variants at a heterozygous state were observed the recessive DHCR7 and SLC2A10. Moreover, there is a significant enrichment of ultra-rare and pathogenic rare variants in PPARGC1A, KIF7, CTBP2 and CREBBP( p<=0.05) suggesting their involvement in the pathogenesis of CDH. The diagnostic yield is 8%. In addition, we identified four new CDH candidate genes which might explain 41% of the CDH. We did not confirm ZFPM2 as major CDH genes and some previously reported variants were considered benign and likely benign. This underscores the challenge of variants interpretation in CDH and the difficulty of assigning causality with certainty.
P39: The TLX1 oncogene modulates the enhancer RNA landscape in T-ALL

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive type of blood cancer resulting from malignant transformation of T-cell precursors. Several driver oncogenes, including the TLX1 transcription factor, have been identified as early events that cooperate with other genetic aberrations in the leukemic transformation of progenitor T-cells. Previously, we established the TLX1 regulome and enhancer landscape integrating TLX1 and H3K27ac ChIP-sequencing with polyA and total RNA-sequencing data of ALL-SIL lymphoblasts upon TLX1 knockdown (Durinck et al., Leukemia, 2015). We revealed amongst others a crucial role of TLX1 in regulation of super-enhancer sites and a key set of T-ALL tumor suppressor genes. We are currently expanding the dissection of the TLX1 regulome towards long non-coding RNAs (lncRNAs). We observed a strong association of TLX1 to enhancer lncRNAs (eRNAs) sites and we are further refining this enhancer landscape through open chromatin mapping (ATAC-sequencing), H3K4me1 and H3K4me3 ChIP-sequencing as well as mapping of MED1 and RNAPII binding to these enhancers. Moreover, we are currently performing differential ATAC-sequencing and total RNA-sequencing in ALL-SIL upon JQ1 inhibition to better define the (super)enhancer chromatin landscape. This approach will support our identification of TLX1 controlled (super)enhancer transcripts implicated in suppression of several key tumor suppressors, currently under further investigation using 4C-sequencing and LNA-mediated transcriptional modulation. This works offers perspectives for developing novel therapies aimed at transcriptional reactivation of these suppressor genes.
P40: Mapping the genomic landscape of retinal dystrophy genes prioritizes regions prone to coding and non-coding copy number variations

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Part of the hidden genetic variation in heterogeneous genetic conditions such as inherited retinal diseases (IRD) can be explained by structural variations including copy number variations (CNV). Here, we mapped the genomic landscape of IRD genes listed in RetNet in order to identify and prioritize those genes susceptible to CNV formation. Hereto, RetNet genes underwent an assessment of genomic features and of CNV occurrence in DGV and in literature, revealing 1,345 reported CNVs. Correlation analysis between rankings of genomic features and CNV occurrence demonstrated the strongest correlation between gene size and CNV occurrence of RetNet genes. Apart from this, we identified and delineated 30 CNVs in 10 RetNet genes in cases with IRD, 13 of which are novel and 3 of which affect non-coding, putative cis-regulatory regions. The breakpoints of fine-mapped CNVs were characterized using Targeted Locus Amplification (TLA), a recently described technology based on the crosslinking of physically proximal sequences in living cells. Here, TLA was performed on extracted genomic DNA, allowing to unravel six complex CNVs in a hypothesis-neutral manner and to elucidate their putative underlying mechanisms. In summary, we identified gene size as the genomic feature most strongly correlated with CNV occurrence in RetNet genes, and we propose a ranking of CNV-prone IRD genes. We demonstrated the efficacy of TLA for the characterization of complex CNVs on extracted DNA. Finally, this IRD-oriented CNV study can serve as a paradigm for other genetically heterogeneous Mendelian diseases with hidden genetic variation.
P41: Novel non-coding homozygous mutation “Ghent +49A>G” in the iron-responsive element of L-ferritin causes hereditary hyperferritinaemia-cataract syndrome

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Background – Progressive cataracts in combination with elevated serum ferritin levels in the absence of iron overload are characteristic of hereditary hyperferritinaemia-cataract syndrome (HHCS). The disorder is caused by heterozygous mutations in the iron responsive element (IRE) located in the 5'UTR of the L-ferritin gene (FTL), disturbing the binding of iron-response proteins (IRP) and consequently the post-transcriptional regulation of ferritin expression. Despite being an autosomal dominant condition, three rare cases of homozygous mutations in the FLT-IRE have been identified in HHCS patients. Here, we report on a novel 5'UTR mutation of FTL occurring in homozygous and heterozygous state in a consanguineous family affected with HHCS, and we demonstrate a correlation between the zygosity of the mutation and the severity of the disease.

Methods – The proband, an 8-year-old boy, was diagnosed with moderate bilateral cataracts and elevated serum ferritin (2770 µg/L) in the absence of iron overload (normal serum ferritin levels: 12 to 300 µg/L). Investigation of the proband’s parents, sister and brother revealed varying degrees of light bilateral cataracts combined with elevated levels of circulating ferritin, which are, however, at least four times lower than those of the proband. Therefore, all available family members were subjected to mutational analysis of the FTL gene using Sanger sequencing. The homozygous state of the mutation identified was confirmed by qPCR. To assess the impact of the FTL mutation on the IRE structure, RNA folding predictions were performed using Sfold (http://sfold.wadsworth.org/).

Results – A novel homozygous variant c.-151A>G (NM_000146.3) was identified in the IRE of FTL in the proband. In line with previous mutation notations, relative to the transcription initiation signal and the place where it was identified for the first time, the alternative notation of c.-151A>G is “Ghent +49A>G”. Segregation of the mutation in a heterozygous state was confirmed in the parents as well as the two siblings. The severity of the phenotype regarding both cataracts and serum ferritin levels shows a correlation with the zygosity of the mutation (mut/mut > mut/wt > wt/wt), a phenomenon not always observed in the previously reported HHCS families with homozygous FTL mutations. Sfold predictions of the secondary structure of the FTL 5'UTR indicate that the mutation is likely to disturb the wild-type IRE conformation. The +49A>G substitution is expected to induce a broad rearrangement of base pairing in the IRE, resulting in the loss of specific secondary structures involved in the direct contact with IRPs, experimental validation of which is ongoing by electrophoretic mobility shift assays.

Conclusions – The Ghent +49A>G FTL mutation adds to the repertoire of homozygous mutations in the HHCS, demonstrating a genotype-phenotype correlation of the zygosity of the mutation and the severity of the ophthalmological and hematological manifestations. The impact of this novel mutation on the binding affinity between the FTL-IRE en IRPs is being evaluated experimentally.
P42: BRD3 as a specific vulnerable therapeutic target in neuroblastoma.

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Introduction: BET inhibitors have raised high expectations for cancer treatment given their anti-proliferative effect by inhibiting BRD4 and enhancer activity of highly transcribed genes such as MYCN. However, current inhibitors also target BRD2 and BRD3 which are not functionally redundant with BRD4 and in neuroblastoma only MYCN amplified tumors respond well to these drugs.

Methods: We performed an integrated bioinformatics approach to identify candidate epigenetic regulators as targets for novel therapies in neuroblastoma.

Results: First we performed a time-resolved expression data analysis of week 1 and 2 hyperplastic lesions and tumors derived from the TH-MYCN transgenic mouse model and confirmed dynamic regulation during tumor development for established neuroblastoma oncogenes and tumor suppressor genes. Next, we filtered within the highest upregulated genes for Cancer Gene Census (CGC) genes and identified 21 upregulated CGC genes mainly involved in chromatin remodeling and DNA repair. Finally, after further selection based on expression in CCLE and survival in neuroblastoma patients, BRD3 was identified as the top-ranked candidate. BRD3 exhibits drastic upregulation during tumor formation, elevated expression is associated with very poor prognosis and BRD3 is the highest expressed in neuroblastoma in the CCLE cell line panel. To explore the non-redundant functions of BRD3 in relation to BRD4, we are currently performing RNA-sequencing after stable knockdown of BRD3 in neuroblastoma cell lines and are comparing it to the downstream effects on the transcriptome as well as the impact on cell viability upon knockdown of BRD4. In addition, we dissected the BRD3 protein complex by means of label-free mass spectrometry analysis to gain further insights into the BRD3 specific functions in relation to control of gene transcription and putative interaction with transcription factors such as MYCN. Current efforts are ongoing to test cooperative interaction of BRD3 versus BRD4 in dbh-MYCN driven neuroblastoma formation in zebrafish as well as BRD3 and BRD4 ChiP-sequencing in neuroblastoma cells.

Conclusion: We identified BRD3 as a candidate novel driver gene in neuroblastoma and present our data on differential transcriptional control and protein interactions of BRD3 versus BRD4 in order to gain insight into BRD3 specific oncogenic functions and as prelude to promote efforts towards developing BRD3 specific inhibitors for neuroblastoma and other BRD3 overexpressing cancer such as T-ALL and small cell lung carcinoma.
P43: Expanding preimplantation genetic diagnostic options

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Genome-wide haplotyping of preimplantation embryos has been used as a generic approach for preimplantation genetic diagnosis of inherited mutations. We have developed haplarithmis, an algorithm that allows for concurrent haplotyping and copy number profiling. Deducing haplotypes present in the embryo requires family member for phasing the parental genotypes of the embryos. There are currently two modules available: (1) both grandparents from the parental side burdened with the mutation or (2) an affected or unaffected child of the couple. Given that these genotypes are not always available, here we present a novel approach where a sibling of the parent carrying the mutation of interest can be used as seed for genotype phasing. This is possible by determining the shared allele between the extended family members. This new option is suitable for either healthy or affected seeds. Twelve extended families have been analyzed using the different modules. The consistency of the results as well as possible challenges are discussed.
P44: Neurofibromatosis type 1-related pseudarthrosis: beyond the pseudarthrosis site

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder affecting 1/2,700 individuals. NF1 is caused by an inactivating mutation in NF1 that encodes neurofibromin, a negative regulator of the RAS-MAPK pathway. Around 5% of NF1 children present with congenital bowing of a long bone resulting in fracture and pseudarthrosis (PA). The current treatment methods for NF1-PA are unsatisfactory, if union remains elusive the affected limb is amputated. Therefore improved understanding of NF1-PA is crucial.

Periosteum is an important mesenchymal stem cell (MSC) source during fracture repair. We performed NF1 mutation analysis and qPCR on cultured periosteal-derived cells (PDCs) from NF1-PA patients.

We found bi-allelic NF1 inactivation in PDCs of PA-tissue in 9/9 NF1 patients. In 3 patients sampled extensively we found a range of NF1+/− and NF1−/− cells in the PA. Periosteum surrounding the PA contained ±20% (proximal) to ±100% (distal) NF1−/− PDCs. One patient even had 30% NF1−/− PDCs at the osteotomy site, far above the PA. Combined with the observation that NF1-PA is always localized in the same skeletal regions, suggests involvement of additional local factors in the development of NF1-PA.

MSCs are characterized by trilineage differentiation potential. NF1+/+ and NF1−/− PDCs were subjected to qPCR for trilineage differentiation. NF1−/− PDCs showed a trend towards the adipogenic and myogenic lineages, which could contribute to poor bone formation and persistence of NF1-PA.

NF1 inactivation in the periosteum and aberrant differentiation are involved in the development and maintenance of NF1-PA. However, to uncover the full pathogenesis more research is needed.
P45: Genome-wide haplotyping uncovers mixoploidy in blastocysts of human preimplantation embryos

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Recently, we identified bovine cleavage stage embryos carrying distinct parental genomic blastomeres. We traced back the causal event to the first zygotic division and therefore coined the zygotic division leading to the segregation of parental genomes into distinct blastomere lineages with the term heterogoneic. These heterogoneic divisions resulted in biparental, androgenetic and/or gynogenetic blastomeres (i.e. paternal and maternal only, respectively) both in presence and absence of fertilization errors. Whether heterogoneic divisions in human exist remains unknown. By applying this novel methodology for concurrent single-cell haplo-typing and copy number profiling, which we termed haplarithmisis, on human blastocysts we demonstrate the existence of heterogoneic cell divisions in human. Specifically, a single blastomere displayed paternal monosomy. Additionally, some chromosomes showed diploidy, maternal monosomy, nullisomy or uniparental disomy. Upon blastocyst dissociation nine biopsies, ranging from a single cell to clumps of 2 to 5 cells, were analyzed with haplarithmisis. An identical profile to the aberrant biopsy was uncovered in a single cell and additionally, two single cells displayed a reciprocal maternal monosomic profile. Surprisingly, the remaining biopsies displayed diploid blastomeres except one blastomere carrying a paternal trisomy for a single chromosome. This is the first case of a heterogeneic division in a human cleavage preimplantation embryo. Moreover, we show the persistence of distinct blastomere lineages until blastocyst stage. This observation pinpoints the mechanisms underlying chimerism/mixoploidy and aneuploidy of human preimplantation embryos. In addition, we believe that paternal cell lines occasionally survive and can cause molar pregnancies.
P46: The presence of amplifications and distal 6q loss is associated with extremely poor survival in high-risk neuroblastoma patients

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Neuroblastoma is a pediatric solid tumor that is characterized by substantial clinical heterogeneity. Despite intensive treatment, the survival rates of high-risk neuroblastoma patients are still disappointingly low. To improve outcome prediction in this subgroup of aggressive neuroblastoma, we aimed at designing a prognostic classification method based on copy number aberrations.
Normalized high-resolution array-CGH data of diagnostic tumor samples from 551 high-risk neuroblastoma patients were collected from eight collaborative groups and uniformly processed. A general classifier based on aberrations across the whole genome was trained on data from 131 samples, but could not be validated in the two validation cohorts.

In addition, we assessed the prognostic value of individual aberrations. We identified two types of copy number aberrations associated with extremely poor survival: (i) in 31 patients, of which 90% died of disease, we found a distal 6q deletion and (ii) 36 patients, of which 92% died of disease, harbored an amplification other than MYCN. The association of these amplicons with survival not only aids in better prognostic stratification, but at the same time identifies patients that could benefit from treatments with inhibitors that target the amplified genes.

In conclusion, while it was not possible to classify patients based on a genome-wide classifier, we did identify a small subset of high-risk neuroblastoma patients with extremely poor outcome that might be eligible for inclusion in clinical trials of new therapeutics.
P47: A CARD9 founder mutation p.Arg70Trp in Belgian and French patients of Turkish origin with a spectrum of chronic mucocutaneous and invasive fungal infections

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Purpose: Biallelic CARD9 mutations predispose to autosomal recessive recurrent mucocutaneous and invasive fungal infections. Here, it was our aim to investigate the origin of a known CARD9 mutation c.208C>T p.(Arg70Trp) found in five Belgian and French families of Turkish origin with these phenotypes. In addition, we aimed to investigate the phenotypic features associated with the p.Arg70Trp mutation in these families.

Methods: Identity-by-descent (IBD) mapping was carried out in two affected individuals from one family by single nucleotide polymorphism (SNP) arrays. Starting from a homozygous region encompassing CARD9, microsatellite and SNP markers were genotyped for haplotype analysis in ten affected patients and eleven healthy family members from the four Belgian and one French families originating from the same region in Turkey (Emirdağ/Eskişehir). Immunologic characterization was done through routine immunologic assessments and immuno-functional assays on peripheral blood mononuclear cells. Clinical information was collected from all patients. Results: We investigated six patients from two unrelated Turkish families with distinct clinical phenotypes of chronic mucocutaneous and invasive fungal infections. Based on the presence of CARD9 in the second-largest IBD region, their clinical phenotype and defective fungal recognition, measured as IL-6 secretion upon Candida stimulation of peripheral blood mononuclear cells, the candidate gene CARD9 was sequenced. All patients carried biallelic p.Arg70Trp mutations in exon 3, which co-segregated with disease in all five families. This variant is rare in population databases (MAF 0.003%) and is predicted to affect protein function by in silico predictions and CADD (PHRED score 25.3). A decrease in CARD9 protein expression and association with impaired NF-kB activation was also shown experimentally for this allele (Lanternier, JACI 2015). Two patients from one of these families were already described earlier. Subsequent analysis of different cohorts and literature revealed four additional patients from three Turkish families. Since all ten patients originated from the Emirdağ/Eskişehir region in the Turkish province of Anatolia, we suspected the p.Arg70Trp to be a founder mutation. Earlier research had already shown a common founder origin for CARD9 p.Gln289* mutations (Lanternier, NEJM 2013), but this has not yet been suggested for the p.Arg70Trp mutation. Segregation analysis of four microsatellite markers and five SNPs indeed revealed a common haplotype of 1.03 Mb surrounding the mutation, suggesting a Turkish founder mutation. Targeted resequencing of the p.Arg70Trp mutation in 68 healthy Turkish individuals from the Istanbul region showed that the allele...
was absent in this population. This suggests that the p.Arg70Trp allele is geographically confined to the rural Anatolian province, where a tradition of consanguineous marriages promotes passing on of recessive disease. Despite all carrying biallelic CARD9 p.Arg70Trp mutations, the clinical phenotype of these ten patients varies widely. Chronic mucocutaneous candidiasis (CMC) was observed in six patients, together with Candida encephalitis and/or meningitis or osteitis in three of them. One patient suffered from pytiriasis versicolor. Treatment-resistant cutaneous dermatophytosis and deep dermatophytosis occurred in another patient. One patient developed abdominal C. albicans with lymphadenitis. Striking was the presence of another patient without known immunodeficiency who developed retroperitoneal aspergillosis, a rare infection in primary immunodeficiency and mostly limited to lung involvement. In addition, some patients also have endocrinopathy. It should be noted that most patients with mild clinical manifestations are still young and may develop more severe infections later in life. Conclusion: Haplotyping results are suggestive for a founder effect of the p.Arg70Trp CARD9 mutation in families with CMC and/or invasive fungal infections originating from the same region in Turkey. This implicates that targeted testing of the this mutation could be valuable in this specific population. Moreover, we expanded the clinical presentations of CARD9 mutations, varying from mild to severe infections, endocrinopathy or a combination of these conditions. Therefore, CARD9 screening can be recommended in patients with recurrent and/or invasive fungal infections in the absence of known immunodeficiencies. Improved identification of patients and family members at risk for severe fungal infections is important to guarantee a regular medical follow-up, especially since disease manifestation seems to aggravate with age.
**P48: ZFMP2 (FOG2) is a new candidate disease gene for primary ovarian insufficiency**

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Background: Primary ovarian insufficiency (POI) is a cause of female infertility affecting around 1% of women under the age of 40. Diagnosis is based on a triad of symptoms: amenorrhea for at least four months, sex steroid deficiency and follicle stimulating hormone (FSH) serum levels higher than 40IU/L in at least two measurements at least one month apart. POI can be the consequence of either follicle dysfunction or depletion and numerous causes are known, ranging from chemo- and radiotherapy to autoimmune disorders. For a subgroup of patients POI is related to genetic defects. Here, we hypothesized that mutations in the Zinc Finger protein multitype 2 (ZFPM2) gene (or FOG2) might contribute to POI pathogenesis, based on the identification of a heterozygous ZFPM2 deletion in a boy with 46, XY DSD and his mother with low AMH, suggestive of a decreased follicle reserve. As ZFPM2 mutations have previously been associated with 46,XY DSD, this is reminiscent to the situation for NR5A1, known as disease gene for 46,XY DSD, 46,XX POI and more recently for 46,XX DSD.

Methods: Copy number analysis of ZFPM2 was performed with array-CGH followed by qPCR validation. Targeted resequencing of ZFPM2 was conducted in 24 Belgian and 357 French patients with POI. Functional validation of the identified variants was performed by luciferase assays in HEK293T cells.

Results: We identified a maternally inherited ZFPM2 deletion in a boy with isolated 46,XY DSD. Hormonal investigations of the mother revealed a low AMH serum concentration, suggestive for a limited follicular reserve and POI risk. Subsequently, we identified seven novel heterozygous ZFPM2 variants in the POI cohort, five of which are missense variants, one in-frame duplication and one frameshift. Prediction algorithms suggest a deleterious effect of these variants and all variants are either absent in publicly available databases like Exac or occur at a population frequency below 1%. The activity of wild type and mutated ZFPM2 constructs on the AMH promoter is being assessed by luciferase assays in HEK293T cells.

Conclusion: We identified seven novel ZFPM2 variants in a large POI cohort, putting forward ZFPM2 as novel disease gene for POI and expanding the phenotypic spectrum of ZFPM2 mutations, so far only associated with cardiac malformations and 46,XY DSD.
P49: Clinical and molecular diagnosis of mosaic Neurofibromatosis type 1: next generation

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Individuals with segmental neurofibromatosis show typical features of neurofibromatosis type 1 (NF1) in one or more segments of the body. They can transmit the NF1 phenotype to their children if gonosomal mosaicism is present. In most cases of segmental NF no NF1 mutation can be detected in peripheral blood leukocytes and prenatal testing cannot be offered. Melanocytes and Schwann cells are affected by the segmental NF phenotype and should contain the NF1 mutation if the gene is involved. To allow counseling of these individuals we set up a method in our diagnostic lab to culture Schwann cells from neurofibromas and melanocytes from café-au-lait spots (CALS).

In our cohort of mosaic NF1 patients in general the phenotype is milder, however severe complications can be present. Segmental NF results mostly from a mosaic NF1 mutation that can be detected by sequencing and deletion testing of melanocytes from CALS or Schwann cells from neurofibromas. NF1 gene deletions and exonic deletions are overrepresented in our cohort and are not easily detected by next generation sequencing techniques. It is important in one patient to study more than one CALS and/or neurofibroma to distinguish between the mosaic NF1 mutation and the “second hits” in NF1. Prenatal diagnosis can be offered if a mosaic NF1 mutation is detected. Parent-offspring transmission has been noticed. In conclusion if mosaic NF1 is clinically recognized personalized follow up and genetic testing of affected tissues can be offered. If the mosaic NF1 mutation is detected prenatal testing can be proposed.
P50: Pre- and post-testing counseling considerations for the provision of expanded carrier screening: Perspectives of European geneticists

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Background: Carrier screening for recessive (autosomal and X-linked) genetic disorders is performed to identify healthy couples at risk of conceiving an affected child. Carrier screening allows carrier couples to make informed reproductive decisions and provides them with the option to prevent the birth of an affected child if they wish to do so. Expanded carrier screening (ECS) is a new paradigm in carrier screening, brought about by recent advances in targeted genotyping and next-generation sequencing technologies, and involves screening for a large number of disorders (usually more than 100) in a single test. Due to its ability to inexpensively include additional disorders on the screening panel, ECS has recently grown in popularity and could soon be implemented in the context of reproductive healthcare. However, large-scale adoption of ECS would have major implications for the practice of reproductive healthcare and is associated with important challenges.

Methods: During the period of April to September 2014, we conducted semi-structured interviews with sixteen European clinical and molecular geneticists with expertise in carrier screening to explore their views on the implementation of ECS in the clinical setting. The interviews were audio-recorded, transcribed, and analyzed using inductive content analysis.

Results: Our participants believed ECS would ideally be targeted at couples before pregnancy, although they were of the opinion that screening during pregnancy should also be accessible. They were worried about the limited awareness of carrier screening in the general public, as well as among non-genetically trained healthcare providers, suggesting that the implementation of a population ECS should be preceded by efforts to improve knowledge about carrier screening. All of our participants agreed it was essential to ensure informed and voluntary participation in ECS, recommending measures to minimize external pressure on prospective parents to undergo screening. To this end, participants strongly favored the provision of ECS services in the clinical setting through healthcare professionals, as opposed internet-based ECS offers by commercial companies. Additionally, several geneticists proposed utilization of carefully constructed audio-visual educational tools to improve pre-test information, and suggested a repeat-visit to the clinic for testing to ensure that those undertaking the test were sufficiently motivated. Furthermore, all participants emphasized the need to provide adequate genetic counselling and follow-up for couples identified to be at risk, in order to facilitate informed, autonomous reproductive decision-making and ensure necessary psychological support.

Conclusion: Population-wide ECS offers substantial benefits, but its successful implementation is conditional on the provision of the availability of adequate pre-test information and post-test counselling.
P51: The micro-RNA miR-210 is expressed by cancer cells but also by the tumor microenvironment in triple negative breast cancer

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The triple-negative breast cancer (TNBC) subtype occurs in about 15% of breast cancer and is an aggressive breast cancer with poor outcome. Furthermore, treatment of patients with TNBC is more challenging due to the heterogeneity of the disease and the absence of well-defined molecular targets. MicroRNA represents a new class of biomarkers that are frequently dysregulated in cancer. It has been described that the microRNA miR-210 is highly expressed in TNBC and its over-expression had been linked to poor prognosis.

TNBC are often infiltrated by immune cells which play a key role in cancer progression. The techniques traditionally used to analyse miR-210 expression like next generation sequencing or quantitative real-time PCR don’t allow the precise identification of the cellular subtype expressing the microRNA. In this study, we have analysed miR-210 expression by in situ hybridization in TNBC samples. The miR-210 signal was detected in the tumor cells, but also the tumor microenvironment, particularly in region positive for the pan-leucocyte marker CD45-LCA. Considering that miR-210 has numerous functions in the tumor microenvironment, the role of miR-210 in the infiltrating cells in TNBC clearly warrants further investigations.

Our results also highlight the necessity of using more standardized RNA extraction procedures and complementary approaches like ISH to take into account the cellular context of microRNA expression.
PS2: Identification of a 2nd form of Capillary Malformation–Arteriovenous Malformation (CM-AVM2)

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Capillary Malformation–Arteriovenous Malformation (CM-AVM) is an autosomal dominant disorder manifesting multifocal CMs together with high risk for fast-flow vascular malformations, especially in the head and neck. We have detected RASA1 heterozygous loss-of-function mutations in about 50% of screened families. This suggests genetic locus heterogeneity. We performed a genome-wide linkage study in a large family with autosomally inherited CMs without a RASA1 mutation, and identified a candidate locus. Whole exome sequencing was performed including an additional 11 blood samples of patients from 9 unrelated families. Subsequently, a candidate gene was screened by targeted massively parallel sequencing for mutations in 365 patients with CMs associated or not with fast-flow vascular malformations. A damaging mutation was identified in 5 out 9 families. Moreover, targeted massive parallel sequencing unraveled mutations in an additional 49 families with CMs with/without fast-flow vascular malformations. In total, 54 mutations were identified: 50% were non-sense, frame-shift, or splice site mutations, and 50% were substitutions predicted to strongly impact protein function. Expression studies of selected variants demonstrated loss of protein function. Mutations were identified altogether in 103 individuals: > 95% had capillary malformations, usually multifocal, and 20% an associated fast-flow vascular malformation. The cutaneous capillary malformations in these patients are more telangiectatic than in CM-AVM, and thus a clinical distinction can be possible. We suggest to call this new entity CM-AVM2. As the fast-flow lesions can cause severe morbidity and even mortality, it is important to recognize for entities. Miikka.Vikkula@uclouvain.be)
**P53: Zebrafish modeling of the \( \beta 4\)GalT7-deficient type of Ehlers-Danlos syndrome**

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Proteoglycans consist of glycosaminoglycan (GAG) side chains attached to a core protein through a tetrasaccharide linker region. Biallelic mutations in B4GALT7, the gene encoding galactosyltransferase I (\( \beta 4\)GalT7) which is an essential enzyme for the biosynthesis of the linker region, are the cause of the rare autosomal recessive variant of the Ehlers-Danlos syndrome (EDS). This disorder is mainly characterized by short stature, hypotonia and skeletal abnormalities, in addition to the typical features of EDS such as joint hypermobility and skin hyperextensibility. Our current knowledge about this severe and disabling disease is very limited, in part due to the lack of a relevant in vivo model. The aim of this study was to create a zebrafish model for the \( \beta 4\)GalT7-deficient type of EDS as there is a need for thorough, functional research of this disorder.

We developed and characterized a knockdown (KD) zebrafish model for the \( \beta 4\)GalT7-deficient type of EDS by using morpholino injections targeting the \( b4galt7 \) gene, as this model mimics the hypomorphic effects patients are suffering from. Embryos injected with a standard control morpholino were used as negative control.

Morphant embryos showed morphological abnormalities such as a small, round head, withdrawn jaw, more front-facing eyes, short stature and mild developmental delay compared to wild-type and control morpholino injected embryos. The total amount of sulfated GAGs, using the Blyscan assay, was severely reduced in morphant embryos and whole-mount immunohistochemistry showed that heparan and chondroitin sulfate proteoglycans were severely diminished in the heads of \( b4galt7 \) morphants. In addition, alcian blue staining demonstrated that cartilage structure in the heads of morphant embryos are absent or strongly misshapen and alizarin red staining indicated a lack of head bone structures. The \( Tg(Col2a1aBAC:mCherry) \) reporter line, which is cartilage specific, confirmed the impaired cartilage pattern and showed an impaired chondrocyte organization in \( b4galt7 \) KD embryos. Furthermore, morphant embryos suffered from a lack of muscle tone and immunohistochemical staining revealed a disturbed filamentous actin pattern in head and tail. The specificity of these results was confirmed by injection of a different morpholino targeting \( b4galt7 \) and by FO \( b4galt7 \) CRISPR/Cas9 injected embryos.

To conclude, a \( b4galt7 \) morphant zebrafish model has been developed, which partly phenocopies the human phenotype of patients suffering from \( \beta 4\)GalT7-deficient EDS. This model enables the in vivo investigation of the pathogenesis of this condition.
PS4: Incidence of uncommon fetal aneuploidies detected by non-invasive prenatal testing

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Non-invasive prenatal testing (NIPT) enables risk estimation for common fetal autosomal aneuploidies with high sensitivity and specificity. NIPT by genomic imbalance profiling (GIPseq) offers the unbiased sampling of cytotrophoblast cells in the maternal blood. Therefore, it allows to detect also uncommon aneuploidies present in the placenta. To inventorize the frequency of pregnancies with placental aneuploidies and to correlate those imbalances with fetal growth characteristics, we performed a multicentric cohort analysis. We included 22,416 pregnant women who were referred for NIPT to three Flemish Centres of Human Genetics during the past three years. We show uncommon aneuploidies in 71 cases (0.3% of pregnancies) with the highest incidence for trisomy 7 (16/71), trisomy 16 (14/71) and trisomy 22 (7/71) respectively. Not surprisingly, we demonstrate that they mostly occur as confined placental mosaicism, since uncommon trisomies are unviable. We investigate the associated risk for intra-uterine growth retardation. One fetus was found to be mosaic for trisomy 15 and additionally showed a maternal heterodisomy of chromosome 15, causing Prader-Willi syndrome. Our study illustrates that detection of uncommon aneuploidies by NIPT can improve pregnancy management and ultrasound follow-up.
P55: Single-cell genome sequencing to characterize chromosome instability in human cleavage stage embryos

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Chromosome instability (CIN) affects the first mitotic divisions of human embryo development following in vitro fertilization. DNA copy number analysis of individual cleavage stage blastomeres has revealed frequent missegregations of whole chromosomes as well as rearrangements of chromosome segments. To further characterize the nature and mechanism of CIN at this stage of human embryogenesis, we applied single-cell whole genome sequencing to all available individual blastomeres of 13 early cleavage stage embryos, and for 6 of these embryos we collected live imaging data of the divisions up to day 3, which we used to interpret the genome reorganization in light of the observed cell divisions. Profiling the copy number landscape of these genomes at unprecedented resolution—including the cell’s genome-wide haplotypes for part of the embryos—we uncover novel natures of CIN, including blastomere-polar body fusions as well as the creation of cell lineages with only the paternal or maternal haplotype.
BACKGROUND
Genetic counselors have been key members of multidisciplinary teams specialized in genetic healthcare in different European countries for more than 30 years. In Belgium, although a working group of genetic counselors was established in 2015, genetic counseling is still not recognised as a profession in its own right. There is currently no core curriculum for the training of individuals working within this role. Also, as the function of non-medically trained healthcare providers working as genetic counselors in Belgium has not been described, their scope of practice remains unclear.

OBJECTIVES
This study explores this scope of practice of paramedically trained healthcare providers operating in the eight genetic centers in Belgium in order to get a better understanding of the specific role of genetic counselors and to identify which additional training and support are necessary for the individuals working within these positions.

METHODS
This study involved administering a questionnaire to all 24 individuals operating within the psychosocial teams within the eight genetic centers in Belgium March 2016. This questionnaire was based on the Delphi method that was used by Skirton et al. (2013) in order to determine the European core curriculum for Masters programs in genetic counseling. These questionnaires asked participants to rate the relevance of a range of aspects related to genetic counseling to their current practice across seven areas: 1) counseling, 2) psychological issue, 3) medical genetics, 4) human genetics, 5) ethics, law and sociology, 6) professional practice and 7) education and research. Participants were also asked to nominate the aspects in which they would benefit from more training.

RESULTS
Twelve participants returned the questionnaire. 11/12 of the potential participants who did not return the questionnaire were psychologists and therefore were not working in a genetic counseling capacity. The participants listed their current titles as genetic counselor (n=5), nurse (n=2), midwife (n=2), psychologist (n=1), social nurse (n=1) and lab technician (n=1). All participants indicated that they frequently use appropriate communication and counseling skills and communicate effectively with the patient and family. Although one participant never assesses the patient's psychological state, 50% of participants do so occasionally and 42% do so frequently. 92% of participants agreed that knowledge about psychosocial aspects such as the impact of family history on the individual and their family, the impact of positive and negative test results on the individual and their family, the impact of living with a disease and test results, and the potential
reactions of the family to genetic risk or test results were very relevant to their practice. Yet participants gave differing opinions concerning the relevance of some aspects of human genetic knowledge.

CONCLUSIONS
The results shown a higher concordance between the participants in relation to the importance and relevance of psychosocial knowledge and skills, rather than of knowledge regarding human and medical genetics. By understanding the scope of practice of genetic counselors in Belgium, the working group can better assist these counselors and assess the need for additional training/education programs.
PS7: Phenomics analysis of zebrafish type I collagen mutants reveals a spectrum of skeletal phenotypes mimicking the clinical variability in human brittle bone disease

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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 very mild forms with minimal fractures, to severely deforming or even lethal forms. The underlying genetic basis of this variability between, but also within 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. We conducted a phenomics analysis on a large set of zebrafish mutants representing different forms of OI by mapping and quantifying skeletal parameters. 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.

Mice models are laborious and expensive for large scale genetic studies. Additionally, bone phenotypes in mice often cause perinatal lethality, making many bone mutants unavailable for the study of later stages. Zebrafish overcomes this challenges as a model. 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.
PS8: RRM2 is an essential and druggable component of the FOXM1 driven replicative stress DNA damage response in neuroblastoma

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Ribonucleotide reductase provides the essential nucleotides during DNA replication with the RRM2 subunit being its primary regulator of enzymatic activity. RRM2 overexpression has been shown to be an important predictor of poor prognosis in various cancer entities and was part of a 4-gene prognostic signature for neuroblastoma patients.

The aim of my study is to investigate the regulatory processes controlling RRM2 levels in neuroblastoma development and to investigate possible options for pharmacological intervention in children with high risk neuroblastoma for which currently prognosis is still very poor.

First, we propose a CHD5-WEE1-RRM2 regulatory axis based on in silico analysis of a large data set of primary neuroblastomas. This is in keeping with the previously reported regulation of RRM2 by the CHD5 chromatin remodeler and the WEE1 kinase. Second, correlation analysis also supports the negative regulation of RRM2 by several let-7 miRNAs. Finally, we also find evidence for the previously reported BRCA1 direct regulated RRM2 expression. As expected, RRM2 mRNA levels were also highly correlated with elevated MYCN levels, MYCN being the main oncogenic driver in neuroblastoma. Remarkably, several key components of the RRM2 regulatory network are located in regions that are recurrently affected by DNA copy number alterations such as 1p deletion (CHD5), 2p amplifications and gains (MYCN, RRM2) and 17q gain (BRCA1), in keeping with the notion that neuroblastoma is a copy number driven cancer entity. Of further interest, we also performed a correlation analysis for RRM2 across the data set for the entire transcriptome and observed a remarkable enrichment of DNA damage response genes, most notable FOXM1 and FOXM1 target genes. This is in keeping with our current hypothesis that neuroblastoma cells establish a replicative stress resistance phenotype during early MYCN driven tumor development ensuring timely and smooth DNA replication by hyperactivation of cell cycle checkpoints, replication fork stabilization and processing, resolving transcription-replication conflicts and mediating mitotic DNA synthesis. Further functional assays are ongoing to validate our in silico analysis, including the generation of a dbh-RRM2 overexpressing zebrafish model for further studies.

In a second part of the study, we explored RRM2 inhibitors in a series of neuroblastoma cell lines and observed high drug sensitivity, thus validating RRM2 as a new possible drug target in this cancer type. In addition, we tested a WEE1 inhibitor and observed the expected inhibition of RRM2 expression levels and high sensitivity for the drug in most cell lines tested.

In conclusion, we show our first results on RRM2 regulation as one of the component driving the replicative stress resistance phenotype in neuroblastoma and propose RRM2 as a novel future drug target for high risk neuroblastomas.
PS9: Screening for genetic and structural variation in the UCP1 gene in obese children and adolescents

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Objective
Obesity is a highly heritable complex and heterogeneous disorder, characterized by an excessive amount of adipose tissue. Numerous studies have shown that 40-70% of the interindividual variability in BMI is attributed to genetic factors, of which approximately 2.7% are currently explained by associated polymorphisms. Copy number variants (CNVs) account for a major proportion of human genetic variation and have been predicted to have an important role in genetic susceptibility to common disease. Genomewide copy number variation (CNV) analyses have associated the 4q31 CNV, containing the UCP1 gene, with obesity. As UCP1 is the most interesting candidate gene in this region, we hypothesized that both genetic and structural variation in UCP1 may be implicated in the pathogenesis of obesity.

Design and methods
In the first part of this study, Multiplex Amplicon Quantification (MAQ) analysis was used to identify CNVs in the UCP1-containing chr.4q31 region in 306 obese children and adolescents. In the second part of this study, we performed a mutation screen for variants in the UCP1 coding region in 643 obese children and adolescents, and 445 healthy lean adults.

Results and conclusion
In our CNV analysis we could not identify CNV in the UCP1 region in our population. Mutation analysis resulted in the identification of three rare non-synonymous heterozygous variants, 2 of which could only be found among obese individuals and 1 that was only identified in a lean subject. By performing in silico analysis, we determined that these 3 variants are probably damaging to the protein structure and might have a disease causing effect. Further functional testing will be necessary to fully understand the impact on UCP1. As we could not identify any CNV in the UCP1 region, structural variation in this gene is unlikely to majorly contribute to the obese phenotype.
P60: Genomes of patients with malignant pleural mesothelioma show frequent copy number variations in regions containing previously reported and unreported cancer-associated genes.

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Objectives:
The genetic background of malignant pleural mesothelioma (MPM) is incompletely known. Using comparative genomic hybridization techniques and arrays, an heterogeneous set of copy number variations (CNVs) was previously demonstrated. These techniques however have a limited resolution compared to next-generation sequencing platforms.

Methods:
The genomes of 21 MPMs and matched normal samples were analysed using low-pass whole genome sequencing on an Illumina HiSeq platform. CNVs were detected using in-house developed analysis pipelines and frequencies of copy number loss and gain were calculated. Results were validated using an MPM-cohort (N=85), for which array data were available through The Cancer Genome Atlas (TCGA).

Results:
Chromosomal regions with recurrent gains and losses were identified in our sample set. Losses of regions on chromosomes 1, 3, 4, 6, 9, 13, 17 and 22 were found in at least 25% of cases, with some regions being lost in up to 60% of all MPMs. Gains occurred less frequently, with regions on chromosomes 1, 3, 5, 7, 15 and 17 exhibiting gains in more than 15% of MPMs. In general, these results were confirmed in the TCGA-dataset. The frequency of CNVs in regions harbouring ‘Cancer census genes’ was determined. Apart from regions exhibiting known MPM-associated genes (e.g. BAP1, CDKN2A and NF2), genes that were not previously associated with MPM were located within the most frequently involved regions. In the TCGA-set, preliminary analyses identified a statistically significant correlation between an overall survival shorter than 36 months and the presence of copy number loss in the region containing CDKN2A.

Conclusion:
Recurrent CNVs were detected, occurring in regions harbouring both known MPM-associated genes and genes that were not previously linked to MPM. Further analyses will determine which CNVs are correlated with clinicopathological parameters and hence are of potential interest in a clinical setting.
Inversion polymorphisms between low copy repeats (LCRs) often predispose to meiotic non-allelic homologous recombination (NAHR) events causing genomic disorders. However, for the 22q11.2 deletion syndrome (22q11.2DS), the most common genomic disorder, no such inversions have been uncovered as of yet. Using fiber-FISH, we demonstrate that parents transmitting the de novo 3 Mb LCR22A-D 22q11.2 deletion, the smaller 1.5 Mb LCR22A-B 22q11.2 deletion or the reciprocal 22q11.2 duplication, carry inversions between LCR22B or LCR22C and LCR22D. Hence, the inversions predispose to meiotic 22q11.2 rearrangements and increase the individual risk over two-fold for transmitting rearrangements when compared to the population. Interestingly, the inversions are nested or flanking rather than coinciding with the deletion/duplication sizes. This finding raises the possibility that inversions are a prerequisite not only for 22q11.2 rearrangements but for most NAHR mediated genomic disorders. Additionally, the lack of individuals or patients homozygous or hemizygous for the inversion suggests negative selection.
P62: Case report: Aberrant Sexual Behavior in two adults with the recurrent 16p11.2 deletion

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Background: Deletion of the recurrent ~600 kb BP4-BP5 chromosomal region 16p11.2 has been associated with a wide range of neurodevelopmental and physical characteristics. An increased frequency of autism spectrum disorder (ASD), intellectual and learning disabilities, behavioral difficulties and psychiatric disorders has been observed (Hanson et al. 2015, Miller et al. 2015). However, the description of this behavioral phenotype remains vague and incomplete.

Methods: From 2005 up to 2015, 35 intellectually disabled adults were admitted to the inpatient psychiatric unit because of out-of-control sexual behavior. Two of them (5.7 %) were diagnosed with the recurrent 16p11.2 deletion. A detailed description of their cognitive functioning, psychiatric history and aberrant sexual behavior will be provided. In addition, possible environmental factors contributing to the this aberrant sexual behavior will be explored.

Results: The first male was diagnosed with mild intellectual disability, epilepsy and hypersexual disorder defined as “excessive sexual behavior accompanied by personal distress and social and medical comorbidity” and for which he was treated with anti-androgens. The other male was diagnosed with moderate intellectual disability, ASD, aggression and pedophilia. Neither of these men had a history of possible provoking environmental factors such as a history of sexual abuse.

Discussion: To our knowledge, this is the first report of severe aberrant sexual behavior in adults with the recurrent 16p11.2 deletion. Although the etiology pedophilia and hypersexual disorder remains unknown, recent studies have suggested a possible genetic contribution toward pathological sexual interest and behavior (Jakubczyk et al. 2017). The fact that out-of-control sexual behavior has not been reported before in adults with 16p11.2 deletions may be explained by the fact that hypersexual disorder and pedophilia are not included in the standardized psychiatric questionnaires used in most studies. Another explanation may be that the diagnosis of aberrant sexual behavior may be missed in 16p11.2 del adults with normal or borderline intelligence. Although these results have limited value by the small sample and are exploratory, they may be an indication for including evaluation of sexual behavior in adolescents and adults with 16p11.2 deletion.

References:
cognitive and behavioral phenotype of the 16p11.2 deletion in a clinically ascertained population. Biol Psychiatry. 2015 May 1;77(9):785-93.


P63: MAGT1-deficiency: novel insights into a controversial protein with a key role in N-glycosylation

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Congenital Disorders of Glycosylation (CDG) are a rapidly growing and heterogeneous group of rare metabolic diseases caused by inborn defects in glycosylation. Since the discovery of the first cases of CDG in 1980, almost 100 different genetic defects causing these disorders have been discovered. In the past five years, a large number of CDG genes were found using Next Generation Sequencing (NGS) techniques.

In our lab a Custom Capture Assay (CCA) was optimized for 79 known CDG- and candidate genes. With this technique two patients with hemizygous mutations in the candidate gene MAGT1 were picked up. A missense mutation was identified in an 11-year-old boy (P1). Further investigations showed that his mother was carrier of the same mutation, but fully skewed the MAGT1-deficient X-chromosome. Second, a de novo nonsense mutation was found in a 9-year-old boy (P2). Both children displayed similar clinical features with Intellectual Disability (ID), developmental delay, macrocephaly and hepatomegaly without splenomegaly.

However, mutations in MAGT1 (c.859_997del, p.N287*fs*1; c.172G>A, p.W37*; c.409C>T, p.R137*; c.598delC, p.R200Gfs*13) have also been described to cause an X-linked immunodeficiency disease (XMEN), characterized by chronic Epstein-Barr virus infections. These studies claim that MAGT1 is a plasma membrane localised Mg2+ transporter required for Mg2+ uptake in vertebrate cells.

This contradicts reports showing that MAGT1 is localised at the Endoplasmic Reticulum (ER) and a subunit of the oligosaccharyltransferase (OST) complex. The latter plays a very important role in N-glycosylation since it catalyses the transfer of the preassembled oligosaccharide precursor to nascent proteins. The two identified patients with a mutation in MAGT1 offer us the opportunity to confirm the causal role of the gene in CDG and to study the function of the protein in glycobiology. Our hypothesis is that MAGT1 is a component of the human OST complex, and is thus involved in the glycosylation of (a subset of) N-glycoproteins. We speculate that one of these substrates could be an Mg2+ transporter, which would explain the phenotypes observed in the XMEN patients.

To test this hypothesis, we have conducted confocal microscopy experiments, which show that the localisation of the protein is not affected in the patients and that it is localised at the ER, confirming the OST complex hypothesis. Second, the expression levels of the different subunits were investigated by transcript analysis. A reduction of more than 50% in MAGT1 expression was observed in the patient presenting a stop mutation. Interestingly, TUSC3 (the homologue of MAGT1) transcript level was also upregulated in both patients-derived cells (2 to 3-fold). Furthermore, also the expression of the catalytic subunits of the OST-complex were altered in P2. These results point towards a rescue mechanism in this patient. Moreover, our study of MAGT1 dependent substrates by western blot confirms that these proteins are hypoglycosylated in the patients’ cells.
At last, preliminary results show that the Mg2+ homeostasis is affected in the patients’ fibroblasts. This could be due to aberrant glycosylation of one (plasma membrane-localised) Mg2+ transporter, to date unknown. Further investigation will allow us to assess (1) the impact of the identified MAGT1 mutations on glycosylation and (2) to elucidate the controversial link between MAGT1 deficiency and magnesium homeostasis.
P64: Analytical and computational performance of variant calling pipelines for targeted NGS gene panels

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The simultaneous analysis of a large set of genes by targeted NGS has become an important tool in clinical diagnostics. Implementation and validation of these novel technologies in a clinical setting requires a good understanding of their analytical performance. This performance is determined by the chosen wetlab protocols and the applied bioinformatic pipeline. We evaluated the performance of different bioinformatic pipelines on data that were generated for two well characterized cell lines (Illumina Platinum Genomes, NA12877 and NA12878).

The exonic regions of 5,811 genes (UZL Mendeliome) were sequenced with an average coverage of 171x and 144x for NA12877 and NA12878, respectively (Illumina HiSeq2500 PE 126 bp). Both datasets were analysed with multiple pipelines combining several mapping, bam-post processing and variant calling tools. The pipelines were run on the same computing infrastructure (Ivy Bridge Xeon E5-2680v2 CPU (2x10 cores), 128GB RAM). If possible, the tools were ran multithreaded or in parallel. The analytical performance was assessed by comparing the variant calls to the platinum genotypes. All pipelines produced calls (variant and reference) for every position in the target (16.35Mb). These call sets were filtered using different criteria and compared to the platinum calls.

BWA-mem was slightly faster than Bowtie2 and produced on average higher mapping quality scores. The call sets based on BWA-mem mapping showed higher congruence with the platinum calls than the call sets based on Bowtie2 mapping. Varying the bam-post processing tools (sorting and duplicate marking) had a minor impact and resulted in almost identical call sets, but elPrep was substantially faster. The choice of variant caller (GATK and FreeBayes) had a strong impact on the resulting call sets and speed performance. GATK was four times faster than FreeBayes and twice as fast when base-recalibration was included. The analytical performance of the variant caller depends on the criteria used for deciding whether a call is reliable/included or not. These criteria determine the reportable range of your experiment, i.e. the fraction of target positions where a call is made. An optimal filter setting will maximize the reportable range, specificity and sensitivity. Since depth of coverage is often used for identifying reliable positions and as such for determining the reportable range, it was used to compare the performance of both callers. Unlike other potential filter parameters, depth is independent of the variant caller. The analytical performance of both callers was similar but we observed that tool specific misclassified calls tended to cluster together, suggesting that sequencing context is important and affects the performance of both variant callers differently.

The combination of BWA-mem and elPrep to prepare the bam files performed best for this application. When speed is important, GATK is the prefered variant caller. However, when choosing a variant caller for a particular application it is important to evaluate the analytical performance for different filtering criteria (not only depth) and take into account the trade-off between reportable range, specificity and sensitivity.
P65: 1q21.1 copy number variants

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Introduction: Array-Comparative Genomic Hybridization (array-CGH) has become a first-tier test in the diagnostic evaluation of children with developmental delay. Since the implementation of array-CGH in the clinical practice, small changes in the amount of DNA, so-called copy number variants (CNV’s) are sometimes detected. The clinical implications of these CNV’s is often unclear. We present a subset of three patients with a 1q21.1 microduplication and three patients with a 1q21.1 microdeletion, referred to the department of genetics.

Aim: We tried to find a common phenotype for patients presenting with a 1q21.1 CNV, which could be implemented in the screening and clinical follow-up of these patients.

Methods: Six patients showed a 1q21.1 CNV by array-CGH. The literature was reviewed using PubMed. The patients were re-invited to the consultation in order to discuss the results obtained by array-CGH. The counseling of the patients included the clinical presentation to be expected and was based upon the data found in the literature. A segregation analysis in parents/siblings was proposed.

Results: Review of literature showed that patients with 1q21.1 CNV’s more frequently have lower intelligence and a delayed motor- and/or language development, microcephaly (in case of a deletion) or macrocephaly (in case of a duplication). Other neuropsychiatric symptoms include autism spectrum disorders (ASD), seizures and behavioral problems. A range of comorbidities (cardiac, ophthalmic, auditory problems) are linked with 1q21.1 CNV’s. The patients in our population presented similar neuropsychiatric findings, deviations in head circumference and severity of developmental delay as those described in literature. Other comorbidities tend to be diverse and unspecific. The most frequent observed comorbidities include hearing loss and vision problems. Segregation analysis showed that a large number of the parents of these children are also carrier of these CNV’s and are without (obvious) symptoms. This suggests, in accordance with literature, that 1q21.1 CNV’s should be seen as predisposing factors but they cannot be considered as a distinct syndrome.

Conclusion: 1q21.1 CNV’s are predisposing factors for developmental delay, microcephaly or macrocephaly, and various neuropsychiatric symptoms. In the follow-up of these children we ask vigilance for hypotonia, feeding problems, behavioral problems/ADHD/ASD and other psychiatric disorders (schizophrenia). In case of the detection of a 1q21.1 CNV, we advise referral to an ophthalmologist, cardiologist and ENT specialist in order to exclude associated comorbidities. Genetic counseling of these patients and their families is difficult, given the wide range of symptoms and the variation in severity. This complexity gives also rise to ethical problems regarding whether or not performing pre-implantation genetic diagnosis (PGD) or invasive prenatal diagnosis in families in whom a 1q21.1 CNV was detected in a proband and an (asymptomatic) parent.
P66: Study of homologous recombination repair in brca2 knockout zebrafish embryos

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Aims:
Since the introduction of next generation sequencing, the challenge for genetic testing moved from developing mutation detection methodologies towards adequate variant interpretation. In preparation of a novel in vivo approach to study the functionality of BRCA2 missense variants in zebrafish, we thoroughly studied the capacity of homologous recombination (HR) in wild type zebrafish embryos and embryos with a knockout/knockdown of endogenous brca2.

Methods:
We induce DNA double strand breaks (DSB) in zebrafish embryos by irradiation. We use γH2AX and RAD51 foci assays as markers for DSB and HR repair respectively. We generated zebrafish brca2 knockdown models by morpholino injection and obtained 3 stable brca2 knockdown lines, one of which obtained through Crispr-Cas9 mutagenesis.

Results:
We developed a protocol for visualising and quantifying RAD51 foci in zebrafish embryonic tissue. Brca2 knockdown by a splice blocking morpholino resulted in almost complete absence of RAD51 foci. Similar results were obtained in the three brca2 knockout lines. Interestingly, we also observed a small but statistically significant decrease in HR in heterozygotes. Besides protocols using microscopy for visualisation of RAD51 foci, we also applied a qPCR-based HR assay, which allows for a higher throughput. This assay also accurately quantified knockdown. Currently we are optimising several protocols to rescue the phenotype by microinjection of wild type human and zebrafish BRCA2 mRNA/protein. In a next step VUS will be introduced to study their effect on HR capacity.

Conclusions:
The zebrafish genome contains nearly all genes involved in different DNA repair pathways in eukaryotes, including HR, in which BRCA2 plays a major role. Therefore, zebrafish provides an ideal in vivo model for studying (variants in genes involved in) DNA damage and repair.
P67: Phenotype in siblings with homozygous mutations of TRAPPC9 and MCPH1.

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The TRAPPC9 defect is characterized by severe intellectual deficiency (ID), postnatal microcephaly, abnormalities of the corpus callosum, cerebellum and white matter. It is inherited as an autosomal recessive trait and all disease-associated mutations have been truncating.

MCPH1 causes autosomal recessive primary microcephaly, with mild to moderate ID and a normal brain except for a small brain size. MCPH1 is expressed at the centrosome and plays roles in mitotic spindle alignment and in the mitotic spindle checkpoint. It contains 3 BRCT domains which are believed to be essential for MCPH1 function.

We report on two siblings with severe ID, microcephaly, and hypoplasia of the corpus callosum. Exome sequencing showed a homozygous mutation in exon 2 of TRAPPC9 (p.Leu178Pro), and a homozygous mutation in exon 13 of MCPH1 (p.Arg741*). This premature stop codon truncates the third BRCT domain of MCPH1, predicted to be important for MCPH1 function. Nonetheless the MCPH1 homozygous mutation was observed in an unaffected sister, revealing its harmless character. Causality of the TRAPPC9 mutation is likely considering the severe ID phenotype with corpus callosum hypoplasia, concordant in two siblings.

Our observation shows that truncation of the third BRCT domain of MCPH1 is consistent with a normal phenotype, and describes the first ID-associated missense mutation in TRAPPC9. It emphasizes that caution must be exerted when interpreting the clinical effect of stop codons in exome sequences, and allows to speculate on possible digenic interactions between MCPH1 and TRAPPC9 in the affected siblings.
P68: Second hit landscape in BRCA-associated breast cancer

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Objective
The literature states that in BRCA1/2-associated breast tumors somatic loss of the wild type allele (LOH) is the most prevalent mechanism leading to the absence of a functional BRCA tumor suppressor gene product. However, little is known about the prevalence of other somatic events (including point mutations). This study's objective was to paint a detailed picture of the somatic alterations in breast tumors from patients with a germline BRCA1/2 mutation.

Methods
We obtained 88 formalin fixed and paraffin embedded (FFPE) breast tumors from proven germline BRCA1/2 mutation carriers, diagnosed between 1989 and 2014. Using NGS libraries constructed from multiplex PCR products, analysis of the complete coding region of BRCA1/2 was performed in FFPE breast tumors and in the matching blood samples to evaluate loss of heterozygosity and the presence of somatic nucleotide variations. In addition, exon-spanning deletions/duplications were investigated using MLPA and methylation-specific MLPA was used to unravel the methylation status of both BRCA promoters.

Results
Loss of the wild type allele was observed in a large number of the tumors. The LOH region spans variable proportions of the gene. In addition, several somatic inactivating point mutations were observed. Methylation of the promoter region however was found to be a rare event. Interestingly, in 9 tumors loss of the mutant allele was observed. We are currently correlating histological, pathological and clinical data to the molecular data to gain more insight in the events that may have led to the unexpected finding of loss of the mutant allele.

Conclusions
We observed both loss of the wild type and mutant allele in the tumors. In addition a large number of somatic BRCA1/2 variations were detected. The results of this study point out that determining the BRCA functionality within breast tumors from BRCA1/2 germline mutation carriers is challenging. This implicates that LOH analyses in breast tumors to determine a role for variants of unknown clinical significance is not informative. The complex combination of somatic events in this cohort could potentially also help explain the less successful treatment effect of PARP inhibitors in breast cancer.
P69: Trisomy 21/mosaic Turner detected in fetus by non-invasive prenatal testing

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Non-invasive prenatal testing (NIPT) is a screening method for the early detection of foetal aneuploidies in pregnant women. While originally developed for the detection of trisomy 13, 18 and 21, it is becoming clear that NIPT can be used for the identification of rare foetal aneuploidies and mosaic aneuploidy as well. Here we describe a female foetus with trisomy 21 in combination with mosaicism X0/XX, detected during follow-up of an abnormal ultrasound (enlarged NT: 3,3 mm). Microarray analysis and FISH on chorion villi cells confirmed trisomy 21, and mosaicism X0/XX (~29% of cells (n=63)). NIPT convincingly detected the presence of trisomy 21 with a Z-score of 22.9. (Partial) monosomy X on NIPT was revealed by the absence of a clear second X chromosome on the sex plots and the high discrepancy between the foetal fraction of chromosome X and the foetal fraction of chromosome Y. Likely the high seqFF value (16%) helped to more confidently identify these aneuploidies. While the debate as to whether or not aneuploidies of the sex chromosomes should be reported continues, this study shows that these aneuploidies can nevertheless be picked up, and probably other rare genetic abnormalities as well.