A comprehensive cardiomyopathy gene panel

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"Genetics & Society"

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Leuven, Belgium

Multiplicom
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SOPHIA GENETICS

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Welcome

Dear Participants to the First Joint Meeting of the Belgian and Netherlands Human Genetics Societies,

It is with great pleasure that we welcome you to this Meeting. This joint project had been wished by the NVHG for more than 5 years, and over a year ago Frank Baas and Hans Kristian Ploos van Amstel of the NVHG came to their first Local Organizing Committee Meeting in Leuven – they have both been back since, and once together with John Engelen.

"Genetics and Society" is the title of this year's Meeting and we do hope that you will actively participate the following 2 days in the Science:

- Today you will be stimulated not only by issues in "molecular archaeology", "miniaturization of biosensors" and "GWAS to panels" but also by "Genetics and Genomics, from Backroom to Centre Stage", "Rare Disease Patient Organisations/ Centres of Expertise" and "Positive Exposure".
- Tomorrow we start with the Herman Van den Berghe Foundation Lecture From New genes, pathways and subtypes in Autism, and will move on to hear the Role of Gut Bacteria in Health and Disease, look at Single Cell Technology in the Biology of Cellular Heterogeneity in Health and Disease, to close with the the Galjaard Prize laureate talking on "maintaining Nature's Perfection".

Thursday afternoon you will be able to partake in informal presentations and discussions in the Speciality Group of your choice. Our Young Investigators are also showing the outcome of their scientific endeavours in both the 25 platform presentations and the numerous posters.

This diverse Meeting would not be possible without the generous support from our sponsors. A big Thank You to all of them. I encourage all participants to show their appreciation of this support by talking to the representatives from all the stands.

The AGM of the BeSHG on Friday morning will highlight some of the activities of this Society.

Welcome to Leuven. We thank you for your scientific support.

Frank Baas & Thomy de Ravel

On behalf of the Boards of both Societies as well as the Local Organizing Committee

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GENOME DIAGNOSTICS NIJMEGEN

Invited speakers

Peter Van Loo
Winton Group Leader – Cancer Genomics, Francis Crick Institute, London, UK

Peter Van Loo is a group leader at the Francis Crick Institute in London, and a part-time assistant professor at the Department of Human Genetics, University of Leuven. He did his PhD at the University of Leuven, where he developed computational approaches to detect *cis*-regulatory modules and to prioritise candidate disease genes. Through initial studies in breast cancer and lymphoma, he developed an interest in cancer research. After his PhD, he developed approaches to study copy-number alterations in cancer genomes, at the Institute for Cancer Research, University of Oslo. The potential created by these methods laid the foundation for many further studies in cancer genomics and prompted him to focus on massively parallel sequencing of cancers and to move to the Wellcome Trust Sanger Institute. There, he developed a strong focus on "molecular archaeology" approaches, studying the subclonal architecture of cancers and disentangling the life history of cancer genomes. His research group at the Francis Crick Institute aims to leverage the wealth of data from massively parallel sequencing efforts to understand carcinogenesis and cancer evolution. Dr. Van Loo has been awarded a Cancer Research UK Future Leaders in Cancer Research Prize in 2015.

Liesbet Lagae
R&D Manager IMEC Life Science Technologies, Belgium

Professor Liesbet Lagae received her PhD degree from the KU Leuven, Belgium for her work on Magnetic Random Access Memories in 2003. As a postdoctoral researcher she pioneered life science technologies based on silicon based biochips at IMEC, Belgium. IMEC is a world-leading independent research centre in nanoelectronics and nanotechnology. Liesbet Lagae is currently R&D manager of IMEC's Life Sciences programme. The programme aims to find solutions for healthcare needs by using silicon (nanoelectronics) based smart technology. In her current role, Liesbet is the scientific leader of a multidisciplinary team of >30 researchers working on miniaturization aspects of biochips, microfluidics and integration of bio-assays. The technology building

blocks are used in diverse applications related to next generation sequencing, cytometry, cell sorting, PCR-on-chip, implants and bioreactors.
Liesbet has (co-) authored 297 publications and holds 16 patents in the field. She coordinated several EU and regional projects. She holds a prestigious ERC Consolidator Grant that deals with an innovative cell sorter-on-chip technology. She is also part-time professor in nanobiotechnology at the KU Leuven/Phyrsics department and is or has been (co-)promotor of >20 PhD students.

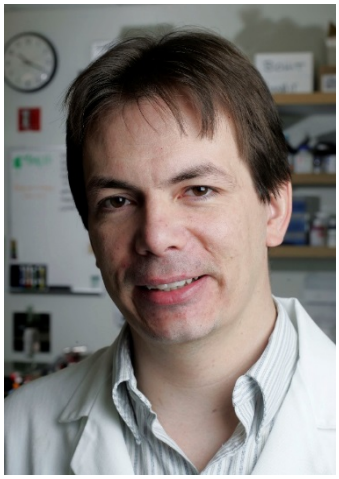
Doug Easton
Professor/Director, Centre for Cancer Genetic Epidemiology, University of Cambridge, UK

Douglas Easton graduated in mathematics from the University of Cambridge and gained his PhD at the Institute of Cancer Research in London. He returned to Cambridge in 1995, where he is currently Professor of Genetic Epidemiology and Director of the Centre for Cancer Genetic Epidemiology, based at Strangeways Research Laboratory. His main research interests are in the genetic epidemiology of cancer and related statistical methods. His group carries out research into inherited predisposition to cancer, with a particular emphasis on hormone-related cancers. In 2007 he led the first genome-wide association study in breast cancer. He co-ordinates the Breast Cancer Association Consortium, a collaborative group that has collected data from over 200,000 individuals from over 70 studies and has identified approximately 100 genetic loci for breast cancer. His group has developed the BOADICEA model, widely used for genetic counselling.

Dian Donnai
Manchester Centre for Genomic Medicine, University of Manchester and Central Manchester University Hospitals NHS Foundation Trust, UK

Dian Donnai is Professor of Medical Genetics and Consultant Clinical Geneticist in the University of Manchester. She trained in paediatrics and clinical genetics and has a major interest in the care and investigation of children with developmental disorders. Her research has focussed on elucidating the underlying causes of these disorders. She was awarded the Lifetime Achievement Award in Genetics by the March of Dimes in 2010. She is past president of the Clinical Genetics Society (1997-9) and the European Society for Human Genetics (2009-10). She is now Head of Saint Mary's Hospital where the Manchester Centre for Genomic Medicine is based.

Evan Eichler
Professor of Genome Sciences, University of Washington, USA



Evan Eichler, Ph.D., is a Professor and Howard Hughes Medical Institute Investigator in the Department of Genome Sciences, University of Washington School of Medicine. He graduated with a B.Sc. Honours degree in Biology from the University of Saskatchewan, Canada, in 1990. He received his Ph.D. in 1995 from the Department of Molecular and Human Genetics at Baylor College of Medicine, Houston. After a Hollaender postdoctoral fellowship at Lawrence Livermore National Laboratory, he joined the Faculty of Case Western Reserve University in 1997 and later the University of Washington in 2004. He was a March of Dimes Basil O'Connor Scholar (1998-2001), appointed as an HHMI Investigator (2005), awarded an AAAS Fellowship (2006) and the American Society of Human Genetics Curt Stern Award (2008), and elected to the National Academy of Sciences (2012). He is an editor of *Genome Research* and has served on various scientific advisory boards for both NIH and NSF. His research group provided the first genome-wide view of segmental duplications within human and other primate genomes and he is a leader in an effort to identify and sequence normal and disease-causing structural variation in the human genome. The long-term goal of his research is to understand the evolution and mechanisms of recent gene duplication and its relationship to copy number variation and human disease.

Cisca Wijmenga

Professor of Human Genetics, Univesity Medical Centre Groningen, The Netherlands

Professor Cisca Wijmenga has been Professor of Human Genetics at the University of Groningen and Head of the Genetics Department of the University Medical Center Groningen since 2007. The aim of her research is to understand the molecular basis of chronic diseases such as autoimmune and metabolic diseases. Her work combines genetic, functional genomic and microbiome research to understand the intricate relation between genetic and environmental factors. For her research she includes and integrates clinical cohort studies and population biobanks such as LifeLines, the largest prospective cohort study in the Netherlands.

Ingrid Jageneau

President, RADIORG, the Belgian Alliance of Rare Disease Patient Organisations

Ingrid Jageneau has a grown up son with Epidermolysis Bullosa, a rare hereditary skin disease. In 1985 there was no information available in Belgium, no EB-centre, no reimbursement of wound dressings. Together with other families she founded Debra Belgium in 1998. Thanks to Debra, new families now have access to specialized care. Six years ago Ingrid joined Radiorg, the Belgian Alliance for Rare Diseases - recognized as such by Eurordis - the last 4 years as president.

Radiorg is a patient driven umbrella organisation and mainly works with volunteers. The main goal is to serve as the voice of the Belgian population affected by a rare disease.

Ingrid has a Master degree as Translator in Dutch/ English/ French and a Bachelor degree in Family Science.

Thierry Voet
Laboratory of Reproductive Genomics, Centre for Human Genetics, University of Leuven, Belgium

Thierry Voet holds a Master of Science in Bioscience Engineering: Cell- and Gene Biotechnology from the University of Leuven (KU Leuven, Belgium), and an inter-university post-graduate in Human Genetics. He obtained his PhD in the Department of Human Genetics (KU Leuven), and performed postdoctoral research within the VIB (Flemish Institute for Biotechnology) and SymBioSys (KU Leuven) – pioneering single-cell microarray analyses. In 2010, he joined the Cancer Genome Project at the Wellcome Trust Sanger Institute (WTSI, UK) to explore next-generation sequencing technologies for single-cell genomics. Since 2011, he is an Associate Faculty member at WTSI, and a founding member of the Sanger-EBI Single-Cell Genomics Centre. Since 2014, he is associate professor at KU Leuven following a 5-year tenure track. His research focuses on (1) the development of wet-lab and computational methods for single-cell (epi)genomics and transcriptomics. (2) The application of these methods to study functional genetic heterogeneity, as well as DNA-mutation, in normal development and in disease processes.

Jan Hoeijmakers
Professor of Genetics, Erasmus University, Rotterdam, The Netherlands

Our DNA is constantly damaged by influences from inside and outside our body. Damage in our DNA can cause diseases, like cancer, and it can make our body age. Professor Hoeijmakers researches our DNA repair mechanisms. By modulating DNA repair, he recently succeeded in largely controlling the process of ageing in mammals like mice.

Background: Professor Jan Hoeijmakers is an expert in the field of DNA repair and ageing. He studied biology and joined the Genetics Department of Erasmus MC after taking his doctoral degree in 1981. Since 1993 he is Professor in Molecular Genetics at Erasmus University Rotterdam. Under his leadership, a brand new molecular biological research started in the field of ageing and cancer. Professor Hoeijmakers has received various prices and grants for his scientific work.

Photography Exhibition

Rick Guidotti, a former award-winning fashion phographer in New York, sees a rich beauty in genetic diversity and works to expose that vision to everyone.

He is the founder and Director of POSITIVE EXPOSURE, an innovative arts, education, advocacy and non-profit organisation. He has spent the past fifteen years working internationally with advocacy organizations/NGOs, medical schools, universities and other educational institutions to effect a sea-change in societal attitudes towards individuals living with genetic, physical and behavioural difference – from albinism to autism. He utilizes photography and video to transform public perceptions and as such his programmes promote a more inclusive, compassionate world where differences are celebrated.

Positive Exposure provides new opportunities to see individuals living with a genetic difference first and foremost as a human being with his/her own challenges rather than as a specific diagnosis/disease entity. This underlies the theme of this Meeting, “Genetics & Society”. This is the first exposure of his photographic works in Europe. We are delighted to have Rick here with us on this occasion. He shall also be carrying out various photographic projects whilst here is Belgium – in the Sensory Garden of the Marguerite-Marie Delacroix Foundation, at the Home Marjorie (vzw Emmaüs) and with members of the NF Kontakt vzw.


CHANGE
HOW YOU
SEE, SEE
HOW YOU
CHANGE
POSITIVE
EXPOSURE

Programme

Thursday 4 February 2016	
09.00	Registration opens + sponsor support Welcome coffee
10.15	**Welcome** Frank Baas & Thomy de Ravel
10.30-12.30	**Plenary session** Co-Chairs: Hélène Antoine-Poirel & Hans van Bokhoven 1. Invited Speaker: Peter Van Loo "Molecular Archaeology of Cancer" 2. Invited Speaker: Liesbet Lagae "Miniaturization of Biosensors and Fluidics for truly Personalized Healthcare" 3. Selected Speakers (3 x 15 minutes) O1: Parveen Kumar "Tracing the origin of disseminated tumor cells in breast cancer using single-cell sequencing" O2: Birgit Sikkema-Raddatz "Rapid screening of severely ill newborns and infants using whole genome sequencing" O3: Renate Hukema "Steps towards a targeted therapeutic intervention for FXTAS"
12.30-13.45	Lunch + poster viewing + sponsor support

13.45-15.00	**Parallel Sessions – Speciality Groups** **1.** Clinical Genetics/Dysmorphology Co-chairs: Mieke van Haelst/ Kathelijn Keymolen + Hilde Van Esch **2.** Laboratory Genetics Co-Chairs: Frank Baas/ Kathleen Claes + Joris Vermeesch/ Gert Matthijs **3.** Oncogenetics Chair: Peter Vandenberghe 4. Community Genetics/Genetic Counsellors Co-Chairs: Lidewij Henneman/ Sylvia De Nobele + Maureen Holvoet
15.15-16.45	**Plenary Session** Co-Chairs: Catheline Vilain & Lidewij Henneman 1. Invited Speaker: Doug Easton "Breast Cancer Genetics, common and rare, BRCA to GWAS to Panels" 2. Invited Speaker: Dian Donnai "Genetics and Genomics: from Back Room to Centre Stage!" 3. Invited Speaker: Ingrid Jageneau "The Role of Rare Disease Patient Organisations in the Centres of Expertise" 4. Invited Speaker: Rick Guidotti "Positive Exposure"
16.45-18.00	**Opening Reception** Poster viewing + Photography Exhibition viewing + drinks + sponsor support
19.00-01h00	**Dinner at Faculty Club**

Friday 5 February 2016	
8.00	Registration opens + sponsor support Coffee
9.30-10.50	**Plenary session** Co-Chairs: Joris Vermeesch & Joris Veltman Herman Van den Berghe Foundation Invited Speaker: Evan Eichler "Autism: New genes, Pathways and Genetic subtypes" Invited Speaker: Cisca Wijmenga "The Role of Gut Bacteria in controlling Health and Disease"
10.50-11.15	**Annual General Meeting BeSHG**
11.15-11.45	Coffee break + poster viewing + sponsor support
11.45-13.15	**Parallel Sessions: Selected Speakers** VENUE 1: (10 + 5 min. x 6) Co-Chairs: Sonia Van Dooren & Erik Jan Kamsteeg Diamond Sponsor: Sophiagenetics O4: Matthew Hestand "Single Molecule Variant Detection: From Heteroduplexes in a Single DNA Molecule to Whole Chromosome Rearrangements" O5: Annekatrien Boel "CRISPR/Cas-mediated gene editing in zebrafish: an optimized workflow" O6: Lennart Johansson "CoNVaDING: single exon variation detection in targeted NGS data"

	O7: Muhammad Imran Khan "Molecular inversion probe based sequence analysis of 108 genes associated with non-syndromic inherited retinal disease in 4,000 probands" O8: Bart Appelhof "tRNA Processing Mutations in Pontocerebellar Hypoplasia" VENUE 2: (10 + 5 min. x 6) Co-Chairs: Cécile Libioulle & Hans Kristian Ploos van Amstel Diamond Sponsor Multiplicom O9: Lidewij Henneman "TRIDENT: or monitored NIPT implementation in the Netherlands" O10: Nathalie Brison "Maternal incidental findings during non-invasive prenatal testing for fetal aneuploidies" O11: Christodoulos Christodoulou "Preimplantation genetic diagnosis (PGD) for translocation carriers using whole genome screening by microarray analysis at the blastocyst stage" O12: Cindy Melotte "Genome-wide haplotyping of preimplantation embryos in the clinic: principles guiding embryo selection in Leuven" O13: Helger Yntema "Experiences with the dissemination of secondary findings by diagnostic exome sequencing"

VENUE 3: (10 + 5 min. x 6)

Co-Chairs: Lut Van Laer & Raoul Hennekam

O14: Mala Isrie
"Mutations in Either TUBB or MAPRE2 Cause Circumferential Skin Creases Kunze Type"

O15: Dorien Baetens
"Dysregulation of NR5A1 is a novel and recurrent cause of 46,XX (ovo)testicular disorder of sex development"

O16: Frauke Coppieters
"Identification of RCBTB1 as a novel disease gene for autosomal recessive isolated and syndromic inherited retinal dystrophy"

O17: Hans Van Bokhoven
"Identification and spatio-temporal mapping of 30 Novel Candidate Genes for Autosomal Recessive Intellectual Disability"

O18: Joris Veltman
"A clinical utility study pediatric neurology: exome sequencing improves patient care significantly"

O19: Jan Jongbloed
"Whole exome sequencing identifies ALPK3 as a new disease gene causing both severe pediatric and 'milder' adult-onset cardiomyopathies"

	VENUE 4: (10 + 5 min. x 6) Co-Chairs: Paul Coucke & Frank Baas O20: Filippo Zambelli "Comprehensive method for the analysis of low-frequency mitochondrial SNVs and small and large deletions in DNA samples and single cells" O21: Lieselot Croes "Analysis of DFNA5 methylation and expression in primary breast adenocarcinomas using large sample numbers on the basis of The Cancer Genome Atlas" O22: Matthias Beyens "Ultra-deep targeted resequencing of 38 pancreatic neuroendocrine tumors reveals tumor heterogeneity for actionable mutations" O23: Tom Van Nieuwenhuysen "Modeling human cancer syndromes using TALEN and CRISPR/Cas9 mediated genome editing in Xenopus tropicalis" O24: Suzanne Vanhauwaert "The BRIP1/FANCJ DNA helicase is a 17q driver oncogene protecting neuroblastoma cells from MYCN induced replicative stress at G-quadruplexes" O25: Annelynn Wallaert "The lncRNAomes of T-cell acute lymphoblastic leukemia and normal immature thymocyte subsets through combined poly-A and total RNA-sequencing"
13.15-14.30	Lunch + Poster viewing + sponsor support

14.30-16.30	**Plenary Session + Awards** Co-Chairs: Frank Baas & Thomy de Ravel **1.** Invited Speaker: Thierry Voet "Single-cell Sequencing to Study the Biology of Cellular Heterogeneity in Health and Disease" **2. Prizes** a. Best Oral Presentation by Young Investigator ESHG Netherlands Prize b. Best Oral Presentation by Young Investigator 1 ESHG Belgium Prize c. Best Oral Presentation by Young Investigator 2 BeSHG Prize d. Best Poster by Young Investigators NVHG Prize e. Best Posters by Young Investigators BeSHG Prizes 1 & 2 **3. Galjaard Prize** Laureate: Jan Hoeijmakers "Maintaining Nature's Perfection: DNA Repair, Cancer, Aging and Longevity" **4. Closure**

Selected Oral Presentations By Young Investigators

O1: Tracing the origin of disseminated tumor cells in breast cancer using single-cell sequencing

Parveen Kumar[1], Elen Moller[2], Jonas Demeulemeester[3], Silje Nord[2], David Wedge[4], April Peterson[5], Randi Mathiesen[2], Renathe Fjelldal[2], Masoud Zamani Esteki[1], Jason Grundstad[5], Anne-Lise Børresen-Dale[2], Kevin White[5], Bjorn Naume[2], Vessela Kristenen[2], Peter Van Loo[3] & Thierry Voet[1]

[1] University of Leuven
[2] University of Oslo
[3] Francis Crick Institute
[4] Welcome Trust Sanger Institute
[5] University of Chicago

Background
Single-cell micro-metastases of solid tumors often occur in the bone marrow. These disseminated tumor cells (DTCs) may lay dormant and resist therapy, yet are potent precursors of overt bone and visceral metastases. Unfortunately, the molecular nature of DTCs remains elusive, as well as when and where they spring from the tumor. Here, we apply single-cell sequencing to identify and trace the origin of DTCs in breast cancer.

Results
We sequenced the genomes of 40 single cells that were isolated from bone marrow of one metastatic and five primary breast cancer patients using established immunocytochemical and morphologic tumor cell markers. Comparison of the cells' DNA copy number aberration (CNA) landscapes with those of the primary tumors and lymph node metastasis established that a quarter of the cells disseminated from a tumor clone. Among the remaining cells were non-aberrant 'normal' cells and 'aberrant cells of unknown origin' that have discordant CNA landscapes compared to the tumor. Genotyping somatic mutations called on bulk tumor exomes in the single-cell sequences confirmed that these cells do not derive from tumor cell lineages. Evolutionary reconstruction analysis of bulk tumor and DTC genomes enabled ordering CNA events in molecular pseudo-time and tracing the origin of the DTCs to specific tumor clones. This revealed both linear and parallel tumor progression patterns.

Conclusions
Single-cell sequencing of bone-marrow epithelial-like cells, in parallel with intra-tumor genetic heterogeneity profiling from bulk DNA, is a powerful approach to identify and study DTCs, yielding insight into metastatic processes. A heterogeneous population of CNA-positive cells deriving from non-tumor cell lineages is prominent in bone marrow.

O2: Rapid screening of severely ill newborns and infants using whole genome sequencing

B. Sikkema-Raddatz[1], C.C. van Diemen[1], W.S. Kerstjens-Frederikse[1], T.J. de Koning[2], R.J. Sinke[1], J.D.H. Jongbloed[1], K.M. Abbott[1], J.C. Herkert[1], P.B.T. Neerincx[1], G. de Vries[1], M. Meems-Veldhuis[1], M. Viel[1], A.J. Scheper[1], K. de Lange[1], J. Dijkhuis[1], J. van der Velde[1], M. de Haan[1], M.A. Swertz[1], K.A. Bergman[2], C.M.A. van Ravenswaaij-Arts[1], I.M. van Langen[1], R.H. Sijmons[1] & C. Wijmenga[1]

[1] Dept of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[2] Beatrix Children's Hospital, University Medical Center Groningen, Groningen, The Netherlands

For severely ill newborns quick molecular diagnoses are of utmost importance for clinical decision-making and can prevent unnecessary and sometimes invasive diagnostics. To date, immediate molecular testing is not a routine procedure for all patients since this is available only for few diseases.
Here we describe a procedure and present the first results to analyze 2800 genetic disorders in severely ill newborns and infants by rapid whole-genome sequencing (WGS). WGS is carried out in parallel to standard diagnostic procedures (including imaging, SNP-arrays, biochemical testing, mutation analysis, etc.). The entire procedure from inclusion to reporting is completed in approximately 2 weeks. The final evaluation of the results is done by a multidisciplinary team of pediatricians, clinical geneticists, technicians, clinical genetic laboratory specialists, researchers and bio-informaticians. Thus far we have included 21 patients in the study and have provided a diagnosis of a monogenic disease for three patients. These patients presented with different clinical characteristics and that could be explained by mutations in the EPG5, KLHL41 and RMND1 genes. One patient was diagnosed with a 1p36 microdeletion upon routine diagnostic testing.
We also tested 8 patients, who died within the first year of life, and their parents with rapid clinical exome sequencing, focusing on the same set of 2800 genes. We found compound heterozygous mutations in BRAT1 in one child with an unexplained severe seizure and rigidity disorder. In two other patients a de novo mutation in KMT2D was detected which causes Kabuki syndrome.

In 12 patients without a diagnosis we got informed consent from the parents for further analysis in research setting. We prioritized genes using Network analysis based on gene co-expression and patient- specific Human Phenotype Ontology (HPO) terms. In addition RNA sequencing has been performed in a subset of child- parent trios to assess whether aberrant expression patterns can help interpreting possible pathogenic variants. This research follow up has led to three potential new diagnoses.

Currently we are evaluating the procedure, comparing our results with those from studies of the Kingsmore's group, who published diagnostic yields of 80% (in a prospective study, Saunders et al. Sci Transl Med. 2012;4:154ra135) and 57% (in a retrospective study, Willig et al. Lancet Respir Med. 2015 35:377-387). We will emphasize on phenotype selection, technical aspects of coverage and filtering methods. One preliminary conclusion explaining the lower yield in our studies, may be that we excluded patients with a clear clinical diagnosis for which targeted sequencing was available.

O3: Steps towards a targeted therapeutic intervention for FXTAS

Ronald A. Buijsen, Lies-Anne W. Severijnen, Helen de Boer, Rob Willemsen & Renate K. Hukema

Department of Clinical Clinical Genetics, Erasmus MC Rotterdam

Fragile X-associated tremor/ataxia syndrome (FXTAS), a late-onset neurodegenerative disorder, is caused by a CGG-repeat expansion (55-200) in the 5?UTR of the fragile-X mental retardation 1 gene. FXTAS is characterized by progressive development of intention tremor, ataxia, parkinsonism, and neuropsychological problems. The neuropathological hallmark of the disease are ubiquitin positive intranuclear inclusions in neurons and astrocytes. The current hypothesis is that FXTAS is caused by an RNA gain-of-function mechanism. Recently, it has been shown that repeat-associated non-AUG (RAN) translation plays a role in inclusion formation and disease progress. Indeed, we and other have found that a polyGlycine containing protein, FMRpolyG, is present in inclusions in post-mortem brain from FXTAS patients.

To understand the contribution of repeat bearing RNA and FMRpolyG in FXTAS, we generated mouse and cellular models for FXTAS. In both the in vitro and in vivo models, FMRpolyG-positive inclusions were shown in neurons and astrocytes and their numbers increase over time and correlate with functional tests in an inducible mouse model. Using this model, we have shown that the formation of the inclusion is reversible if you stop expression of the pathogenic trigger.

The contribution of FMRpolyG to toxicity and pathology opens new avenues for therapeutic intervention studies for FXTAS. In our primary neuron culture we tested drugs that block this aberrant translation. Our first results suggest that it is possible to prevent inclusion formation by using small chemical compounds binding the repeat.

O4: Single Molecule Variant Detection: From Heteroduplexes in a Single DNA Molecule to Whole Chromosome Rearrangements

Matthew S. Hestand[1], Jeroen K. Van Houdt[1], Martin A. Mensah[2], Heleen Masset[1], Maarten H. Larmuseau[3], Damien Sanlaville[4], Peter N. Robinson[5] & Joris Vermeesch[1]

[1] Department of Human Genetics, KU Leuven
[2] Department of Human Genetics, KU Leuven; Institut fur Medizinische Genetik und Humangenetik, Charite – Universitatsmedizin Berlin
[3] Department of Biology, KU Leuven
[4] Laboratoire de Cytogénétique Constitutionnelle, Hospices Civils de Lyon
[5] Institut fur Medizinische Genetik und Humangenetik, Charite – Universitatsmedizin Berlin

The PacBio single-molecule sequencing platform produces long (avg 12-15kb) error-prone reads, though the errors are randomly distributed. Therefore, combined with read coverage or circularizing a DNA molecule and repeatedly sequencing both strands produces highly accurate consensus sequences. We have utilized this circular sequencing approach to determine error rates and profiles across six commonly used polymerases. Besides accurately determining mutations in double strands, the platform permits the identification of heteroduplexes, where a base on one strand is not complimentary to a base on the other strand. Interestingly, we observed that Watson-Crick base-pairing errors are not equally distributed, but that across most polymerases there is a bias for pyrimidine transitions over purine transitions. Moving from single molecule errors to chromosome spanning errors, the long reads also provide a unique resource to identify structural variation, including sequencing across repetitive elements. Indeed, we used PacBio to demonstrate an insertional translocation of chrX sequence into chrY, generating an extended pseudoautosomal region (PAR). The insertion is generated by non-allelic homologous recombination between a 548 bp LTR6B repeat within the chrY PAR1 and a second LTR6B repeat located 105 kb from the PAR boundary on chrX. PacBio phasing within the duplicated region also enabled identification of the paternally inherited insert sequence and findings of multiple haplotypes from ancestrally related individuals, demonstrating X/Y recombination. In a separate cohort, aCGH identified three patients containing distinct clusters of only copy number gains across a single chromosome 18 or 22. A combination of Illumina, PacBio, and Sanger sequencing was used to identify and characterize the breakpoints in these patients. For these highly rearranged chromosomes, breakpoint sequences lead to the hypothesis of an origin different from traditional chromothripsis and chromoanasynthesis, possibly a repair process driven by non-canonical non-homologous end joining mediated by polymerase theta. In conclusion, we demonstrate the PacBio platform provides unique capabilities to detect variation, from single molecules to whole chromosomes rearrangements.

O5: CRISPR/Cas-mediated gene editing in zebrafish: an optimized workflow

Annekatrien Boel, Wouter Steyaert, Nina De Rocker, Björn Menten, Anne De Paepe, Paul Coucke & Andy Willaert

Ghent University, Center for Medical Genetics, Ghent, Belgium

In the last decade, zebrafish has emerged as a popular model organism, due to its low cost, ex utero embryogenesis and ease of genetic modification. Zebrafish has proven to be a valuable tool in both forward and reverse genetic approaches. Reverse genetics has previously been carried out by the use of morpholinos, antisense molecules that transiently knock down gene function and therefore rapidly provide researchers with gene function information. However, the usefulness of this approach is hampered by its transient nature and by the potential for off-target effects. These issues have directed the zebrafish community to the use of genome editing approaches with a prominent role for the CRISPR/Cas system. The CRISPR/Cas method is based on the co-injection of a single guide RNA (sgRNA), harbouring a complementary sequence to the target site and a Cas9 nuclease, that cleaves the DNA at the region of interest.

In this work, we present an optimized workflow for CRISPR/Cas-mediated gene editing in zebrafish, for both generating knockout zebrafish models as for precise genome editing via homology-directed repair (HDR). First, we introduced a rapid method of sgRNA and HDR template synthesis, using synthetic double-stranded DNA molecules. Secondly, we show that the determination of indel rates can be dramatically simplified by the use of next-generation sequencing followed by sequence analysis using an in-house developed bioinformatics tool. This tool takes advantage of existing free open-source toolkits and is able to generate results in batch mode. The generated results are threefold: aligned sequencing reads are visualized in UCSC, the global indel and repair rates are calculated and an overview of the detected variants together with their concordant frequency is generated. The tool was evaluated in zebrafish, analyzing the streamlined workflow for the generation of knockout and knockin zebrafish mutants. The tool will not only contribute to the evaluation of CRISPR/Cas9-based experiments in zebrafish, but will be of use in any genome editing experiment and has the ability to analyze data for any organism with a sequenced genome.

O6: CoNVaDING: single exon variation detection in targeted NGS data

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We have developed a tool for detecting single exon copy number variations (CNVs) in targeted next-generation sequencing data: CoNVaDING (Copy Number Variation Detection In Next-generation sequencing Gene panels).

The method is based upon a read depth comparison of selected target regions between a sample of interest and a set of control samples. Existing tools, such as XHMM [1] and CoNIFER [2], consider all control samples equally informative even though there are sample to sample variations caused by differences in PCR and capturing efficiency. CoNVaDING, however, selects the control samples showing a coverage pattern most similar to that of the sample analysed. Data is then normalized in two different ways, using within the sample all autosomal targets or all targets within the same gene. Based on the normalized data, for each target the ratio of the normalized average read depth of the sample to that of the controls and a distribution analysis using a Z-score are calculated. Based on the calculated ratio and distributions a prediction is made for each target to determine whether a CNV is present or not. CoNVaDING provides for each sample quality metrics to distinguish high quality samples and targets from low quality ones.

We compared the performance of CoNVaDING with XHMM and CoNIFER in 320 samples captured with two different targeted gene-panels (Agilent Sure Select custom design 0421101 and 0679001) containing in total 308,574 exons. For all CNV calls made by one of the three methods MLPA was performed. CoNVaDING detected all known CNVs in high quality targets, giving 100% sensitivity, at a 99.998% specificity for 308,574 exons. Thereby outperforming XHMM and CoNIFER by exhibiting a higher sensitivity and specificity and by precisely identifying low-quality samples and regions, which have a high risk of false positive or false negative results. Since the quality control metrics show exactly which exons can be reliably analyzed and which exons are in need of an alternative analysis method, CoNVaDING can not only be applied for CNV detection in a research setting, but also in clinical diagnostics.

CoNVaDING software is available under the GNU GPL open source license and can be freely downloaded from https://github.com/molgenis/CoNVaDING.

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O7: Molecular inversion probe based sequence analysis of 108 genes associated with non-syndromic inherited retinal disease in 4,000 probands

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Purpose: Inherited retinal diseases (IRDs) are clinically and genetically very heterogeneous, as ~125 genes have been associated with non-syndromic IRDs. The purpose of this study was to develop a flexible, comprehensive and cost effective sequencing procedure for IRDs.

Methods: In total, 6,200 molecular inversion probes (MIPs) were designed to capture ~1600 protein-coding exons and flanking intronic sequences of 108 IRD-associated genes published up to October 2013. About 4,000 probands with non-syndromic IRDs were ascertained by partners of the European Retinal Disease Consortium (ERDC). The most prevalent phenotypes were retinitis pigmentosa, cone-rod dystrophy, and Leber congenital amaurosis. To analyze the performance of the MIPs, three tests were performed. The captured targets were sequenced on a NextSeq500 Illumina sequencer and the data were analyzed using an in-house pipeline to find the causal genetic variants. The over- and poor-performing probes were rebalanced and used to capture the genomic targets in 4,000 individuals.

Results: In pools of 120 samples the average coverage per probe was ~500X, where 95.6% of the probes were covered >10X. Probes targeting 272 (4.4%) regions were either poorly covered (2.3%) or not covered (2.1%). Analysis was completed for 2,500/4,000 samples. Sanger validation was performed for a representative set of variants identified in 290 probands from Nijmegen. Material costs (including MIPs synthesis, library preparations and sequencing costs) for sequencing 108 IRD genes, was € 65 per sample, which makes it very cost-effective. We identified the causal variants in 59% (172/290) of the cases, 36 of 118 unsolved cases (i.e. 12% of total) carry one likely pathogenic variant in an autosomal recessive gene.

Conclusion: Taken into consideration that the Nijmegen cohort was previously prescreened using various genotyping methods, the corrected yield would be ~71%. At 1/10th of WES cost, this efficiency is equal or superior to other published gene-panel (36 - 62%) or WES-based (49 - 66%) sequence analysis. Based on preliminary results, we estimate to identify causal variants in at least 2,000 IRD probands.

O8: tRNA Processing Mutations in Pontocerebellar Hypoplasia

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Pontocerebellar hypoplasia (PCH) represents a heterogeneous group of neurodegenerative disorders with a prenatal onset. So far, ten subtypes are described (PCH1-10), based on genetic and clinical features. Coinciding symptoms are hypoplasia and/or atrophy of the pons and cerebellum and patients suffer from severe cognitive and motor defects. Currently, only symptomatic treatment is available and most patients die before adulthood. Multiple aberrations in different genes have been associated with PCH thus far. The majority of these genes participate in RNA processing, e.g. the tRNA splicing endonucleose (TSEN) genes, arginyl tRNA synthetase 2 (RARS2), cleavage and polyadenylation factor 1 subunit 1 (CLP1) and exosome component 3 (EXOSC3). However, there is still a substantial group of patients where the pathogenic mutation is not yet identified.

We performed whole exome sequencing on 25 PCH patients and 5 trios with an unknown genetic cause, aiming to identify novel genes involved in PCH which explain the disease mechanism. So far, we identified over ten candidate mutations of which one is located in RNA 3'-terminal phosphate cyclase (RtcA).

The mutation in RtcA is further assessed in a zebrafish model. Knockdown of RtcA by using morpholinos results in microcephaly and abnormal brain development. Also aberrant movement is seen in the knockdown animals. This fits a PCH phenotype.

RtcA is a cyclase involved in the tRNA processing pathway. After intron removal by TSEN complex, a tRNA exon with a terminal 2,3-cyclic phosphate is generated. RtcB cleaves the phosphate resulting in either a 2'- or 3'- phospate. The latter is a substrate for the ligation reaction, whereas the 2'-phosphate is a dead end product. RtcA can rescue this molecule by again forming a 2,3-cyclic phosphate, enabling proper ligation and mature tRNA syntheses. Cyclase activity was shown to be absent in fibroblast lysate of the patient.

RtcA is the third PCH related gene directly involved in tRNA processing next to TSEN and CLP1. This finding invigorates the importance of tRNA processing for normal cerebellar development and enables us to start revealing the underlying mechanism further.

O9: TRIDENT: or monitored NIPT implementation in the Netherlands

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In many countries, Non Invasive Prenatal testing (NIPT) has been introduced commercially, without governmental guidance. In the Netherlands the Population Screening Act regulates the introduction of screening programs for untreatable diseases such as Down syndrome. The Dutch NIPT consortium, consisting of all relevant stakeholders, obtained a license for 2 years for a nationwide NIPT implementation study called TRIDENT-1 (Trial by Dutch laboratories for Evaluation of Non-Invasive Prenatal Testing). The study started on April 1st 2014. Inclusion criteria are an increased risk (>1:200) for trisomy (T) 21, 18 or 13 based on the first trimester combined test, or because of medical history. After one year of study, 3306 pregnant women have been tested and 3278 (99,2%) reports issued. We found 87 cases of T21 (2,7%), 9 cases of T18 (0,3%) and 11 cases of T13 (0,3%). Follow-up was completed for 102 cases of which 95 were confirmed, 7 were false positives, presumably due to confined placental mosaicism. Data on pregnancy outcomes are currently being collected and will be presented, together with data on findings other than T21, T13 or T18. A license for a TRIDENT-2 follow up study to offer NIPT to all pregnant women as a first tier test has been requested.

The Netherlands are the first country where NIPT is incorporated into a governmentally supported and health care funded prenatal Down syndrome screening program. The incorporation of the test in a university hospital laboratory and clinical service guarantees appropriate counseling and allows for proper follow up including thorough exploration of biological causes of false positive and false negative findings including detailed placental examination.

O10: Maternal incidental findings during non-invasive prenatal testing for fetal aneuploidies

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Non-invasive prenatal testing (NIPT) for common fetal aneuploidies by either targeted or whole genome sequencing of circulating free DNA (cfDNA) has become standard prenatal care. Random genome-wide cfDNA sequencing enables not only the detection of fetal chromosomal imbalances but also of maternally derived copy number variants (CNVs). Following routine clinical analysis of over 10.000 prospective pregnancies using an accredited in-house developed analysis pipeline we identified five different clinically relevant constitutional CNVs and imbalanced translocations of maternal origin which were reported back to the mother because they (i) were either relevant for the pregnancy management, (ii) had potential consequences for the fetus or (iii) could have consequences for future reproductive choice. Hence, genome wide cfDNA profiling of maternal plasma improves not only fetal but also more general pregnancy management.

O11: Preimplantation Genetic Diagnosis (PGD) for translocation carriers using whole genome screening by microarray analysis at the blastocyst stage

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Introduction:
Recent technical advances in the field of PGD can improve success rates for couples with chromosome rearrangements. In this study, we investigated the value of whole genome screening by array comparative genomic hybridization (array CGH) for PGD in embryos of translocation carriers. This is a retrospective data analysis contacted from October 2013 to December 2015 on blastocysts from couples carrying a chromosome rearrangement.

Methods:
A total of 187 blastocysts originating from 43 PGD oocyte collection cycles (OCCs) were investigated. These cycles involved 33 couples with a mean maternal age of 32,8 years. In eight couples one of both parents was carrier of a Robertsonian translocation, 21 were carriers of a reciprocal translocation, two of inversions, one of an insertional translocation and one with a double two-way exchange reciprocal translocation. Trophectoderm biopsy was performed on the 5th or 6th day of embryo development. Whole genome amplification (WGA) was performed on all samples, while the amplified DNA material from 176 blastocysts was analyzed with array CGH via the 24sure+ microarray platform (Bluegnome, Illumina). All embryos were vitrified and after warming embryos with normal chromosome content were transferred to the patients in non-stimulated cycles.

Findings:
We detected chromosome abnormalities in 114/176 embryos (65% of successfully amplified) while 62 showed a normal microarray profile (35%). In 42 of the 114 abnormal embryos (37%), an unbalanced rearrangement originating from the parental translocation was identified. Interestingly, 34% of abnormal embryos (39/114) harbored de novo chromosome aneuploidies that could not be directly related to the parental translocation in question. We also detected a combination of unbalanced – parental derived – rearrangements and de novo aneuploidies in 21/114 abnormal embryos (18%).
More specifically, from a total of 193 chromosome abnormalities found, we identified 71 aneuploidies and 19 structural abnormalities (a combined 47%), unrelated to the parental rearrangement. Due to technical limitations, results were not generated in 12/176 (7%) of the samples.

Conclusions:
The use of trophectoderm biopsy at the blastocyst stage leads to a more reliable estimate of the genomic content of the embryo compared to single or double cell biopsy at the cleavage stage. Moreover, it has been shown that trophectoderm biopsy is less detrimental to the

survival of the embryo when compared to blastomere biopsy. Use of array CGH for PGD on trophectoderm cells at the blastocyst stage has multiple advantages and is more convenient than fluorescent in situ hybridization (FISH) on single cells of cleavage stage embryos.
Besides the detection of chromosome aberrations due to the parental translocation, this approach revealed a high occurrence of de novo aneuploidies and structural rearrangements unrelated to the parental translocation. Our study describes the successful implementation of array CGH analysis on blastocysts for PGD in patients with structural chromosome rearrangements. With a positive hCG and ongoing pregnancy rate per ET of 44,7% and 31,6% respectively, our study confirms that array CGH analysis for PGD on blastocysts is a beneficial clinical application to identify viable euploid embryos for transfer.

Support:
This study was supported by a concerted research actions funding from BOF (Bijzonder Onderzoeksfonds) Ghent University, grant number BOF15/GOA/011.

O12: Genome-wide haplotyping of preimplantation embryos in the clinic: principles guiding embryo selection in Leuven

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Preimplantation genetic diagnosis by genome wide haplotyping provide a general overview of the embryonal genome and enable the simultaneous detection of multiple variants, mutations and imbalances genome-wide. The introduction in a diagnostic settings raises novel ethical questions. Here we present the principles guiding embryo selection and prioritization that are applied at our center according to the chromosomal content and mutational load of the embryos. Our embryo selection principles are based not only on technical and biological, but also on ethical criteria and have a profound impact on the organization of PGD operations and on the information that is transferred amongst the genetic unit, the fertility clinic and the patients. Those principles are also important for the organization of pre- and post-counselling and influence the way of interpreting and reporting preimplantation genotyping results. From June 2014 until November 2015, 300 embryos from 47 couples had been tested in 79 cycles, leading to 17 clinical pregnancies (47.2 % clinical pregnancy rate per cycle for which pregnancy outcome information is available) and the birth of 3 healthy babies so far. Thirty-one different indications, 27 for monogenic disorders, 3 chromosomal aberrations and 1 case of combined monogenic disorder with chromosomal aberration have been included. As novel genome-wide approaches for embryo selection are revolutionizing the field of reproductive genetics, national and international discussions to set general principles are warranted.

O13: Experiences with the dissemination of secondary findings by diagnostic exome sequencing

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In the Genome Diagnostics laboratory at the Radboudumc Nijmegen, whole exome sequencing (WES) has been performed for more than 4,000 patients. The WES diagnostic process is a two-tiered approach, in which tier 1 is limited to the analysis of genes known for the respective disease of the patient (in silico gene panel approach) and tier 2, performed if tier 1 is negative and informed consent is given, the analysis of the entire protein coding sequence. This second tier has the intrinsic risk of identifying secondary findings: mutations in disease genes not implicated in the aetiology of the disease the patient is tested for.

As part of WES implementation as a diagnostic test, we set up a procedure on how to deal with secondary findings. Upon identification, a multidisciplinary committee of experts can be assembled on an ad hoc basis to discuss the dissemination a secondary finding. The committee consists of a clinical laboratory specialist, a clinical geneticist, a molecular geneticist, a social worker, a lawyer, and an ethicist. Other specialists with specific expertise are consulted upon indication. The committee checks informed consent, evaluates the molecular finding in the patient's clinical context, and discusses the potential medical and social impact of the finding. When it is decided (by majority vote) that the finding should be returned, a separate report is issued to the requesting clinician, not only reporting the mutation, but also the arguments for reporting the finding, as well as guidelines on how to further proceed.

To date, WES beyond the gene panel of approximately 1,500 cases has been finished. Thirty-five (2.3%) potential secondary findings have been discussed by the committee. For 21 (1.4%) of these, mostly involving hereditary cancer and cardiac disease, it was (unanimously) decided to report the finding, based on the clear pathogenicity of the mutation and relevant medical actions that could be taken. The main reason not to report the remaining 14 findings was the unclear pathogenicity of the variant.

In our hands, 1.4% of patients receiving WES is confronted with a secondary finding. The expert committee judging these findings is considered beneficial for all laboratory specialists and clinical geneticists of our department. Furthermore, this committee is now also participating in most research projects within our hospital, suggesting that there is a wider need for discussing secondary findings than in a diagnostic setting alone.

O14: Mutations in Either TUBB or MAPRE2 Cause Circumferential Skin Creases Kunze Type

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Circumferential skin creases Kunze type (CSC-KT) is a specific congenital entity with an unknown genetic cause. The disease phenotype comprises characteristic circumferential skin creases accompanied by intellectual disability, a cleft palate, short stature, and dysmorphic features. Here, we report that mutations in either MAPRE2 or TUBB underlie the genetic origin of this syndrome. MAPRE2 encodes a member of the microtubule end-binding family of proteins that bind to the guanosine triphosphate cap at growing microtubule plus ends, and TUBB encodes a ?-tubulin isotype that is expressed abundantly in the developing brain. Functional analyses of the TUBB mutants show multiple defects in the chaperone-dependent tubulin heterodimer folding and assembly pathway that leads to a compromised yield of native heterodimers. The TUBB mutations also have an impact on microtubule dynamics. For MAPRE2, we show that the mutations result in enhanced MAPRE2 binding to microtubules, implying an increased dwell time at microtubule plus ends. Further, in vivo analysis of MAPRE2 mutations in a zebrafish model of craniofacial development shows that the variants most likely perturb the patterning of branchial arches, either through excessive activity (under a recessive paradigm) or through haploinsufficiency (dominant de novo paradigm). Taken together, our data add CSC-KT to the growing list of tubulinopathies and highlight how multiple inheritance paradigms can affect dosage-sensitive biological systems so as to result in the same clinical defect.

O15: Dysregulation of NR5A1 is a novel and recurrent cause of 46,XX (ovo)testicular disorder of sex development

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Background. While in the last decades many causes of 46,XY differences of sex development (DSD) have been elucidated, the mechanisms leading to 46,XX testicular and ovotesticular DSD are still poorly understood. It has been hypothesized that both conditions may represent a phenotypic spectrum resulting from a common underlying genetic defect.

Methods Whole exome sequencing was applied to identify the underlying molecular cause in ten unrelated patients with 46,XX (ovo)testicular DSD. Immunohistochemistry was performed on gonadal specimens of all patients for relevant markers of gonadal development. The transcriptional activation capacity of variant versus wild type NR5A1 was assessed using luciferase assays. For transcriptome profiling RNA-seq on patient-derived lymphocytes was used.

Results We identified a novel heterozygous NR5A1 variant c.274C>T p.(Arg92Trp) in three out of ten unrelated cases with 46,XX (ovo)testicular DSD. This missense variant could not be found in genomic databases and is predicted to have a deleterious effect on protein function. The Arg92 residue is conserved up to zebrafish and is located in the RGGR motif formed by R89-R92 in the loop before the C-terminus helix in the highly conserved Ftz-F1 box, involved in DNA-binding stability. Immunohistochemistry confirmed SRY-independent SOX9 expression and absent FOXL2 in testicular parts of XX gonads whereas ovarian differentiation showed to be associated with absent SOX9 and consistent FOXL2 expression. Luciferase assays in different cell lines did not show altered transcriptional activation capacities of the p.(Arg92Trp) variant. Structural analysis and transcriptome profiling to assess the consequences of this mutation are ongoing.

Conclusions NR5A1 is one of the transcription factors involved in the upregulation of early male specific genes like SRY and SOX9. Previously identified NR5A1 mutations have a loss-of-function effect, disrupting normal male sex development and resulting in a variable degree of male undervirilization. Here, a novel NR5A1 mutation c.274C>T, p.(Arg92Trp) was found to be recurrent in three unrelated cases of 46,XX (ovo)testicular DSD, an ultra-rare condition. We hypothesize this variant may result in gain-of-function and triggers testicular differentiation in 46,XX individuals. Finally, we propose NR5A1 as new disease gene for 46,XX (ovo)testicular DSD.

O16: Identification of RCBTB1 as a novel disease gene for autosomal recessive isolated and syndromic inherited retinal dystrophy

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Purpose: The aim of this study was to identify and functionally study a novel disease gene mutated in a Turkish consanguineous family with a severe syndromic inherited retinal dystrophy (iRD).

Methods: Genome-wide SNP arrays were used for homozygosity mapping in three affected and one healthy sibling. Two affected individuals underwent whole exome sequencing (WES) (HiSeq2000, Illumina). WES data of over 1000 iRD patients were inspected for RCBTB1 mutations, and targeted next-generation sequencing of the coding region of RCBTB1 was performed in 281 iRD patients. Flanking SNPs and microsatellite markers were genotyped for haplotyping. RCBTB1 qPCR expression analysis was performed in cDNA from human retina and retinal pigment epithelium (RPE). RCBTB1 immunostaining was performed on human and murine sections. As RCBTB1 has previously been identified as a Cullin3 substrate adaptor, different components of the Cullin3 and Nrf2 pathway were quantified using qPCR.

Results: Homozygosity mapping revealed a single 11 Mb homozygous region on chromosome 13 shared by the three affected individuals in the first family. WES identified a novel missense variant, c.973C>T p.(His325Tyr) (rs200826424), in RCBTB1 (NM_018191.3). Subsequently, additional homozygous missense mutations were identified in 5 families with isolated and syndromic iRD. All changes segregate with disease, affect highly conserved amino acids and in silico predictions are suggestive for a deleterious effect. Three mutations are located in the 6th repeat of the RCC1 domain, while two variants localize in the first BTB domain. A founder haplotype was identified for mutation c.919G>A, p.(Val307Met), occurring in two families of Italian and Greek origin, respectively. Ocular phenotypes range from typical RP starting in the second decade to a chorioretinal dystrophy with a later age of onset. RCBTB1 mRNA expression was demonstrated in human retina and RPE and protein immunostaining was observed mainly in the inner retina. Different components of the Cullin3 and Nrf2 pathway displayed decreased mRNA expression in patients' lymphocytes.

Conclusions: Hypomorphic RCBTB1 missense mutations clustering in two protein domains were identified in families with non-syndromic and syndromic IRD respectively, putting forward RCBTB1 as a new IRD disease gene. A founder effect was demonstrated for one RCBTB1 mutation in two Mediterranean families. Finally, our data suggest a potential role of the ubiquitination pathway in the pathogenetic mechanism underlying RCBTB1-associated IRD.

O17: Identification and spatio-temporal mapping of 30 Novel Candidate Genes for Autosomal Recessive Intellectual Disability

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Intellectual disability (ID) is a clinically and genetically heterogeneous disorder, affecting 1-3% of the general population. Although research into the genetic causes of ID has recently gained momentum, identification of pathogenic mutations that cause autosomal recessive ID (ARID) has lagged behind, predominantly due to non-availability of sizeable families. Here, we present the results of exome sequencing in 121 large consanguineous Pakistani ID families. In 60 families, we identified a single homozygous DNA variant, 30 affecting reported ID genes and 30 novel candidate ID genes. Potentially pathogenicity of these alleles was supported by co-segregation with the phenotype, frequency in control populations and the application of stringent bioinformatics analyses. In another eight families segregation of multiple pathogenic variants was observed, affecting 19 genes that were either known or are novel candidates for ID. Transcriptome profiles of normal human brain tissues showed that the novel candidate ID genes formed a network significantly enriched for transcriptional co-expression ($p<0.0001$) in the temporal-parietal and sub-cortex during infancy to adulthood. In addition, proteins encoded by 12 novel ID genes directly interact with previously reported ID proteins in six known pathways essential for cognitive function ($p<0.0001$). These results suggest that disruptions of temporal parietal and sub-cortical neurogenesis during infancy are critical to the pathophysiology of ID. These findings further expand the existing repertoire of genes involved in ARID, and provide new insights into the molecular mechanisms and transcriptome map of ID.

O18: A clinical utility study pediatric neurology: exome sequencing improves patient care significantly

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Implementation of novel genetic tests into routine diagnostic practice is generally driven by technological advances as they promise to be faster and/or lead to increased diagnostic yield. Yet, this yield is rarely systematically compared between traditional and novel tests. In addition, other aspects including time and cost to diagnosis as well as information and communication needs of families involved are often not assessed prior to implementation. Here, we have addressed these aspects for the implementation of whole exome sequencing (WES) in pediatric neurology. We have collected 150 patients (and their parents) presenting with complex neurological problems of suspected genetic origin. Typically, finding a diagnosis in these patients is not easy, nor fast or cheap, and often involves burdensome procedures. In a unique parallel study design, all patients underwent both the standard genetic diagnostic procedure, dominated by gene-by-gene based testing in sequential order, as well as WES. This unique set-up allowed for direct comparison of diagnostic yield, time-to-diagnosis and costs involved in obtaining this diagnosis. Our data show that WES identified significantly more conclusive diagnosis (n=44) than the standard genetic care pathway (n=11), in a shorter time span (9.9 months vs. 44.2 months). In addition, the costs associated with genetic testing by WES was reduced by 20% (€ 921) compared the standard genetic care pathway. Caretakers involved had a realistic perception of the potentials and limitations of the WES technology after receiving adequate information. No major issues were raised that should argue against implementation of diagnostic WES in this patient group. Thus our data strongly support implementation of WES as first tier genetic test in patients presenting with pediatric neurology of presumed genetic origin.

O19: Whole exome sequencing identifies *ALPK3* as a new disease gene causing both severe pediatric and 'milder' adult-onset cardiomyopathies.

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Pediatric cardiomyopathies are a heterogeneous group of disorders characterized by structural and functional cardiac myocardial abnormalities. Up to 40% of affected children die or undergo cardiac transplantation within five years of diagnosis. While the understanding of the molecular basis of pediatric cardiomyopathy has greatly improved over the last two decades, in many cases the underlying cause remains unknown. Currently available (targeted/custom based) diagnostic tests do not represent the whole spectrum of genetic etiologies and lead to a correct genetic diagnosis in a subset of pediatric CMP patients only. Therefore, we decided to apply duo/trio-based exome sequencing to identify causal mutations in known or novel disease genes.

Using homozygosity mapping and exome sequencing in two consanguineous families with idiopathic pediatric cardiomyopathy, we identified homozygous truncating mutations in a new disease gene: alpha-kinase 3 (*ALPK3*). This gene encodes a nuclear kinase that is essential for early differentiation of cardiomyocytes, being involved in essential transcription factor pathways. A third family carrying mutated *ALPK3* was identified upon cohort screening. Patients with biallelic mutations presented with severe cardiomyopathy leading to early lethality or biventricular dysfunction in childhood. Interestingly, some heterozygous family members showed adult-onset cardiomyopathy with atypically distributed hypertrophy, indicating that this gene may also play a role in dominantly inherited cardiomyopathies. We provide microscopic evidence of intercalated disc remodelling, as previously observed in

Alpk3 knockout mice. Electron microscopy and protein expression experiments on heart tissue of the patients and studies on the role of the gene in adult-onset cardiomyopathies as well as in cardiac hypertrophy in general are ongoing.

In conclusion, biallelic truncating mutations in *ALPK3* cause severe pediatric cardiomyopathy in humans. Our findings highlight the importance of transcription factor pathways in the molecular mechanisms underlying human cardiomyopathies and underscore the high genetic heterogeneity of pediatric cardiomyopathies.

This work has been accepted by the Journal of the American College of Cardiology. Further research is ongoing

O20: Comprehensive method for the analysis of low-frequency mitochondrial SNVs and small and large deletions in DNA samples and single cells

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The mitochondrial DNA (mtDNA) can harbor structural rearrangements and single nucleotide variants (SNVs) that may be associated with inherited and acquired disease, and can be linked to the natural process of ageing. Studying mtDNA changes can be technically challenging, especially for variants and deletions with low heteroplasmic loads. Massive parallel sequencing (MPS) has been shown to be useful to detect SNVs at very low frequencies, but no methods are currently available to simultaneously and accurately establish the breakpoints and mutation loads of large deletions.

In this work, our aim was to develop a method, from wet lab to bioinformatic processing, to identify and quantify low frequency SNVs and large deletions in the mtDNA of DNA samples and single cells.

We used a PCR-based approach for mtDNA enrichment, and used two primersets that generate two amplicons of 8kb and 12kb. The smaller amplicon was contained in the larger. MPS was carried out on a Miseq with 2x150 bp sequencing length and approximately 5000x sequencing depth. The fastq files were aligned and processed by 2 distinct algorithms for the detection of SNVs (GATK + CLCBio Genomic Workbench) and large deletions (custom pipeline). Very briefly, our custom pipeline identifies split reads aligning to two different parts of the mtDNA, suggesting the presence of a deletion breakpoint. The reads containing alignments of the same breakpoint are counted and the mutation load is calculated by dividing the number of reads by the coverage depth at the breakpoint position. First, we used our setup to study DNA samples of three patients known to carry mtDNA deletions. Two patients carried a single deletion, and one patient carried multiple deletions. The results obtained with the two different primersets were concordant, and showed a large deletion at a high frequency in two patients (m.10223-12589del at 80% load and m.12112-14412del at 87% load), while the third patient showed multiple deletions at individual loads ranging from 0.5% to 25%. Next, to establish to lowest detection limit and the accuracy of our protocol, we analyzed a set of artificial heteroplasmic samples, with loads ranging from 0.1% to 25%, obtained by mixing a control full-length mtDNA with the DNA sample containing the m.10223-12589del. The experiments were carried out in triplicate. Given that the sequencing error of the system can be as high as 0,6%, we set a lower detection limit for SNVs at 1%,

while the deletions could be detected at frequencies down to 0.1%. The observed heteroplasmic values correlated with the expected values for both primersets (R2=0,991 and R2=0,996 for deletions and R2=0,991 and R2=0,998 for SNVs). Regarding the absolute quantification, one primerset consistently underestimated the frequency of the deletions, and the other set gave different results depending on the run. This illustrates that a given PCR setup may perform well at quantifying mutations at high loads, but may result in biased results when working at low heteroplasmic loads. Together with the expected deletions, we observed sequences strongly resembling bona fide deletions, appearing at very low frequencies. To establish if these sequences were generated by the PCR, we carried out single-molecule PCR. We found that indeed the PCR can generate false rearrangements, but at loads of maximum 0.06%. Taking this into consideration, we raised the lower detection threshold for deletions to 0.5% load. Finally, we downscaled the method to the single cell level. First, we tested the impact of an increase of 15 PCR cycles on the results by using 10 pg of one of the artificial mixes, simulating a single cell with a known genetic content. We observed no changes in the deletion and SNVs calls, while the noise remained below the 0.5% frequency in all the conditions. Next, we sequenced six single fibroblasts from a control individual and two COX-negative muscle fibers from the multiple deletion patient tested earlier. The single fibroblasts were matched to the sequencing results of the bulk DNA of the same fibroblast culture. This allowed us to analyze the differences between single cells and bulk DNA, particularly for the SNVs; for instance, we observed how the variant m.12071T>C, present in bulk DNA at a constant level of around 13%, is present in a homoplasmic state in one out of six cells sequenced, while absent in the others. The muscle fibers showed a low number of large deletions at a high frequency, and most of them were also identified in the blood sample of the same patient.

The method developed proved to be reliable in detecting SNVs and large deletions in the mtDNA at heteroplasmic levels as low as 1% and 0.5%, respectively. The method is applicable also to single cells and allows, for instance, for the study of cellular diversity, and is a promising tool to use in diagnostic applications in which the analysis of a single cell is required, such as preimplantation genetic diagnosis.

O21: Analysis of DFNA5 methylation and expression in primary breast adenocarcinomas using large sample numbers on the basis of The Cancer Genome Atlas

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Introduction: Methylation of promoter CpG islands is frequently associated with transcriptional silencing and may serve as a mechanism to inactivate tumor suppressor genes in breast cancer. Identification of methylation markers that are sensitive and specific for breast cancer may improve early detection, which is of tremendous importance in achieving a better prognosis. We hypothesize that DFNA5 promoter methylation may be a valuable epigenetic biomarker, based upon strong indications for its role as tumor suppressor gene, its function in programmed cell death and its potential role as biomarker in cancer. In this study we analyzed DFNA5 methylation in a high number of samples using publicly available data from The Cancer Genome Atlas (TCGA).
Materials and methods: All data for this study were downloaded from the TCGA data portal. Infinium HumanMethylation450k data from 668 unique, primary, untreated, female breast adenocarcinoma samples (496 ductal and 172 lobular carcinomas) and 79 paired normal breast samples were obtained. The Infinium HumanMethylation450 BeadChip covers 22 different CpGs in the DFNA5 gene. Agilent 244K Custom Gene Expression G4502A-07 data were obtained for 476 unique, primary, untreated, female breast adenocarcinoma samples (435 ductal and 41 lobular carcinomas) and 55 paired normal breast samples. For 191 of these breast adenocarcinomas and 36 of the normal samples, both DFNA5 methylation and expression values were available.

Results: We first analyzed the effect of age, race and menopausal state on DFNA5 methylation. The methylation status of 11 out of the 22 covered CpGs were associated with age. Because this age effect has been described in literature before, downstream analyses were always corrected for age. A statistical significant difference in DFNA5 methylation (N = 79) between primary tumor (T) and paired normal breast samples (N) was found for all 22 CpGs analyzed ($p < 0{,}001$). This was also the case for DFNA5 expression (N = 55; p = 1,79.10-09). No linear correlation was found between DFNA5 methylation and expression (T: N = 191; N: N = 36). Next, a physical map was constructed to correlate the chromosomal location of these 22 CpGs with the average methylation values of the different subgroups (T vs. N). A clear clustering of the methylation values at the different positions was observed. The methylation values of the first 6 CpGs, which are located in the gene body, were always higher in the normal tissue compared to the tumor samples. In contrast, for CpG7 till CpG20, located in the gene promoter region, the average methylation values of the normal samples were always lower than the tumor samples. For CpG21 and CpG22 methylation values were again higher in normal tissue. Next, the methylation analysis was repeated taking the different clinical subgroups (ductal-lobular) into account. A statistical significant difference in DFNA5 methylation between ductal and lobular adenocarcinoma was found in 11 out of the

22 CpGs ($p < 0,05$). The lobular carcinomas showed higher methylation than the ductal carcinoma in all 22 CpGs. DFNA5 expression was also statistically significant different between both subgroups ($p = 1,37.10\text{-}03$). Finally, a multiple linear regression model that included age, histological diagnoses (ductal-lobular), tumor stage, estrogen receptor (ER), progesterone receptor (PgR) and HER2 state, showed that age and histological diagnosis had a statistically significant effect on the methylation of respectively 11 and 5 out of the 22 CpGs. Moreover, the model demonstrated that there was no effect of HER2 state nor PgR state and little effect of tumor stage on DFNA5 methylation. The most interesting finding was a statistically significant effect of ER state in 18 of the 22 CpGs ($p < 0,05$). Overall and progression free survival analysis on these data is ongoing.

Conclusion: These preliminary data suggest an interesting and promising role of DFNA5 in breast cancer. The difference in DFNA5 methylation between lobular and ductal carcinoma and the observed association with ER status are noteworthy. Further research is needed to elucidate the cause of the associations, because this could lead to more insights into tumorigenesis, or a better molecular subclassification. In addition, this analysis shows the power of initiatives such as TCGA, providing genetic data for large sample numbers, for the analysis of individual genes involved in cancer.

O22: Ultra-deep targeted resequencing of 38 pancreatic neuroendocrine tumors reveals tumor heterogeneity for actionable mutations

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Introduction
Pancreatic neuroendocrine tumors (PNETs) are rare tumors arising from the endocrine pancreas. Low-coverage exome sequencing of primary PNET tumor samples revealed PI3K-AKT-mTOR pathway genes to be mutated in 16% of all PNETs, in addition to highlighting mutations in other genes, including MEN1 (44% of all patients), DAXX (25%), ATRX (18%). Recently, intra-tumor heterogeneity has been described as a driving factor for tumor progression and therapy resistance in different tumor types. Our pre-clinical research suggests that intra-tumor heterogeneity plays a role in PNET therapy resistance. To study tumor heterogeneity and identify subclones within tumors, high coverage next generation sequencing data sets from PNETs are needed and currently lacking.

Materials and methods
Formaline-fixed paraffin-embedded paired tumor-normal tissue from 38 invasive grade 1-2 PNETs was collected at Erasmus MC and University Hospital Antwerp. After DNA extraction and enrichment with an in-house-developed Agilent Haloplex 24-gene-panel, all tumor samples were ultra-deep sequenced on the Illumina HiSeq 1500 platform. Single-nucleotide variants (SNVs) and insertions and deletions (indels) were detected using the Genome Analysis ToolKit using a ploidy setting of 40, allowing detection of alterations present in 5% of tumor cells. VariantDB was used for variant annotation and filtering. SNVs and indels, predicted to be damaging by PolyPhen2, SIFT, PROVEAN or MutationTaster, were filtered to have an allelic fraction (AF) >0.025, alternative allele depth >20, mapping quality >50, Fisher-scaled strand bias <20, snpEff annotations ? noncoding, only RefSeq stopgain, stoploss, and nonsynonymous SNVs.

Results
Average target base coverage over all samples was 2602-fold. A total of 3572 mutations were identified, with 70,3% of these mutations only present in less than 30% of all reads, pointing to subclonal tumor cell populations containing specific mutations.

All genes in the panel, but KRAS, showed mutations in at least one tumor sample. All tumors showed mutations in PTCH2, CYFIP2, MUC17 & MUC 16. The mutation load was highest in MUC16, PTCH2 and TSC2. DAXX and ATRX mutations were seen in 89,5% and 73,7% of all tumors, respectively. MEN1 was found mutated in 94,7% of included tumors. Components of the PI3K-AKT-mTOR pathway, including PIK3C2A, MTOR and PTEN, were mutated in sub-clonal cell populations in more than 80% of all tumors, possibly explaining limited efficacy of mTOR-inhibitor everolimus in PNETs.

Conclusions

This first study using ultra-deep targeted sequencing in PNETs reveals genetic tumor heterogeneity. Known PNET mutations, such as in ATRX/DAXX and MEN1 were seen in higher fractions in our study population than reported in literature. Additionally, actionable mutations in the PI3K-AKT-mTOR pathway were found. Next, the variants will be categorized using an assumption ranking system. This categorization step allows us to distinguish between contradictional findings of ambigious prediction program annotations. Furthermore validation and correlation with clinical data is ongoing.

O23: Modeling human cancer syndromes using TALEN and CRISPR/Cas9 mediated genome editing in Xenopus tropicalis.

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The recent developments in genome editing via guided nucleases such as CRISPR/Cas9 and TALENs (Tal effector nucleases) are creating a true revolution in the use of non-mammalian vertebrate model organisms. For the first time it is now possible to mutate specific genes (i.e. performing factual Reverse Genetics), rather than relying on random mutagenesis approaches (Forward Genetics) or transient knock-down of gene expression (e.g. via RNAi or Morpholino injections).

Especially for the Xenopus and zebrafish research communities, this is starting an exciting new era of genome editing that creates unique opportunities for modeling human disease. Several cancer models have already been established in zebrafish, but Xenopus has lagged behind, primarily due to the absence of genetic mutants and the much smaller size of the research community. However, thanks to the new techniques for genome editing, Xenopus is now well positioned to quickly fill this gap.

The tetrapod species Xenopus tropicalis is extremely well positioned for modeling human disease due to some specific advantages. (1) Unlike Xenopus laevis and zebrafish, Xenopus tropicalis has a true diploid genome. Hence, gene disruption studies are not suffering from redundancy. (2) The Xenopus tropicalis genome shows a high degree of synteny with humans, which greatly facilitates the identification of orthologs of human disease genes. (3) It shares with zebrafish an aquatic habitat, which allows the continuous and simple administration of chemical compounds, including candidate cancer drugs. (4) It also shares with zebrafish an external development, large brood size and a short life cycle. This allows very efficient, very cheap and large scale genomic manipulations and housing.
In a first study we modeled human familial adenomatous polyposis (FAP) syndromes, which are caused by nonsense mutations in the tumor suppressor gene adenomatous polyposis coli (APC). Tadpoles and froglets derived from embryos injected with TALEN or CRISPR/Cas9 targeting the mutation cluster region in the apc gene rapidly developed intestinal hyperplasia and other neoplasms observed in FAP patients, including desmoid tumors and medulloblastomas (1). Bi-allelic apc mutations causing frame shifts were detected in the tumors, which displayed activation of the Wnt/?-catenin pathway and showed increased cellular proliferation. We further demonstrate that simultaneous double bi-allelic mutation of apc and a non-relevant gene is possible in the neoplasias, opening the door for identification and characterization of effector or modifier genes in tumors expressing truncated apc. Additional genetic cancer models have meanwhile been established by targeting other tumor suppressor genes. Our results demonstrate the power of modeling human cancer in Xenopus tropicalis using mosaic bi-allelic gene disruption via TALEN and CRISPR/Cas9.

O24: The BRIP1/FANCJ DNA helicase is a 17q driver oncogene protecting neuroblastoma cells from MYCN induced replicative stress at G-quadruplexes

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Introduction: Chromosome 17q gain is by far the most common DNA copy number alteration in aggressive neuroblastoma (NB) but thus far the causal 17q drivers remain to be identified due to the large size of the recurrently involved chromosome segments.

Methods and Results: Integrated mRNA/CNV analysis of 211 NBs using the CONEXIC algorithm identified BRIP1 (alias FANCJ) as top-ranked candidate 17q driver gene. Stable BRIP1 knock down in NB cell lines significantly reduced cell viability and colony forming capacity. In keeping with its role in DNA damage repair, knock down induced DNA damage as evidenced by increased ?H2AX. Given that BRIP1 also unwinds G-quadruplex (G4) DNA structures, we hypothesized that increased BRIP1 levels could protect NB cells from MYCN induced replicative stress. Knock down increases RPA32 protein levels and decreases sensitivity to hydroxy urea induced replication fork stalling as measured by DNA combing. The G4 stabilizer TMPYP4 strongly affected viability. Gene expression profiling after BRIP1 knock down confirmed enrichment for gene sets implicated in DNA replication and repair. Preliminary results indicate that overexpression of BRIP1 in dbh-MYCN-eGFP transgenic zebrafish accelerates tumor formation. Finally, administrating various drug in combination with TMPYP4 by oral gavage in zebrafish is ongoing to assess possible synergistic effects as a prelude to novel therapies for high risk NB.

Discussion: We propose BRIP1 as a major 17q cooperative driver oncogene in NB by protecting NB cells from MYCN induced replicative stress at G4s offering a new entry point for drugging of aggressive high risk NB.

O25: The lncRNAomes of T-cell acute lymphoblastic leukemia and normal immature thymocyte subsets through combined poly-A and total RNA-sequencing

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T-cell acute lymphoblastic leukemia (T-ALL) is a hematologic malignancy caused by uncontrolled proliferation of immature thymocytes that arrested during differentiation. T-ALL can be classified into molecular genetic subgroups that are defined by overexpression of transcription factor oncogenes including TLX1, TLX3, TAL1/LMO2 or HOXA. A fifth subgroup has been added with an immature immunophenotype, a high mutational load and poor prognosis, but without an obvious single driver gene event. Long noncoding RNAs (lncRNAs) are emerging as important players in cancer development. We and others previously demonstrated a contribution of long noncoding RNAs to the leukemic phenotype (Trimarchi et al, Cell, 2014; Durinck, Wallaert et al, Haematologica, 2014).

Here, we present a unique resource of establishing the lncRNAomes of T-ALL and immature thymocytes. We performed poly-A RNA sequencing on a cohort of 60 primary T-ALL patient samples and total RNA sequencing of 25 samples from this cohort. First, we did a comparison of poly-A versus total RNAseq. This revealed that total RNAseq detected over 20 % more lncRNAs then poly-A RNAseq, but only 5 % more protein coding genes. Next, we showed that the T-ALL molecular genetic subgroups, as defined by protein coding gene expression profiles, are recapitulated by the lncRNA datasets. Thirdly, we profiled stage-specific normal developing thymocytes and identified, upon comparison with T-ALL profiles, several ectopically expressed, thus putative oncogenic lncRNAs. Finally, we examined the top 50 upregulated lncRNAs in the immature T-ALLs for lncRNAs that are higher expressed in the immature T-ALL samples compared with the CD34+ thymocytes, depicting potential oncogenic lncRNAs in this specific subgroup.

Using 4C-sequencing and guilt-by-association analysis, lncRNAs are currently selected for further functional analysis to decipher their role in normal T-cell development and T-ALL formation.

Poster Presentations

P1: Clinical and molecular lessons from targeted next generation sequencing of 51 genes involved in primary electrical disease

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Primary electrical disease (PED) is characterized by cardiac arrhythmias, which can lead to sudden cardiac death in the absence of detectable structural heart disease. PED encompasses a diversity of inherited syndromes, predominantly Brugada syndrome, early repolarization syndrome, long QT syndrome, short QT syndrome, arrhythmogenic right ventricular cardiomyopathy/dysplasia and catecholaminergic polymorphic ventricular tachycardia. To overcome the diagnostic challenges imposed by the clinical and genetic heterogeneity, we developed a targeted gene panel for next generation sequencing of 51 genes involved in PED. Twenty CEPH samples and 20 positive control samples were used to validate the panel. A technical sensitivity and specificity of 100% respectively 99.9% was obtained.

After validation, we applied the assay to 114 PED patients. We identified 107 variants in 36 different genes, 18 of which were classified as pathogenic or likely pathogenic, 54 variants were of unknown significance and 35 were classified as likely benign. In the patient groups of BrS, ARVC/D and LQTS we reached causal mutation detection rates of 23% (18/80), 37.5% (3/8) and 36.4% (4/11) respectively. In total, 46.5% of patients (53/114) had a variant, either (likely) pathogenic or VUS, that required additional molecular and clinical follow-up. We hypothesize that several VUS will also be causal because we identified more variants per gene in our PED patients compared to what could be expected by chance, based on the number of variants present in the ExAC database for these genes (eg. 3.7 times more SCN5A variants (MAF ? 0.001) in our PED patients compared to the ExAC database). Our data also support a putative oligogenic nature as in 17 probands we identified more than one (possible) pathogenic variant or VUS. Even more intriguing is the observation that within one family, two different pathogenic mutations cause the identical phenotype in different individuals. The latter advocates for the application of the panel testing in different affected individuals to identify to full underlying genetic architecture. Finally, we identified the first SCN5A founder mutation in 17 Belgian families.

In conclusion, the PED MASTR Plus assay is a proficient, highly reliable and reproducible technique to routinely screen patients suffering from primary arrhythmias.

P2: Clinical delineation of the recurrent de novo c.607C>T mutation in PACS1

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We have recently described two unrelated boys with a strikingly similar facial appearance and intellectual disability in whom an identical de novo mutation in PACS1, c.607C>T, was detected by exome sequencing (NM_ 0180026.2, OMIM 615009: Mental Retardation, Autosomal Dominant 17) [1]. In vitro and in vivo studies showed that the mutant protein forms cytoplasmic aggregates in vitro with concomitant increased protein stability. Further, expression studies of mutant PACS1 mRNA in zebrafish embryos suggest that the mutant zebrafish phenotype is driven by aberrant specification and migration of SOX10-positive cranial neural crest cells, probably explaining the craniofacial phenotype in patients. Since this initial report, we have collected clinical information on 19 individuals with this identical de novo mutation. Social media (facebook) has shown its value in collecting additional patients with this rare genetic disorder. There is a distinctive facial appearance (19/19), characterized by arched eyebrows, hypertelorism with downslanting palpebral fissures, long eye lashes, ptosis, low set and simple ears, bulbous nasal tip, wide mouth with downturned corners and a thin upper lip with an unusual 'wavy' profile, flat philtrum and diastema of the teeth. Intellectual disability, ranging from mild to moderate, was present in all. Hypotonia is common in infancy (9/19). Seizures are frequent (12/19) and respond well to anticonvulsive medication. Structural malformations are common, including heart (10/19), brain (12/15), eye (9/19), kidney (3/19) and cryptorchidism (6/12 males). Feeding dysfunction is presenting in infancy with failure to thrive (6/19), gastroesophageal reflux (6/19), and gastrostomy tube placement (5/19). There is persistence of oral motor dysfunction. In summary, this recurrent de novo c.607C>T mutation in PACS1 gives rise to a clinically recognizable intellectual disability syndrome with multiple congenital anomalies. Syndromes with overlapping facial characteristics are Baraitser Winter syndrome (OMIM: #243310), Cornelia de Lange syndrome (OMIM: #122470), Mowat-Wilson syndrome (OMIM: #235730) and Kabuki syndrome (OMIM: #147920). We provide suggestions for clinical work-up and management. Given the recognizable facial gestalt, we hope that the present study will facilitate clinical recognition of further cases.

1. Schuurs-Hoeijmakers et al. AJHG, dec 2012

P3: Novel mutation in a patient with Canavan disease

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Canavan disease (CD) is a rare fatal childhood neurological autosomal recessive genetic disease caused by mutations in the ASPA gene, which lead to catalytic deficiency of the ASPA enzyme that catalyzes the deacetylation of NAA. It is a severe progressive leukodystrophy characterized by spongiform degeneration of the white matter of the brain. CD occurs frequently among Ashkenazi Jewish population, however it has been reported in many other ethnic groups with significantly lower frequency. Here, we report on a 2 year-old Egyptian child with severe CD who harbors a novel homozygous missense variant (c.91G > T, p.V31F) in the ASPA gene. The clinical, radiological, and molecular genetic, and structural profiles are reviewed in details.

P4: Mutations in the E-subunit of the vacuolar ATPase complex cause a novel type of autosomal recessive cutis laxa

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Introduction
Cutis laxa denotes a heterogeneous group of rare heritable connective tissue disorders, characterized by the presence of loose, sagging, and inelastic skin that may present with systemic manifestations of variable severity. In this study we describe a novel type of cutis laxa in an Iranian consanguineous pedigree with severe and generalized cutis laxa, arthrogryposis, congenital hip dysplasia, and severe aortic root dilatation and biventricular hypertrophy.

Methods
Genomic DNA (gDNA) from the index patient was enriched using the Agilent SureSelect XT Human All Exon kit and whole exome sequencing (WES) was performed on the Illumina NextSeq 500 platform. Functional studies, including homology modeling, transmission electron microscopy (TEM) and Brefeldin A (BFA) studies, were performed as described previously.

Results
Using WES, we identified the homozygous p.(Leu128Pro) (c.383T>C) missense mutation in ATP6V1E1, encoding the E-subunit of cytoplasmic V1 part of the vacuolar ATPase (V-ATPase) complex. This complex is directly implicated in intracellular trafficking pathways and exocytosis through acidification of vesicular compartments. The p.(Leu128Pro) substitution is predicted to destabilize the head of this peripheral stalk subunit and interfere with proper V-ATPase function. Retrograde translocation of Golgi membranes to the endoplasmic reticulum was severely delayed in BFA treated fibroblasts from affected individuals, and ultrastructural analysis showed showed abnormal swelling and fragmentation of the Golgi apparatus. TEM analysis of the dermis from a skin biopsy showed severe changes in the amount, structure and organization of elastic and collagen fibers.

Conclusion
This study describes a novel type of cutis laxa caused by mutations in the E-subunit of the V-ATPase complex.

P5: Deciphering the genetic background of high-risk BRCA 1/2 mutation-negative breast cancer patients

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Background
Breast cancer is the most frequent malignant disease and the second cause of cancer death 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. Different technologies enabled to identify several other breast cancer predisposition genes of high, moderate or low penetrance. It is however considered that these variants only explain 40% of the inherited risk of breast cancer.
We used whole-exome sequencing to investigate BRCA 1/2-negative high-risk familial breast cancer patients, aiming to identify their patterns of genetic variation within the breast cancer predisposition genes.

Methods
Germline exomes of 48 breast cancer patients with a strong familial history and a BOADICEA lifetime risk score above 20% were sequenced on a 5500 SOLiD(™) System. Variants were called within a panel of 236 genes already associated to cancer or to DNA repair. Synonymous variants and variants with minor allele frequency in a default global population above 1,5% were discarded.
Candidate variants were validated with Sanger sequencing. Familial validation through co-segregation with the disease is ongoing.

Results
Quality and gene-panel filtering could narrow the list of variants to 308 from the 3.628.494 identified. Of these, 123 were kept after alignment validation and literature study. 116 were validated by Sanger sequencing. Out of them, 11 variants affected a splice site region and 105 resulted in an amino acid substitution. Nineteen variants were known in the COSMIC database.
Most patients (46 out of 48) had at least one validated variant; mean number of variants per patient was 2.4 (range: 0 to 6). Each variant was found in 1 to 5 patients (mean 1.4). Variants were detected in the known high-to-moderate penetrance breast cancer susceptibility genes ATM, PALB2, MSH2, MSH6, PMS2, RAD51C, MRE11A and RECQL for 9 patients. Co-segregation has so far been revealed for altogether 18 variants.

Conclusion

In these high-risk BRCA 1/2 mutation-negative breast cancer patients, massively parallel whole exome sequencing enabled us to detect several germline variants in genes linked to breast cancer or related to DNA repair. Further segregation analysis is needed to corroborate their clinical significance.

P6: Copy Number Variation (CNV) analysis and mutation analysis of the 6q14.1 – 6q16.3 genes SIM1 and MRAP2 in Prader Willi like patients.

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Background: Prader-Willi syndrome (PWS), caused by a paternal defect on 15q11.2 – q13, is the most common form of syndromic obesity. However, patients clinically diagnosed with PWS do not always show this defect on chromosome 15q and are therefore molecularly categorized as Prader Willi like (PWL). Deletions at 6q14.1 – q16.3 encompassing MRAP2 and SIM1 were reported in some individuals with a PWL phenotype. In addition, a few mutations in SIM1 and MRAP2 were also previously identified in cohorts of obese individuals. Therefore, we decided to perform copy number variation analysis of the 6q14.1 – 6q16.3 region followed by mutation analysis of SIM1 and MRAP2 in a PWL cohort.

Methods: A genome-wide microarray analysis was performed in a group of 109 PWL patients. Next, we screened 94 PWL patients for mutations in SIM1 and MRAP2 using high-resolution melting curve analysis and Sanger sequencing. Additionally, 363 obese children and adolescents were screened for mutations in MRAP2.

Results: No gene harboring deletions were identified at the 6q14.1 – q16.3 region in the 109 PWL patients. SIM1 mutation analysis resulted in the identification of one very rare nonsynonymous variant p.P352S (rs3734354). Another nonsynonymous variant, p.A40S, was detected in the MRAP2 gene. No variants were identified in the 363 obese individuals.

Conclusions: In contrast to literature reports, no gene harboring deletions were identified in the SIM1 and MRAP2 regions in our PWL cohort. Secondly, taking into account their very low minor allel frequencies in public sequencing databases and the results of in silico prediction programs, further functional analysis of p.P352S found in SIM1 and p.A40S found in MRAP2 is useful. This would provide further support for a possible role of SIM1 and MRAP2 in the pathogenesis of the PWL phenotype albeit in a limited number of patients.

P7: Combination of CNV analysis and mutation screening indicates an important role for the NPY4R gene in human obesity.

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Objective
Obesity is a highly heritable complex disorder that has reached epidemic proportions worldwide. Its increasing prevalence and associated morbidity imposes an enormous burden on human health. Although an obesogenic environment and a passive lifestyle are main contributors to obesity prevalence, numerous studies have shown that 40-70% of the interindividual variability in BMI is attributed to genetic factors. Recently, 97 BMI-associated loci were identified that account for approximately 2.7% of BMI variation, leaving a substantial component of the heritability unexplained. Other forms of variation such as epigenetic marks and structural variation might contribute to the missing heritability. Genomewide copy number variation (CNV) analyses have associated the 10q11.22 CNV with obesity. As the NPY4R gene is the most interesting candidate gene in this region, we hypothesized that both genetic and structural variation in NPY4R might be implicated in the pathogenesis of obesity.

Design and Methods
In the first part of this study, we screened 326 obese children and adolescents, and 298 healthy lean individuals for CNV in the NPY4R-containing chr.10q11.22 region. In the second part of this study, we performed a mutation screen for variants in the NPY4R coding region in 356 obese children and adolescents, and 337 healthy lean adults.

Results
In our CNV analysis we demonstrated a significantly higher frequency of NPY4R containing 10q11.22 CNV loss in the patient population ($p=0.0003$), while CNV gain in this region was more prevalent in our control population ($p=0.031$). Mutation analysis resulted in the identification of fifteen rare non-synonymous heterozygous variants. For two variants that could only be identified in our patient population, we were able to demonstratie receptor dysfunction and thus a pathogenic effect.

Conclusion
Taken together, the presence of structural (12 10q11.22 CNV loss carriers) and genetic variation (2 carriers with NPY4R dysfunction) within the NPY4R gene at least partially explains the obese phenotype of approximately 4.3% of our patient population. In conclusion, these data support an essential role for genetic and structural variation within the NPY4R gene in the pathogenesis of obesity.

P8: 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/.

P9: In Vivo Modeling of Copy Number Variants in Marfan Syndrome and Autosomal Dominant Polycystic Kidney Disease-Associated Phenotypes

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Thoracic aortic dissections are among the most life threatening forms of cardiovascular disease. Thoracic aortic aneurysm, preceding dissection, is a prominent clinical feature of several heritable connective tissue disorders, including Marfan syndrome (MFS). MFS is caused by mutations in FBN1, which encodes fibrillin-1, an important extracellular matrix protein. Mutations in PKD1 or PKD2, two polycystin encoding genes, are responsible for autosomal dominant polycystic kidney disease (ADPKD). Aortic and arterial aneurysms also occur in ADPKD. Vice versa, kidney cysts have also been observed in MFS. This clinical overlap suggests a mechanistic link between ADPKD and MFS.
Here we describe a four generation family with nine affected individuals presenting with both thoracic aortic aneurysm and mild cystic kidneys. FBN1, PKD1 and PKD2 were excluded as disease causing genes by linkage analysis and/or sequencing. Subsequently, whole genome linkage analysis resulted in the delineation of a unique linked region on chromosome 16q21-q24.1. Exome sequencing was performed but no putative causal variants were found in the linked region. Copy number variation analysis identified two duplicated regions in the linkage interval, one (chr16: 86862531-870228808) gene-less and the other (chr16: 86357163-86725305) containing seven genes, including three genes encoding transcription factors of the FOX gene family (FOXC2, FOXF1, FOXL1), one gene encoding a methenyl tetrahydrofolate synthetase containing domain protein (MTHFSD) and three long non coding RNAs (LOC732275, FENDRR, FLJ30679). The presence of the first duplication and its segregation in the family was confirmed using Multiplex Amplicon Quantification analysis. By overexpressing these genes separately and in combination with each other in zebrafish, we will explore the pathogenic mechanisms underlying not only MFS and ADPKD, but aneurysm and cyst formation in general. Preliminary results of these experiments, revealing a potential role for FOXF1 in cyst formation, will be presented.

P10: Somatic Activating GNAQ Mutations explain the Cause of Only Half of Capillary Malformations

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Vascular anomalies are localized defects of the vascular system. They are most commonly observed on the skin, but can affect any body part. They are divided according to affected vessel type into arterial, capillary, venous, lymphatic and combined malformations. Capillary malformation (CM) is the most common vascular malformation affecting cutaneous capillary vessels in 0.3% of newborns. These lesions are usually unifocal or diffuse. CM most often occurs as an isolated and sporadic feature. Syndromic forms also exist, such as Sturge-Weber syndrome (SWS), Klippel-Trenaunay syndrome, and Parkes Weber syndrome (Boon et al., 2005). The etiology has remained unknown. We reported that the rare multifocal and familial CM-AVM (capillary malformation-arteriovenous malformation) is caused by RASA1 haploinsufficiency, most likely combined with a tissular second-hit, of p120-RasGAP, the protein product of RASA1.

A series of Sturge–Weber syndrome and capillary malformations were recently shown to harbor a non-synonymous somatic single-nucleotide variant in GNAQ, encoding guanine nucleotide binding protein (G protein). We assessed by Sanger sequencing for the presence of the c.548G?A hot-spot mutation on cDNA from 14 lesions with similar vascular phenotype. The activating somatic mutation was detected in 50 % of the lesions. The fact that some tissues were negative in the screen could be due to a low proportion of mutated cells in the resected lesion. To increase our detection rate, targeted massive parallel sequencing of GNAQ was performed on a set of 49 CM-lesion derived DNAs (5 SWS patients and 44 sporadic isolated CMs) using Ion AmpliSeq Panels on PGM. Twenty-four tissues with a GNAQ mutation were identified, including the 5 SWS samples, leaving 25 without a GNAQ mutation. This suggests that there is locus heterogeneity, and that other genes should be involved in the etiopathogenesis of sporadically occurring isolated CMs. The identification of GNAQ-mutated and GNAQ-wild type tissues (patients) allows already to study genotype/phenotype correlations. (miikka.vikkula@uclouvain.be).

P11: Hypotrichosis-lymphedema-telangiectasia-renal defect associated with a truncating mutation in the SOX18 gene

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SOX18 mutations in humans are associated with both recessive and dominant hypotrichosis – lymphedema – telangiectasia syndrome (HLTS). We report two families with affected children carrying a SOX18 mutation: a living patient and his stillborn brother from Canada and a Belgian patient. The two living patients were diagnosed with HLTS and DNA analysis for the SOX18 gene showed that both had the identical heterozygous C > A transversion, resulting in a pre-mature truncation of the protein, lacking the transactivation domain. Both living patients developed renal failure with severe hypertension in childhood for which both underwent renal transplantation. To our best knowledge this is the first report of renal failure associated with heterozygous mutations in the SOX18 gene. We conclude that this specific mutation results in a new, autosomal dominant condition and propose the acronym HLT-renal defect syndrome for HLTRS.

P12: Mutations in VEGFR3 signaling pathway explain a third of familial primary lymphedema

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Background: Lymphedema, caused by dysfunction of lymphatic vessels, leads to disabling swelling that occurs mostly on the extremities. Lymphedema can be either primary (congenital) or secondary (acquired). Familial primary lymphedema commonly segregates in an autosomal dominant or recessive manner. It can also occur in combination with other clinical features. Twenty-three mutated genes have been identified in different isolated or syndromic forms of lymphedema (Brouillard et al., 2014). However, the prevalence of primary lymphedema that can be explained by these genetic alterations is unknown. In this study, we investigated systematically 11 of these genes.
Methods: We screened 503 index patients, including a third from families with inherited primary lymphedema. A targeted next generation sequencing panel for Ion Torrent (Personal Genome Machine, PGM) was designed. Data analysis was performed with the in-house developed Highlander software (Helaers et al., in prep). The filtering criteria were: pass GATK filters (Genome Analysis Tool Kit, Broad Institute), alternative allele proportion (?0.25), number of patients with the change (<50), in 1000 genomes (<10) / goNL (?5) / ExAC (?0.0014), impact consensus determined damaging by at least 3 programs of prediction of 6 used, and checked in silico with IGV. Validations and co-segregations were done using Sanger sequencing.

Results: The filters allowed us to keep only 147 variants among the approximately 15,241 detected. We limited this number at 104 after validation and co-segregation analyses, explaining 20,7% of the cases. The most frequently mutated genes are VEGFR3, FOXC2, KIF11, CCBE1, SOX18, GATA2, and GJC2 in our cohort. No mutation was found in GJA1, PTPN14, IKBKG, and VEGFC. We are currently continuing co-segregation analyses and more detailed clinical phenotyping for those patients, and try to characterize further 29 additional variants of unknown significance.

Discussion: The genetic cause of primary lymphedema remains unexplained at around 60% of patients with a family history and 85% of sporadic or with unknown origin cases. Identification of those genes is important for understanding etiopathogenesis, stratification of treatments and generation of disease models. Interestingly, most of the proteins that are encoded by the genes mutated in primary lymphedema 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.

P13: Molecular landscape of early Crohn's disease using an integrated approach of mRNA/miRNA profiling and genomics

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BACKGROUND
Crohn's disease (CD) is a chronic inflammatory condition of the gut, characterized by a progression to stricturing and/or penetrating complications in most patients. Effective intervention before the onset of bowel damage, and thus in the early phase of the disease, will be required to optimize patient outcomes. We aimed to define the molecular landscape of early CD, looking at microRNA (miRNA)- and SNP-mediated gene regulation. An eminent model to study this early phase is that of early post-operative recurrence (POR) in CD, in which the lesions recur within months to weeks after ileo-colonic resection.

METHODS
Ileal biopsies were obtained from inflamed mucosa of 25 CD patients with POR (Rutgeerts' score i2, i3 or i4), and from normal mucosa of 12 controls. Total RNA was used to study mRNA and miRNA expression via Affymetrix Human Gene 1.0 ST and Affymetrix miRNA 2.0 arrays, respectively. Data were analyzed with R/Bioconductor. A false discovery rate (FDR) <5% and >2-fold change (mRNA) or >1.5-fold change (miRNA) were considered biologically significant. Gene and miRNA expression profiles were integrated using the Ingenuity microRNA Target Filter, and experimentally validated interactions were annotated using TarBase. To identify expression quantitative trait loci (eQTL) for mRNAs, Matrix eQTL (FDR <5%) was applied to a subset of 18 POR CD patients and to a different set of 15 CD patients in different stages of disease. Genotype data were obtained from the Immunochip.

RESULTS
When comparing POR CD patients with controls, 333 (222 up and 111 down) gene probe sets and 24 miRNAs (7 up and 17 down) gave significantly different signals. We identified 92 miRNA-mRNA pairs with negative correlation in expression profiles (17 different miRNAs, and 72 different mRNAs). Of these, there are 54 pairs where the miRNA is predicted to repress the expression of its target mRNA to 40% of its normal level. Four pairs are experimentally supported: let-7a-5p is known to target PRDM1 and PTGS2, and miR-30c-5p targets SLC7A11 and WNT5A. There were no cis-eQTLs (within 1 Mb of their target gene) and 114 trans-eQTL signals. In contrast, in another dataset with CD patients in different stages of their disease, we found 66 cis-eQTL and 1367 trans-eQTL signals.

CONCLUSION
Integrated analysis of gene and miRNA expression profiles in POR in CD patients revealed potential miRNA targets that alter the expression of many genes related to CD pathogenesis.

Let-7a-5p is a promising target, as it regulates the expression of PRDM1, which is a susceptibility gene for CD and encodes a ?-IFN repressor. The lack of cis- and trans-eQTL signals within the POR patient group could reflect the homogeneity in gene expression in POR CD patients, and highlights its usefulness as a model to study early disease.

P14: Implementation of Molecular Inversion Probes for routine diagnostics of BRCA1 and BRCA2

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Despite the great success of exome sequencing, there is still a need for improved (NGS-based) individual gene testing. Molecular inversion probes (MIPs) have shown to be a cost-effective enrichment technology, particularly if used in large sample cohorts. Single molecule (sm) tags thereby further enhance data quality, by removal of duplicate reads thereby constructing consensus sequences free of experimental artifacts. Single molecule molecular inversion probes (smMIPs) therefore proof excellent for usage in highly sensitive tests, such as breast cancer genetic testing. We have developed a workflow for re-sequencing BRCA1, BRCA2 and CHEK2 c.1100del (p.(Thr367fs)) using single molecule MIPs (smMIP) in combination with NextSeq500 sequencing. 527 overlapping smMIPs on both strands were designed, with every base being targeted by at least two independent smMIPs. More than 150 BRCA1 and BRCA2 mutation positive cases were sequenced for both genes with an average coverage of >130-fold (unique reads), yielding in 100% coverage of all targeted bases. Analysis of known pathogenic mutations and SNPs in 152 samples resulted in an analytical sensitivity and specificity of 100%, with no false positives and no false negatives. Based on manual protocols that produced these data, the workflow was completely automated. Next to automation of pre- and posthybridization pipetting, also file handling, data transfer and analysis of sequencing data was automated, delivering fast sequencing data of highest quality. In addition, our setup is such that each analysis is performed in duplicate to make the analysis more robust and to have an independent confirmation of mutation positive cases in the same run. Sequencing in duplicate leads to less loops in the workflow, to a reduction of rework, and accordingly reduces turnaround times. In conclusion, genetic testing of breast cancer susceptibility genes BRCA1 and BRCA2 works exceptionally well using smMIPs. Although ordering costs of smMIPs are relatively high, probes can be highly multiplexed, allowing time- and cost-efficient sample preparation. We have successfully replaced our former amplicon based diagnostic NGS-test for germline and formalin fixed paraffin embedded BRCA1 and BRCA2 testing. Together with automation of both library preparation and data analysis, this resulted in a reduction of 10-15 days in turnaround times which is now stable at ~10-12 days. Implementation of additional genes is ongoing.

P15: Two novel MYLK nonsense mutations causing thoracic aortic aneurysms/dissections in patients without apparent family history

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Aims. Thoracic aortic aneurysm and dissection (TAAD) is a genetically heterogeneous disorder representing a frequent cause of morbidity and mortality in the western world. To date, only two heterozygous loss-of-function mutations have been described in MYLK (myosin light chain kinase) causing familial aortic disease with little to no aortic enlargement prior to dissection. Here, we have aimed to expand the phenotypical spectrum associated with MYLK mutations.

Methods and results. After the application of a next-generation sequencing based TAAD gene panel in a cohort of 359 syndromic and non-syndromic TAAD patients, we identified two novel heterozygous MYLK mutations leading to a premature stop codon. Two female patients, with nonsense mutations at amino acids p.Arg1458 and p.Arg1487, presented with type B aortic dissection at ages 47 and 49 years, respectively. Both patients had a longstanding history of hypertension. Physical exam revealed unilateral iris flocculi in one, whereas the other woman presented with several systemic connective tissue findings. Remarkably, none of the patients had a family history of aortic aneurysms or dissections.

Conclusion. Two novel heterozygous loss-of-function MYLK mutations have been identified. In addition to vascular findings, patients showed variable systemic features. The current report doubles the number of known MYLK mutations and significantly informs the further clinical delineation of the MYLK phenotypic spectrum.

P16: Somatic activating PIK3CA mutations cause venous malformation

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Somatic mutations in the endothelial cell tyrosine kinase receptor TIE2/TEK cause more than half of sporadically occurring unifocal venous malformations (VMs). Here, we report that somatic mutations in PIK3CA, the gene encoding the catalytic p110? subunit of PI3K, cause 54% (27 out of 50) of VMs with no detected TEK mutation. The hotspot mutations c.1624G>A, c.1633G>A and c.3140A>G (p.Glu542Lys, p.Glu545Lys and p.His1047Arg), frequent in PIK3CA-associated cancers, overgrowth syndromes and lymphatic malformation (LM), account for >92% of individuals carrying mutations. Like VM-causative mutations in TEK, the PIK3CA mutations cause chronic activation of AKT, dysregulation of certain important angiogenic factors, and abnormal endothelial cell morphology when expressed in HUVECs. The p110?-specific inhibitor BYL719 restores all abnormal phenotypes tested, in PIK3CA- as well as TEK-mutant HUVECs, demonstrating that they operate via the same pathogenic pathways. Nevertheless, significant genotype-phenotype correlations in lesion localization and histology are observed between individuals with mutations in PIK3CA vs. TEK, pointing to gene-specific effects.

P17: Heterozygous Loss-of-Function Mutations in DLL4 Cause Adams-Oliver Syndrome

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Adams-Oliver syndrome (AOS) is a rare developmental disorder characterized by the presence of both aplasia cutis congenita (ACC) of the scalp vertex and terminal limb reduction defects, such as brachydactyly, oligodactyly, syndactyly, hypoplastic nails or transverse amputations. Cardiovascular anomalies, comprising pulmonary hypertension, ventricular septum defects, tetralogy of Fallot and anomalies of the great arteries and their valves are also frequently observed. Mutations in five genes have been identified as a cause for AOS prior to this report. Mutations in EOGT and DOCK6 cause autosomal recessive AOS, whereas mutations in ARHGAP31, RBPJ and NOTCH1 lead to the autosomal dominant form of AOS. As RBPJ, NOTCH1 and EOGT are all involved in the Notch signaling cascade, we hypothesized that mutations in other genes involved in this pathway may also be implicated in AOS pathogenesis. Using a candidate gene based approach, we prioritized DLL4, a critical Notch ligand, due to its essential role in vascular development and angiogenesis in the context of cardiovascular features in AOS patients. Targeted resequencing of the DLL4 gene using a custom enrichment panel was performed in 89 independent families, in which we found seven mutations. In addition, a defect in DLL4 was also detected in two pedigrees with whole exome/genome sequencing.

In total, nine heterozygous mutations in DLL4 were identified, including two nonsense variants, which are predicted to lead to nonsense mediated decay, and seven missense variants. These missense variants encompass four mutations that replace or create cysteine residues, which are likely critical for maintaining the structural integrity of the protein and three mutations that affect conserved amino acids in two functional domains of the protein. These functional domains include the DSL domain and the MNNL domain, which are both involved in binding of the ligand to the Notch receptor. Affected individuals with DLL4 mutations present with variable clinical expression and incomplete penetrance with no emerging genotype-phenotype correlations. Our findings demonstrate DLL4 mutations as an additional cause of autosomal dominant AOS or isolated ACC and provide yet further evidence for a key role of Notch signaling in the etiology of this disorder.

P18: Molecular and Genetic Determinants of Infantile Hemangioma Pathogenesis

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Infantile hemangioma (IH) is a common, 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 will use two complementary approaches to identify molecules and pathways that contribute to IH pathogenesis. (1) We will carry 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. Sequences will be analyzed on an in-house pipeline (« Highlander », Dr. R. Helaers, unpublished) for variant detection, followed by extensive variant annotation, filtering, and visualization. (2) In order to determine if somatic mutations contribute to sporadic IH pathogenesis, we will carry out WES on a pilot-series of snap-frozen tumor samples and paired blood-DNA. We will also WES DNAs extracted from isolated cell lines from IHs.

We have so far WESed 11 patients and 4 unaffected individuals from 4 families. Bioinformatic analyses are ongoing. The candidate genes from these approaches will be screened in an 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 molecules implicated.

P19: What should be the criteria for the development of expanded carrier screening panels? An interview study with clinical and molecular geneticists

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Carrier screening for autosomal and X-linked recessive disorders allows identification of couples in the general population who are at risk of having an affected child. Recent advances in molecular diagnostic technologies have lead to the development of expanded carrier screening (ECS) panels that include mutations associated with hundreds of recessive disorders at little additional cost. With the potential to continuously expand the scope of carrier screening, it is crucial to devise suitable criteria for the inclusion of disorders and specific pathogenic mutations on ECS panels.

To address this issue, we conducted semi-structured interviews with 16 European geneticists with expertise in carrier screening, to investigate their views on the composition of ECS panels. Most participants favored limiting carrier screening to disorders that, due to their severity, would clearly justify altering reproductive plans by at-risk couples. Some geneticists considered the impact of the disease on the family, suggesting screening should also be performed for severe conditions with effective, yet burdensome therapeutic interventions. All 16 participants were strongly in favor of limiting screening to known disease-causing variants with clearly established genotype-phenotype associations. Our participants generally favored mutation-based panels over next-generation sequencing approaches that aim to identify previously unreported mutations with possible deleterious effects.

Geneticists in our study were cautious about the clinical implementation of ECS and attached great value to screening for mutations with the highest positive predictive value and clear medical benefits. The insights gained from our study contribute to the growing body of empirical literature centered on ECS and may be valuable for developing guidelines towards ethically sound implementation of ECS in reproductive healthcare.

P20: Novel microdeletions on chromosome 14q32.2 suggest a potential role for non-coding RNAs in Kagami-Ogata syndrome

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In approximately 20% of the individuals with Kagami-Ogata syndrome (KOS, MIM 608149), characterized by a small bell-shaped thorax with coat-hanger configuration of the ribs, joint contractures, abdominal wall defects and polyhydramnios during the pregnancy, the syndrome is caused by a maternal deletion of the imprinted gene cluster in chromosome 14q32.2. Most deletions reported so far included one or both of the differentially methylated regions DLK1/MEG3 IG-DMR and MEG3-DMR. We present two unrelated families with two affected siblings each, with classical KOS due to maternally inherited microdeletions. Interestingly, all four patients have lived through to adulthood, even though mortality rates for patients with KOS due to a microdeletion are relatively high. In the first family, the deletion eliminates the expression of the non-coding transcripts MEG3, MEG8 and RTL1as only. None of the differentially methylated regions (DMRs) is included in the deletion and the methylation patterns in the region are identical to those observed in controls. A deletion which does not encompass the DMRs in this region is thus sufficient to elicit the full KOS phenotype. In the second family, a partially overlapping deletion including both DMRs and MEG3 was detected. In summary, our findings show that patients with KOS can live into adulthood, that deletions do not always include the IG-DMR and the MEG3-DMR and that hence a normal methylation pattern does not exclude KOS.

P21: Molecular study of the MFRP gene in patients with posterior microphthalmia (MCOP) supports its role in autosomal recessive MCOP pathogenesis

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Purpose: Posterior microphthalmia (MCOP) is a rare developmental disease restricted to the posterior segment of the eye. To date, mutations in the MFRP gene, encoding a frizzled-related protein, have been reported in autosomal recessive MCOP (arMCOP), often found in consanguineous families. Here, we aimed to identify the underlying genetic cause of arMCOP in seven unrelated patients from different ethnic origins.

Methods: All patients underwent detailed ophthalmological evaluations and Sanger sequencing of the coding region of MFRP (NM_031433.2). Two patients originating from a consanguineous marriage underwent homozygosity mapping using SNP arrays.

Results: In the patients who underwent homozygosity mapping MFRP was found in a homozygous region of 10.2 and 6.2 Mb respectively. Overall, eight distinct MFRP mutations were found in the patients studied. Five patients were homozygous for an MFRP mutation: two missense variants with predicted pathogenic effect (c.1231T>C p.Y411H,novel; c.1549C>T p.R517W, known) and three frameshift mutations (c.1090_1094del p.T364*, novel; c.498del p.N167T*25 and c.498dup p.N167fs*, known). Moreover, a sixth patient was compound heterozygous for nonsense mutation (c.955C>T p.Q319*, novel) and novel deletion of 6,2 kb (c.1-6088_54+40delinsA), predicted to abolish the transcription initiation site. The seventh patient was heterozygous for a known frameshift mutation (c.491_492insT p.N167Qfs*34), no second mutation was found so far. All patients had short axial length (13-16.5 mm), reduced visual acuity (0.15-0.8 logMAR) and hyperopia (+13D to +17.25D). Crowded optic discs were noticed in 7/7 and macular folds in 3/7 patients. Optical coherence tomography showed intraretinal cysts in 5/7 patients. Peripheral pigmentary changes were observed in 5/7 patients. Sight threatening complications such as angle closure glaucoma were seen in one patient.

Conclusions: Eight distinct MFRP mutations were identified in all patients studied. Four of these mutations were novel, including a genomic rearrangement, reported here for the first time. No clear genotype-phenotype correlations could be observed. A recent report by Dinculescu et al (2012) suggested that MFRP-associated retinal dystrophy might be a target for gene therapy. The identification of new families with MFRP mutations might offer opportunities for potential gene-based therapies.

P22: Coding and non-coding copy number variations explaining unsolved retinal dystrophies: role of genomic architectural features and underlying mechanisms

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Purpose: It was our aim to gain insight into susceptibility factors for the formation of copy number variations (CNVs) affecting retinal dystrophy (RD) genes, and to identify and assess the underlying mechanism of coding and noncoding CNVs in RDs.

Methods: Genomic architectural features contributing to CNV formation were investigated in regions containing known RD genes listed in RetNet (https://sph.uth.edu/retnet/). Detection of CNVs in a diagnostic cohort of RD patients without a molecular genetic diagnosis after sequence-based mutation screening was performed by SNP arrays, multiplex ligation-dependent probe amplification (MLPA) or qPCR. For breakpoint mapping we used conventional PCR, qPCR, targeted arrays, long-range PCR and sequencing of junctions. Targeted Locus Amplification (TLA) on extracted DNA was used to characterize CNVs at the nucleotide level.

Results: Genomic architectural features like gene size, intron length, repetitive elements, sequence motifs, non-B DNA conformations were assessed for all RetNet genes. A hypothetical ranking of RD genomic regions prone to CNV formation was proposed. This was first tested by extensive mining of reported CNVs in known RD genes. Secondly, 17 distinct newly identified CNVs including 14 deletions and three duplications in nine different RD genes (BEST1, EYS, KCNV2, MERTK, OPA1, PCDH15, PRPH2, SPATA7 and USH2A) were further studied here. Eleven of these are novel, including two deletions in PRPH2 in which no CNVs have been reported previously. Three of these (two in EYS, one in PCDH15) affect non-coding, putative regulatory regions of their target gene. Fine-mapping of the breakpoints was performed for all CNVs. TLA, a recent strategy based on the crosslinking of physically proximal sequences, was used to map six CNVs at the nucleotide level, for the first time on extracted human DNA instead of living cells. Finally, bio-informatic analyses contributed to the underlying genetic mechanisms of all delineated CNVs studied here.

Conclusions: This study proposed a ranking of CNV-prone RD disease genes, which was validated by investigating genomic data of reported and newly identified RD-associated CNVs, of which 11 are novel. In addition, we demonstrated the efficacy of TLA on extracted genomic DNA to characterize CNVs in a hypothesis neutral manner.

P23: The HBP1 tumor suppressor is an epigenetic regulator of MYCN driven neuroblastoma through interaction with the PRC2 complex

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Introduction:
The MYCN oncogene signaling plays a key role in initiation and progression of neuroblastoma (NB), a pediatric tumor of the sympathetic nervous system, and represents a major potential target for development of novel drugging strategies. Through previous work, we established HBP1 as an important additional regulator of MYCN activity in ALK mutant tumors. In addition, we showed that MYCN controls HBP1 expression levels through a complex regulatory loop.
Methods:
Pathway analysis of HBP1 regulation was done by in vitro evaluation of transcriptional response of NB cells to compounds targeting ALK, PI3K/AKT and MAPK signaling. The SHEP cell line with inducible miR-17?92 was used to investigate HBP1 regulation. HBP1 was modulated in the NGP cell line in order to study transcriptional networks. Data mining was performed in R using available algorithms.

Results:
In this study, we explored the transcriptional consequences of HBP1 modulation in NB in vitro and in vivo model systems. Initial pathway analysis marked SUZ12, together with EZH2 a component of the repressive PRC2 epigenetic regulatory protein complex, as a key mediator in HBP1 controlled gene regulation. Subsequently, we explored the overlap of HBP1 regulated genes versus Myc/Miz-1 repressed gene targets and genes upregulated upon EZH2 inhibition. Based on our current insights of the role of HBP1 in NB, we selected several novel drug combinations for further testing. The green tea component EGCG, known to upregulate HBP1, was shown in vitro and in vivo to act synergistically with the BET inhibitor JQ1. Secondly, we combined the HDAC inhibitor SAHA, in view of the presence of HDAC in HBP1 regulatory complexes, with the PI3K/AKT dual inhibitor BEZ-238, in view of the implication of this pathway in HBP1 regulation and MYCN stabilization, and also observed very strong synergism. Finally, our most recent data point at CTSL2 as a crucial HBP1/EZH2 target. CTSL2 is a pro-apoptotic target of E2F1 and sensitizes cells to HDAC inhibition. Given that EZH2 inhibition upregulates CTSL2, we are currently testing combined pharmacological targeting of EZH2 and HDAC in MYCN transgenic zebrafish and mouse models.

Discussion:
In conclusion, our data provide fundamental new insights into the complex regulation of MYCN activity in NB cells and offers various novel entry points for further phase I clinical trials of precision combination therapies.

P24: An optimized protocol for homozygosity mapping based on whole exome sequencing: HOMWES

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Homozygosity mapping is an effective approach for detecting molecular defects in consanguineous families by delineating stretches of genomic DNA that are identical by descent. Constant developments in the next generation sequencing created possibilities to combine whole exome sequencing (WES) and homozygosity mapping in a single step. Currently, there is no consensus in defining the optimal parameters for autozygosity search based on WES analysis. In a group of autosomal recessive families, with both SNP array and WES data available, we performed a basic optimization of homozygosity mapping parameters. The criteria for SNP extraction and PLINK thresholds were varied in order to estimate their effect on the accuracy of homozygosity mapping based on WES. Our protocol showed high specificity (82%) and sensitivity (84%) for homozygosity detection. Filtering and mapping with optimized parameters was integrated into the HOMWES (HOmozygosity Mapping based on WES analysis) tool in the GenomeComb package for genomic data analysis. The performance of our protocol surpassed those of Homozygosity mapper and H3M2, two available methods for detection of identical-by-descent regions. Additionally the tool facilitated the identification of novel mutations in GAN, GBA2 and ZFYVE26 in four families affected by hereditary spastic paraplegia or Charcot-Marie-Tooth disease. Finally, we present recommendations for detection of homozygous regions based on WES data and a bioinformatics tool for their identification, which can be widely applied for studying autosomal recessive disorders.

P25: Genetic alterations in MYC-rearranged aggressive B-cell lymphomas: differences between pediatric and adult Burkitt lymphomas

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Dysregulation of MYC is the genetic hallmark of Burkitt lymphoma (BL) but it is encountered in other aggressive mature B-cell lymphomas. MYC dysregulation needs other cooperating events for BL development. We aimed to characterize these events and assess for differences between adult and pediatric BLs which may explain the different outcomes.

We studied 34 MYC-rearranged lymphomas: 24 BLs (11 adults/13 children), 7 B-cell lymphomas, unclassifiable, with features intermediate between DLBCL and BL (DLBCL/BLs) and 3 DLBCLs. We analyzed patterns of genetic aberrations: genomic imbalances (CNV), copy-neutral loss of heterozygosity (CN-LOH) and mutations in TP53, CDKN2A, ID3 (exon 1), TCF3 (exon17) and CCND3 (exon 6).

Young patients displayed more frequent 13q31.3q32.1 amplification, 7q32q36 gain, 5q23.3 CN-LOH, while 17p13 and 18q21.3 CN-LOH were only detected in adult BLs. ID3 mutations were present in all adult samples, but only in 42% of childhood ones. CCND3 and ID3 double-hit mutations, as well as 18q21 CN-LOH, seemed to associate with poorer outcome.

For the first time, we report different genetic anomalies between adult and pediatric BLs suggesting age-related heterogeneity in Burkitt lymphomagenesis. This may explain the poorer prognosis of adult BLs. Additional studies are needed to confirm these results in the setting of clinical trials.

P26: Identification of rare ZNF469 variants in 4 out of 19 Iranian keratoconus families

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Keratoconus (KC) is a noninflammatory corneal ectasia, characterized by the typical conical shape of the cornea due to thinning of this structure. This conical shape leads to a drastic decrease in visual perception and, with a prevalence of approximately 1/2000, affects a large group of patients.
There are multiple indications that genetic factors underlie KC but the identification of disease-causing genes has been scarce. Recently, multiple publications reported that heterozygous ZNF469 variants contribute to the risk of developing KC.

Using whole exome sequencing (WES) in 19 Iranian families, 4 variants in ZNF469 were identified which are highly interesting given the described link between this gene and KC.
The 4 variants in ZNF469 were all validated in the patients that were included in WES and segregated with the disease (under the hypothesis of reduced penetrance in all 4 families, which has been described frequently for KC). In two of these families, two different genetic causes seem to underlie KC and the ZNF469 variant can only explain the phenotype in one branch of the pedigree.

These results provide additional indications for the pathological role of ZNF469 in KC. However, this gene cannot be a full explanation for the inheritance of the disease in these 4 families, thus indicating that the inheritance may be more complex and that additional disease-causing genes remain to be identified. Further research needs to be performed to establish the actual contribution ZNF469 has to the pathogenesis of KC. To clarify the true role ZNF469 plays in KC and to evaluate the role of 46 other genes, we performed NGS targeted resequencing analysis on a large population of 623 patients and an equal number of controls. The analysis of these data is ongoing but will provide much needed insights into the molecular basis of KC. The analysis of this experiment is currently ongoing and the preliminary results will be discussed further on the poster.

P27: Segmental chromosomal imbalances arise at high frequency in human fibroblasts.

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Preservation of chromosome integrity is key for the survival of any organism. To assure segregation of intact chromosomes, cell division is presumed to be under strict control of cell-cycle checkpoints. Estimates of chromosomal mutation rates per generation based on cytogenetic analyses of newborns and products of conception, range between 4.57x10-5 and 3.42x10-4. In contrast, the high incidence of segmental imbalances detected by single-cell genome-wide copy number profiling indicates that the error rate per cell division might be more than an order of magnitude higher. To directly measure the de novo incidence of segmental chromosomal imbalances, we plated a single fibroblast and analyzed the genomes of the two sister cells following a single cell division. Analysis of 89 pairs of sister cells (178 single cells in total) from 5 different cell lines revealed megabase-sized chromosomal imbalances in 21 fibroblasts, 14 of which derived from 7 mitoses with complementary segmental aneuploidies in the two daughter cells. The mutation rate of segmental imbalances is thus at least 7.9%, indicating that compared to the per generation chromosome stability is at least 100 times lower in vitro and likely underestimated in vivo.

P28: Incidental finding of X-linked adrenoleukodystrophy in a male patient and gonosomal mosaicism in his mother

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In a 5-year old boy, demyelination of the splenium of the corpus callosum was observed on brain MRI after prolonged headache complaints after minor head trauma. Elevated plasma VLCFA levels and adrenal insufficiency were consistent with the diagnosis of X-linked adrenoleukodystrophy (X-ALD). Sequencing analysis identified a novel intronic ABCD1 mutation (c.1866-11C>A), creating an novel splice acceptor site. Carrier testing in the mother showed a low level of heterozygocity of the mutation, suggestive of gonosomal mosaicism and was confirmed by pyrosequencing, restriction enzyme assay and subsequently sequencing of the restriction fragments.

This is the second report on gonosomal mosaicism in X-ALD. Although the level of mosaicism is low (estimated 10%), it is not possible to predict the clinical outcome in the mother, as it is currently unknown to what extent X-chromosome inactivation and modifier genes play a role in the development of the AMN-like phenotype in female carriers.

P29: Functional characterization of novel deleterious MFSD8 mutations found by whole exome sequencing in early-onset isolated maculopathy

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Purpose: Non-syndromic autosomal recessive macular dystrophies are associated with mutations in a number of genes, of which ABCA4 is the most frequently mutated one. Sequencing of ABCA4 and whole exome sequencing
(WES) was performed to identify the molecular cause of suspected atypical Stargardt disease in an eight-year-old girl.

Methods: The entire ABCA4 locus was enriched with a custom Haloplex panel followed by next-generation sequencing (NGS) (Miseq, Illumina). WES data was generated and analyzed (SureSelectXT HumanAllExonV5+UTRs, Agilent; NextSeq 500, Illumina; Ingenuity Variant Analysis, Qiagen). Mini-gene assays and cDNA sequencing on patient's lymphocytes were used to study the effect of a donor splice variant in MFSD8. MFSD8 expression was assessed in lymphocytes with qPCR. Transmission electron microscopy (TEM) was performed on a patient's skin biopsy. The patient underwent a neurological examination and brain magnetic resonance imaging (MRI).

Results: Sequencing of the entire ABCA4 locus revealed one heterozygous variant p.(Ala1038Val). WES revealed two novel heterozygous MFSD8 variants: c.590del p.(Gly197Valfs*2) and c.439+3 A>C p.(=), occuring in trans. In vitro mini-gene assays and cDNA sequencing on patient's lymphocytes demonstrated an out-of-frame skip of exon 5 p.(Ile67Glufs*3) resulting from c.439+3A>C. Expression of MFSD8 in patient's lymphocytes was significantly reduced. TEM on a patient's skin biopsy showed lipopigment inclusions with characteristic and mixed lamellar profiles, as can be seen in cases with neuronal ceroid lipofuscinosis (NCL). The clinical neurological examination of the patient was normal, MRI of the brain showed slight cerebellar atrophy and discrete signs of cerebral cortical atrophy.

Conclusions: Two deleterious MFSD8 mutations were identified in a young patient with an isolated maculopathy. Roosing et al. (2015) reported a combination of mild and severe MFSD8 mutations in late-onset non-syndromic maculopathy. The combination of two severe MFSD8 mutations has only been seen in severe variant late-infantile NCL, unlike the presentation here. Through WES and downstream functional characterization we uncovered a potential syndromic maculopathy with poor outcome, despite the current absence of neurological manifestations. This study illustrates the power of WES to refine clinical diagnoses and to anticipate disease progression.

P30: CHEK2 mutations other than c.1100delC are not that rare

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Introduction: As of September 2014 patients suspected of hereditary breast cancer are not only tested for mutations in BRCA1 and BRCA2 but also for the c.1100delC founder mutation in CHEK2. We started testing for this mutation by sequencing exon 11 of CHEK2, since March 2015 we sequence the complete CHEK2 gene as part of our NGS breast cancer panels.

Aim: To investigate in what percentage of families mutations other than c.1100delC in CHEK2 are detected (VOUS, likely pathogenic (LP) or pathogenic (P)), and to assess possible differences between the families with these mutations compared to the c.1100delC families.

Patients and methods: Patients included in diagnostic mutation scanning because of suspected hereditary breast cancer since the introduction of screening for c.1100delC. Method: Sanger sequencing of exon 11 of CHEK2 or NGS.

Results: In 361 cases only exon 11 was sequenced. In 15/361 cases we detected heterozygosity for c.1100delC (4,2%). In two cases two different LP mutations were found in exon 11. Another 522 cases were analyzed by NGS. Heterozygosity for c.1100delC was detected in 30/522 cases (5,7%). In 8/522 cases in total 6 different mutations were detected: 2 mutations were classified as VUS and 4 were clasified as LP/P. Taken together 17% of the (likely) pathogenic mutations in CHEK2 in Dutch patients suspected of HB(O)C is NOT c.1100delC. Index and family characteristics will be presented.

Discussion: Mutations in CHEK2 other than c.1100delC are not as rare as was suggested in some publications. It can be expected that the biological/phenotypical effect of other truncating mutations is comparable to that of c.1100delC.

Conclusion
By limiting the mutation analysis of CHEK2 to c.1100delC, relevant causative mutations in a number of familial breast cancer families will be missed. Pooling of data on penetrance is needed to enable cancer risk estimations

P31: Whole Exome Sequencing (WES) to Analyze the Genetic Basis of Cleft Lip and Palate

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Background: Cleft lip with or without cleft palate (CL/P) is the most common craniofacial birth defect with an incidence of ~ 1/700 live births, varying with ethnicity and cleft type. It is a debilitating condition requiring an expensive and lifelong treatment. CL/P can occur isolated or as part of a syndrome. The etiology is still largely unknown.
Methods: We performed whole exome sequencing (WES) on 85 CL/P subjects. The cohort predominantly consisted of non-syndromic (n=21/25) families. We selected at least two most distant relatives per family for WES (n=36). We also included few sporadic CL/P cases (n=12). The sub-phenotypes of these 73 patients ranged from a full- blown bilateral CL/P to a subtler velopharyngeal insufficiency (VPI). In addition, we WESed 8 unrelated individuals all affected with the rare Cerebro-oculo-nasal syndrome that has cleft lip and high arched palate as part of its spectrum. For two of the eight, parents were also WESed.

Results: After bioinformatic processing of raw data with an in-house developed pipeline, we analyzed our samples on Highlander (a software developed in-house by R. Helaers). We retained variants for further analysis if they met the following criteria: (i) passed the GATK filter, (ii) reported allele frequency of ?1% in the population from the 1000 genomes project, (iii) not reported in the population from the GO-NL (genome Netherlands) project, (iv) ?1% reported in Exome Aggregation Consortium (ExAC), (v) and not occurring in our in-house WES controls (subjects affected with =different condition than CL/P). As "likely pathogenic" variants, we considered those with a high impact (premature termination codon=PTC) or a moderate impact (non synonymous-NS), as predicted by SnpEff software. On average, each family harbored 1 PTC and 30 NS variants. All NS variants were predicted as damaging by at least 3 softwares. Approximately 5 changes co-segregated with the phenotype. This led to identify the causative mutation in 3 cases in known CL/P genes (TP63 ,GRHL3 and TBX1). For the remaining samples characterization of variants of interest is ongoing.
Discussion: To be able to distill out the causal gene from the aforementioned data, we will WES additional affected subjects from multiplex families, for which continued collection is also ongoing.
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P32: Copy number variations in psychiatric patients with intellectual disability and catatonia: an exploratory study

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Introduction: Catatonia is a motor dysregulation syndrome co-occurring with a variety of psychiatric and somatic disorders. The good response to treatment with benzodiazepines and electroconvulsive therapy indicates a neurobiological background. Environmental factors (intoxication, infection) as well as genetic factors play a role in the etiopathogenesis. Research on the genetic etiology is limited. We hypothesize that copy number variations known to be risks factors for neurodevelopmental disorders may play a role in the etiology of catatonia. The aim of this study is to describe the CNVs in a population of psychiatric patients with an intellectual disability and catatonia.
Methods: Fifteen intellectually disabled adults admitted to a psychiatric inpatient unit and diagnosed with catatonia were selected for genetic examination. Medical files were analysed retrospectively to collect data on cognitive functioning and psychiatric diagnosis. A clinical genetic examination was performed. Blood samples were taken for molecular karyotyping (Comparative Genomic Hybridisation).
Results: CNVs, including 5 duplications and 3 deletions, were detected in 8 of the 15 patients (53%). In 2 of these patients a microdeletion including SHANK3 was found. Psychiatric diagnoses in these patients are autism, psychotic and affective disorders. Intellectual disability ranged from borderline to severe disability.
Conclusion: CNVs occured in half of intellectually disabled psychiatric adults with catatonia. These findings suggest that SHANK3del may play a role in the etiology of catatonia in intellectually disabled patients. Cognitive functioning ranged from borderline to severe intellectual disability. Genetic research in children as well as in adults with intellectual disabilities and psychiatric comorbidity is important and meaningful.

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P33: Deletion of TBX1 is not responsible for facial dysmorphology in patients with 22q11.2 deletion syndrome

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22q11.2 deletion syndrome or velocardiofacial syndrome (VCFS, OMIM #192430) is the most common microdeletion syndrome with a prevalence of 1 in 4000 live births. The phenotype associated with the syndrome is very diverse and not linked to the size of the deletion, with a variable combination of over 190 phenotypical characteristics, such as congenital heart defects, immunological deficiencies, cleft palate, hypernasal speech and psychiatric problems. Sometimes, facial dysmorphology, characterized by a long face, prominent forehead, prominence of the supraorbital ridges, prominence of the midface, prominence of the premaxilla, narrow jaw, retrognatia, upward displacement of the eyes, mild hypertelorism, downslanting palpebral fissures with slight narrowing of the eyelids, upward and slight forward displacement of the nose, increase of nasal length, bulbous nasal tip, narrowing of the nasal base, thickness of the vermillion, open mouth posture and a downslant of the mouth, can be seen in patients with VCFS. Haploinsufficiency of the transcription factor TBX1 is thought to be responsible for the majority of the symptoms associated to VCFS (such as craniofacial dysmorphology) because of its presence in various pathways and tissues relevant for the symptomatology, such as the pharyngeal apparatus.

In this study, a family (nine subjects) with autosomal dominant velopharyngeal insufficiency is described. Since velopharyngeal insufficiency is one of the most prevalent symptoms of VCFS, FISH analysis to discover a deletion of the 22q11 region was previously performed and found normal. Other symptoms associated with VCFS were not present. Using micro-array, a small and atypical deletion in the 22q11 region was found in the affected family members, including a partly deletion of TBX1. However, objective 3D analysis revealed that no characteristic facial features associated with VCFS are present in these subjects. Further, a 14 year old male expressing facial features of VCFS was found to have another atypical 22q11 deletion, with TBX1 completely preserved but including a deletion of CRKL.

Based on these observations, we can conclude that the craniofacial phenotype in VCFS is not caused by TBX1 haploinsufficiency, but rather by haploinsufficiency of CRKL.

P34: Association of variants in the VEGFA (Vascular Endothelial Growth Factor) gene with severe retinopathy in pseudoxanthoma elasticum: implications for molecular screening, counseling and management

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AIMS: one of the most incapacitating symptoms of pseudoxanthoma elasticum (PXE) - a connective tissue disease with remarkable variability in clinical severity - is subretinal (choroidal) neovascularization and subsequent blindness, due to increased VEGFA activity. We aim to validate single nucleotide polymorphisms (SNPs) in the VEGFA gene as prognostic biomarkers for the PXE retinopathy which could be used for ocular risk stratification and hence counseling and follow-up of PXE families.

METHODS: the VEGFA coding and non-coding (intronic and promotor) regions were Sanger sequenced in 65 molecularly confirmed PXE patients with a mild, respectively severe retinopathy. The latter was defined as unilateral best corrected visual acuity (BCVA) < 5/10 (with an impact on quality of life) and/or the need for anti-angiogenesis treatment with multiple anti-VEGF injections. Associations of VEGFA SNPs with disease severity and anti-VEGF therapeutic outcome were evaluated.

RESULTS: a significant association of 5 VEGFA SNPs with severe retinopathy - but not with therapeutic outcome - was found, 4 of which had been previously suggested to be involved in the PXE ocular phenotype. Importantly, in several patients severe eye disease was characterized by an increased need for anti-VEGF therapy due to neovascularization, even with (near) normal BCVA. Hence, carriers of these SNPs have a more active eye disease but will not necessarily go blind if followed and treated sufficiently strict.

CONCLUSIONS: the association of 4 VEGFA SNPs with a severe PXE retinopathy could be validated in an independent patient cohort, providing reliable data to stratify PXE patients for the ophthalmological complications and individualize counseling and management in the high-risk group. Prospective evaluation is ongoing to evaluate the outcome of such a personalized regime. Apart from the benefits for patient management, the need for validated prognostic biomarkers for the different phenotypic features of PXE is also essential for the design and interpretation of clinical trials. This is a pressing need, as novel pathophysiological and molecular insights in PXE have recently paved the way for human trials with allele-specific treatment using 4-phenylbutyrate or anti-mineralization therapy using bisphosphonates. Based on our results, genotyping of these VEGFA genetic biomarkers is now done in the design of such trials in PXE patients.

P35: Implementing non-invasive prenatal testing for aneuploidy in a national healthcare system: global challenges and national solutions

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Since the introduction of non-invasive prenatal testing (NIPT) in 2011, mainly by commercial companies, a growing demand for NIPT from the public and healthcare professionals has been putting pressure on healthcare systems of various countries. This study identifies the challenges of establishing a responsible implementation of NIPT for aneuploidy in prenatal healthcare, by looking at the Netherlands. We used a mixed methods approach involving 13 stakeholder interviews, document analysis and (participatory) observations of the Dutch NIPT consortium meetings. The Diffusion of Innovation Theory and a Network of Actors model were used to interpret the findings. Implementation of NIPT was facilitated by several factors. The set-up of a national NIPT consortium enabled discussion and collaboration between stakeholders. Moreover, it led to the plan to offer NIPT through a nationwide research setting (TRIDENT study), which created a learning phase for careful implementation. The Dutch legal context was perceived as a delaying factor, but eventually gave room for the parties involved to organise themselves and their practices. This study shows that implementing advanced technologies with profound effects on prenatal care benefit from a learning phase that allows time to carefully evaluate the technical performance and women's experiences and to enable public debate. Such a coordinated learning phase, involving all stakeholders, will stimulate the process of responsible and sustainable implementation.

P36: Replacing MLPA CNV detection with XHMM on targeted panel NGS data

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Currently MLPA and arrays are routinely used for the clinical diagnosis of large DNA duplications and deletions (copy number variations, CNVs). Recently several tools for the detection of Copy Number Variations (CNVs) on NGS read-depth data have been developed. CNV detection by read-depth methods - such as XHMM (eXome-Hidden Markov Model) - is economically advantageous, as this type of analysis uses the NGS data already available from the SNP/INDEL detection without any additional laboratory costs. In this study we validated the use of XHMM for the detection of CNVs in a NGS based targeted gene panel.

Method
XHMM was optimized on 1210 samples sequenced with the Connective Tissue Disorder (CTD) panel version 1; 1176 exons captured in solution by a custom Mycroarray MYbaits library. The default parameter setting proved a good choice, except for single exon CNVs. A separate optimal parameter setting for detecting single exon CNVs was validated and implemented next to the default run.

The performance of XHMM was tested on 318 samples sequenced with CTD panel version 2; 1977 exons captured in solution by a custom Roche Nimblegen SeqCap EZ Choice Library (IRN4000018830, Nimblegen). In this set were 35 previously MLPA or array confirmed CNVs: 12 duplications, 24 deletions – all heterozygous – and 1 homozygous deletion. Both the duplications and deletions range in size from single exon to over 60 exons.

After exploratory runs with all 191 samples at both parameter settings, the panel was cleaned by excluding 30 samples where XHMM found CNVs and 17 samples with outlier average read-depths. 12 exons in the TNXB gene, a known pseudogenic region, were excluded from further analyses. Next, all excluded samples were each in turn analyzed against the cleaned panel at both parameter settings.

Results and conclusion
XHMM correctly called all 35 known CNVs, including 8 single exon deletions and 4 single exon duplications. XHMM detected an additional 68 CNVs that remain unconfirmed. Roughly half of these CNVs appear false positives, the other half plausible. The unconfirmed CNVs were all filtered out when results were filtered on frequently observed CNVs and requested gene-panel.
CNV detection by XHMM was demonstrated to be at least as sensitive as the current methods. After filtering, the specificity proved sufficient. VUmc clinical genetics has implemented XHMM analysis has replaced MLPA for the clinical diagnosis of exon DNA duplications and deletions in CTD samples.

P37: Influence of Pregnancy on Cardiovascular Disease in Marfan syndrome

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Background: Mouse models for Marfan syndrome (MFS, caused by fibrillin-1 mutations) are instrumental for the study of the pathogenetic mechanism of thoracic aortic aneurysms and dissections (TAAD). Previously, pregnancy in MFS has been regarded as a risk factor for TAAD. The precise effect of pregnancy on the aorta remains elusive.

This study aims to (1) perform in depth cardiovascular phenotyping of Fbn1 GT8/+ male and both nulliparous and multiparous female mice and (2) correlate these findings to the human phenotype.
Methods: Aortic diameters of male GT8/+ mice aged 1-12 months and female GT8/+ mice aged 8 or 12 months that were either nulliparous or used for breeding, were assessed by means of ultrasound. Aortic histology was studied with light microscopy. Findings were compared to those of sex-matched wild-type littermates. In addition, we conducted a retrospective study of aortic dimensions in a cohort of 20 women with MFS. Progression of aortic root dilation during a mean follow-up time of 5,6 years (1,4-9,8) was compared between 10 pregnant women (total of 13 pregnancies) and 10 nulliparous women. Age-matched non-pregnant women were selected from our population of MFS patients.

Results: Male GT8/+ mice showed progressive dilatation of the thoracic aorta at the level of the sinuses of Valsalva, ascending aorta and aortic arch. Aortic histology revealed progressive fragmentation of the elastic lamellae and the presence of major breaks, affecting at least 3 consecutive elastic lamellae, in GT8/+ mice. Female GT8/+ mice that were used for breeding showed a significantly more pronounced aortic phenotype than nulliparous GT8/+ females. In concordance, more severe fragmentation of the aortic elastic lamellae was observed in the bred females compared to the nulliparous females.

Aortic dilatation in humans was restricted to the level of the sinuses of Valsalva. At baseline, aortic root diameters were significantly higher in the non-pregnant group (median 33 mm vs 37,5 mm; p=0,043) possibly reflecting the influence of aortic diameter in the decision to carry a pregnancy. No aortic dissection was observed during pregnancy. The diameter of the aortic root increased significantly in the pregnant group in comparison to the non-pregnant group (mean 1,25 mm/yr vs 0,04 mm/yr, p=0,001). Maximal aortic diameter progression was observed in the pregnant group during the first 2,5 years after delivery and stabilized thereafter.

Conclusions: Analogous to other MFS mouse models and in contrast to humans, GT8/+ mice display more extensive thoracic aortic dilatation. Pregnancy-related changes in female mice influence aortic disease severity. These results are corroborated by the findings in MFS patients.

P38: A novel FLNA mutation in a 5-year old boy with Ehlers-Danlos syndrome, periventricular nodular heterotopia type.

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Ehlers-Danlos syndrome (EDS) comprises a group of hereditary connective tissue disorders characterized by hyperextensible skin, joint hypermobility, and tissue fragility. Periventricular heterotopia (PNH) is a neuronal migration disorder, of which the neuropathological hallmark encompasses the formation of grey matter nodules. Despite the fact that EDS and PNH appear clinically different, mutations in the X-linked FLNA gene, which encodes for the actin binding protein filamin A, have been designated the genetic cause of the so called EDS-PHN syndrome. FLNA mutations are lethal in the majority of the hemizygous males, however rare survivors have been reported. In these, disease was caused by either hypomorphic missense, C-terminal truncating, or mosaic FLNA mutations.

Here, we report the identification of a novel, de novo, FLNA missense mutation (p.Leu80Val) in the N-terminal part of the protein after whole exome sequencing in an Indian EDS patient. The mutation is predicted to alter protein activity, though without complete loss of function. The 5-year-old boy presented with distinct facial features, including a flat face, periorbital fullness, brachycephaly, and low set ears. Systemic signs comprised nodular heterotopia, mitral valve prolapse with regurgitation, skin laxity, joint hypermobility and tall vertebral bodies. The neuromotor and language milestones were normal.

Comparison of the described phenotypic characteristics and corresponding genotype in our patient to the existing literature allows to classify this patient as an Ehlers-Danlos variant of periventricular heterotopia due to FLNA mutation.

P39: Targeted massive parallel sequencing for arrhythmias and cardiomyopathies in a diagnostic setting

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Cardiomyopathies (CMP) and primary arrhythmia syndromes (PAS) are characterized by a significant genetic heterogeneity, rendering classical sequencing expensive and very laborious. As a proof of concept, we evaluated targeted massive parallel sequencing (MPS) as a fast and efficient diagnostic method.

An in-solution NimbleGen SeqCap EZ capture was designed in order to capture the coding region of 75 genes associated with arrhythmias and cardiomyopathies. The probe design covers approximately 850 kb of genomic sequence, corresponding to 94% of the targeted region. Targeted sequencing was performed on the Illumina Hiseq 2500 platform as 150bp paired-end reads. Variant annotation and classification into 5 classes was achieved according to a stringent scoring system, taking into account different in silico analyses, population frequencies, paralogous/orthologous conservation and supportive literature.

From January 2014 till June 2015, 532 patients suspected of CMP or PAS were screened with targeted sequencing. In total, 25% and 18% had a pathogenic mutation in a gene related to CMP or PAS respectively. In addition, 27% and 17% showed variants of unknown significance (VUS) for CMP or PAS. For patients carrying a VUS, segregation analysis will be performed (if possible) in order to elucidate their role.

Our results show that targeted sequencing for CMP and PAS works as an efficient and cost-effective tool for genetic diagnosis of these heterogeneous disorders in a diagnostic setting. The mutations are mostly located in the so-called 'core genes', indicating that the extra yield of additional genes is limited. The lower yield of 'solved' cases in comparison with the literature is mainly due to the fact that genetic testing for CMP or PAS is easy accessible for medical specialists in Belgium. Given the huge amount of information generated by MPS, a rigorous filtering strategy of variants together with multidisciplinary collaboration is crucial to determine the potential pathogenic role of identified variants in the cause of CMP and PAS.

P40: Targeted massive parallel sequencing for Primary Immune Deficiencies in a diagnostic setting

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Primary immune deficiencies (PID) are a group of congenital disorders affecting various components of the immune system. Correct diagnosis remains difficult due to the genetic heterogeneity and the complexity of clinical symptoms. In a minority of cases, there is a clear genotype-phenotype correlation. As a consequence, individual genes can be screened by Sanger sequencing in order to confirm the diagnosis. However, in most cases, no prior selection of the candidate genes can be made. Therefore, massive parallel sequencing (MPS) is the ideal tool to explore PID in a diagnostic setting.

An in-house developed capture based method (NimbleGen SeqCap EZ capture) enables us to identify mutations in 174 known PID related genes, categorized in 16 different subpanels, including e.g. the innate immunity deficiency (IID) panel. Approximately 1385kb or 91% of the targeted region is covered with probes. Sequencing was performed on the Hiseq 2500 rapid mode (Illumina) as 150bp paired-end reads. With an in-house created bio-informatics pipeline using the GATK package base calling, alignment and variant calling was done. Variant annotation and classification was performed within the Cartagenia BENCHlab NGS module (Cartagenia v3.2).

Forty-eight patients with an unknown cause of their PID were subjected to the targeted capture. Here, we describe two cases in which the genetic defect could be pinpointed.

The first patient was phenotypically diagnosed with hyper IgE syndrome (HIES). Unfortunately, this could not be confirmed on a molecular level. She was included in the PID capture in order to identify the underlying cause of the disease. After sequencing and variant filtering, a homozygous stop mutation was identified in IRAK4 (c.877C>T, p.Gln293*). In retrospect, this patient was misdiagnosed, due to an atypical course of disease, which is not uncommon in PID.

Secondly, a child suffered from a severe sepsis following a pneumococcal infection. The innate immunity deficiency (IID) panel was analyzed, but no pathogenic variants were retained. Subsequently, the remaining variants in the full PID panel were explored and revealed a novel missense variant in GATA2 (c.1415C>T, p.Pro472Leu). This variant was considered to be likely pathogenic, based on population frequencies, in silico analysis, conservation and segregation in the family.

In conclusion, our center now offers thorough genetic testing for primary immune deficiencies, through Sanger sequencing of a handful individual genes and MPS of a PID panel containing 174 PID related genes, recently expanded to 218 genes. Nevertheless, the genetic heterogeneity of PID and remarkable variability of expression make it challenging to interpret the large number of identified variants in patients.

P41: Targeted massive parallel sequencing for inherited kidney diseases in a diagnostic setting

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Proteinuric kidney diseases are a heterogeneous group of disorders with variation in clinical features, disease progression and response to therapy among patients. Many kidney diseases have been revealed as monogenic disorders and 'single gene' diagnostic tests have become available. However, due to the genetic heterogeneity and remarkable variability of expression in inherited kidney diseases, massive parallel sequencing (MPS) was implemented in order to identify the causal mutation.

An in-house developed capture based method (NimbleGen SeqCap EZ capture) was designed and enables us to identify mutations in 64 known kidney related genes. Sequencing was performed on the Hiseq 2500 rapid mode (Illumina) as 150bp paired-end reads. An in-house pipeline was used for base-calling, alignment and variant calling. Variant annotation and classification was performed within the Cartagenia BENCHlab NGS module (Cartagenia v.3.2).

MPS was completed for 48 patients in 30 families with a chronic proteinuric kidney disease of unknown origin. Here, we describe two familial case studies from our cohort.

The first patient is an eight-year-old boy with typical features of X-linked recessive Dent disease. His mother presented with proteinuria during pregnancy. Analysis of the genes related to Dent disease (OCRL and CLCN5) revealed no causal point mutation. Consequently, the raw sequencing data for these patients were scrutinized. This revealed a partial deletion in CLCN5 in the index patient which perfectly correlates with the phenotype. His mother also carried this deletion.

In a second family, two siblings, 62 and 68 years old, presented with focal segmental glomerulosclerosis (FSGS). A specific gene panel was analyzed and showed that both patients were heterozygous for a c.652C>T (p.Arg218Trp) mutation in INF2, which is associated with FSGS and Charcot-Marie-Tooth disease. This mutation has been described in literature and is pathogenic. Moreover, segregation analysis of affected and non-affected family members showed that the c.652C>T (p.Arg218Trp) mutation in INF2 segregated perfectly in this family.

In conclusion, targeted massive parallel sequencing is a powerful tool to diagnose familial kidney diseases in children and adults. Our results demonstrate the utility and success of using MPS in a diagnostic setting for the identification of causal mutations in families with a kidney disease.

P42: FOXP1-related intellectual disability syndrome: a recognizable entity

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Aim: Intellectual disability (ID) represents a major challenge in clinical genetics. Associated characteristics such as typical facial morphology or neurological features such as specific language impairment (SLI) might provide clues to the underlying genetic defect. Array-CGH and next generation sequencing (NGS) made reverse genetics a mainstream approach in clinical genetics, identifying recurrent phenotypes in patients with similar molecular defects. Forkhead box protein P1 (FOXP1) is a transcriptional regulator associated with ID and SLI, with or without autistic features (MIM: 613670). Fourteen patients with FOXP1 related ID mutation have been described, though so far, no specific phenotype emerged. We hereby report fourteen novel patients with a FOXP1 defect and delineate this rare condition.

Methods: We described fourteen novel patients with a FOXP1 defect detected through array-CGH analysis, direct Sanger sequencing and/or NGS and compare the clinical and molecular data with the fourteen previously reported patients with FOXP1 defects.

Results: Phenotypic analysis of all 22 FOXP1 patients showed a combination of neuromotor developmental delay, ID, SLI and typical facial features including a high and broad forehead, a frontal hair upsweep, bent and downslanting palpebral fissures, ptosis, and a large mouth. Additional features are variable and include relative macrocephaly, strabismus, progressive spasticity, behavioural abnormalities including autism and aggression, and urogenital malformations.

Conclusion: Our data show that the FOXP1-related ID syndrome is a recognizable entity that is more frequent than expected based on the rare case reports. The delineation of this condition will enable clinicians to make a gestalt diagnosis and will be helpful to evaluate genotype-phenotype correlations when interpreting large NGS data obtained in patients with ID.

P43: Chromosome Fragility at FRAXA in Human Cleavage Stage Embryos at Risk for Fragile X Syndrome.

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Fragile X mental retardation syndrome (FXS), the most common inherited mental retardation syndrome, is caused by expansion and hypermethylation of the CGG repeat in the 5' UTR of the FMR1 gene. This expanded repeat, also known as the rare fragile site FRAXA, causes X chromosome fragility in cultured cells from patients but only when induced by perturbing pyrimidine synthesis. We performed preimplantation genetic diagnosis (PGD) on 595 blastomeres biopsied from 442 cleavage stage embryos at risk for FXS using short tandem repeat (STR) markers. In six blastomeres from five embryos an incomplete haplotype was observed with loss of all alleles telomeric to the CGG repeat. In all five embryos the incomplete haplotype corresponds with the haplotype carrying the CGG repeat expansion. Subsequent analysis of additional blastomeres from three embryos by array comparative genomic hybridization (aCGH) confirmed the presence of a terminal deletion with a breakpoint close to the CGG repeat in two blastomeres from one embryo. A blastomere from another embryo showed the complementary duplication. We conclude that a CGG repeat expansion at FRAXA causes X chromosome fragility in early human IVF embryos.

P44: REVEAL: De-novo assembly based construction and analysis of a human population reference graph

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The increase in read length of PacBio sequencing offers the prospect of routine de-novo assembly of human genomes in the near future. We developed a method called REVEAL which is aimed at transforming multiple (human) genomes into single reference graph representations. Analysis of the resulting graphs provides us with an overview of the entire spectrum of genetic variations, from heterozygous single nucleotide polymorphisms to kilobases of inserted, deleted or inverted sequence.

Here we discuss the variations that REVEAL detects when multiple independent assemblies of a PacBio sequenced human hydatidiform mole (CHM1) are aligned to recent reference genomes (hg38/hg19). We compare these results to previously published results based on a method that mapped the underlying PacBio reads to the reference genome without de-novo assembling them.

We find large concordance between both sets of variant calls, confirming the applicability of our approach. We furthermore find that, depending on the version of the reference genome, we detect an enrichment of inversions on the X chromosome (which is confirmed in all independent de-novo assemblies of the same dataset).

These results hint on an enrichment of common inversions specific to the sex chromosomes. We explain this observation from a biological perspective, confirming the role of inverted repeat structures (LCRs, segmental duplications) and LINE elements in the formation of structural variations like inversions.

P45: BIOMEDICAL TEXT MINING FOR DISEASE-GENE DISCOVERY: SOMETIMES LESS IS MORE

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Biomedical text is increasingly being made available online in either abstract or full article formats. This goes in parallel with the knowledge desire to extract information from such text (e.g. finding links between diseases and genes). Consequently text mining is very popular in the biomedical domain given that it provides the possibility to automatically analyze these texts in order to extract knowledge. One of the big challenges in text mining is recognizing named entities (e.g. disease and gene entities) inside a given text, which is widely known as Named Entity Recognition (NER). We studied two biomedical taggers that apply different NER methods on MEDLINE abstracts. Here, we compare the contribution of each of the two taggers in associating genes with diseases. We show that with fewer recognized entities we gain more knowledge and we better associate genes with diseases.

P46: A genome-wide association study for tinnitus

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Tinnitus, the perception of an auditory phantom sound in the form of ringing, buzzing, roaring or hissing in the absence of an external sound source, is perceived by approximately 15% of the population of which 2.5% experiences a severely bothersome tinnitus. The cause of tinnitus may be otologic (hearing loss, noise trauma, Ménière's disease, acoustic neurinoma, ototoxic medications or substances), neurologic (multiple sclerosis, head injury), metabolic (thyroid disorder, hyperlipidemia, vitamin B12 deficiency) or psychogenic (depression, anxiety, fibromyalgia). However, despite thorough clinical examination, tinnitus etiology often remains unclear.

We performed a genome-wide association study on 916 independent individuals, of whom 18% reported episodes of tinnitus lasting for longer than 5 minutes. After imputation and quality checking, we tested the association between the tinnitus phenotype and 4,000,000 SNPs using PLINK (v1.07) assuming an additive model.

None of the SNPs reached the threshold for genome-wide significance ($p<5E-8$). The most significant SNPs, which are situated outside coding genes, reach a p-value of 3.4E-7. Using the Genetic Analysis of Complex Traits (GACT) software, we estimated the percentage of the variance explained by all SNPs in the GWAS. This showed a very low heritability of tinnitus (3.2%).

P47: Diagnosis of sickle cell disease by innovative PCR without DNA extraction.

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Background: Sickle cell disease is an important inherited blood disorder. Universal screening and early intervention have significantly helped to reduce childhood mortality in industrialized countries. However in low-resource settings children are often not diagnosed until late childhood, when clinical symptoms are presenting. Current diagnostic techniques are highly accurate and mostly based on isoelectric focusing, HPLC or mass spectrometry, which require advanced laboratory equipment. A simple and rapid molecular diagnostic test could be implemented in small laboratories and developing countries where such advanced equipments are not available.

Methods: The Human Hemoglobin S/C kit uses loop-mediated isothermal amplification (LAMP) with melting curve analysis for rapid and accurate detection of hemoglobin S and C. The test is performed directly on fresh or frozen blood samples, or on dried blood spots. The kit contains a ready to use mastermix, to which samples are added after lysis. The complete protocol has only two manipulation steps and results are obtained within 45 minutes.
In this study 50 fresh blood samples and 100 dried blood cards from routine diagnostics have been tested with the proposed LAMP method. Molecular results were compared with the corresponding phenotypic assays performed in the genetics lab at the university hospital of Liège. Capillary electrophoresis was used as reference method for whole blood samples while tandem mass spectrometry was preferred for dried blood cards.

Results: Genotypes HbSS, HbAS, HbAC, HbAA and HbSC are correctly identified with the Human Hemoglobin S/C kit. Other hemoglobin variants like Hb D or Hb E and ?-globin production defects (?-thalassemia) could not be identified with this technique, as expected. The assay performs well on both fresh blood samples and dried blood spots, with limited input volumes.

Conclusion: Based on the study results this technique is robust and accurate and can be used for sickle cell disease screening from blood and dried blood spots, with minimal hands-on time and minimal laboratory equipment. Further validation of the Human Hemoglobin S/C kit is needed to confirm these results.

P48: Golgi maintenance and function: lessons from one congenital disorders of glycosylation, MAN1B1-CDG

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Background:
Protein glycosylation is one the most important post-translational modifications. Congenital Disorders of Glycosylation (CDG) refer to a rapidly growing and heterogeneous group of rare inherited errors of metabolism, due to deficient protein glycosylation. CDG patients present with an extremely variable and complex phenotype, including severe developmental delay and a multiorgan involvement, often accompanied by neurological symptoms and dysmorphic features.

Rationale and results:
Our group recently identified MAN1B1-deficiency as a frequent type of CDG, associated with impaired Golgi glycosylation, intellectual disability and obesity [1]. MAN1B1 has long been considered as an endoplasmic reticulum (ER)-localized mannosidase, key actor in the ER-associated protein degradation (ERAD) pathway. However, our findings together with the ones of collaborators suggest that MAN1B1 is rather localized in the Golgi apparatus, and may have a gatekeeping role in retrieving escaped misfolded glycoproteins back to the ER for degradation [1,2].
It is well known that the Golgi apparatus has a central role in the trafficking and processing of membrane and secretory proteins. Still, how its various functions are integrated to ensure appropriate membrane assembly and distribution of cargo effectors is poorly understood. While perturbations in ER homeostasis are known to create a condition termed ER stress and leading to the activation of a complex signalling cascade, the mechanisms regulating Golgi capacity still remain unclear. Very limited evidence indeed exists so far to argue for the presence of a Golgi stress response. Nevertheless, we hypothesize that similarly to the mechanism of ER stress, MAN1B1-deficiency would lead to an accumulation of escaped misfolded proteins in the Golgi, overwhelming its capacity.

We show that Golgi trafficking and morphology are impaired in MAN1B1-depleted cells. In addition, we monitored an altered transcription of Golgi-related genes including structural proteins and regulators described for their role in regulating Golgi secretory capacity. Our data suggest as well that Golgi stress could be mediated by altered phosphatidylinositol (PI) signalling, since we observed altered levels of the PI(4)P effector protein GOLPH3 and of specific PI(4) kinases. Interestingly, PI(4)P have been described to be required to coordinate Golgi functions [3].

Conclusion:
We propose that MAN1B1-defciiency is associated with an altered transcription of a set of specific genes, likely triggered by stimuli compromising Golgi capacity and involving PI(4)P signalling. Ongoing RNA sequencing experiments should allow us to identify the transcription factor(s) involved in regulating Golgi capacity. Moreover, we believe that beyond the pathophysiology of MAN1B1-deficiency, we have the unique opportunity to investigate the

molecular mechanisms by which Golgi homeostasis is maintained, and identify how altered homeostasis can impact on Golgi function and lead to disease.

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P49: Exome Sequencing and Linkage Analysis as Tools in solving Syndromic Cardiopathies in Small Families

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Introduction: Congenital heart defects (CHD) are a major cause of infant morbidity and mortality. Reaching an etiological diagnosis in patients with a syndromic cardiopathy is important, not only to gain insight into their pathogenesis and genetic counseling on recurrence risks, but especially with regard to providing information on the future prospective, based on knowledge of the natural course of the disorder. In syndromic cases, an exact etiological diagnosis can be reached in an estimated 50-60%, following careful clinical evaluation, complemented by various genetic tests, including array-CGH.
We present three small families with syndromic cardiopathies diagnosed in two children of each family. The parents are non-consanguineous and have a normal phenotype. In one family with three children one sibling is unaffected. An autosomal recessive hypothesis is most likely, as these are very rare and unique phenotypes.
Methods: Analysis using linkage analysis and exome sequencing was performed. Genomewide parametric linkage analysis was performed, SNP typing platform was used in a recessive model. Genotyping was done in parents and both the unaffected and affected siblings. Data analysis was done using commercial and in-house developed software. Only variants in genes from the linkage regions were retained. All homozygous calls were excluded in the parents and the unaffected sibling, reference calls were excluded in the affected siblings. Only exonic and splicing variants were included, synonymous variants were excluded. Variants occurring with a frequency of <1% in the 1000 genomes project or with an unknown frequency were included.
Results: After variant filtering, candidate genes are identified in the linkage regions with homozygous mutations in the patients, inherited from both parents, and for which the unaffected sibling is heterozygous or reference. Results of this analysis is currently still ongoing and will be presented.
Conclusions: Reaching a genetic diagnosis in rare disorders and small families remains a challenge. Identification of the underlying mutation is sometimes possible, using a combination of sophisticated genetic tools.

P50: Clinical exome sequencing for improved diagnostics and treatment of patients visiting a multidisciplinary nephrogenetic outpatient clinic

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Single gene disorders are estimated to account for ~30% of children and ~10% of adult patients attending renal outpatient services. In the Radboudumc diagnostic exome sequencing for a broad spectrum of isolated- and syndromic renal (ciliary) disorders has been developed. The approach consists of a two-tier analysis in which the first step is to screen for pathogenic variants in genes that are known to be mutated in renal diseases (187 genes) or (renal) ciliopathies (125 genes). If causative mutations are not identified in the first step, the complete exome data set can be analyzed with informed consent. The first results with the renal disease gene panel in 120 unrelated patients with undiagnosed renal disease led to pathogenic mutations in 21 cases (17%) and in 22 other cases (18%) likely pathogenic variants needed follow-up studies. In addition, copy number variation analysis of exome data revealed a pathogenic deletion three cases, confirming the clinical diagnosis. We conclude that the combination of the multidisciplinary outpatient clinic with diagnostic exome sequencing provides a powerful tool for detecting causative mutations in up to 46 out of 120 patients (38%) with a renal disease.

P51: Zebrafish as a model for skeletal disease: insights into zebrafish type I collagen expression and composition

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Osteogenesis imperfecta (OI) is a rare congenital disorder, which is characterized by bone fragility and fractures. Genetic defects in OI are mainly related to abnormal synthesis of type I (pro)collagen, a protein that, in mammals, mostly exists as a heterotrimer composed of two (pro)?1 and one (pro)?2 chain, encoded by the COL1A1 and COL1A2 genes, respectively.
To better understand the pathogenesis of OI, animal models are indispensable. Murine models have already delivered valuable insights but their use is often limited by low viability of bone mutants at adult stage. Zebrafish overcomes this limitation and has hence imposed itself over the last few years as a powerful model to study skeletal diseases. Zebrafish mutants that model human OI have already been generated. However, in zebrafish not two but three type I collagen genes exist, namely col1a1a, col1a1b and col1a2 coding for the ?1, ?3 and ?2 chains respectively. The exact composition of type I (pro)collagen and the spatiotemporal expression pattern of the encoding genes has not yet been determined in zebrafish tissues. Because this knowledge is essential for proper interpretation of molecular analyses in zebrafish models for OI, we have performed an extensive molecular and biochemical analysis of zebrafish collagen type I.
The three collagen type I genes showed a similar spatiotemporal expression pattern starting from the oocyte stage until the adult age, demonstrated by RT-qPCR and whole mount in situ hybridization (WISH). The presence of all three ?(I) chains at protein level was demonstrated in embryos as well as in adult skin, scales and bone using SDS-PAGE and mass-spectrometry. A developmental or tissue specific collagen type I composition was found in embryos, whereas in adult tissues no significant differences were evident in collagen type I electrophoretic migration, amino acid composition and thermal stability. In conclusion, our data will be useful to properly interpret results and insights gained from zebrafish models for OI and other diseases affecting collagen type I.

P52: Zebrafish modeling of the ?4GalT7-deficient type of Ehlers-Danlos syndrome

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Proteoglycans are found on the surface of animal cells and in the extracellular matrix (ECM). They are involved in many vital functions including cell-cell and cell-matrix interactions, cell proliferation and migration, cytokine and growth factor signaling, and embryonic and postnatal development and are implicated in several pathological processes such as viral infection. Proteoglycans consist of glycosaminoglycan (GAG) side chains attached to a core protein through a linkerregion. Biallelic mutations in B4GALT7, the gene encoding galactosyltransferase I (?4GalT7) which is an essential enzyme for the biosynthesis of the tetrasaccharide linker region, are the cause of the rare progeroid variant of the Ehlers-Danlos syndrome (EDS). This disorder is characterized by short stature, hypotonia, a progeroid facial appearance and skeletal abnormalities, in addition to the typical features of EDS such as joint hypermobility and skin hyperextensibility. Our current knowledge about this potentially severe and disabling disease is very limited, in part due to the lack of a relevant in vivo model.

In this study we developed and characterized a knockdown (KD) zebrafish model for the progeroid type of EDS by using morpholino injections targeted against b4galt7. Morphant embryos showed morphological abnormalities such as a small, round head, withdrawn jaw, more front-facing eyes and mild developmental delay compared to wild-type and negative control embyos. The impact of ?4GalT7-deficiency on proteoglycan synthesis was quantitatively and qualitatively assessed. As expected, the total amount of sulfated GAGs was severely reduced in these morphant embryos. Whole-mount immunohistochemistry showed that heparan sulfate proteoglycans seem to be randomly distributed in the heads of b4galt7 KD embryos while chondroitin sulfate proteoglycans were barely present. In addition, b4galt7 knockdown affects normal cartilage and muscle development. Alcian blue staining showed that cartilage structures in the head of b4galt7 KD embryos are absent or severely misshapen. Both structural and functional muscle abnormalities appeared in the knockdown embryos. Immunohistochemical staining for actin demonstrated a disturbed filamentous actin pattern in the head and in the tail of the b4galt7 KD embryos.

In conclusion, a b4galt7 knockdown zebrafish model has been developed which seems to be specific as it mimics partly the human phenotype of patients suffering from progeroid EDS. Therefore, this model could make it possible to investigate the pathogenesis of the progeroid type of EDS in vivo. Additional experiments on this model are planned and in the near future a b4galt7 knockout zebrafish model will be generated using the CRISPR/Cas technology.

P53: GEVACT : GENOMIC VARIANT CLASSIFIER TOOL

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INTRODUCTION
With the emergence of new screening techniques, targeted, whole exome and genome screening are becoming standard diagnostic norms in clinical settings to identify the variants causative of a genetic disease. However, development of bioinformatics solutions for pathogenic classification of the variants still remains a big challenge and henceforth, making the process ponderous for geneticists and clinicians. In this work, we describe GEVACT (Genomic Variant Classifier Tool), a tool for classification of genomic single nucleotide and short insertion/deletion variants. The aim of this study was to design and implement a variant classification algorithm, based on a literature review of cardiac arrhythmia syndromes and existing knowledge of clinical geneticists.

METHODS
The algorithm we propose for GEVACT is based on a published variant classification schema for cardiac arrhythmia syndromes (Hofman et al., 2013). It proposes two varying approaches: one to classify missense variants and another to classify nonsense and frameshift variants. The algorithm is implemented in two phases: pre-processing and classification.

In the pre-processing phase, an annotated tab-delimited variant file (.vcf.ann) retrieved from Alamut-batch (Interactive Biosoftware) can be refined based on the gene list for the disease-of-interest, so as to reduce the number of variants for the analysis. Filters are applied to look for variants that have already been reported in the Human Genome Mutation Database (Stenson et al., 2003) and in ClinVar (Landrum et al., 2014), or that have previously been detected and classified in an internal patient population. And lastly, the variants are filtered based on their location in the genome and their coding effect, followed by the check for minor allele frequency of the variant in a control population (Sherry ST et al. 2001).

Thereafter, in the classification phase, the filtered variants are classified as missense or nonsense/frameshift variants. For missense variants the classification is based on the parameters: amino acid substitution and its impact on protein function (Adzhubei et al., 2010; Kumar et al., 2009), biochemical variation (Mathe et al., 2006), conservation (Pollard et al., 2010), frequency of variant alleles in a control population (ESP6500), effects on splicing (Desmet et al., 2009), family and phenotype information and functional analysis. Whereas, for the nonsense and frameshift variants, it is based on: effects on splicing, frequency of variant alleles in a control population, family and phenotype information and functional analysis. For each parameter, a score is given to the variant, which is subsequently cumulated. Conclusively, based on the cumulative score each variant is classified into one of the five categories: Class I - Non-Pathogenic; Class II - VUS1 (unlikely pathogenic); Class III - VUS2 (unclear); Class IV - VUS3 (likely pathogenic); Class V - Pathogenic (Sharon et al., 2008).

In this study, we report a Java based tool called GEVACT, developed for classification of genomic variants. Input for the tool is an annotated vcf file, while the output depicts the cumulative classification score along with the class label for a variant. The tool was tested on a dataset of 130 cardiac arrhythmia syndrome patients, available at UZ Brussel. The results of the variant classification made by the tool were validated by manual curation, performed by the clinical geneticist. Definitively, the study indicates the tool to be promising but needs to be further validated on datasets from other diseases. In addition to, we are working on the tool to be adaptable for file inputs from other annotation software.

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P54: The first Belgian SCN5A founder mutation in a cohort of Brugada syndrome patients

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The Brugada syndrome (BrS) is a heritable primary electrical disorder of the heart characterized by ventricular arrhythmias and typical ECG alterations. It accounts for up to 20% of sudden cardiac death cases in young individuals (<45 years) without apparent structural cardiac abnormalities. BrS is genetically heterogeneous and so far roughly 20 genes have been associated with the disorder, but at present a causal mutation is identified in only 20-30% of the patients. The majority of these mutations (75%) is detected in the SCN5A gene encoding a cardiac sodium channel. Loss of function mutations will lead to a decreased inward sodium current and disturbed cardiac action potential.

Here, we present 31 BrS patients of 18 different Belgian families carrying the identical c.4813+3_4813+6dupGGGT mutation in intron 27 of SCN5A. This mutation has previously been described in two families of Western European descent and functionally characterized (Hong et al., 2005; Rossenbacker et al., 2005). It results in the use of a cryptic splice site in exon 27, leading to a 32 amino acid deletion at protein level and complete loss of function of the sodium channel. In the 18 Belgian families we identified a shared haplotype around the mutation, consisting of seven genetic markers spanning a region of 5.35 Mb. Based on the haplotype size, we can estimate that the founder mutation arose 20 generations ago, so the common ancestor lived approximately 400 years ago. As expected with a founder mutation, the 18 families originate from one geographical region in Belgium, namely Campine (de Kempen). The clinical spectrum of mutation carrying individuals ranges from asymptomatic to abrupt syncopes, and a significant number of sudden cardiac death has been observed.

In conclusion, we have identified the first Belgian BrS founder mutation. These families will be instrumental for the future identification of BrS phenotype modifying genes.

P55: Mutations in the ABCC6 gene are associated with an increased risk for ischaemic stroke

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Background. Evidence is emerging that ischemic stroke (IS) results from a complex interplay between environmental and genetic risk factors. One method to identify genetic factors, is a candidate gene approach. Because of the increased incidence of IS in pseudoxanthoma elasticum (PXE) – an autosomal recessive connective tissue disease with skin, eye and cardiovascular (CV) symptoms due to mutations in the ABCC6 gene – and the higher CV risk in carriers of one ABCC6 mutation, it was hypothesized that ABCC6 is a candidate risk factor for IS. To date, studies have evaluated the association of one frequent p.R1141X mutation in ABCC6 with IS, but could not confirm this hypothesis.

Methodology. We studied segregation of an ABCC6 mutation in a large family with an apparent autosomal dominant pattern of cerebrovascular disease. Further, we investigated the frequency of ABCC6 mutations through direct sequencing of the whole coding region and exon-intron boundaries of ABCC6 in 424 consecutive cryptogenic IS patients and 250 healthy controls. Allelic frequency differences were analyzed using a two-tailed Fisher's Exact test. Logistic regression analysis assessed modification of mutation-stroke interaction by CV risk factors.

Results. In a large three-generation family, we established segregation of a known ABCC6 missense mutation (p.Arg1314Gln) in 18 family members who suffered repetitive ischemic stroke and/or cardiovascular disease at young age. During this study, two additional family members were identified as having PXE, due to co-inheritance of a second ABCC6 mutation from the family in laws.
In an independent cohort of 424 IS patients, we identified 18 carriers of one ABCC6 mutation compared to 2 carriers in controls. None showed typical clinical features of PXE. Carriers were heterogeneous in age, familial history and stroke type. When only considering the p.R1141X mutation, found in 6 patients, the calculated Odds Ratio was 1.898 (p=0.434; 95% CI 0.38-9.47). However, for all 18 mutations the Odds Ratio was 5.4975 (p =0.023; 95% CI 1.2-23.8). No interaction with other CV risk factors was noted.

Conclusion. The perfect segregation of an ABCC6 mutation in affected members of a multi-generation family with cerebro- and cardiovascular disease suggests heterozygous ABCC6 mutations to be a significant risk factor for IS. This was further confirmed by a relatively high incidence of heterozygous ABCC6 mutations in an independent cohort of patients with cryptogenic IS compared to controls. Molecular analysis of ABCC6 in IS should not be limited to the mutation hotspots, but involve the whole coding region. As also demonstrated by the diagnosis of two novel PXE patients in our first family, identification of ABCC6 mutation carriers can have important implications for genetic counseling and follow-up of these families.

P56: NGS-Logistics: federated analysis of NGS sequence variants across multiple locations

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As many personal genomes are being sequenced, collaborative analysis of those genomes has become essential. However, analysis of personal genomic data raises important privacy and confidentiality issues. We propose a methodology for federated analysis of sequence variants from personal genomes. Specific base-pair positions and/or regions are queried for samples to which the user has access but also for the whole population. The statistics results do not breach data confidentiality but allow further exploration of the data; researchers can negotiate access to relevant samples through pseudonymous identifiers. This approach minimizes the impact on data confidentiality while enabling powerful data analysis by gaining access to important rare samples. Our methodology is implemented in an open source tool called NGS-Logistics, freely available at https://ngsl.esat.kuleuven.be

P57: A targeted resequencing approach for diagnostics of Congenital Disorders of Glycosylation

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Background: Congenital disorders of glycosylation (CDG) are a rapidly growing group of metabolic diseases, comprising almost 100 distinct disorders in protein and lipid glycosylation. Diagnosis of CDG is challenging because of its clinical and genetic heterogeneity. Furthermore, there is no major contribution of one mutation in a single gene, but rather of point mutations distributed all over the affected genes (coding, splicing, intronic...).

Patients and Methods: We designed a capture assay for a panel of 79 genes associated with CDG type I, CDG type II, congenital muscular dystrophy-dystroglycanopathy and other genes associated to disorders whose phenotype resembles CDG. A total of 95 DNA samples (including 88 CDG cases) were captured and Pair-End sequenced on either a Miseq or Hiseq 2500 (Illumina).

Results: A diagnosis was confirmed in 44 of 88 CDG patients. Almost all coding bases of the genes included in our assay could be reliably genotyped. Overall, the mean coverage in the target region was 1,315 (± 533) and a genotype was called for more than 97% of the targeted bases and more than 99% of all coding bases plus 20 flanking bases of the transcripts used for analysis.

Discussion and Conclusion: We identified pathogenic mutations in 50% of our cohort. Interestingly, eight patients presented mutations in ALG1, a gene that could not be assayed by genomic Sanger sequencing due to the abundance of pseudogenes. In addition, ten patients were picked up with secondary glycosylation defects due to mutations in GALT, GALE and ALDOB.

P58: Studying the functionality of the homologous repair pathway in zebrafish embryos: heading for an in vivo functional test to evaluate the pathogenicity of BRCA2 variants identified in breast/ovarian cancer patients.

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Aims:
Since the introduction of next generation sequencing, the challenge for genetic testing moved from development of mutation detection methodologies towards adequate interpretation of (rare) variants. Here, we propose a novel in vivo approach to study the functionality of BRCA2 missense variants in zebrafish. We aim to develop an in vivo functional assay to measure in zebrafish embryos the capacity of homologous recombination for human BRCA2 mRNA containing variants of unknown clinical significance (VUS).

Methods:
To evaluate the efficiency of HR repair we induce DNA double strand breaks (DSB) in zebrafish embryos by irradiation. We use yH2AX and RAD51 foci assays as markers for DSB and HR repair respectively. We generated zebrafish brca2 knockdown models by morpholino injection and Crispr-Cas9 mutagenesis. After synthesis of human BRCA2 mRNA, rescue experiments will be performed with wild type mRNA and mRNA containing the VUS of interest.

Results:
We developed a protocol for visualising and quantifying RAD51 foci in tissues of wild type zebrafish embryos. Knockdown of brca2 by a morpholino results in an almost complete absence of RAD51 foci in irradiated embryos. Similar results are currently being generated in the Crispr-Cas9 brca2 knockout model. In a next step we will rescue the phenotype by microinjection of wild type human BRCA2 mRNA and mRNA containing VUS to study the effect of these VUS on the HR capacity.

Conclusion:
The zebrafish genome contains nearly all the genes involved in different DNA repair pathways in eukaryotes, including, homologous recombination (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.

P59: Identification of Congenital Diaphragmatic Hernia genes by Whole Exome Sequencing

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Introduction:Congenital Diaphragmatic Hernia (CDH) is thought to have a strong genetic component. However, CDH genetics is still poorly understood since only about 20% of CDH patients have a well characterized genetic cause. In roughly 70% of CDH patients, the etiology remains unknown. We hypothesize that Whole Exome Sequencing(WES) application will help us to advance our understanding of genes involved in CDH pathogenesis.
Results: We performed WES on 13 patients and on the parents of 6 of them. Dominant and recessive models were taken into account for the variants calling in all the patients. We identified pathogenic variants in known CDH causal genes: ZFPM2(FOG2), KMT2D (MLL2) and PORCN. The former gene was mutated in two different families and was transmitted from an unaffected mother in both cases. The latter genes 'mutations were respectively observed in a fetus displaying Kabuki syndrome clinical features and in 2 male siblings having Goltz-Gorlin Syndrome. In addition, we observed probably pathogenic variants in 3 genes thus far not yet known to cause CDH, including PIGN in a fetus with complex bilateral CDH.
Discussion: While ZFPM2 is known as a major CDH gene responsible for about 5% of CDH cases, KMT2D and PORCN mutations are uncommonly associated with this disorder. Similarly to the previous reports, ZFPM2 mutation is also observed with a reduced penetrance and a variable expressivity in our families. ZFPM2 gene is a modulator of GATA4 activity and are both extremely important for diaphragm and heart development. PORCN gene lesions cause of X-linked Focal Dermal Hypoplasia that was previously considered to be embryonic lethal in male. Interestingly, we are the first to report PORCN variant in non-mosaic males. Defective PORCN, leads to an impairment of WNT trafficking through the cell which affects the downstream genes dependent on this pathway. PIGN is involved in Glycosylphosphatidylinositol anchor biosynthesis. Prior to our report, PIGN mutations were described in association with multiple malformations but seldom with CDH which we suggest to be due to a more severe loss of function than in the other reported mutations. As for the new candidate genes, they are known to cause X-linked Intellectual disability and the confirmation of their implication in diaphragm development is ongoing.

P60: Long term follow-up on a boy with a severe form of spondylometaphyseal dysplasia with cone-rod dystrophy caused by the recurrent p.Ala99Val mutation on the paternal allele and a gene deletion on the maternal allele

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Spondylometaphyseal dysplasia with cone-rod dystrophy (SMD-CRD, MIM 608940) is a very rare autosomal recessive disorder characterized by normal cognition, severe short stature, progressive bowing of the lower limbs, platyspondyly, metaphyseal irregularities and cone–rod dystrophy causing progressive visual impairment. Here, we report on a long term follow-up of an 11-year-old boy who presented at birth with short limbs but normal length (48 cm at 38 weeks gestation). Postnatal radiographs revealed spondylometaphyseal abnormalities and follow-up showed a severe postnatal growth failure. Around the age of 9 months a horizontal nystagmus was noticed by the parents and the presence of a cone-rod dystrophy was found at the age of 2 years, which ultimately lead to the diagnosis of SMD-CRD. Last evaluation at the age of 11 years 4 months revealed a severe disproportionate short stature with a height of 86 cm (- 10SD). The boy is of normal intelligence and follows school for visually impaired children. He is functionally blind. He has short limbs with broadening of the wrists, brachydactyly and genua vara. He is severely restricted in his mobility. The parents are healthy and non-consanguineous. The mother is of Egyptian origin and the father of Moroccan descent.

Exome sequencing of genomic DNA obtained from peripheral blood of the affected boy and his parents was performed using the KAPA library preparation kit (KAPABioscience) and the Nimblegen SeqCap Exome EZ kit (Roche) on a HiSeq1500 (Illumina). Data were analyzed using an in-house developed Galaxy based pipeline and variant filtering was performed using the in-house developed program Variant db. Copy number variation (CNV) analysis on genomic DNA of the patient was performed using HumanCyto12v2.1 chip (Illumina) on the Illumina iScan Reader. Raw data were analyzed using the GenomeStudio from Illumina and further analysis was performed using the CNV webstore.

Analysis of the exome sequencing data, demonstrated the presence of the p.Ala99Val mutation in the PCYT1A gene, encoding for the enzyme choline-phosphate cytidylyltransferase ? (CCT?). In 2014, two independent research groups reported that bi-allelic mutations in PCYT1A (including the recurrent p.Ala99Val mutation) can cause SMD-CRD. Although from the exome data the mutation seemed homozygous in the patient, segregation analysis demonstrated that only the father was carrying the p.Ala99Val mutation. No variants could be identified in the mother, despite the fact that the complete PCYT1A gene was covered by the exome sequencing. Consequently, we performed whole genome SNP-array analysis with specific attention for the PCYT1A gene. This analysis demonstrated that the proband only had one copy of the PCYT1A gene, suggesting that he inherited a

deletion of the PCYT1A gene from his mother. Our data confirm that bi-allelic mutations in PCYT1A can cause SMD-CRD. The finding of a gene deletion reinforces the hypothesis that SMD-CRD alleles have a hypo- or amorphic effect on the biosynthesis or function of the choline-phosphate cytidylyltransferase ? (CCT?) enzyme.

P61: 'de novo' 2q23.3q24.2 deletion in a self-healing collodion baby: co existing conditions ?

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We report the natural history of a 18-month-old boy, second child from non consanguineous parents. The pregnancy as delivery were unremarkable. Birthweight was 2430g (P3-10), height 47 cm (P10) and OFC 31.5 cm (P3). He was transferred to neonatalogy for 'collodion baby'. Severe neonatal erythrodermia was noted and sequencing of genes ALOX12B, ALOXE3 and TGM1 did not identify any mutation. At the age of 18-month-old, he presented global development delay with axial and peripheral hypotonia. Failure to thrive (requiring nasogastric feeding) and postnatal growth retardation were noted. Facial features include a prominent and high forehead and mild hypertelorism. First tier chromosome molecular analysis using CytoScan 750k-array (Affymetrix) revealed a de novo ~9.4Mb interstitial deletion at 2q23.3-q24.2 covering 32 coding genes. To date, ~20 patients with de novo 2q23.3-q24.2 deletion were reported in the literature and in the DECIPHER database. The size and the breakpoints of the deletions reported are highly variable. Although the patients exhibit a heterogeneous phenotype, some features appear to be recurrent: generalized hypotonia, intellectual disability, low-birth weight, bilateral hip dislocation, 'mild facial dysmorphism'. In a previous study, a recurrent deleted overlapping region (SOR) in 2q24.2 encompassing 8 genes was suggested to be responsible for the phenotype. Among these, 4 genes (LY75, PLA2R1, ITG6 and RBMS1) are deleted in our patient, underlying possibly the suggested genotype-phenotype correlation. Additionally, to our knowledge erythrodermia or cutaneous involvement has never been associated with a 2q23.3-q24.2 deletion. At this stage, ichthyosis in our patient may either be a distinctive unrelated condition or is linked to a mutation in the remaining allele of a recessive disease-causing gene.

P62: WNT16 requires G?12, G?13 and G?q subunits as intracellular partners for both its canonical and non-canonical WNT signaling activity in osteoblasts

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Since the time that genetic variation in WNT16 was first associated with bone mineral density and fracture risk by genome-wide and candidate gene association studies, many functional research has been performed to further verify and confirm its role in the pathogenesis of osteoporosis and bone homeostasis in general. Nevertheless, little is known on the precise actions of WNT16 on the WNT signaling pathway in osteoblasts. The aim of this study was thus to further elucidate its effects on both canonical (WNT/?-catenin) and non-canonical (WNT/Calcium; WNT/PCP) WNT signaling pathways in Saos-2 cells, an osteoblast-like cell line.

Hereto, we first performed TCF/LEF-, NFAT- and AP-1-responsive luciferase reporter assays in Saos-2 cells. These reporter experiments demonstrated that Wnt16 activates WNT/Calcium and WNT/PCP signaling in Saos-2 cells. We also confirmed the previously reported activation of WNT/?-catenin signaling by Wnt16 in Saos-2 cells, but additionally demonstrated that this activation occurs in a LRP5/6 independent manner and with a stimulatory effect of sclerostin and Dickkopf1. To further look into these effects of Wnt16 on WNT signaling in osteoblasts, we investigated whether specific G? subunits are recruited towards the Frizzled receptor after Wnt16 binding. Therefore, G?12, G?13 and G?q were selected for knockdown experiments in Saos-2 cells by using gene-specific siRNA duplexes against the GNA12, GNA13 and GNAQ genes, respectively. Repetition of all WNT luciferase reporter assays with knockdown of G?q alone significantly decreased the activity of non-canonical WNT/Calcium signaling. Moreover, combined knockdown of G?12 and G?13 lowered activation of all WNT signaling pathways below baseline levels with great significance. Combined knockdown of G?12, G?13 and G?q resulted in even lower levels of activation for all WNT pathways. Since, after knockdown, canonical WNT signaling activity by Wnt16 also came down to baseline levels, recruitment of specific G? subunits to the Frizzled receptor is a plausible explanation for its non-classic mechanism of activation.

Altogether, these findings demonstrate that Wnt16 activates both canonical and non-canonical WNT signaling in osteoblasts. Moreover, we identified a prominent and orchestrating role of G?12, G?13 and G?q in the upstream and downstream effects of Wnt16 in osteoblasts. In addition to the current knowledge on WNT16, these data can be valuable in the research to new treatment strategies to reduce the risk for osteoporotic fractures in the future, highlighting not only WNT16 but also its intracellular partners, the G? subunits, as potential points of action.

P63: Genomic study of multiple myeloma reveals a potential clinical interest of X chromosome alteration

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-Background
Multiple myeloma (MM) is the third most frequent haematological cancer. This disorder is characterized by an uncontrolled proliferation of mature plasma cells within the bone marrow. Genomic studies allowed the separation of MM into two major groups. Patients of the first one show a hyperdiploid profile (up to 75 chromosomes) which is associated to a good prognosis. Patients of the second group show a normoploid with specific chromosomal rearrangements (t(4;14)(p16;q32), del(13), del(17)(p13), del(1)(p31;p32) and dup(1)(q)) rather associated to a bad prognosis. Nevertheless these prognosis markers still have limited reliability and lots of patients do not belong to one of the currently well-known prognostic groups.

-Material & Methods
In this study we used DNA-microarray technologies on a cohort of 150 MM patients to detect copy number variation (CNV) and loss of heterozygosity (LOH). We tried to detect new genomic markers which could be associated to clinical data leading to a new sub-classification of MM.

-Results
DNA Microarrays have been used to detect common chromosomal aberrations such as deletions, duplications and loss of heterozygosity. After CD-138 (plasma cells marker) antibody selection on bone marrow samples, 150 patients were screened using Genome-wide Human SNP 6.0 arrays (Affymetrix) or SurePrint G3 Human CGH 60K Microarray (Agilent Technologies) and analyzed using Genotyping Console software (Affymetrix) and CytoGenomics software (Agilent Technologies).
Data analysis leads to the detection of the two well-known prognosis groups (normoploid and hyperploid). Moreover, several patients shared alterations coming from both groups and alterations with unknown clinical impact. A partial duplication of the long arm of X chromosome, associated with a loss of heterozygosity in female patients, was observed in 30% of the patients. This duplication of the active X led to high expression of many X-linked genes and was associated with a decreased overall survival for patients with hyperdiploid profile.

-Conclusions
Even if genomic X alterations have been described in many cancers, nothing is known about their implication in MM. In our study, we highlighted the high frequency of these alterations and hypothesized a possible interest of their detection for evaluating MM prognosis.

P64: Genotype-phenotype correlation of the mutations in the LRP4 gene causing Cenani-Lenz syndrome and sclerosteosis- investigating the disease mechanisms.

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The LRP4 protein serves as a co-receptor for the Wnt-signaling pathway, one of the key regulators of bone formation. Not surprisingly, mutations in the gene encoding this protein lead to diseases with strong skeletal involvement, such as sclerosteosis and Cenani-Lenz syndrome.

In this study, we investigate the mechanisms by which mutations in the gene lead to the observed phenotypes. The recently reported R1170Q mutation found in a sclerosteosis patient, and localized in the central cavity of the third ?-propeller domain of the protein is tested against previously reported mutations found in the patients diagnosed with the same condition. We show the impairment of the inhibitory action of sclerostin, the pathway antagonist, in the presence of the mutations. Moreover, mutations underlying Cenani-Lenz syndrome are shown to affect the membrane trafficking of the receptor, decreasing the availability of the functional protein, and preventing it from facilitating its function. In addition, we investigate the circulating sclerostin levels in the serum of a patient suffering from sclerosteosis due to an LRP4 mutation. In this case, we show how impaired sclerostin binding potential of the receptor leads to the drastically elevated levels of circulating sclerostin. These data are in line with recent observations coming from murine models of LRP4 deficiency and provide first evidence of this mechanism in humans, questioning the utility of determining the circulating sclerostin levels as an efficient predictor of other bone parameters.

Although more studies are required to fully understand the mechanism by which LRP4 facilitates the inhibitory action of sclerostin, we believe that our data provide valuable insights into the biology of the receptor and highlight it as a potent drug target in future treatments of osteoporosis.

P65: Prenatal genetic diagnostics in Noonan syndrome

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Noonan syndrome (NS, OMIM163950) is a common, clinically and genetically heterogeneous disorder, transmitted as an autosomal dominant trait. The principal features include dysmorphic facial features, short stature and congenital heart defects. NS shows clinical overlap with LEOPARD syndrome (OMIM 151100), Cardio-Facio-Cutaneous syndrome (CFC, OMIM 115150) and Costello syndrome (OMIM 218040). Together they are considered as a family of disorders, the RASopathy, caused by congenital mutations in the RAS/MAPK (mitogen-activated protein kinase) pathway. Recent studies suggest prenatal testing for Noonan syndrome in fetuses with ultrasound anomalies like an increased nuchal translucency and a normal karyotype.

In a time span of 6 years (2010-2015), we sequenced DNA of 42 fetuses with an increased nuchal translucency and a normal array CGH result. At first, the hot-spot regions of the PTPN11 gene (exons 2, 3, 4, 7, 8, 12, and 13 NM_002834.3) were analysed by PCR en sequencing.Subsequently, if possible, the RASopathy panel was analysed by PCR and sequencing. The RASopathy panel consists of : SOS1 gene (NM_005633.3) all exons; RAF1 gene (NM_002880.3) exons 7, 14 and 17; RIT1 gene (NM_006912.5) all exons; KRAS gene (NM_004985.3) all exons; BRAF gene (NM_004333.4) exons 4, 6, 11-16; MEK1 / MAP2K1 gene (NM_002755.3) exons 2 and 3; MEK2 / MAP2K2 gene (NM_030662.3) exons 2, 3 and 7; HRAS gene (NM_005343.2) exon 2. The number of genes tested in each fetus depends on the amount of DNA that was available.

A diagnosis was confirmed in 8 fetuses (6 fetuses presented mutations in the PTPN11 gene and 2 fetuses in the RAF1 gene). We identified pathogenic mutations in 19% of our cohort. It is significantly lower than the expected diagnostic rate described in literature but our current inclusion criteria are only based on an increased nuchal translucency. We propose to implement guidelines for prenatal genetic screening and to test fetuses with at least one of the criteria: fetus with an increased nuchal translucency and polyhydramnios or hydrops foetalis or jugular lymphatic sacs or hydrothorax or ascites or cardiac anomalies or renal anomalies; fetus with a cardiomyopathie and polyhydramnios; or fetus with a polyhydramnios, fetal overgrowth, relative macrocephaly +/- cardiopathy/arrhythmia. Furthermore, the implementation of a new NGS sequencing method will enable us to screen the complete RASopathy panel for each prenatal request.

P66: MPL S204P Is a Recurrent Mutation in Essential Thrombocythemia

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Introduction: The JAK2 V617F, MPL W515K/L and CALR indels occur in a mutually exclusive pattern in 80-90% of cases with Essential Thrombocythemia (ET), but the driver mutations are unknown in the remaining 10-20%. In this study we aimed to identify driver mutations in the latter group of triple negative (TN) ET by exome sequencing of 10 such cases.

Results: We found 27 somatic variants, including indels, in 6 out of 10 TN ET patients (range: 1-10 mutations/case; mean: 2,7 mutations/case), none of which were recurrent. In one case, we found a MPL c.610T>C (p.S204P) mutation, which is located in the extracellular domain of the MPL receptor. By Sanger sequencing of MPL exon 4 in 20 additional TN ET cases, an additional patient with the MPL S204P mutation was identified.

In order to study the effect of this mutation on the function of MPL, we produced stable Ba/F3 cell lines expressing MPL S204P, MPL W515K or MPL WT, and assessed the dependence of their growth on exogenous thrombopoietin (TPO). The three transduced cell lines grew in the presence of TPO, indicating the proper surface expression and the functionality of the transduced receptors. Only MPL W515K transduced Ba/F3 cells proliferated in the absence of TPO. The levels of phospho-JAK2 and phospho-STAT5 were low in cytokine-deprived MPL S204P cells but increased upon TPO stimulation. In contrast, phospho-JAK2 and phospho-STAT5 were detectable in MPL W515K transduced Ba/F3 in the absence of cytokines as assessed by Western blotting. Culture of MPL S204P transduced Ba/F3 in the presence of TPO over a range of concentrations (0,01-10 ng/ml) yielded growth curves comparable with MPL WT transduced Ba/F3.

Using flow cytometry, we also explored cell surface marker expression on peripheral blood platelets from the two MPL S204P ET patients. Data were compared with healthy donors or ET patients with JAK2 or CALR mutations. MPL S204P ET platelets displayed higher expression of CD61 than platelets from healthy donors or from JAK2 or CALR mutated ET ($p<0,01$). In addition, there was a trend for higher expression of CD36 and CD42b on platelets from the MPL S204P ET cases. Moreover, following platelet activation through the protease activated receptor1, the degranulation response of platelets from MPL S204P ET was decreased in comparison with JAK2 or CALR mutated ET.

Conclusion: The MPL S204P mutation is a recurrent mutation in TN ET, with a frequency of 7% (2/30) in this series, but this mutation does not induce TPO-independent growth nor increased TPO-sensitivity in Ba/F3 cells. However, preliminary phenotypic and functional

evidence supports the notion that MPL S204P platelets display specific characteristics as compared with JAK2 or CALR mutated ET. The mechanisms by which the MPL S204P mutation influences megakaryopoiesis and platelet function remain to be elucidated.

P67: Maternal and fetal micro-rearrangements identified by Non Invasive Prenatal Testing (NIPT).

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Non Invasive Prenatal Testing (NIPT) is a new option in testing for genetic abnormalities during pregnancy. Currently-used fetal diagnostic tests such as amniocentesis or chorionic villus sampling are invasive procedures involving a slight risk of miscarriage. By contrast, NIPT uses a simple blood test to collect cell-free fetal DNA (cfDNA) circulating in a mother's bloodstream, and represents minimal risk to mother and child. Recent clinical trials suggest that tests may be up to 99% accurate in detecting more common chromosomal abnormalities, such as trisomy 21 (Down syndrome), 13 and 18. . Method for non-invasive fetal aneuploidy detection involves counting chromosomes by mapping sequences generated via Massive Parallel Sequencing of cfDNA in maternal plasma. The number of reads produced per patient for this analysis is usually considered as low coverage sequencing : 7 to 25 millions reads depending on the sequencing platform used. This number of reads is sufficient to perform a reliable analysis of aneuploidy for chromosomes 13, 18 and 21. These chromosomes are usually the only ones to be validated for testing in routine analysis but other chromosomal aneuploidies have been identified.

We analyzed during a period of 17 months a cohort of 2196 maternal blood samples to exclude trisomies 13, 18 and 21. We reported 34 T21, 4 T13 and 3 T18. We also observed aneuploidies of chromosomes 2, 4, 7, 9, 16 and 20, each once. Reporting these very rare aneuploidies could be of interest. Even if they are confined to the placenta and if the fetus is euploid, it can in some instances lead to intrauterine growth retardation or Uniparental Disomy (UPD) by trisomy rescue in the fetus : chromosome 6 paternal UPD (neonatal diabetes), chromosome 7 maternal UPD (Silver-Russell syndrome), chromosome 11 paternal UPD (Beckwith-Wiedemann syndrome), chromosome 14 maternal or paternal UPD (MCA/MR), chromosome 15 maternal UPD (Prader-Willi syndrome) or paternal UPD (Angelman syndrome).

During the years 2013-2014, we collected maternal blood samples for NIPT for most of the women asking for an invasive prenatal testing. Therefore we had cell-free DNA from pregnancies of trisomic fetuses for validation of our screening method, but also of fetuses with subchromosomal rearrangements. Sequence coverage in NIPT is usually high enough to perform analysis for copy number variants. We have used the Wisecondor package developped by the university of Nijmegen (Straver et al., 2013 ; NAR) in order to look in maternal plasma for the micro-deletions or micro-duplications observed by CGH-array using DNA extracted from amniotic fluid or chorionic villus sampling. We have tested by NIPT 8 cfDNAs collected from fetuses with CNVs ranging from 30 Mb to 2 Mb. CNVs above 10 Mb were all detected. CNVs below 10 Mb can be suspected from the Wisecondor profile but not with high reliability. We also identified CNVs which turn out to be of maternal origin. Some illustrations of these CNVs discovered by NIPT analysis will be presented.

P68: NEFL mutations are associated with a novel dominant-intermediate Charcot-Marie-Tooth disease phentoype

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In this study, we describe two pedigrees with mutations in the gene encoding the neurofilament light chain (NEFL) that are associated with a heterogeneous dominant-intermediate Charcot-Marie-Tooth disease (DI-CMT) phenotype. In the first family, a c.1186G>A NEFL mutation (p.E396K) is co-segregating with disease in four patients over two generations. Patients are aged between 35 and 59 years, and were serially evaluated since 1993. Their clinical picture is characterized by pes cavus, sensorimotor neuropathy and spastic gait. Electrophysiology shows uniform nerve conduction slowing in the intermediate range. Multimodal evoked potential and blink reflex studies reveal abnormalities indicative of central sensorimotor pathway dysfunction. Imaging studies of lower-limb musculature demonstrate massive atrophy of intrinsic foot muscles and, to a lesser degree, of calves and thighs, predominating in muscles innervated by tibial and sciatic nerves. In both patients exhibiting waddling gait, there is atrophy of pelvic muscles. The second pedigree comprises two patients, the proband and her son, aged 38 and 5 years. In both affected individuals, a c.392A>G NEFL mutation (p.N98S) is co-segregating with disease. The proband showed delayed motor milestones that, as of the second decade, evolved into severe phenotype consisting of sensorimotor neuropathy, pes cavus, clawing hands, gait and kinetic cerebellar ataxia, nystagmus and dysarthria. She is currently wheelchair-bound. Her son shows a mild phenotype characterized by delayed motor milestones, and lower-limb hypotonia and areflexia. Electrophysiology in both patients demonstrates nerve conduction slowing to the intermediate range. However, where compound muscle action potentials are severely attenuated, it slows down to the demyelinating range. In the proband, cranial MRI shows cerebellar atrophy, electromyography discloses active denervation in tibialis anterior. and MRI of lower-limb musculature demonstrates widespread and distally accentuated muscle fatty atrophy. Furthermore, water sensitive MRI sequences illustrate edema of calf muscles. We conclude that NEFL mutations can cause a DI-CMT phenotype characterized by simultaneous involvement of the peripheral and central nervous system. Moreover, NEFL can be associated with an early-onset, severely disabling sensorimotor neuropathy that may evolve into a complex clinical picture including cerebellar ataxia.

P69: A terminal 5 Mb triplication of chromosome 1p in a patient with severe intellectual disability, absent speech and facial dysmorphisms

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Whole genome cytogenetic screening has proven to be instrumental in retrieving the genetic cause of intellectual disability with or without congenital malformations in a significant portion of patients. Routine diagnostics for this type of patients is nowadays performed using SNP array, allowing detection of copy-number imbalances down to approximately 100 kb.
A 39 year old patient with a severe intellectual disability, congenital ptosis, strabismus, nystagmus, exophthalmos, spastic tetraparesis, pectus carinatum and scoliosis was referred for cytogenetic investigation. SNP array analysis using an Infinium CytoSNP-850K BeadChip array (Illumina, inc., San Diego, U.S.A.), showed a terminal amplification of 5,66 Mb (4 copies) of chromosome 1p36.31p36.33, proximally directly flanked by a small gain of 90 kb (3 copies) of 1p36.31. Standard karyotyping was not performed.
To confirm the presence of 4 copies of the distal 1p36 locus and to investigate the location and orientation of the amplified fragments, metaphase FISH was performed using proximally and distally situated BAC clones in the amplified region. The results showed a local triplication on one of the chromosomes 1, with three copies in a row with the middle copy inverted, relative to the other two copies. The orientation of the triplicated fragments and the presence of a flanking duplication match previously published cases with terminal triplications. Microhomology at the junctions was proven in some of these cases, and the proposed mechanism is that inverted repeats can interfere with the replication process leading to rearrangements, in which also microhomology and nonhomologous events play a role (1,2). In the literature, only one patient was published with a terminal triplication of 1p of similar size (3). Overlapping phenotypic characteristics were intellectual disability, absent speech, strabismus, hypertelorism, ptosis and facial characteristics. By presenting a patient with a rare terminal triplication/duplication and comparing with previously published patients, here we add evidence that a specific mechanism is responsible for this event.

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2. Yatsenko SA et al., Hum Genet. 131(12):1895-910 (2012)
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P70: Developmental course of language skills in primary school-aged children with 22q11.2 deletion syndrome

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Background: The last decade there is increasing interest and evidence for syndrome-specific phenotypic transitions in cognitive-behavioral development of children with 22q11.2 deletion syndrome (Swillen & McDonald-McGinn, 2015). In children with 22q11.2 deletion syndrome (22q11.2DS) a decline of verbal intelligence was noticed in one third of the group from nine years old onward (Duijff et al., 2013). Because of the high risk and increased incidence of psychiatric disorders in this syndrome, follow-up of communicative competences seems very valuable.

Method: Structural language skills of 18 monolingual Dutch-speaking children with 22q11.2DS (age 6-13 years old) were re-evaluated after a period of 18 to 24 months and compared to the developmental course of these skills in children with idiopathic intellectual disability. Standardized language assessments were conducted and performance was reported taking into account both chronological and mental age norms.

Results: In children with 22q11.2DS limited progress and 'growing into deficit' trajectories were demonstrated, especially for language comprehension measures. Following complex directions and comprehension of abstract words remained challenging. These difficulties were more common in children with 22q11.2DS than in children with idiopathic intellectual disability.

Conclusion: Dynamic and evolving language profiles should be re-evaluated in order to adapt intervention and remediation guidelines to changing individual needs and environmental demands. Future research should focus on changes in social language use.

P71: Loss-of-function mutations in SCN4A cause severe foetal hypokinesia or "classical" congenital myopathy.

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Congenital myopathies are a clinically and genetically heterogeneous group of muscle disorders characterised by congenital or early-onset hypotonia and muscle weakness, and specific pathological features on muscle biopsy. The phenotype ranges from foetal akinesia resulting in in utero or neonatal mortality, to milder disorders that are not life-limiting. Over the past decade, more than 20 new congenital myopathy genes have been identified. Most encode proteins involved in muscle contraction, however mutations in ion channel-encoding genes are increasingly being recognised as a cause of this group of disorders.

SCN4A encodes the ?-subunit of the skeletal muscle voltage-gated sodium channel (NaV1.4). This channel is essential for the generation and propagation of the muscle action potential crucial to muscle contraction. Dominant SCN4A gain-of-function mutations are a well-established cause of myotonia and periodic paralysis. Using whole exome sequencing, we identified homozygous or compound heterozygous SCN4A mutations in a cohort of 11 individuals from six unrelated kindreds with congenital myopathy. Affected members developed in utero- or neonatal-onset muscle weakness of variable severity. In seven cases, severe muscle weakness resulted in death during the third trimester or shortly after birth. The remaining four cases had marked congenital or neonatal-onset hypotonia and weakness associated with mild to moderate facial and neck weakness, significant neonatal-onset respiratory and swallowing difficulties and childhood-onset spinal deformities. All four surviving cohort members experienced clinical improvement in the first decade of life. Muscle biopsies showed myopathic features including fibre size variability, presence of fibrofatty tissue of varying severity, without specific structural abnormalities. Electrophysiology suggested a myopathic process, without myotonia.

In vitro functional assessment in HEK293 cells of the impact of the identified SCN4A mutations showed loss-of-function of the mutant NaV1.4 channels. All, apart from one, of the mutations either caused fully non-functional channels, or resulted in a reduced channel activity. Each of the affected cases carried at least one full loss-of-function mutation. In five out of six families, a second loss-of-function mutation was present on the trans allele. These functional results provide convincing evidence for the pathogenicity of the identified mutations and suggest that different degree of loss-of-function in mutant NaV1.4 channels are associated with attenuation of the skeletal muscle action potential amplitude to a level insufficient to support normal muscle function. The results demonstrate that recessive loss-of-function SCN4A mutations should be considered in patients with a congenital myopathy.

P72: Discovery of novel candidate genes for normal-pressure glaucoma by whole exome sequencing in a large multiplex family

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Normal-pressure glaucoma (NPG) is characterized by a normal intraocular pressure of the eye and glaucomatous optic nerve damage with corresponding progressive visual field defects. The aim of this study was to identify the causative gene in a large (Dutch) family with NPG by applying whole exome sequencing (WES).

WES was performed on the DNA of four affected family members and shared rare variants were selected. Polymerase chain reaction and Sanger sequencing were used to analyze variants for cosegregation with the disease in additional family members. WES analysis identified two segregating heterozygous variants in the TP53BP2 (c.109G>A; p.Val37Met) and MAPKAPK2 (c.305G>A; p.Arg102His) genes. In silico analysis classified both substitutions as pathogenic. Both variants were absent in public databases and in 180 population-matched controls.

Interestingly, both genes are involved in apoptosis, suggesting a digenic inheritance in this family. Alternatively, the TP53BP2 variant by itself may be pathogenic, since it has been demonstrated that the gene regulates apoptosis in retinal ganglion cells. Additional screening of both genes in patients with NPG from different populations is required to confirm their involvement in the disease.

P73: Delineating the phenotypical spectrum of RARS2 mutations: a report of two cases and a review of the literature

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Pontocerebellar Hypoplasia type 6 (PCH6) is caused by mutations in RARS2, the gene encoding mitochondrial arginyl tRNA synthetase. Due to the clinical variability, making the correct diagnosis might be challenging. 'Missing' the diagnosis negatively affects the patients and their families because they might not get the appropriate medical care or genetic counseling.

The purpose of this study was to answer the following questions. (1) What is the 'typical' clinical profile of PCH6 patients with mutations in the RARS2 gene? (2) What other, 'atypical' phenotypes are described in the literature?

By reviewing original papers we collected clinical data of 20 patients. We described two additional patients with previously unreported RARS2 mutations form our own center.
The phenotype of the patients from our center was characterized by epilepsy, feeding problems and profound progressive cerebral atrophy with relative sparing of the cerebellar structures and pons on brain MRI.

The results of our study suggest that the 'typical' clinical profile of patients with mutations in the RARS2 gene consists of: epileptic seizures (19 of 22 patients, 86%), lactate acidosis in blood, cerebrospinal fluid (CSF) or urine (14 of 17 patients, 82%), respiratory chain defects (8 of 13 patients, 62%) and cerebellar hemisphere and/or vermis atrophy/hypoplasia (19 of 19 patients, 100%).

'Atypical' phenotypes include absence of cerebellar hypoplasia/atrophy at an early stage, cerebellar atrophy less significant compared to supratentorial features and sparing of the ventral pons. Lactate acidosis in blood and CSF and RC defects can be absent. Rarely, other organs like lungs, heart or liver are involved. These are important pitfalls which might lead to an incorrect rejection of PCH6 as possible diagnosis.
This review describes the clinical features of PCH6 and adds two peculiar cases to the literature, thereby further broadening the phenotype.
We propose that consideration should be given to testing for RARS2 mutations in children presenting with epileptic seizures, lactate acidosis and/or respiratory chain defects and brain atrophy, even in the absence of early cerebellar involvement.

P74: ALK-positive Anaplastic Large Cell Lymphoma with the Variant EEF1G-, RNF213- and ATIC-ALK Fusions is Featured by Copy Number Gain of the Rearranged ALK Gene

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Background: Molecular pathogenesis of ALK-positive Anaplastic Large Cell Lymphoma (ALK+ ALCL) is not completely understood. Approximately 80% of ALK+ ALCL cases harbor the t(2;5)(p23;q35)-associated NPM1-ALK rearrangement, while several variant 2p23/ALK translocations involving at least nine partner genes have been identified in the remaining cases. The 5'ALK partners play a key role in the constitutive activation of the chimeric protein by mediating its oligomerization and its subcellular localization. Their role in ALK lymphomagenesis is debated, but experimental studies showed that the partners impact a range of biological activities of ALK chimeras, including proliferation, transformation and metastatic capacities. Comparative analysis of biological properties of ALK oncoproteins, however, is hampered by the relative low frequency of different variant ALK fusions.

Study: To gain more insight into the molecular pathogenesis of ALK+ ALCL, we have characterized ALK fusions in five cases recently diagnosed in our institution. All tumors showed a cytoplasmic expression of ALK indicating the presence of a variant t(2p23/ALK). Intriguingly, FISH analysis of these cases disclosed copy number gain (2-7) of the rearranged ALK gene.

Methods: FISH, high resolution array CGH (Affymetrix CytoScan HD), 5'RACE PCR, low coverage full genome sequencing (LCFGS), Sanger sequencing, QRT-PCR, immunohistochemistry (IHC), and in vitro transformation assay on lL-3 dependent BaF3 cells.

Results: Using 5'RACE PCR, low coverage full genome sequencing and FISH, all five ALK rearrangements were characterized. The identified partner genes included EEF1G, a novel ALK partner located at 11q12.3 (one case), and the already known partners, RNF213/ALO17 (17q25) (one case) and ATIC (2q35) (3 cases). Notably, all five cases displayed a similar LSI ALK break-apart FISH pattern indicative of a reciprocal t(2p23/ALK) associated with copy number gain of the rearranged ALK gene. FISH findings were confirmed by array CGH in two available cases. To assess whether the need of increased copy of ALK hybrid gene is caused by the weaker promoter of EEF1G, RFN213 and ATIC, compared to the NPM1 promoter, we determined at first the relative mRNA expression level of the four ALK partner genes. The study revealed a significantly lower expression of EEF1G, RNF213 and ATIC in nonmalignant lymph nodes when compared to NPM1. In the next step, we compared oncogenic potential of all four fusions in the murine hematopoietic IL-3 dependent Ba/F3 cell line. Following IL3-depletion, all four fusions were able to transform the Ba/F3 cells, however, NPM1-ALK

expressing cells were the first to recover after removing IL3, reflecting its strong oncogenic potential

Conclusions: ALCL driven by EEF1G-ALK, RNF213-ALK and ATIC-ALK is characterized by a copy number gain of the ALK hybrid gene. Occurrence of ? 2 copies of the rearranged ALK in diagnostic samples contrasts with the constant presence of one copy of NPM1-ALK in t(2;5)-positive ALCL. Given that transcriptional potential of EEF1G, RNF213 and ATIC is lower than NPM1, and oncogenic properties of EEF1G-ALK, RNF213-ALK and ATIC-ALK are lower than NPM1-ALK, these lymphomas seem to compensate an insufficient expression of variant ALK fusions by increased dosage of the hybrid gene.

P75: Novel MALAT1-GLI1 Oncogenic Fusion in Plexiform Angiomyxoid Myofibroblastic Tumors of the Stomach

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Plexiform angiomyxoid myofibroblastic tumors of the stomach are extremely rare and probably of benign nature. Diagnosis is mainly based on histopathological features. Characteristically, these tumors have a multinodular/plexiform growth pattern, and contain variable cellular areas of bland myofibroblast type spindle cells embedded in an abundant myxoid matrix that is rich in small vessels. As yet, nothing is known about the molecular and/or genetic features of these recently described tumors. Here, we describe as basic (cyto)genetic finding a recurrent translocation t(11;12)(q11;q13) involving the MALAT1 (metastasis associated lung adenocarcinoma transcript 1) long noncoding gene and the GLI1 (glioma-associated oncogene homologue 1) gene in a subgroup of these tumors. The presence of the fusion transcript in our index case was validated using polymerase chain reaction on genomic DNA followed by Sanger sequencing. We showed that the truncated GLI1 protein is overexpressed and retains its capacity to transcriptionally activate its target genes. A specific FISH assay was developed to detect the novel MALAT1-GLI1 translocation in formalin-fixed paraffin-embedded material. This resulted in the identification of 2 additional cases with this fusion and one additional case with polysomy of (part of) chromosome 12 containing the GLI1 gene. Finally, immunohistochemistry revealed that GLI1 protein is overexpressed in those cases where we detected the novel MALAT1-GLI1 fusion. In conclusion, we detected a recurrent MALAT1-GLI1 fusion resulting in the overexpression of GLI1 protein.

P76: Expanding the phenotype of OPHN1 mutations : two unrelated families with intellectual disability and absence of cerebellar hypoplasia

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The oligophrenin I gene (OPHN1, MIM #300127) is located on Xq12 and encodes a Rho-GTPase-activating protein involved in the regulation of the G-protein cycle. Rho protein members play an important role in dendritic growth and in plasticity of excitatory synapses. Mutations in OPHN1 have been identified in patients with X-linked intellectual disability (XLID) associated with cerebellar hypoplasia and ventriculomegaly, suggesting a recognizable syndromic intellectual disability. Patients often share other clinical findings such as seizures, strabismus, ataxic gait, behavioral difficulties and slight facial dysmorphism with a long face, deep set eyes with pronounced infraorbital creases, short philtrum and prominent chin.

We report on two unrelated families affected by mild to severe intellectual disability due to OPHN1 mutations where brain MRI did not reveal any cerebellar anomaly. We describe clinical, genetic and neuroimaging data of affected patients, we discuss the intrafamilial clinical variability and we compare our patients with those previously reported. We emphasize the power of next generation techniques (X-exome sequencing and/or target multi gene panel) to expand the phenotypic and mutational spectrum of XLID caused by OPHN1.

P77: Towards a Belgian reference set

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Introduction
Next-Generation Sequencing (NGS) is increasingly used to study and diagnose human disorders. The simultaneous sequencing of a large number of genes leading to the detection of a large number of variants, the bottleneck has moved from sequencing to variant interpretation and classification. Publically available databases of variant frequencies provided by, among others, the Exome Sequencing Project (ESP) the 1000 genomes project (McVean et al., 2012) or dbSNP (Sherry et al., 2001) help distinguishing causative mutations from common variants, identifying up to 78% of variants as common for a Belgian exome. However, these data sets often lack population specific variant frequencies and are outperformed by databases of local variants. For example, using GoNL (The Genome of the Netherlands Consortium, 2014) alone allowed the identification of up to 85% of variants as common for the same Belgian exome. The fact that the GoNL is based on only 498 individuals further highlights the importance of building and using population specific databases.
Such population specific data can be retrieved from locally sequenced individuals that underwent Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS). Storing only the frequencies and genotype counts of the variants provides a valuable tool for variant classification while no sensitive information on the individuals is included.

Methods
WES data of 350 unrelated and unaffected individuals have been parsed. All samples were analysed in a similar way i.e. reads were aligned to the reference genome with BWA (Li & Durbin, 2009) and genotyping was performed according to GATK best practices (McKenna et al., 2010; DePristo et al., 2011). All samples were genotyped at all polymorphic positions using GATK HaplotypeCaller and GenotypeGVCFs. For each position, samples with low quality genotype were considered as not genotyped and excluded from the genotype counts. The number of alternate alleles, allele counts and genotypes were compiled in a population VCF file.
Variant frequencies can also be extracted from low coverage WGS. As a pilot we processed the data of chromosome 21 of about 4,000 WGS. The mapping was performed with BWA (Li & Durbin, 2009) and the BAM files were merged per 200 samples. All positions were genotyped using freebayes (Garrison & Marth, 2012). Genotype information of all locations outside low complexity regions were then compiled for all samples using the integration of Apache Hadoop, HBase and Hive. Several models were then used to distinguish real variants from sequencing errors: the Minor Allele Frequency (MAF), the transition/transversion ratio, the expected number of loci with a MAF of 5%, etc.

Results & Discussion
We demonstrated the effect of our reference set on several exomes. The inclusion of only 350 individuals allowed the identification of about 3% additional common variants, not listed as common by ESP, dbSNP (Sherry et al., 2001), 1000 Genomes (McVean et al., 2012) and

GoNL (The Genome of the Netherlands Consortium, 2014). Since only the frequencies of the variants in the screened populations are reported, this file can easily be shared between laboratories. Besides, the procedure used to generate the population VCF file can easily be applied to several genetic centers in order to generate a common population VCF file, as planned within the BeMGI project.

Finally we expect that the data from WGS will further increase the performance of our reference set. A genome-wide variant frequencies file from local population will become worthwhile when WGS is routinely used in diagnostics.

P78: A new method to identify the organ-specific role of biomarkers as oncogene or oncosuppressor in different tumor tissues using TCGAbiolinks package

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Two major genes influence the development of cancer in opposite ways: tumor suppressor genes (TSGs) and oncogenes (OCGs), whose loss or gain of function contributes to cancer cell growth, respectively. Many studies found a substantial number of TSGs and OCGs characterized a particular cancer. However, the underlying mechanisms for TSGs and OCGs to regulate biological processes across different cancer types are still not clear.

In particular we propose a new approach to define TSGs and OCGs according to their molecular functions, and we define a semi-quantitative score (based on Z-Score) to quantify their roles as modulators of these functions.

We address our new package TCGAbiolinks: An R/Bioconductor package for integrative analysis with TCGA data, dealing with our proposed pipeline (https://www.bioconductor.org/packages/release/bioc/html/TCGAbiolinks.html)
The aim of TCGAbiolinks is : i) facilitate the TCGA open-access data retrieval, ii) prepare the data using the appropriate pre-processing strategies, iii) provide the means to carry out different standard analyses and iv) allow the user to download a specific version of the data and thus to easily reproduce earlier research results. In more detail, the package provides multiple methods for analysis (e.g., differential expression analysis, identifying differentially methylated regions) and methods for visualization (e.g., survival plots, volcano plots, starburst plots) in order to easily develop complete analysis pipelines.

The proposed method is described in the workflow showed in Figure 1. It consists of the eight steps detailed below:

1. We collected 5 normal tissues (NT) and 5 cancer tissues (CT) according to TCGA criteria: Breast (BRCA), Gastrointestinal (COAD, READ, LIHC, PAAD), Head and Neck (HNSC, UVM), Thoracic (LUAD, LUSC, MESO), and Urologic (KICH, KIRC, KIRP, PRAD, TGCT, BLCA).

2. A Differential Expression Analysis (DEA) was used to find differentially expressed genes (DEGs) between NT and CT samples for each tissue. We used the edgeR package to select DEGs based on quantile adjusted conditional maximum likelihood (qCML). We corrected p-values for multiple testing using the Benjamini-Hochberg procedure.

3. We inferred gene regulatory networks (GRNs) between each single DEG (sDEG) and all genes using mutual information providing an index of dependence between sDEG and the

other genes. We estimate an appropriate threshold with a permutation test to filter out non-significant dependences and thus obtaining the set of regulated genes (RG) by each sDEG.

4. In order to identify a group of gene sets (with biological functions linked to cancer) significantly enriched by RG, we applied an Enrichment Analysis (EA), using a Fisher's test.

5. For DEGs in each enriched gene set, we applied Z-Score being the ratio between the sum of all predicted effects for all the gene involved in the specific function and the square-root of the number of all genes.

6. We identified sDEG as TSG or OCG. We found a cut-off for Z-score, and we assessed the significance of the sDEG and their genes. sDEG is a possible modulator of that gene set and according to its verse of differentially expression, we defined sDEG as either TCG (down) or OCG (up).

7. We applied the above procedure to the all five tissues to obtain tissue-specific lists of TCG and OCG. We compared the lists for each tissue: if a sDEG was TSG in a tissue and OCG in another we defined it as dual-role TSG-OCG. Otherwise if we found a sDEG defined as OCG or TSG only in one tissue we defined it tissue specific biomarker.

8. We adopted a table of gold standard validated TSG and OCGs in tissues to perform the accuracy of proposed method.

Results: In total, our DEA identified 9591 DEGs.

DEGs were distributed in the different tissue samples, as follows:

- Breast cancer: 1908 up-genes, 1454 down-genes

- Gastrointestinal cancer: 3453 up-genes, 955 down-genes

- Head and Neck cancer: 3438 up-genes, 1042 down-genes

- Thoracic cancer: 2799 up-genes, 1529 down-genes

- Urologic cancer: 1378 up-genes 1190 down-genes.

We found 419 DEGs in five tissues, 762 DEGs in four tissues, 1490 in three tissues.

To summarize the results, for each of the CT analyzed, we identified several DEGs already described by the literature as TSGs or OCGs. For examples, in breast we found 619 DEGs as candidate TSGs and OCGs tissue specific tumor: among others FOX family members (FOXO1, FOXO4, FOXN3) emerged as TSG and OCG already associated to this type of tumor. Therefore, our approach is able to 1) confirm and identify possible TSGs or OCGs in different tumor samples; 2) define possible sDEG with a double role in different types of tumor.

P79: Identification of the pathogenic mutations in three families with distal spinal muscular atrophy

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Three families diagnosed with distal spinal muscular atrophy (dSMA) with unknown genetic cause were subjected to genetic screening using a combination of Sanger sequencing, and exome sequencing after capture of a panel of Charcot-Marie-Tooth associated genes (CMT) or whole exome sequence analysis. CMT is the most common peripheral neuropathy mainly affecting the distal muscles. The clinical picture is highly variable, and sensory complaints may also exist, as well as foot deformities. CMT type 2 is the axonal type of CMT in which the axons are primarily affected ultimately leading to disintegration of the nerve and concomitant wasting of the distal muscles. In CMT2, nerve conduction velocities are (near) normal and when sensory symptoms are absent or mild, the disease resembles distal spinal muscular atrophy that is a purely motor disorder.

Our candidate gene approach yielded a mutation in BSCL2, also known as the Silver mutation, in one family. In the proband of the second family, a pathogenic mutation was found in the HSBP1 gene, in addition to six variations in other CMT-related genes. Both pathogenic mutations showed complete co-segregation with the disease. In the third family, no mutations in known CMT genes were found. Whole exome analysis (WES) at low coverage of six affected and four not-affected family members pointed to a region on chromosome 14 in which most SNPs were located that were shared between affected family members. Previous findings based on linkage analysis had indicated a possible linkage to the same 14q11.2 region (LOD score 2.11) of approximately 30 cM. Sequencing at a higher coverage enabled us to identify a haplotype that was shared between all affected family members confining this region to 11.2 Mb. A detailed analysis of this region demonstrated the presence of a heterozygous three-basepair deletion in exon 33 of the MYH7 gene that was reported in the public databases as a pathogenic allele. Mutations in MYH7 are mostly known for their role in cardiomyopathies but in-frame deletions in this part of the gene have also been reported in Laing distal myopathy, a pure muscle disorder. Muscle biopsies of affected family members had however shown a typical class clustering indicative of a neurologic origin of the disease. Misleading EMGs have been reported twice before for this particular mutation. In view of the expression of this gene also in nerve and brain, albeit at much lower levels than heart and muscle, it is tempting to speculate that MYH7 could have an additional role in neural tissue.

In addition to identification of the genetic cause of the disease in these families, our data demonstrate the overlap of different neuromuscular diseases both at the clinical and genetic level.

P80: Confirmatory invasive testing after a positive NIPT result: chorionic villus sampling or amniocentesis?

Diane Van Opstal & Malgorzata Srebniak

Erasmus MC

Non-invasive prenatal testing (NIPT) for fetal trisomy detection already revealed that there is a small chance of a false positive and false negative result. This is partly due to the fact that the fetal DNA present in the cell free maternal plasma fraction is derived from the cytotrophoblast of chorionic villi (CV). From cytogenetic studies in CV we know that the cytotrophoblast is not always representative for the fetus due to chromosomal mosaicism. Therefore, a positive NIPT should always be confirmed with invasive testing in order to investigate the fetal karyotype. It is generally assumed and recommended that amniocentesis is the preferred technique.

However, the fact that NIPT can be performed from the 10th week of gestation on, makes CV sampling, routinely applied between 11-14 weeks of gestation, a more suitable technique for confirmation studies than amniocentesis, mostly only carried out after 15,5 gestational weeks.

Based on our experience with cytogenetic investigations in CV and on the literature, the choice for CV sampling or amniocentesis will highly depend on the chromosome aberration involved. For trisomy 13, 18 and 21, CV sampling can be an appropriate test, provided that these studies include the cytogenetic investigation of both the cytotrophoblast (for confirmation of the NIPT result) and the mesenchymal core (for verification of the fetal karyotype). The protocol for other chromosome aberrations will be shown as well.

P81: A robust immunohistology method reveals subtle myofiber-type transitions in skeletal muscle

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Background: Skeletal muscle is highly complex and versatile tissue. It is composed of multinucleated long cells expressing a variety of myosin heavy chain (MyHC) protein isotypes, which render contractile properties to healthy skeletal muscles. Predominantly four MyHC isotypes are found in adult muscles and their expression differ between muscles and conditions such as physical training, aging and dystrophies. A robust quantification method is required to measure myofiber-specific changes in expression levels of these isotypes in muscles under different physiological and disease conditions.

Methods: We developed a quantitative immunohistological procedure, which measures the expression levels of four myofiber-types per myofiber in a single staining procedure. We applied this procedure to assess adeno-associated viruse (AAV) serotype specific preferential transduction of myofibers in mouse muscles and to determine myofiber-type transitions in disease and aging affected muscles.

Results: Using this method, we revealed AAV serotype specific preferential transduction for myofiber-types in mouse muscles. Importantly, we were able to capture subtle transitions in expression levels of four MyHC isotypes in muscles of the mdx mouse model for Duchenne muscular dystrophy and in aging human muscles.

Conclusion: We suggest that this newly developed method may provide a robust quantification of disease- and intervention-associated changes in contractile properties of muscles. The quantification of myofiber-type transitions may provide reliable options to statistically assess the contribution of each MyHC isotype to disease severity in multiple muscle dystrophies.

P82: Zygotes segregate entire parental genomes in distinct blastomere lineages causing cleavage stage chimaerism and mixolpoidy

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Chimaerism and mixoploidy define the presence of cell lineages with different parental genomes or different ploidy states in a single individual. Our knowledge on their mechanistic origin results from indirect observations, often when the cell lineages have been subject to rigorous selective pressure during development. Here, we applied haplarithmisis to infer the haplotypes and the copy number of parental genomes in 116 single blastomeres comprising entire preimplantation stage bovine embryos (n=23) following in vitro fertilization. Not only abnormal fertilizations leading to triploid zygotes, but also normally fertilized zygotes can spontaneously segregate entire parental genomes into different cell lineages during cleavage of the zygote. We coin the term "heterogoneic division" to indicate the events leading to non-canonical zygotic cytokinesis segregating the parental genomes into distinct lineages. Persistence of those cell lines during development is the likely cause of chimaerism and mixoploidy in mammals.

P83: Evaluation of Glomulin Function

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Glomuvenous malformations (GVMs) are pink to bluish-purple superficial vascular lesions in which distended venous channels are surrounded by abnormally differentiated vascular smooth muscle cells (vSMC). GVMs are caused by loss-of-function mutations in the Glomulin (GLMN) gene. Due to the nature of GVMs and according to in situ hybridization studies on mice and in vitro data, GLMN is believed to be expressed specifically in vSMCs. Functionally, not much is clearly known about GLMN. Conventional knockout of Glmn in mice results in embryonic lethality at E8.5. Thus, Glmn was conditionally knocked-down (KD) in mouse embryos, using RNAi technology. Smooth muscle-specific Glmn KD (Glmn-RNAi;Tagln-cre) embryos recapitulated the conventional knockout phenotype. Furthermore, Glmn was KD ubiquitously or within endothelial cells (EC) by crossing Glmn-RNAi mice with CAG-CreERT2 or Cdh5(PAC)-CreERT2 mice, respectively, then treating pregnant females with tamoxifen at E10.5 and E11.5. The time of embryonic lethality varied, depending on mouse background (between E13.5-18.5). The ubiquitous and EC-specific Glmn KD embryos exhibited consistent multifocal hemorrhages, with edema in many; however, there was not 100% penetrance of the phenotype. Histological analyses revealed several vessels were dilated. Surprisingly, as embryos in which Glmn was KD in ECs were affected and based on expression analyses, it appears GLMN is essential within ECs, in addition to vSMCs. In all, the Glmn KD models have potential to clarify Glmn's role in angiogenesis and the vasculature and may eventually lead to a GVM mouse model.

P84: Increased NT (> 3.5 mm) in the first trimester – NIPT or array testing?

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Objectives The aim of this study was to evaluate the incidence and size of chromosome abnormalities in fetuses with an isolated increased nuchal translucency (NT ? 3.5mm) in order to determine the most appropriate approach: invasive testing followed by whole genome array or non-invasive prenatal testing (NIPT).

Method 306 patients were tested due to an isolated increased NT (? 3.5 mm). Chromosome aberrations were investigated by using QF-PCR, karyotyping and whole genome SNP array.

Results 120/306 (39%) fetuses showed abnormal results. 114/306 (37%) cases concerned common aneuploidies. In 4/306 (1.3%) a microscopically visible structural unbalanced chromosome aberration was seen and in 2/306 (0.6%) a submicroscopic aberration was found (0.6%).

Conclusion Despite the high prevalence of common aneuploidies, invasive testing in cases of isolated NT ? 3.5 mm in the first trimester of pregnancy still seems to be justified over NIPT since in 3-10% (depending on the NIPT test used) of cases a chromosome aberration will be missed by NIPT. Larger cohorts of fetuses with isolated increased NT or hygroma colli should be tested to investigate the actual prevalence of submicroscopic chromosome aberrations and to assess the additional diagnostic value of array testing in the first trimester.

P85: Loss-of-function mutations in the Parkinsonism/neuronal ceroid lipofuscinosis gene ATP13A2/PARK9 cause complicated hereditary spastic paraplegia

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Hereditary spastic paraplegias (HSP) are heterogeneous neurodegenerative disorders characterized by progressive spasticity of the lower limbs due to degeneration of the corticospinal neurons. Clinically, HSP are classified into pure and complicated forms, the latter being associated with additional neurological or non-neurological symptoms. Mutations in over 60 genes are implicated in HSP, explaining however only ~30-70% of cases depending on the mode of inheritance. Importantly, recent gene discoveries have revealed a genetic overlap between HSP and a spectrum of neuronopathies and axonopathies, supporting their clinical similarity and common pathomechanistic background.

The aim of the current study was to identify new genetic causes of HSP. For that purpose, we have studied a Bulgarian family with three affected brothers presenting an adult onset complex HSP phenotype. Whole exome sequencing (WES) was performed on two affected members, followed by WES-based homozygosity mapping using the HOMWES software. We identified a homozygous missense variant in ATP13A2 co-segregating with the disease and absent in ethnically matched controls. Query of the GEnomes Management Application database (GEM.app) and cohort screening of AR HSP cases identified two additional families carrying different nonsense mutations. In vitro studies demonstrated that all HSP-causing mutations trigger mis-targeting, protein instability and impair autophosphorylation activity of ATP13A2.

ATP13A2 (OMIM 610513) is a lysosomal P5-type transport ATPase, which has been initially associated with the Kufor-Rakeb syndrome (KRS), an autosomal recessive (AR) form of juvenile-onset Parkinsonism. Our findings expand the clinical and genetic spectrum of ATP13A2-associated disorders to include a complicated HSP phenotype.

P86: Exploring the TLX1-PHF6 cooperative interrelationship in T-cell acute lymphoblastic leukemia through zebrafish modeling

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T-cell acute lymphoblastic leukemia (T-ALL) results from the malignant transformation of thymocytes. In T-ALL, different oncogenic genetic lesions (e.g. TLX1/3, TAL1/2, LMO1/2, HOXA) are driving events while further additional mutations in oncogenes and tumor suppressor genes collaboratively contribute to T-cell transformation. PHF6 is one of the most commonly affected tumor suppressor genes in T-ALL and predominantly occurring in TLX1 driven cases. In order to explore this peculiar interrelationship, we first established the TLX1 regulatory landscape. Unexpectedly, this revealed antagonistic interaction between TLX1 and NOTCH1, explaining the observed thymic regression in TLX1+ T-ALL mouse models prior to leukemia formation and high frequency of NOTCH1 mutations in TLX1+ cases (Durinck et al., Leukemia 2015). Upon PHF6 knock down in T-ALL cells and immature thymocytes we observed, amongst others, robust IL7R upregulation. Using CRISPR based genome editing, we generated PHF6 deficient MOHITO mouse T-ALL cells to assess sensitization for JAK inhibitors. To further test the role of PHF6 loss in counteracting TLX1 mediated IL7R repression in vivo, we performed PHF6 gene inactivation by injection of gRNAs and Cas9 protein into the one-cell stage zebrafish embryos yielding varying out-of-frame in/dels. In addition, we will use a TALEN based PHF6 knock out zebrafish line (Moore et al., 2012). We will investigate the genetic interaction between TLX1 and PHF6 either by crossing PHF6 deficient and Rag2-TLX1 overexpressing stable lines or injection of Rag2-TLX1 constructs into PHF6 deficient embryos. Finally, we also showed that upon PHF6 knock down, marked perturbations occurred in maturation of human T- and B-cell and myeloid lineages. Therefore, we are currently investigating altered expression of lineage specific markers for primitive and definitive haematopoiesis in PHF6 deficient versus normal zebrafish embryos.

P87: Unraveling the role of E3 ubiquitin ligase LRSAM1 in Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth (CMT) disease is the most common inherited neuropathy. CMT is clinically and genetically heterogeneous condition and its characterized by peripheral axon degeneration leading to motor and sensory deficits. This disease can be divided into demyelinating and axonal types of disease. CMT2 is an axonal type of CMT with autosomal dominant inheritance. In contrast to the most common form of CMT, where the majority of patients carry a genomic duplication of PMP22 gene, CMT2-associated mutations have been identified in at least 15 different genes, without an obvious link to neuronal-specific function. As such, elucidating the function of these genes is a first necessary step for development of future effective therapies.

We recently identified a frame shift mutation in LRSAM1 gene as a cause of CMT type 2. This novel, dominant mutation causes disruption of the C-terminal RING domain that leads to lack of ubiquitinylation capacity of LRSAM1. To date, three other mutations in LRSAM1 have been identified in CMT patients. A homozygous recessive mutation was reported in a Canadian family leading to a complete absence of the protein. The other, similar to the one we identified, mutations in LRSAM1 gene result in aberrant transcripts in addition to the normal transcript. Of note, all dominant mutations affect the RING domain of the LRSAM1 and its ubiquitinylating capacity.

The function of LRSAM1 and its ubiquitylation targets are still relatively unknown. Therefore, the main aim of this project is to identify endogenous interaction and ubiquitylation targets of LRSAM1, in order to understand the mechanisms underlying CMT2. Since LRSAM1 has also been associated with neuromuscular disorders other than CMT, in view of its neuroprotective role in Huntington's disease, the outcome of this study may potentially be beneficial for the patients who are afflicted with other diseases.

P88: TuneSim - Tunable variant set Simulator for NGS reads

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NGS analysis softwares and pipelines optimization is crucial in order to improve discovery of (new) disease causing variants. A better combination between existing tools and the right choice of parameters can lead to more specific and sensitive calling. Simulated datasets allow the step-by-step generation of new alignment or calling software. Creating a simulator able to insert known human variants at a realistic minor frequency and artificial variants in a tunable controlled way would allow to overcome three optimization limits: complete knowledge of the input dataset, allowing to determine exact calling sensitivity and accuracy; optimization on the appropriate population; and the capacity to dynamically test a pipeline one variable at the time.

Introduction
Identification of anomalies causing genetic disorders is difficult. It can be limited by scarcity of affliction concerned, by disorder genetic heterogeneity, or by phenotypic pleiotropy associated with the anoma-lies in a single gene. Exome and genome sequencing allowed the identification of many genetic diseases causes, whose origin remained inaccessible up to now by the usual techniques of research in genetics (Ng et al., 2009), (Gilissen et al., 2012), (Yang et al., 2013), (Gilissen et al., 2014). Exome and genome sequencing data analysis pipelines are constituted by several steps (roughly: alignment, quality filters, variant calling) and several software are available for those steps. Evaluation and comparison of those tools are crucial in order to improve pipelines accuracy. Exome and genome sequencing simulations should allow to determine the veracity of called variants (false positives and false negatives).

Methods
We implemented TuneSIM, a wrapper around NGS dwgsim (http://sourceforge.net/projects/dnaa/) reads simulator with realistic mutations. Generated reads contain real mutations from 1KG project and dbsnp138. We use existing tool dwgsim for reads generations. In order to generate data as realistic as possible we decided to keep the haplotype blocks structure. We computed blocks using vcf files from 1KG project phase 3 in european individuals with Plink (Purcell et al., 2007). For each block, we obtained a frequency of each combination of variants and we used these frequencies for blocks selection. We also insert variants in an independent way using their frequencies in dbSNP (Smigielski et al., 2000). Using 33 in house samples, we computed global allele frequency variants distributions in coding and non coding regions and we select the variants according to those frequencies. We are developing a web interfaceallowing users to download existing generated datasets. After running their pipelines they can upload their output and see accuracy of their pipelines.

Results & Discussion

Simulations with different coverage, rate of indels have been performed and analysed with different pipelines. Results will be presented.

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P89: Full-length and phased CYP2D6 variant genotyping using the PacBio RSII

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The Cytochrome P450 2D6 enzyme, encoded by the CYP2D6 gene, is among the most important enzymes involved in the metabolism of prescription drugs. Specific variants in the gene are associated with changes in the enzyme's amount and enzymatic activity, which determines the rate at which drugs get metabolized. This metabolic activity has been classified into four metabolizer groups, i.e., poor metabolizers which have no CYP2D6 activity, intermediate metabolizers, extensive metabolizers (wild type) and ultrarapid metabolizers. CYP2D6 metabolic activity can be predicted based on a patient's specific variant profile, allowing a clinician to fine tune the treatment regimen on dose and duration in a personalized genomics approach.

Different technologies exist to determine these sequence variants, such as the Roche AmpliChip CYP450 GeneChip,Taqman qPCR or Next Generation Sequencing (NGS). However, sequence homology between several cytochrome P450 genes and pseudogene CYP2D7 impairs reliable CYP2D6 genotyping, in particular for NGS. Moreover, variant phasing to determine the linkage of heterozygous variants or haplotypes cannot be performed with these assays. The Pacific Biosciences RSII third-generation sequencing platform produces a combination of long reads and high-quality consensus sequences, enabling accurate variant calling and haplotyping. In addition, the long reads are pivotal to accurately identify and exclude off target signals from any homologous sequences, including pseudogenes.

We sequenced CYP2D6 for a total 24 samples with clinically relevant haplotypes (wild type *1; *2; *3; *4; *5; *6; *9; *10; *17; *35; *41) using the PacBio sequencer and obtained high-quality, full-length, phased CYP2D6 sequence reads, enabling accurate variant calling and haplotyping. The previously established diplotype by the Roche amplichip (unphased) was confirmed using the PacBio data for 21 samples, including a duplication of one of the haplogroup sequences for three of the samples. However, the *5 gene deletion and a tandem duplication of the *2 haplogroup could not be detected as a result of the ablation of the primer recognition sites on these alleles, resulting in the detection of a single haplogroup sequence for these samples. In total 62 unique variants were detected across the 24 samples, representing 51 SNPs, 5 insertions and 6 deletions. In addition to known variants associated with changes in the CYP2D6 metabolic activity we detected a range of variants that had not previously been associated with the described haplotypes before.

To further aid genomic analysis using standard reference sequences and HGVS nomenclature we have established a LOVD-powered CYP2D6 gene variant database and added all reference haplotypes and data reported here.

We conclude that our PacBio CYP2D6 genotyping approach produces reliable CYP2D6 genotype calls and haplotype calls when compared to the Roche AmpliChip and reveals information about additional variants, including phasing and copy-number variations.

P90: Performance assessment of targeted re-sequencing experiments using full genome sequenced cell lines.

Luc Dehaspe, Erika Souche, Céline Helsmoortel, Anniek Corveleyn, Valérie Race, Gert Matthijs & Jeroen Van Houdt

UZ Leuven

Combining target enrichment with Next Generation Sequencing (NGS) has become an important strategy for interrogating larger parts (gene panels) of the genome at relatively low cost. This technology is currently used in basic research projects and in diagnostic settings. The size of the enriched target might vary substantially among different applications. The selected target can be a few dozens of genes related to a particular clinical question or thousands of genes possibly involved in inherited diseases (the so called Mendeliome) or even the full humane exome. When setting up a targeted re-sequencing experiment one needs to decide on the used technology and analyses pipeline. The combination of both will determine the performance of the experiment.

We present a strategy for evaluating the performance of targeted re-sequencing experiments by including cell lines for which reliable full genome information is available. We used cell lines for which Illumina Platinum Genome information is available for assessing the performance of targeted gene panels of various sizes.

P91: Exploring the role of the PHF6 tumor suppressor gene in DNA repair in T-cell acute lymphoblastic leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy that is characterized by the uncontrolled proliferation of immature lymphoblasts. Despite improved survival rates of T-ALL patients due to intensive chemotherapeutic treatment, these protocols have severe side effects and T-ALL patients that relapse have poor prognosis, indicating the need to develop more effective and specific antileukemic drugs. To identify novel therapeutic targets, it is crucial to understand the genetics and molecular mechanisms involved in normal T-cell development and T-ALL oncogenesis. During T-ALL development, different genetic lesions cooperatively contribute to the transformation of precursor T-cells. The PHF6 tumor suppressor gene is one the most frequently affected genes in T-ALL, and inactivating mutations or deletions are found in about 16% of pediatric and 38% of adult T-ALL cases.
PHF6 is a presumed chromatin reader controlling gene expression and acting as a key regulator during normal hematopoiesis (Durinck et al., unpublished). Previous work also showed that PHF6 inactivation in HeLa cells increased DNA damage at rDNA loci and resulted in cell cycle arrest (Wang et al, JBC, 2013). Here, we explore in further detail the possible role of PHF6 in regulation of DNA damage repair in the context of T-ALL. First, PHF6 immunoprecipitation coupled to mass spectrometry (IP-MS) analyses in Jurkat T-ALL cells revealed a variety of candidate PHF6-interacting proteins involved in DNA damage signaling and DNA repair. Secondly, using immunofluorescence microscopy and quantification of ?H2AX foci as a marker for DNA damage levels in the nuclei, we observed that PHF6 knockdown ALL-SIL cells showed slower DNA repair kinetics as compared to wildtype PHF6 control cells after removal of the DNA damage inducing compound etoposide (topo-isomerase II inhibitor). Finally, we also performed proof-of-principle experiments indicating that upon PHF6 knockdown, Jurkat T-ALL cells become more sensitive to DNA damage-inducing compounds Olaparib (PARP inhibitor) and etoposide, suggesting that DNA repair is impaired in PHF6 deficient cells. These findings warrant further testing of DNA damage-inducing compounds in PHF6 deficient T-ALLs and could offer novel therapeutic opportunities for T-ALL patients.

P92: Combining time-lapse imaging and genome-wide haplotyping reveals novel mechanisms underlying chimerism, mixoploidy and aneuploidy formation in mono- and tripronuclear human preimplantation embryos

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Recently, we developed haplarithmisis, enabling concurrent haplotyping and copy number profiling of single cells based on SNP arrays and implemented this methodology into routine preimplantation genetic diagnosis (PGD). Most genome wide haplotyping studies have focused on understanding the causes for aneuploidy in normal fertilized embryos which are characterized by 2 pronuclei. In contrast, monopronuclear (1PN) and tripronuclear (3PN) embryos are discarded from being used in IVF cycles and little is known about the genomic constitution, developmental potential and the chromosomal stability of 1PN and 3PN derived human preimplantation embryos which are derived either through in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Here, we apply time-lapse imaging to monitor the cleavage divisions and analyse all cells by haplarithmisis of four 4-6 cell stage embryos. In a first 1 PN embryo containing only a maternal genome the cleavage was shown to be activated. A second 1PN embryo turned out to be diploid, suggesting that pronuclear formation occurred asymmetrically. One of the tripronuclear embryos gave rise to three blastomeres following the first cleavage. Interestingly, two out of three blastomeres underwent fusion, which resulted in a diploid cell containing a different maternal genomic profile in comparison to the remaning blasomere. This suggests the persistence of a polar body with blastomere sized cells in the early-stage embryo. These observations pinpoint novel mechanisms underlying chimerism/mixoploidy and aneuploidy, highlighting the genomic flexibility of human preimplantation embryos during early cleavages.

P93: Elucidation of the molecular cause underlying Sorsby fundus dystrophy in a large Belgian pedigree: N-terminal TIMP3 mutation 15 years later.

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Sorsby fundus dystrophy (SFD) is an autosomal dominant retinal dystrophy in which patients lose central vision during the fourth or fifth decade of life. SFD is caused by mutations in TIMP3. TIMP3 is a member of a family of four secreted proteins inhibiting the activity of matrix metalloproteinases. In 2000, Assink et al. (Br J Ophthalmol, 2000) examined a large Belgian family with typical SFD. Although linkage was shown with the TIMP3 locus, mutation screening using single strand conformational polymorphism, Sanger sequencing and cloning of the fifth exon of TIMP3 did not reveal a mutation in the coding exons, intron-exon boundaries, promotor region and 3'UTR of TIMP3. Here, it was our aim to elucidate the genetic cause of SFD in this family.

Therefore, we first performed microsatellite analysis with four additional markers located closer to the gene. We confirmed linkage with TIMP3, and subsequently performed Sanger sequencing of the coding region, revealing a known mutation in exon 1, c.113C>G; p.(Ser38Cys). This is the only known mutation located in the N-terminal domain of the TIMP3 protein. The mutation segregates with disease in 63 investigated family members. In addition, we identified a second, French family with the same mutation. Microsatellite analysis in both families is ongoing to determine if it concerns a founder mutation.

TIMP3 comprises six disulfide bonds, established by twelve cysteines. The p.(Ser38Cys) mutation results in an additional cysteine at the N-terminus. Arris et al. (Biochimica et Biophysica Acta, 2003) proposed a mechanism whereby mutations adding an extra cysteine create proteins that form abnormal disulfide-bonded dimers, thus reducing protein turnover in the Bruch membrane. However, we could not confirm this hypothesis for the p.(Ser38Cys) mutation using Western blot analysis on patient- and control derived fibroblasts.

The N-terminal domain of TIMP3 alone is sufficient to mediate its metalloproteinase inhibitory activities, whereas the C-terminal domain ensures tight binding to the extracellular matrix. To investigate whether the N-terminal mutation affects this inhibitory activity, EnzChek gelatinase/collagenase (Molecular Probes) activity assays with MMP2, a target of TIMP3, are currently ongoing.

In conclusion, we elucidated the molecular cause underlying SFD in a large Belgian pedigree, previously missed by less sensitive screening methods. Our study confirms the genetic homogeneity of SFD. We could not confirm the hypothesis proposed by Arris et al. on protein dimer formation, and are currently investigating a possible effect on TIMP3 inhibitory activity.

P94: Need for multidisciplinary approach in Klinefelter syndrome: the UZ Brussel experience

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Introduction
Although 47,XXY (Klinefelter syndrome(KS)) is the most frequent sex chromosomal disorder affecting one in 660 newborn boys, it remains a profoundly underdiagnosed condition with only 25% of patients ever being diagnosed. The physiopathological pathways responsible for the wide range of comorbidities remain mostly unknown. It is extremely important to increase the knowledge on the variable phenotype of the syndrome, to be able to offer proper prenatal counselling to the future parents and early diagnosed patients. Since KS is a variable,multiorgan and systemic disorder a multidisciplinary approach is needed . In order to improve and optimise care UZ Brussels has founded in 2013 a KS clinic .

Material and Methods
In 2013, the UZ Brussels has founded its multidisciplinary KS clinic for both children and adult KS patients. The team consists both adult and pediatric endocrinologists, neurologist, urologist,psychiatrist, pschylologist, a geneticist ,a fertility doctor and a nurse coordinator. Patients have a first contact with the nurse coordinator by phone or mail to orient the patients problems. At intake, patients are counselled by a psychologist and geneticist, and seen by some or all members of the team, depending on their individual needs. An individualised yearly follow-up scheme is offered to each patient. Furthermore, the clinic aims at improving early diagnosis of the syndrome, as well as increasing the knowledge on its treatment. It acknowledges the multidisciplinary aspects and needs, and wants to stimulate research frameworks with special interests in fertility preservation and testosterone substitution, Support to existing patient organisations is given.

Results
Since the start of the clinic 153 new patients were recorded. A local register was initiated to document the overall health status of the patients. New studies on fertility preservation in adolescent KS boys were initiated, and newly developed questionnaires to investigate the attitudes of KS parents as well as general pediatricians with regard to neonatal screening for the syndrome and towards early fertility preservation techniques were analysed.

Conclusion

KS is a multi-organ and systemic disorder, under recognised and under diagnosed for which a multidisciplinary approach is needed in order to provide a preventive care. Organising a KS clinic were patients are seen on a regulary individual base , at one moment by the different specialists and by an experienced team is an added value in optimalising care for these patients.

P95: The pathogenesis of neurofibromatosis type 1-related pseudarthrosis

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder affecting 1 in 2.700 individuals. NF1 is caused by an inactivating mutation in the NF1 gene that encodes neurofibromin, a negative regulator of the RAS-MAPK pathway. Besides the common dermal abnormalities, around 5% of NF1 children present with congenital bowing of a long bone (usually the tibia) resulting in a fracture. This fracture often develops into a non-union or pseudarthrosis, due to impaired healing. The current treatment methods for NF1-related pseudarthrosis are unsatisfactory and if union remains illusive the affected limb is amputated. Therefore improved understanding of NF1-related pseudarthrosis is crucial.

Recently an additional somatic mutation in the NF1 gene was identified in pseudarthrosis tissue. This concurs with our results. We found bi-allelic NF1 inactivation in periosteal-derived cells (PDCs) of the primary pseudarthrosis tissue in seven NF1 patients.

Two of these seven patients were sampled more extensively, including periosteum sampling surrounding the pseudarthrosis region and at the osteotomy site. Mutation analysis of these samples indicates that the pseudarthrosis site contains a mixture of NF1+/- and NF1-/- PDCs, ranging from entirely NF1+/- to 100% NF1-/- at different sites in the same pseudarthrosis region. Furthermore, samples from the periosteum surrounding the pseudarthrosis site were found to contain 20% to almost 100% NF1-/- PDCs. Additionally, one patient was found to have NF1-/- cells at the osteotomy site (30%), far above the pseudarthrosis site. The additional somatic mutation identified in this patient was caused by mitotic recombination. This mechanism has not previously been described in NF1-related pseudarthrosis. These results indicate that the NF1-/- cells are more widespread than expected and previously found in NF1-related pseudarthrosis cases. This illustrates the probability of additional factors involved in the development of NF1-related pseudarthrosis.

P96: Large-scale single-molecule sequencing of tandem repeats on the human X chromosome

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Tandem repeats are short DNA sequences that are repeated head-to-tail with a propensity to be variable. They constitute a significant proportion of the human genome, also occurring within coding and regulatory regions. Variation in these repeats can alter the function and/or expression of genes allowing organisms to swiftly adapt to novel environments. Importantly, some repeat expansions have also been linked to certain diseases. Unfortunately, due to the nature of short read sequencing technologies, tandem repeats are not analyzed during whole genome or exome sequencing studies. We developed a novel capture assay for large-scale genotyping of tandem repeats (Duitama J., Zablotskaya A. et al., Nucl. Acids Research, 2014) and extended the assay for the identification of X linked disease-related repeats using long read (averaging 12 kb) PacBio RS II technology. For 837 (83% of all) potentially functional repeats, unique capture baits were designed, as well as for 1000 intronic and intergenic repeats. Of these, a full tandem repeat length sequence was obtained for 88-90% of the targets in male DNA samples. Sequencing read length and analysis pipeline allows to detect cases of tandem repeat expansion. We are currently implementing this assay to screen for potentially causal variation underlying X-linked disorders that are not explained following array and exome sequencing. A candidate variant for miR-222 deregulation has been revealed in one of the families with X-linked intellectual disability.

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P97: STIL compound heterozygous mutations detected prenatally cause of microcephaly, brain abnormalities and embrionic lethality?

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Microcephaly (MC) is a condition characterized by reduced brain volume that can be appear as a sporadic trait or in combination with other features in the so called syndromic forms. MC is defined as primary when observed pre-or peri-natally as opposed to secondary which develops after birth. Currently 15 genes have been associated to primary recessive forms of non-syndromic MC. Amongst them, STIL (SCL/TAL1-interupting locus) mutations are responsible for MCPH7 (OMIM # 612703). Both homozygous recessive and a single case of compound heterozygous mutations have been identified in patients with MC alone or in combination with other brain abnormalities. STIL is required to initiate centriole duplication during the centrosome cell cycle. In G1, it is recruited to the proximal end of a nascent procentriole from each disengaged parental centriole, promoting the formation of two distinct centrosomes that at the beginning of mitosis will nucleate the mitotic spindle and astral microtubules.

We describe a couple with two healthy siblings, recurrent miscarriages and two pregnancy terminations after ultrasound identification of MC, simplified gyral pattern and corpus callosum abnormalities in the fetuses. Using the Agilent OneSeq Constitutional Research Panel we identified compound heterozygous novel missense mutations in STIL. No homozygous recessive or putative de novo variants suggesting parental germinal mosaicism were identified. Functional studies analyzing amniocytes from the affected fetuses for centriole number abnormalities or other centrosomal defects might confirm further allelic pathogenicity.

P98: Molecular diagnosis of segmental neurofibromatosis.

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Individuals with segmental neurofibromatosis show typical features of neurofibromatosis type 1 (NF1) in one or more segments of the body and 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 leucocytes 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). We investigated 14 individuals with segmental NF. In 3 cases both CALS and discrete neurofibromas were present, in 2 cases a large hyperpigmentation spot was present in the lumbosacral region overlying a plexiform neurofibroma and in 9 cases only CALS were present. In 10 cases freckling was seen. In 3/14 cases no result was obtained due to no or poor cell growth. In 8 cases we identified the responsible NF1 mutation. No NF1 or SPRED1 mutation was found in the remaining 3 cases. Total NF1 gene deletions were seen in 4 cases and single- or multi-exon deletions in 2 cases representing 75% of mosaic NF1 mutations. In NF1 patients who are carrier of a germline NF1 mutation we observe these gene- and exon mutations only in 7%. In 14 of 18 separate cultures from these 8 individuals a neurofibroma or CALS specific "second hit" in the NF1 gene was detected. We did not detect any of the NF1 mutations in peripheral blood leucocytes or in fibroblasts from CALS or neurofibromas, pointing towards the developing neural crest as the origin of the mosaic NF1 mutation. We performed 5 times prenatal diagnosis in 3 patients with a normal result each time.

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 segmental NF 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 mutation is detected.

P99: Elucidating the role of coding and noncoding genes in neuronal development using human embryonic stem cells and the CRISPR/Cas9 system

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One of the main limiting factors in the study of genes involved in neuronal development is the availability of relevant tissue and cellular model systems for functional analysis. Being pluripotent, human embryonic stem cells (hESCs) can be differentiated to the neural lineage, which opens an attractive perspective to generate an in vitro neural model system. Hence, efficient genome editing is necessary in these hESCs for the functional elucidation of candidate neuronal genes. The CRISPR-Cas9 system, originally a bacterial RNA-mediated adaptive immune system, has become a popular tool in genome-editing due to its ease of use and relative cheap design and construction. It relies on a single guide RNA (sgRNA) to direct site-specific chromosome breakage mediated by the Cas9 endonuclease, resulting in deletions or insertions at the target region.

We set up a pipeline to generate knockouts for coding as well as non-coding genes in hESCs using this system. First, hESCs are transfected with a plasmid containing the Cas9 endonuclease and the sgRNA through nucleofection. Subsequently, to obtain a clonal cell line, the transfected hESCs are single cell isolated through serial dilutions. In a next step, DNA is isolated and the target site is amplified. Using next-generation sequencing (NGS) the genome-edited clonal cell lines are identified and these are then further expanded. Next to the target sites, in silico predicted off-target sites are also amplified and screened for genomic aberrations by NGS.

After obtaining a knockout model for the gene of interest, the wildtype and genome-edited hESCs can be differentiated into relevant neuron types for downstream characterization. For this we successfully set up the differentiation towards neuronal progenitor cells and subsequently towards a mix of neuronal subtypes (Denham and Dotorri, Methods Mol Biol, 2011).

Human embryonic stem cells are hard-to-transfect cells, but with optimized nucleofection conditions, respectively a transfection efficiency and viability of 54% and 90% was seen. The CRISPR/Cas9 assays we designed were for both coding and non-coding genes. The Cas9 protein cleaves at the on-target site with an editing efficiency of 2-40%, showing that the mutation frequency depends on the target locus and/or sgRNA. No disruptions were noted at the in silico predicted off-target sites.

In conclusion, we established a workflow for efficient genome editing of human stem cells through the CRISPR/Cas9 system, ideal for human neural differentiation research. After obtaining a knockout for a candidate coding or non-coding gene, the hESCs can be differentiated to the desired neuron type to functionally investigate the target gene and/or to identify underlying genetic networks.

P100: Long Read Sequencing of Single Microdissected Chromosomes enables targeted genotyping

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Genotyping, or assigning genetic variation into alleles, is becoming increasingly important in order to complete the full description of the human genome. We aim to assemble a genotype map for chromosome 22q11.2 and unravel the region's high susceptibility for rearrangements. Furthermore, we hypothesize we could find an answer in structural variation to explain wide phenotypical spectra of the 22q11 Deletion syndrome, man's most frequent recurrent microdeletion.

We developed a novel method to isolate, amplify and sequence single chromosomes. We isolated single chromosomes using glass needle microdissection, assisted by fluorescence in situ hybridization to pinpoint regions of interest. Microdissected fragments are amplified by multiple displacement amplification and prepared for long read, single molecule sequencing.

Initial tests show we can fully cover the sequence of a chromosome, starting from a pool of 10 chromosome fragments. Mean read lengths were over 8Kb, and sequencing depth was 2.3 on average. The latter is 10 times higher than what can be achieved from a full genome, without enriching, using the same resources. Future experiments aim to target chromosome 22q11 during microdissection, and provide us with the necessary data to resolve the region's genotype.

P101: Detection of AGG interruptions in FMR1 premutation females by Single-molecule sequencing

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The Fragile X mental retardation gene (FMR1) contains an unstable CGG repeat in the 5' untranslated region. This repeat is expanded from around 30 (normal) to a range of 55-200 repeat units in female premutation carriers. Premutations occur in the population with an estimated frequency of about 1 in 200. Those females are at risk for CGG expansion resulting in Fragile X tremor/ataxia syndrome (FXTAS) and Premature Ovarian Failure (POF). Furthermore, the germline transmission of the CGG repeat is highly unstable, and therefore premutation females will often transmit a full mutation (>200 CGG repeats) to their offspring. Full mutations cause the Fragile X syndrome (FXS) which is the most common form of inherited X-linked Intellectual Disability (XLID) and the leading single cause of autism.

The risk that a premutation female will transmit a full mutation to her offspring is variable. The larger CGG repeats expand faster to full mutations. In addition, AGG triplets interrupting the CGG repeat stabilize repeat lengths and reduce the risk for expansions. For example, the difference in risk of transmitting a full mutation is more than 60% for a female with 75 repeats and 0 AGG's as compared to the same female with 2 AGG interruptions. Despite its importance, AGG measurement is not yet a standard feature of FMR1 diagnostic work-up. If determined, AGG interruptions are detected by a Triplet-Primed PCR (TP-PCR). Unfortunately, those AGG interruptions can be obscured because both the normal and premutated allele camouflage each other's interruptions.

Here, we explore single-molecule sequencing (Pacific Biosciences) to determine the AGG interruptions in a cohort of premutation females. This "third" generation sequencing technology is perfectly equipped to read through large and GC-rich repeats. Moreover, the insert of each single molecule is sequenced several times resulting in accurate trinucleotide size determination. We demonstrate that single molecule sequencing correctly determines the size of both the normal and premutated allele for each female of the cohort as compared with standard approaches. More interestingly, the single-molecule sequencing also allowed the unambiguous separation of the normal from the premutated allele, and enables detection of the location and number of AGG interruptions for each allele. We foresee that this technology will replace current tests and that the identification of the location and number of AGG interruptions will improve risk estimates allowing for improved genetic counseling and better risk estimate studies.

P102: Hidden Markov Model based CNV calling in whole exome sequencing data.

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Next Generation Sequencing is rapidly entering routine molecular diagnostics. As whole exome sequencing (WES) becomes cost-efficient for complex disorders, it is being applied to large patient cohorts. While well established pipelines are available for single nucleotide variant detection, copy number variants are often overlooked. High inter-bait coverage variability, due to variable capture efficiencies, obfuscates true copy number differences in coverage depth analysis.

In microarray technology, the integration of genotype data in copy number calling using two-layer hidden markov models increased the resolution significantly for SNP-array platforms. Similarly, WES data provides high confidence SNP-genotypes, in addition to a total intensity signal. Measuring total intensity signal as the average coverage of exons is prone to capture efficiency bias. Here, we define it as the coverage of non-genic regions by aspecific enrichment, collated in 50kb bins. This coverage shows homogeneous profiles after removal of recurrent coverage peaks and exonic regions. LogR ratios are constructed between the sample coverage and average coverage over a reference population. To obtain confident genotype information, we selected 90,000 exonic SNPs present in dbSNP with a minor allele frequency of at least 10% over at least 500 individuals. These SNPs are then genotyped in the sample under study. Reliable calls are converted to a b-allele ratio and assigned to the corresponding bins used for coverage analysis, giving preference for heterozygous calls if multiple SNPs are present in a bin.

We applied segmentation using a two layer hidden markov model, implement in the VanillaICE library in R. Validation on 30 samples analysed on an Illumina HumanCytoSNP12 showed a minimal cut-off of 10 bins, corresponding to 500kb, yields reliable CNV calls. We further showed that application of a two-layer HMM outperforms HMM or circular binary segmentation applied to only Depth of Coverage data in both sensitivity and specificity.

P103: Identification of lncRNAs involved in neuronal differentiation and intellectual disability

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Recent studies have assigned important functions to long non-coding RNAs (lncRNAs) in gene regulation and protein interactions. Since many of these lncRNAs emerged recently during vertebrate and primate evolution, a crucial role in the human brain is anticipated. Here, we aimed at identifying candidate lncRNAs associated with neuronal development and intellectual disability (ID).

To do this, we combined the latest genomic annotations of lncRNAs (i.e. LNCipedia database) with functional data (neuron-specific H3K4 trimethylation, REST binding & DNaseI hypersensitivity). These three datasets were applied as filters, both to RefSeq protein-coding genes and LNCipedia lncRNA transcripts. To assess the specificity of these potential filters, we performed an enrichment analysis of ID genes and genome wide association study (GWAS) hits for central nervous system (CNS) disorders, on the resulting sets of protein-coding genes (ID genes & GWAS hits) and lncRNA transcripts (GWAS hits only). We found the neuron-specific H3K4me3 mark to confer the highest specificity for genes involved in ID and neurodevelopment. Applying this mark as a filter for all LNCipedia transcripts resulted in a set of 4188 lncRNAs, of which 53 harbour a GWAS hit for CNS disorders. As the presence of such a SNP directly implicates these lncRNA loci in neuropathogenesis, we focused during subsequent analyses on this set of 53 lncRNAs.

This approach was complemented by extensive expression profiling of all protein-coding genes and ca. 23,000 lncRNA transcripts in 15 human tissues, among which 8 different brain samples. This allowed us to construct coexpression profiles for 30 of the lncRNAs that were identified by our filtering strategy (no unique probes could be designed for the other 23 transcripts). Using Gene Set Enrichment Analysis (GSEA) we evaluated the involvement of the selected lncRNAs in neuronal processes. For 19 out of the 30 selected lncRNAs, gene sets linked to synaptic transmission, nervous system development or neurogenesis were highly enriched among the top positively correlated genes. Five lncRNAs were negatively correlated to genes involved in these neuronal processes, suggesting that these lncRNAs may be involved in suppressive regulation.

In conclusion, we set up a strategy to identify lncRNAs involved in neuronal development and 30 interesting lncRNA transcripts remained. The relevance of our strategy was underscored by the fact that at least 24 of these lncRNAs are implicated in neuronal processes through correlated expression profiles. In our further research we will validate these top candidates by targeted functional analysis.

P104: Thorough Second hit analysis in BRCA1/2 associated breast cancer

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Female carriers of a germline BRCA1/2 mutation have a highly increased risk for breast cancer. Loss of the remaining functional allele leads to carcinogenesis in agreement with Knudson's second hit hypothesis. According to the literature somatic loss of heterozygosity (LOH) is the most prevalent mechanism leading to the absence of a functional tumor suppressor gene product. Here the functional allele is lost by either incorrect mitosis or a deleterious chromosomal event. However, thorough studies on the prevalence of other somatic events (including point mutations) have not been published. Therefore, we optimized a targeted enrichment and next generation sequencing approach to perform mutation analysis of the complete coding region of BRCA1/2 in formalin fixed and paraffin embedded (FFPE) breast tumors. We collected 133 formalin fixed and paraffin embedded (FFPE) breast tumors from germline BRCA1/2 mutation carriers diagnosed between 1989 and 2014.

To enhance tumor cell percentages in each sample as much as possible, we optimized a Laser Guided Macrodissection (LGM) approach. DNA extraction following LGM was successful in 99 tumors. We performed sequencing in DNA extracted from tumor and blood in parallel. Data analysis is still ongoing, but preliminary results confirm loss of the wild type allele in a considerable number of the tumors. The LOH region spans variable proportions of the gene. Interestingly, in several tumors loss of the mutant allele was observed, suggestive for more than 2 hits. The results on the frequency of somatic point mutations will be presented at the meeting.

The results of our study can be used to determine the value of performing LOH analysis in tumors from patients with VUS (variants of unknown significance) to gain insight in the clinical role of such variants. In addition, thorough knowledge on the second, third, etc. hits in tumors will be important to better understand therapy resistance for e.g. Parp inhibitors which are now being applied in clinics for the treatment of hereditary tumors with DNA repair defects.

P105: Efficient diagnostic routing using clinical exome sequencing for families with an unexplained genetic disease

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Clinical exome sequencing (CES) can provide a molecular diagnosis in families with an unexplained genetic disease. When using this technique in a diagnostic setting it is important to follow a well documented diagnostic routing. Here we present an effective routing and the results of the first 322 cases/families. Clinicians can choose to offer CES directly or to first discuss candidate families in a multidisciplinary team. Sequencing was performed on a HiSeq 2000/2500 and data processed using an in house pipeline. Prioritization of variants is performed with Cartagenia Bench Lab NGS using validated variant filtering trees. A number of dedicated filtering trees is used depending on the availability of (both) parents and for more distantly related individuals affected with dominantly inherited phenotypes. The CES procedure was evaluated for the first 322 cases/families. A conclusive molecular diagnosis (class 4/5 variants) was made in 83/322 patients (26%); phenotype including intellectual disability (ID, 61/248, yield 25%) and phenotypes without ID (n=22/74, yield 30%). A possible diagnosis (class 3 variants) was made in an additional 40/322 patients (12%); ID 32/248 (13%), no ID 8/74 (11%). In total 33 incidental findings were detected; 8 (2.5 %) secondary findings (clinical diagnoses) and 25 (7.8%) carriers of autosomal recessive diseases.

If applicable, targeted pre-analysis, such as ID gene panel analysis (~850 genes, updated regularly), was performed prior to whole exome analysis. In cases where no conclusive molecular diagnosis was identified by targeted analysis, or in case this was not applicable, whole exome analysis was performed. This procedure increases efficiency and reduces the chance of incidental findings. In a substantial number of cases, 14/61 ID cases (23%), the conclusive molecular diagnosis was made in the second analysis step (whole exome analysis). This is mainly due to the fact that such genes have only recently been linked to disease in the literature. In conclusion, we have implemented an efficient strategy for CES with an average diagnostic yield of 26%.

P106: Targeted next generation sequencing gene panel for patients with epilepsy

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Many genes are known to be associated with epilepsies. We developed a targeted next generation sequencing (NGS) gene panel based on Agilent Sure Select Target Enrichment® for mutation detection of 144 genes. The gene panel has been validated for diagnostic purposes.

Within this panel nine sometimes overlapping subpanels were designed based on different epilepsy phenotypes. The smallest subpanel "Benign familial neonatal/infantile seizures" contains 5 genes and the largest "Syndromes with epilepsy and intellectual disability" 63 genes. The other subpanels are: epileptic encephalopathy (39 genes), focal epilepsy (12 genes), fever related seizures (12 genes), progressive myoclonic epilepsy (19 genes), assumed metabolic disorder with epilepsy (43 genes), idiopathic generalized epilepsies (14 genes), and epilepsy in combination with other paroxysmal disorders (11 genes).

Sometimes the patient can not be allocated to only one particular subpanel. In that case two or three subpanels are analysed. In about 10% of the patients a disease causing mutation was identified. The results of the first 125 patients, in whom a disease causing mutation, a likely disease causing mutation or a variant of unknown significance was identified, will be presented.

P107: Disruption of a remote putative novel enhancer in the cis-regulatory domain of FOXL2 in a multigenerational Polynesian family with BPES

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Background: Loss-of-function mutations in FOXL2 lead to the rare, autosomal dominant, developmental disorder blepharophimosis syndrome (BPES; OMIM 110100), associating a typical eyelid malformation with premature ovarian insufficiency (POI). Apart from coding changes in FOXL2, the only known disease gene so far, upstream translocations and cis-regulatory deletions have been described in BPES cases, emphasizing the need for a strictly regulated spatiotemporal expression in development. Despite extensive genetic studies, the molecular causes remain unexplained in at least 12% of typical BPES patients, assuming the presence of more subtle defects in the cis-regulatory region of FOXL2.

Patients and Methods: A multigenerational BPES pedigree of Polynesian origin in which linkage to FOXL2 (LOD of 3.8) was shown, remained without a molecular diagnosis after having excluded coding defects and non-coding CNVs. The goal of this study was to unravel the underlying pathogenetic mechanism in this family. To this end, we resequenced FOXL2 and its putative cis-regulatory domain (chr3:138652808-139067278; GRCh37) using HaloPlex target enrichment (Agilent technologies) followed by next-generation sequencing (MiSeq, Illumina) in five individuals of this family. Data-analysis was performed using CLC bio Genomics Workbench. After mapping, variants were called, annotated and filtered. Next, filtered variants were uploaded into the Ensembl Variant Effector Predictor (VEP) tool to annotate the variants with the regulatory build.

Results: We identified a heterozygous non-coding variant, Chr3(GRCh37):g.138954755G>A, that is not present in genomic databases. The variant is present in all four available affected family member but absent in an unaffected individual. It is located in a 200-bp non-conserved sequence predicted by Ensembl to function as an enhancer. In addition, this predicted enhancer is located in a shortest region of overlap of previously delineated regulatory deletions. Interestingly, an interaction of this fragment with the FOXL2 promoter has been demonstrated using chromosome conformation capture (3C) in human granulosa-like tumor KGN cells (D'haene et al. PLoS Genetics 2009).

Conclusions: This is the first report of a non-coding sequence variant in a putative novel enhancer of FOXL2 leading to BPES. Our study adds to increasing number of Mendelian developmental disorders caused by subtle genetics defects of cis-regulatory elements, such as the ZRS and SIMO elements in the SHH and PAX6 regions respectively. Further in vitro and in vivo functional studies of this putative wild type and mutated enhancer will provide more insights into the underlying mechanisms.

P108: Differential expression of type III collagen in male and female mice

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Collagens are the most prevalent component of the extracellular matrix; they represent one-third of the total body protein content, and occur in a wide array of tissues where they provide structural integrity and strength. Collagen synthesis occurs continuously throughout life to repair and replace damaged collagen and build new cellular structures. However, collagen levels drop with age due to decreased production and increased degradation. Recent evidence demonstrated that next to age, also gender is an important factor for differences in collagen levels and other extracellular matrix components. A study on broilers showed that total collagen content of male skin is higher than the content of female skin, resulting in a higher tensile strength of male skin. This was confirmed in mice where significant differences in total collagen content were observed between the skin of wild type male and female mice. This observation became even more evident at puberty. It is known that the endocrine system is one of the most important factors involved in the initiation of aging. For skin, estrogens affect skin thickness, wrinkle formation and skin moisture. Estrogens can increase collagen production in the skin, where they maintain epidermal thickness and allow skin to remain plump, hydrated and wrinkle-free. Thus, the effects of the estrogen:testosterone ratio may be a key mediator in pathways influencing collagen content.

As these gender differences have only been described for type I collagen, we focused our study on type III, which is a major fibrillar collagen consisting of three ?1(III)-chains and is encoded by the COL3A1 gene. Type III collagen is expressed throughout embryogenesis and in a wide variety of adult tissues, including skin. The skin of wild type male and female mice was subjected to a biomechanical test, where males showed a significant higher tensile strength. Type III collagen was then isolated from skin tissue to test the quantity and biochemical characteristics. Male skin yielded significantly more type III collagen than female skin, which was consistent with the observed biomechanical differences among male and female skin. Furthermore, amino acid analysis revealed a novel observation where methionine was differentially modified in type III collagen of male skin.

Herewith, we show remarkable gender differences in biomechanical properties of the skin and biochemical characteristics of type III collagen in wild type mice. These findings highlight the importance of factors such as gender and age in studies involving collagen and other extracellular matrix components.

P109: Detection of acquired genetic abnormalities when using SNP array analysis for constitutional copy-number detection

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Introduction: SNP array analysis has replaced conventional karyotyping for retrieving the cytogenetic causes of intellectual disability (ID) and/or congenital malformations (MCA), also allowing the detection of large copy-number neutral regions of homozygosity (ROH), that might be indicative for the presence of recessive traits. In routine practice, SNP array analysis is also used, either simultaneously or subsequently, to investigate the parents of an affected index for carrier status of possibly relevant observed copy-number variants (CNVs) to determine the inheritance and significance of the abnormality. Using DNA isolated from peripheral blood, it is also possible to detect the presence of acquired abnormalities e.g. numerical abnormalities and acquired ROH, for instance caused by the presence of a (haematological) malignancy. In addition, the acquired abnormalities might be of help in elucidating the genetic cause of disease in the patient. We will report 8 individuals whose DNA showed acquired copy number abnormalities when referred for ID, MCA or both or for carrier status using Illumina SNP arrays (IL-C12 or HCS850K), from a total cohort of approx. 13.000 investigated individuals analyzed for CNVs (0.06%).

Abnormalities observed included numerical changes and ROH, both present in mosaic state. ROH predominantly involved an entire chromosome arm. Two newborns, investigated for microcephaly, both showed loss of chromosome 7 in 20-85% of cells, which was subsequently confirmed in the bone marrow, indicative for the presence of Juvenile MyeloMonocytic Leukemia (JMML) or Myelodysplastic Syndrome (MDS). One of these children was diagnosed with Fanconi Anemia. The presence of acquired abnormalities helped in elucidating the genetic cause of the disease in this patient. For one of the 8 patients successive samples were available showing the emergence of the CNLOH at the time point when the patient started developing an anemia.

Conclusion: Detecting acquired abnormalities when carrying out SNP analysis for postnatal referral reasons is very rare. The presence of acquired abnormalities might be of help in elucidating the genetic cause of the disease in the patient. In addition, it being present has to be taken into account when carrying out the analysis and when informing a patient or parent about possible additional findings.

P110: Fatigue in adults with a 22q11.2 deletion syndrome.

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Introduction:
The 22q11.2 deletion syndrome (22q11.2DS) is a genetic microdeletion syndrome with a high phenotypic variability. Recurrent pathology includes somatic disorders such as congenital heart diseases and velopharyngeal insufficiency as well as psychiatric diseases including schizophrenia spectrum disorders, anxiety disorders and mood disorders. After noticing a recurring complaint of fatigue in adolescents and adults with the 22q11.2DS during clinical consultations, we started administering the multidimensional fatigue inventory in a systematic manner to objectify these complaints.

Methods:
29 adults (mean age 27,8; SD 5,3) with a confirmed 22q11.2 DS using FISH or micro-array, filled in the multidimensional fatigue inventory (MFI). The MFI consists of 20 items and scores on 5 subscales: general fatigue, physical fatigue, reduced activity, cognitive fatigue and reduced motivation. In a subgroup these results were compared with results of the Beck Depression Inventory (BDI) (n=17) and the WHO Quality of Life questionnaire (n=13).

Results:
Total MFI scores and subscale scores seemed to be higher in adults with 22q11.2DS. 80 % had a total MFI score above the mean of the norms. For all 5 subscales we saw the same trend. We found a significant correlation between depressive symptoms scored on the BDI and MFI total score. MFI total score was also significantly associated with quality of life scores, specifically the general score, psychological health and environment.

Discussion:
This is the first report and description of high levels of fatigue in adolescents and adults with the 22q11.2 DS. Fatigue is a common complaint in this age group and should get the necessary attention since it does seem to have an impact on quality of life. Taking into account the multisystem nature of 22q11 DS present knowledge about 22q11.2DS and the findings of this explorative study, we recommend a systematic clinical examination to exclude underlying causes of fatigue in order to start an appropriate treatment of these complaints and the associated psychiatric symptoms. Further studies are needed on the prevalence of fatigue in 22q11 DS across age, and on the possible underlying mechanisms.

P111: A comprehensive cardiomyopathy gene panel

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Secondary cardiac arrhythmias, or cardiomyopathies are characterized by structural abnormalities of the cardiac muscle. These diseases are associated with arrhythmias, heart failure and sudden cardiac death. The cardiomyopathies are a heterogeneous group of diseases mainly comprising of hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and arrhythmogenic right ventricular dysplasia/arrhythmogenic cardiomyopathy (ARVD/ACM). The prevalence of these diseases ranges from 1/500 in HCM to 1/5000 in ARVD/C and in more than 50% of the cases there is evidence of familial inheritance. The structural arrhythmias are mainly associated with mutations in genes encoding desmosomal, sarcomeric, cytoskeletal and nuclear envelope proteins.

We developed a gene panel comprised of 184 genes associated with secondary cardiac arrhythmias. This gene panel, based on capturing probes, provides us with a flexible solution to perform next generation sequencing-based mutation detection in patients diagnosed with secondary arrhythmias.
For validation purposes 5 control samples (2 healthy, in-house control samples, 2 HapMap samples and 1 patient with known mutations) were sequenced using enriched exome sequencing (Illumina HiSeq) by complementing standard exome probes with the cardiomyopathy specific gene panel probes in order to obtain a diagnostics grade coverage of the genes included in the gene panel. In parallel the same samples were sequenced using only the secondary arrhythmia gene panel probes (Illumina MiSeq).

In a first step the coverage data was analyzed. Out of the 184 genes, covering about 3400 exons, there were only 15 exons that did not reach our cut-off value of 30x complete exonic coverage in both experimental set-ups. For these exons Sanger sequencing based gap filling was developed. In a second step all exonic variants identified in the cardiomyopathy gene panel in both experimental set-ups were compared. There was a complete concordance between the identified variants irrespective of the sequencing and enrichment methods used. All known variants present in the patient sample were also detected in both experiments.

We are currently evaluating this comprehensive gene panel in a pilot study of patients with a known (likely) pathogenic mutation in one of the core cardiomyopathy genes, identified either through single gene analysis or limited gene panel analysis. This study will help us on

the one hand in the further validation of the gene panel; on the other hand we simultaneously want to evaluate the additional diagnostic yield of this comprehensive cardiomyopathy gene panel as we expect to identify combinations of (likely) pathogenic variants that probably contribute to the complexity of cardiomyopathy development.

P112: Amplicon-based NGS analysis of a gene panel in a cohort of 200 HBOC patients

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Introduction
At the Center for Human Genetics of UZ Leuven, genetic testing of hereditary breast and ovarian cancer (HBOC) is offered in high risk families. This test included the analysis of the BRCA1 and BRCA2 genes, and the detection of the c.1100delC CHEK2 variant. This combination allowed to identify pathogenic mutations in 11% of the families. Recently, this strategy was adapted to include genes that are known to be associated with a high or moderate penetrance-risk for HBOC.

Methods
A cohort of 200 patients, that were eligible for HBOC genetic testing, was selected. Constitutional DNA from blood samples was extracted. The library consisted of a pool of 40 patients' samples that was prepared with the BRCA Hereditary Cancer MASTR Plus (Multiplicom) and sequenced on the Miseq platform (Illumina, 250bp-v2).

The data were analysed with the SeqNext module of the SeqPilot software (JSI Medical Systems). The thresholds were defined as a minimum sequencing depth of 40x in combination with a double filtering for variant frequency at 20% and 15% respectively. The core genes (BRCA1, BRCA2, CHEK2, TP53 and PALB2) were analysed.

Results
Out of the 200 samples, 7 samples (3.5%) presented a general drop out, i.e. the whole workflow had to be repeated. On average, 5 amplicons out of 1000 amplicons (0.5%) presented either of low coverage or a drop out. Pathogenic mutations were identified in BRCA1, BRCA2, CHEK2 and PALB2.

Conclusions
Gene panel analysis of HBOC patients with an amplicon-based approach is a robust method. We optimized the workflow to get an optimal output and so we were able to find a good balance between the efficiency of the test and the turn-around-time.

P113: Redefining the Charcot-Marie-Tooth type 2G disease: reassessment of clinical and linkage data leading to a new genetic diagnosis

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Charcot-Marie-Tooth disease (CMT) encompasses a highly phenotypically and genetically heterogeneous group of peripheral sensory and motor neuropathies. An estimated 20% of all patients are suffering from axonal type of CMT (CMT2). Mutations in over 40 genes involved in energy metabolism, cytoskeleton integrity, and vesicle transport have been show to cause CMT2. Yet, the known genes explain only 25% of all CMT2 cases. We have analysed a Spanish CMT2 family first described by Berciano et al. in 1986, whose genetic defect was initially linked to 12q12-q13.3 by Nelis et al., 2004. Unfortunately, neither genome sequencing nor copy number analyses could identify the culprit mutation within this region so far.

The negative results prompted us to clinically re-evaluate the patients and their siblings, especially in the youngest generations of the family. The genetic linkage analysis using the novel affection statuses re-defined the location of the disease-causing mutation to a 25Mb interval on chromosome 9q31.3-q34.3. Using a combination of whole exome and genome sequencing we identified a single coding missense variant (p.Cys694Tyr) in LRSAM1 that co-segregates with the disease. LRSAM1 is an E3 ubiquitin ligase with an important role in cargo sorting during receptor recycling. The identified missense variant is located in the RING domain of the protein, which is detrimental for its ubiquitylation function. Several mutations disrupting the RING domain have been previously reported to cause Autosomal Dominant CMT type 2P (CMT2P). Furthermore, another missense variant affecting the same residue (p.Cys694Arg) has been recently reported by Zhu et al., 2015 as causing CMT2P in two American families.

Our findings suggest that p.Cys694 is a hotspot residue and highlight the pivotal role of the RING domain of LRSAM1 in CMT development. Furthermore, this report emphasizes the pitfalls of genetic analysis and the difficulties in establishing the correct clinical diagnosis in late onset peripheral neuropathies.

P114: NOVOPlasty: In silico assembly of plastid genomes from whole genome NGS data and beyond.

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Thanks to the evolution in next-generation sequencer (NGS) technology, whole genome data can be readily obtained from a variety of samples. There are many algorithms available to assemble these reads, but few of them focus on assembling the plastid genomes. Therefore we developed a new algorithm that solely assembles the plastid genomes from whole genome data, starting from a single seed. The algorithm is capable of utilizing the full advantage of very high coverage, which makes it even capable of assembling through problematic regions (AT-rich). The algorithm has been tested on several whole genome Illumina datasets and it outperformed other assemblers in runtime and specificity. Besides the assembly of plastids, the algorithm is also capable to assemble targeted regions in the human genome. Therefore NOVOPlasty will be modified to assemble human genomic loci which are linked to genetic disorders.

Introduction
Chloroplasts and mitochondria are both responsible for generating metabolic energy within eukaryotic cells. Genetic defects within the mitochondrial genome can be linked to several disorders in humans. But assembling these plastids genomes is not always that straightforward with the currently available tools. Therefore we developed a new algorithm, specifically for the assembly of plastid genomes from whole genome data.

Methods
The algorithm is written in Perl. All assemblies were executed on Intel Xeon CPU machine containing 24 cores of 2.93 GHz with a total of 96,8 GB of RAM. All non-human samples were sequenced on the Illumina HiSeq platform (101 bp paired-end reads). The human mitochondria samples (PCR-free) were sequenced on the Illumina HiSeqX platform (150 bp paired-end reads). The Gonioctena intermedia sample was also sequenced on the PacBio platform.

Results & Discussion
Algorithm
The algorithm is similar to string overlap algorithms like SSAKE (Warren et al., 2007) and VCAKE (Jeck et al., 2007). It starts with reading the sequences into a hash table, which facilitates a quick accessibility. The assembly has to be initiated by a seed that will be extended bidirectionally in iterations. The seed input is quite flexible, it can be one sequence read, a conserved gene or even a complete mitochondrial genome from a distant species. Every base extension is determined by a consensus between the overlapping reads. Unlike most assemblers, NOVOPlasty doesn't try to assemble every read, but will extend the given seed until the circular plastid is formed.

Assemblies

NOVOPlasty has currently been tested for the assembly of 8 chloroplasts and 6 mitochondria. Since chloroplasts contain an inverted repeat, two versions of the assembly are generated. The differ only in the orientation of the region between the two repeats; the correct one will have to be resolved manually. Besides the mitochondrion of the leaf beetle Gonioctena intermedia, all assemblies resulted in a complete circular genome. A comparative study of four assemblers for the mitochondrial genome of G. intermedia clearly shows the speed and specificity of NOVOPlasty (Table 1).

Discussion

Despite the many available assemblers, many researchers still struggle to find a good assembler for plastids genomes. NOVOPlasty offers an assembler specifically designed for plastids that will deliver the complete genome within 30 minutes. The algorithm will be tested on more datasets and a comparative study with other assemblers is in progress. The algorithm can assemble limited regions of high quality in a short time frame, what makes it suitable for targeted assembly of certain loci in the human genome.

P115: The splicing factor SRSF2 is a dosage sensitive candidate oncogene in neuroblastoma

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Neuroblastoma (NB) is a pediatric cancer arising from the sympathetic nervous system and responsible for ~15% of all childhood deaths. MYCN plays a key role in development of aggressive NB and is amplified in about half of these cases. Gain of chromosome 17q often accompanies MYCN amplification indicating that one or most probably several genes on 17q cooperate with MYCN driven tumor formation due to dosage effects. In order to identify candidate cooperative driver genes, we established the transcriptomes of hyperplastic lesions and tumors as well as normal ganglia in a MYCN neuroblastoma mouse model in order to determine dynamic regulation of gene expression during the process of tumor initiation and progression. Amongst the top 20 ranked upregulated genes, we identified the Serine/Arginine Splicing Factor 2 (SRSF2). While SRSF2 is a well-known tumor suppressor in myelodysplastic leukemia, SRSF2 was recently also shown to be implicated in regulation of transcription. More specifically, SRSF2 binds to promoter-associated nascent RNA thus enabling the switch of P-TEFb from the 7SK complex to RNAPII leading to transcription pause release and transcription activation. In view of this function and given the strong SRSF2 upregulation during MYCN tumor formation, we propose SRSF2 as a key 17q cooperative oncogene. To this end, shRNA knock down and CRISPR knock out is currently being tested in a panel of NB cell lines to monitor effects on growth, clonogenicity, cell cycle, apoptosis and differentiation. In parallel, we make use of a recently successfully validated zebrafish MYCN neuroblastoma model as tool for testing candidate cooperative oncogenes. To this end we have injected embryos with SRSF2 tissue specific overexpression constructs and are currently performing tumor watch to monitor accelerated tumor formation. Further studies will include gene expression profiling of cell lines after SRSF2 knock down or in vitro and in vivo pharmacological inhibition of P-TEFb controlled transcription initiation using CDK7 and CDK9 inhibitors.

P116: Our Sequenced Lives. Living together in European biosocieties

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KU Leuven

As of the turn of the 21st century, the life sciences have begun to probe virtually all aspects of the human condition. This has far-reaching and unprecedented implications not just for science, but, increasingly, also for society. Genomic innovations are expanding our ability to control our own biology, changing our ideas of 'normality' and 'abnormality', transforming our understanding of humanness, identity and social relations, while generating important ethical, social, and regulatory dilemmas. Dealing with these dilemmas and challenges calls for new means of addressing them, beyond disciplinary boundaries. This requires not only collaboration between the social sciences and the humanities, but also between these disciplines and the life sciences. Such cross- and interdisciplinary efforts are crucial if we are to develop new ways of thinking about, and intervening in, the relations between biology and sociality and between the life sciences and society.

The Life Sciences & Society Lab at KU Leuven studies the social aspects and implications of the life sciences, including its profound effects on the making of sociality and social life, the very subject of social scientific research. Using a range of qualitative methods, the group conducts politico-sociological research into biomedical knowledges and their appropriation within diverse societal practices, ranging from insurance markets, public health, welfare institutions, and family lives to citizens' lived realities and the European polity. Our goal is to conduct robust social science research that reveals the challenges and opportunities of present-day biomedicine and seeks to articulate them as new vistas for living together in European biosocieties.

P117: Why do parents want carrier testing for their children? Perspectives of parents and genetic health professionals

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Introduction: The majority of international guidelines recommend carrier testing should not be performed in childhood. Although some parents want to know their other children's carrier status following the diagnosis of a child with a genetic condition, whether testing is ever facilitated is unclear. In addition, the reasons why this information is of interest to parents are relatively unstudied.

Methods: To answer these questions, semi-structured interviews were conducted with genetic health professionals (n=17), and parents of children with one of three genetic conditions (cystic fibrosis, haemophilia and Duchenne muscular dystrophy) (n=33) in Australia. Inductive content analysis was used to categorise responses and then the reasons discussed by both groups were compared.

Results: Interviews with both parents and genetic health professionals identified that some carrier testing does take place, although practices vary. Parents discussed multiple reasons underlying their desire to know their child's carrier status. Most commonly they expressed their desire to communicate the information to their children to provide them with reproductive options or prepare them for having an affected child. For some parents, information seeking was their way of coping. On the other hand, many genetic health professionals believed parents primarily want carrier testing because they are curious, or to reduce their own anxiety and be reassured of their child's health.

Conclusions: The misalignment between genetic health professionals' and parents' responses suggests that health professionals make assumptions about parents' motivations for requesting carrier testing for their children. This is likely to influence their responses to parents' requests for testing, which has important implications for genetic counselling practice.

P118: Dual targeting of ALK and RET: establishing a novel basis for the treatment of neuroblastoma.

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Background
Neuroblastoma (NB) is a pediatric cancer of the developing sympathetic nervous system for which survival rates for high-risk patients are still unsatisfactory. Moreover, current treatment is very harsh and toxic, causing severe short and longterm side effects. Therefore, the search for more effective and less toxic targeted drugs remains at the forefront of NB research. Activating mutations in the tyrosine kinase domain of the ALK transmembrane receptor are found in the majority of hereditary NB and occur as somatic defects in 7–10% of sporadic cases. Recently we showed that ALK mutations emerge or are selected for in relapsed NB. Small molecule inhibitors are available for targeted therapy in mutant ALK positive NB patients and open new possibilities for understanding the functional pathways through which ALK exerts its oncogenicity.

With the aim of selecting novel nodes for therapy intervention in NB, we have generated a 77-gene signature list reminiscent of mutant ALK activation in NB cells. Cross species genomics analysis of MYCN and ALKF1174 driven tumors and targeted ALK inhibition studies in vitro indicated that ALKF1174L leads to upregulation of the oncogenic tyrosine kinase RET in mouse models of NB. Inhibition of mutant ALK in NB cells results in robust downregulation of RET, indicating a strong functional relationship between these two oncogenes. We have further determined the efficacy of dual versus single targeting of mutant ALK and RET in NB cell lines in vitro and in vivo.

Material and Methods
Single and combinatorial targeting of mutant ALK and RET was performed on a panel of wild type, ALKF1174L and ALKR1275Q NB cell lines. Cell proliferation and apoptosis were assessed using Cell Titer Glow and Caspase Glo assays (Promega). Targeting compounds were selected based on their clinical applicability. Crizotinib (ALK-inhibitor) was combined with vandetanib, cabozantinib or sorafenib (RET-inhibitors). The combination index method by Chou and Talalay was used to determine an additive or synergistic effect between the drug combinations. In vivo experiments were performed using xenografted ALKR1275Q NB cells, carrying luciferase and GFP as reporter genes (CLBGA/Luc-GFP). Tumor response to drug treatment was determined by daily caliper measurements and bioluminescence imagining.

Results

Our preliminary results indicate that dual RET and ALK inhibition by crizotinib and vandetanib in vitro has a very strong anti-proliferative effect on cells compared to either drug alone. We have established the growth parameters and dose response curve to crizotinib of the xenografted NB cell line CLBGA/Luc-GFP in immunocompromised mice. We show that targeting ALKR1275Q in vivo results in initial regression of the tumor, followed by regrowth upon discontinuation of the treatment. Further experiments are ongoing to determine the activation status of RET in the resistant cells and to establish the appropriate experimental parameters for combinatorial ALK and RET inhibition in vivo.

Conclusions

Single compound treated tumors typically relapse following an initial response as is also the case for mutant ALK cells, either through acquisition of novel ALK mutations or mutations in downstream effectors of the ALK or other interfering signaling pathways. While higher affinity ALK inhibitors are underway, targeting the RET signaling pathway emerges as an important option for novel combination therapy which may render higher efficacy to future treatment in primary and relapsed NB patients.

P119: ETV5 functionally connects ALK and CXCR4 signaling in neuroblastoma

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Background: Neuroblastoma (NB) is an agressive pediatric tumor of the sympathetic nervous system, displaying a high metastatic potential and high relapse risk after initial therapy response. Understanding the molecular basis of this aggressive behaviour and the underlying events causing therapy failure are crucial towards identifying novel therapeutic strategies to more succesfully combat this disease. Activating mutations in the tyrosine kinase domain of the ALK transmembrane receptor occur in as much as 10% of sporadic cases and recently we also showed that ALK mutations emerge or are selected for at relapse. Moreover, we previously also identified ETV5 as one of the most robustly downregulated genes upon mutant ALK inhibition in NB cells. ETV5 is an oncogene belonging to the ETS group of transcription factors known to act as regulators of cell invasion and motility, and to control stem cell properties and neuronal cell fate decisions during development. Here we present important novel insights into the putative role of ETV5 in the ALK driven aggressive phenotype of NB cells. Importantly, these novel insights also provide a rationale for the design of innovative combination therapy to avoid or treat failure of conventional therapy.

Materials and methods: We validated ETV5 as ALK downstream target gene using activating ALK monoclonal antibodies and overexpression constructs. MAPK, PI3K and PLCgamma inhibitors were applied to test ETV5 upstream regulating pathways controlled by ALK. The effect of ETV5 knockdown on cellular growth and invasion capacity was analysed in ALK mutant NB cell lines in vitro and in xenografted SH-SY5Y ALKF1174L NB cell line. The ETV5 regulated transcriptome was determined by shETV5 knockdown in NB cell lines and xenografts by RNA sequencing.

Results: Stimulation of ALK activity resulted in robust accumulation of ETV5 protein in NB cells and we showed that ETV5 expression is under control of the MAPK signaling pathway downstream of ALK. Importantly, knockdown of ETV5 in ALK mutant NB cell lines alters their clonogenic potential and reduces the growth of NB tumors in vivo. Data mining analysis of gene expression changes in shETV5 xenografted cell lines allowed to generate an ETV5 signature score which correlated with overall survival of NB patients suggesting that ETV5 impacts on tumor aggressiveness. This ETV5 signature also overlaps with a transcriptional signature that predicts sensitivity to MEK inhibition in keeping with the recent view that MAPK activation contributes to therapy failure in NB. Additionally, gene set enrichment analysis (GSEA) of the ETV5 transcriptome identified gene sets controlling epithelial-to-mesenchymal transition and cell cycle. Finally, the chemokine receptor CXCR4 emerged as a crucial ETV5

target thus opening unexpected novel opportunities for drugging, as CXCR4 inhibitors are available.

Discussion: Our data highlight ETV5 as an intrinsic component of ALK downstream and RAS/MAPK signaling in neuroblastoma. The presence of RAS/MAPK and acquired ALK mutations in relapsed NB tumors highlights the significance of the ETV5 signaling pathway in NB pathogenesis. Moreover, ETV5 provides a functional link between the ALK and chemotaxis pathways, involved in cancer metastasis and identifies CXCR4 as novel drug target.

P120: An embryonic stem cell activated FOXM1 transcriptional program marks ultra-high-risk primary neuroblastoma patients for FDI-6 small molecule inhibition

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Introduction: Chemotherapy resistance is responsible for high mortality rates in high-risk neuroblastoma patients. MYCN is a major oncogenic driver in these tumors controlling pluripotency genes including LIN28B. Therefore, we hypothesized that enhanced embryonic stem cell (ESC) gene regulatory programs could mark tumors with increased risk for therapy failure enabling the selection of patients for novel targeted therapies.

M&M: A microRNA expression ESC-signature was established based on publically available data. In addition, an mRNA ESC-signature of top 500 protein coding genes with highest positive correlation with the microRNA ESC-signature score was generated.

Results: High ESC-signature scores were significantly correlated with worse neuroblastoma patient survival, both in the global patient cohort as well as in the subset of stage 4 tumors without MYCN-amplification. In addition, both in neuroblastoma and other embryonal tumors exhibiting MYCN-activation, the scores were significantly higher. This was confirmed in MYCN cell model systems where the scores altered upon MYCN-overexpression/knock-down. Using GSEA, we identified that genes implicated in DNA damage response and cell cycle control were strongly enriched in the signature. One of the genes in the signature is the transcription factor FOXM1, which is a master regulator driving those pathways. The upstream activator of FOXM1, MELK, was also part of the signature. Inhibition of FOXM1 in neuroblastoma cells using the small molecule FDI-6 significantly reduced cell viability. In addition, MELK inhibitors are currently tested in vitro and both FOXM1 and MELK inhibitors are evaluated in MYCN transgenic zebrafish models.

Conclusion: A novel ESC-signature score marks neuroblastomas with poor prognosis enabling the identification of ultra-high-risk neuroblastoma patients that may benefit from targeted therapies using FOXM1 or MELK inhibitors.

P121: HYPERTROPHIC REMODELLING IN CARDIAC REGULATORY MYOSIN LIGHT CHAIN (MYL2) FOUNDER MUTATION CARRIERS

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PURPOSE
Phenotypic heterogeneity and incomplete penetrance are common in patients with hypertrophic cardiomyopathy (HCM). We aim to improve the understanding in genotype-phenotype correlations in HCM, particularly the contribution of a MYL2 founder mutation and risk factors to left ventricular hypertrophic remodelling.

METHODS AND RESULTS
We analysed 14 HCM families of whom 38 family members share the MYL2 c.64G>A [p.(Glu22Lys)] mutation and a common founder haplotype. In this unique cohort we investigated factors influencing phenotypic outcome in addition to the primary mutation. The mutation alone showed benign disease manifestation with low penetrance. The co-presence of additional risk factors for hypertrophy such as hypertension, obesity or other sarcomeric gene mutation increased disease penetrance substantially and caused HCM in 89% of MYL2 mutation carriers (P = 0.0005). The most prominent risk factor was hypertension, observed in 71% of mutation carriers with HCM and an additional risk factor.

CONCLUSION
The MYL2 mutation c.64G>A on its own is incapable of triggering clinical HCM in most carriers. However, the presence of an additional risk factor for hypertrophy, particularly hypertension, adds to the development of HCM. Early diagnosis of risk factors is important for early treatment of MYL2 mutation carriers and close monitoring should be guaranteed in this case. Our findings also suggest that the presence of hypertension or another risk factor for hypertrophy should not be an exclusion criterion for genetic studies.

P122: A high-quality reference panel reveals the complexity and distribution of structural genome changes in a human population

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Structural variation (SV) represents a major source of differences between individual human genomes and has been linked to disease phenotypes. However, current studies on SVs have failed to provide a global view of the full spectrum of SVs and to integrate them into reference panels of genetic variation.

Here, we analyzed 769 individuals from 250 Dutch families, whole-genome sequenced at an average coverage of 14.5x, and provide a haplotype-resolved map of 1.9 million genome variants across 9 different variant classes, including novel forms of complex indels and retrotransposition-mediated insertions of mobile elements and processed RNAs. A large proportion of the structural variants (36%) were discovered in the size range of 21 – 100bp, a size range which remains under reported in many studies. Furthermore, we detected 4 megabases of novel sequence, extending the human pangenome with 11 new active transcripts. We show 191 known, trait-associated SNPs to be in strong linkage disequilibrium with a structural variant and demonstrate that our panel facilitates accurate imputation of SVs into unrelated individuals, which is essential for future genome-wide association studies. Finally, we used the family-based setup to analyse the spectrum and frequency of de novo SVs in the human genome.

P123: Whole genome sequencing of a dizygotic twin suggests a role for the serotonin receptor HTR7 in autism spectrum disorder

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Whole genome sequencing of a severely affected dizygotic twin with an autism spectrum disorder and intellectual disability revealed a compound heterozygous mutation in the HTR7 gene as the only variation not detected in control databases. Each parent carries one allele of the mutation, which is not present in an unaffected stepsister. The HTR7 gene encodes the 5-HT7 serotonin receptor that is involved in brain development, synaptic transmission and plasticity. The paternally inherited p.W60C variant is situated at an evolutionary conserved nucleotide and predicted damaging by Polyphen2. A mutation akin to the maternally inherited pV286I mutation has been reported to significantly affect the binding characteristics of the receptor. Therefore the observed sequence alterations provide a first suggestive link between a genetic abnormality in the HTR7 gene and a neurodevelopmental disorder.