BeSHG 2020
BRUSSELS

20th annual meeting of the
Belgian Society of Human Genetics

Genome for all?
THE SQUARE
Brussels Convention Centre
March 6th, 2020
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<td>Non-invasive prenatal diagnosis by genome-wide haplotyping of cell-free plasma DNA</td>
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<td>Lucia Lorenzi</td>
<td>The RNA Atlas, a single nucleotide resolution map of the human transcriptome</td>
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<td>Dale Annear</td>
<td>A Catalogue of the CGG Short-Tandem Repeats in the Human Genome</td>
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<td>Lore Pottie</td>
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<td>Two novel but unfinished genetic tales: 1. Transgenerational inheritance of an epigenetic effect in humans 2. The consequence of primate specific intergenic mutation</td>
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Dear Colleagues, Friends,

Welcome to the 20th BeSHG Meeting. Twenty, that’s right! It is 20 years since the BeSHG was created. Hence, we have an anniversary. Our journey started together with the new millennium – unless you want to argue the millennium started in 2000. It is our pleasure to host this Meeting in the heart of Brussels.

From the start, the organization of the Annual Meeting has been a rotating task between the Belgian genetic centres. Interestingly, it was ULB that organized the first meeting in 2001, and again today.

On this occasion, I sincerely wish to thank the Presidents, the Board members and all the participants of the Working Groups that have served the Society, and have helped it to grow. We have achieved a lot, but there are still lots of endeavours in human genetics and genomics. We are a young society after all, and the best is likely still to come. Basic and clinical research are thriving on a wave of genomic tools and discoveries. We are at the verge of a genomic world, with transitions from classical medicine to genomic health care, from one treatment suits all to personalized medicine, from cure to prevention.

Just as a reminder, please find below the short mission statement of our Society:

‘The aim of the Society is to allow all scientists involved in the field of human genetics, working in all Belgian universities, and all independent research institutes or genetic centres in Belgium to have an official representative Society in Belgium. The BeSHG is aimed at promoting Human Genetics in its wider sense, by supporting genetic research, improving exchanges between Belgium and foreign countries, organizing scientific meetings, and enhancing collaboration between Belgian labs. The BeSHG will represent the community of Belgian geneticists at the other national and international Societies of Genetics, and is intended to become a discussion forum on scientific, professional, social and ethical issues linked to the practice of human genetics.’

You see, we have come a long way! And there is still room for growth. So spread the news, and invite your colleagues and students to join us next year.

Enjoy this 20th anniversary meeting!

Sincerely,

Prof. Gert Matthijs,
President of the BeSHG

Prof. Guillaume Smits,
Chair of the 2020 organising committee
Gold Sponsors

Agilent Technologies

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t

Roche
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- PerkinElmer
  For the Better

- Sanbio
  Research & Diagnostics

- TWIST
  Bioscience

- Thermo Fisher Scientific
Invited Speakers
Stylianos E. Antonarakis

The search for high impact variants in the Population

University of Geneva

There are 4485 protein-coding genes in OMIM that if mutated with pathogenic (high impact variants) variants cause a fraction of the mendelian phenotypes. It has been estimated that the majority of protein-coding genes causing mendelian disorders have not yet been identified. Thus, an important objective in Genetic Medicine is to identify all genes (coding and non-coding) and all functional genomic elements that relate to the myriad traits of phenotypic variability. The “dominant” genes could be studied in outbred population by analyzing sporadic, denovo variants, and much progress has already been made in this regards. The “recessive” genes, however, require larger families with more than one affected individual and large genomic areas of identity by decent that could be found in consanguineous families. Research efforts and current achievements will be discussed. The accurate molecular cause of each mendelian disease, and the elucidation of the molecular pathophysiology substantially contributes to the diagnostic efforts, and provide the means for rationalistic treatment options.
Bjarni V. Halldórsson

Long read sequencing of 1,817 Icelanders provides insight into the role of structural variants in human disease.

Long-read sequencing (LRS) promises to improve characterization of structural variants (SVs), a major source of genetic diversity. We generated LRS data on 1,817 Icelanders using Oxford Nanopore Technologies, and identified a median of 23,111 autosomal structural variants per individual (a median of 11,506 insertions and 11,576 deletions), spanning cumulatively a median of 9.9 Mb. We found that rare SVs are larger in size than common ones and are more likely to impact protein function. We discovered an association with a rare deletion of the first exon of PCSK9. Carriers of this deletion have 0.93 mmol/L (1.36 sd) lower LDL cholesterol levels than the population average (p-value = 2.4·10⁻²²). We show that SVs can be accurately characterized at population scale using long read sequence data in a genome wide non-targeted fashion and how these variants impact disease.

Preprint: https://www.biorxiv.org/content/10.1101/848366v1
Flash Presentations

P47: A wide range of protective and disease causing variants in Aggrecan influence the susceptibility for otosclerosis

Lisse Tavernier

P64: WiNGS: A widely integrated platform for federated interpretation of whole genome sequencing data

Geert Vandeweyer

P15: Tyrosine kinase-inactivating mutations in EPHB4 cause severe forms of primary lymphedema and hydrops fetalis

Nassim Homayun Sepehr

P49: Transcriptome analysis of skin fibroblasts leading to a first somatic mutation in the catalytic domain of MAP2K1 in a melorheostosis patient.

Raphaël De Ridder

P4: Delineating Ehlers-Danlos syndrome, the classical subtype: molecular and clinical characteristics of a large patient cohort.

Marlies Colman

P44: The zebrafish as a model for reverse genetic screening of osteoporosis candidate genes

Jan Willem Bek

P31: Modeling PIK3CA-related overgrowth syndromes in Xenopus tropicalis.

Dionysia Dimitrakopoulou

P32: Exome-based panel testing as an efficient method to diagnose the highly heterogeneous ocular disorder spectrum Microphthalmia, Anophthalmia, Coloboma and Anterior Segment Dysgenesis (MAC-ASD)

Hannah Verdin
Oral Presentations
O1: A scoping review of forensic population genetics research in China: involvement of surveillance organizations and ethics of surveillance genomics

Yves Moreau¹ & Michiel Van Der Haegen²

¹ KU Leuven ESAT-STADIUS
² KU Leuven

We investigate the development of surveillance genomic technology in China by carrying out a scoping review of forensic population genetics (FPG) research in China. Because of ongoing concerns about abuses of forensic genetics technology in China, we review applicable ethical standards and relevant legislation, such as the 2013 Declaration of Helsinki, the US Common Rule, the Convention on Human Rights on Biomedicine, and publication ethics standards (which include explicit commitments to abide by the Declaration of Helsinki). We then assess the involvement of surveillance organizations in FPG research in China, as well as the intensity of this research against different ethnic populations (Tibet, Uyghur, other Muslim minorities, other Chinese minorities, and Han). We first conclude that FPG research on most-vulnerable populations (from Tibet, Xinjiang, and other Muslim minorities) involving surveillance organizations is highly unethical. Such research should not have been authorized, carried out, or published. The responsibility of scientific journal editors and publishers is also engaged. Moreover, our analysis shows that the problem is not limited to those most-vulnerable populations. Indeed, because the rapid growth of human identification DNA databasing across China suffers from a lack of privacy and human rights safeguards, all Chinese populations—including the Han population—should be considered vulnerable. Our analysis shows that about half of all recent FPG publications in China involve at least one co-author from surveillance organizations. It also shows a tight web of collaboration between surveillance organizations and other FPG researchers that makes carrying out research independently from those surveillance organizations essentially impossible.

The past two decades have seen the rapid development of total surveillance technology (i.e., the combination of internet censorship, GPS tracking, camera surveillance, facial recognition, social credit scoring, DNA profiling, etc.) in China. Moreover, surveillance technology has been a central element of ethnic and religious repression in Tibet and Xinjiang. DNA profiling is one of the key surveillance technologies. In 2016, forensic blood samples of the entire Xinjiang population aged between 12 and 65 were systematically collected. Chinese police forces in Xinjiang also purchased at least 40 capillary DNA sequencers in 2016, on top of the infrastructure available at the 20 existing forensic DNA laboratories in Xinjiang, together with a correspondingly large number of human identification reagent kits.
This infrastructure is sufficient to DNA profile at least a couple of millions of people per year, out of a population of about 25 million people.

The growth of DNA profiling in China has been extremely rapid with the objective of creating a national DNA database of at least 100 million DNA profiles by 2020. It contained 41 million profiles in mid-2016, 68 million profiles by the end of 2017, and 80 million in mid-2019. The Chinese national DNA database is thus already much larger than the next biggest national DNA databases (US, 16 million profiles; UK, 6 million profiles). While in terms of fraction of the population (UK: 9%, China: around 6%, US: 5%), it is still lagging behind the United Kingdom, it can be expected to even the UK percentage within two years. Moreover, DNA profiling has not been limited to sentenced criminals or people suspected of a specific crime, but the DNA database specifically targets “focus” persons, which includes activists, internal migrants, and sex workers, next to the targeting of ethnic minorities described above. Thus, DNA profiling is being used much more widely than can be justified for legitimate forensic purposes. Current use against vulnerable minorities and targeted groups cannot be considered to be proportionate law enforcement measures, but instead as an instrument of social control, especially because it is often associated with other forms of surveillance. Notwithstanding law enforcement successes, DNA profiling and databasing across China should thus be regarded primarily as genomic surveillance, rather than law enforcement.

Segmental duplications or low copy repeats (LCRs) are amongst the most complex architectures in the human genome. Scrutinizing these structures unraveled their important role during primate and human evolution. LCRs play an important role in the disease mechanism of genomic disorders as well. Misalignment of the LCRs during meiotic homologue pairing can result in non-allelic homologous recombination resulting in recurrent deletions and duplications. One of the regions with the highest proportion of segmental duplications in the genome is the 22q11.2 region. Deletions between LCRs on this chromosome 22 (LCR22s) cause the 22q11.2 deletion syndrome, the most common microdeletion disorder in humans. Research to this phenomenon was strongly hampered by the presence of three reference genome gaps in LCR22-A, the most proximal and largest LCR22. By using an LCR22-specific fiber-FISH method, interindividual hypervariability of the LCR22-A haplotype was discovered in the normal population. However, it remains unknown whether such variability is human specific or is also a feature of other primates.

Scrutinizing the reference genomes of the Great Apes uncovered that the orthologous loci of human chromosome 22 were enriched for sequence gaps in all species. As a consequence, it was not possible to rely on the reference. To chart the inter-species variability of these LCR22s in the Great Apes, we performed a de novo assembly of the haplotypes, using an LCR22-specific fiber-FISH, with probes designed to human DNA. The structures of five chimpanzees (Pan troglodytes), one bonobo (Pan paniscus), two gorillas (Gorilla gorilla), and six orangutans (Pongo pygmaeus) were analyzed to assess the level of variation between and within each species. The customized human LCR22-specific probe set was supplemented with BAC probes mapping to the regions between the LCR22s to examine the overall structure of the 22q11.2 region. The exact allele probe compositions were deduced by probe color changing experiments. In addition, we used optical mapping to chart the structure of the orangutans.

Compared to human, the chimpanzee, our closest ancestor, harbored the smallest LCR22-A allele ever observed in humans, containing core duplicons. There was no inter-individual structural variation between the investigated chimpanzees (n=5). The same haplotype was assembled for the bonobo (n=1). The gorilla haplotype had
small expansions compared to the chimpanzee, without intra-species variation (n=3). In contrast, the overall 22q11.2 region is different in the orangutan, including rearrangements in the sequence between the LCR22s, smaller LCR22s, and an LCR22-A haplotype not resembling the human one. In addition, structural variation between the six orangutans was present at the LCR22-A level.

For the first time, the LCR22s are evolutionary visualized at subunit level. This uncovered lineage-specific differences in LCR22 composition as well as in the unique surrounding region. Based on these results, we hypothesize that the LCR22s started to evolve to their human structures in the common Great Ape ancestor after the orangutan branch. The second noteworthy feature is the human specificity of the LCR22-A duplication expansions, since no structural variation was observed in the chimpanzee samples, harboring the smallest human-observed LCR22-A allele. This suggests an important role of the locus in human adaptation and evolution.
O3: Whole genome sequencing and 4C techniques provide novel insights into the genetic architecture and mechanisms underlying North Carolina macular dystrophy, a cis-regulatory disease

Stijn Van de Sompele, Eva D’haene, Thijs Van der Snickt, Sarah Vergult, Petra Liskova, Carlo Rivolta, Jenneke van de nEnde, Arthur A. Bergen, Irinia Balikova, Julie De Zaeytijd, Fadi S. Shaya, Kent W. Small & Elfride De Baere

1 Center for Medical Genetics, Ghent University and Ghent University Hospital, Ghent, Belgium
2 Research Unit for Rare Diseases, Charles University and General University Hospital, Prague, Czech Republic
3 Institute of Molecular and Clinical Ophthalmology, Basel University Hospital, Basel, Switzerland
4 Center for Medical Genetics, Antwerp University Hospital, Antwerp, Belgium
5 Department of Ophthalmology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
6 Department of Ophthalmology, Queen Fabiola Children’s University Hospital, Brussels, Belgium
7 Department of Ophthalmology, Ghent University and Ghent University Hospital, Ghent, Belgium
8 Macula and Retina Institute, Los Angeles and Glendale, California, USA

AIMS: North Carolina macular dystrophy (NCMD) is a rare autosomal dominant disease, characterized by loss of central vision. With the identification of noncoding sequence variants and duplications overlapping with a DNase I hypersensitive site near PRDM13 or IRX1 as underlying genetic cause, NCMD can be considered to be a cis-regulatory disease. These known genetic defects can only explain a fraction of all NCMD cases. Moreover, the mechanisms through which these defects disturb retinal development are still unknown.

METHODS: A cohort of 25 NCMD families underwent targeted testing of known genetic defects followed by whole genome sequencing (WGS) in the unsolved families. In addition, chromosome conformation capture techniques (4C-seq and UMI-4C) were applied on retinas of postmortem human donor eyes to fine-map interactions of cis-regulatory elements with the PRDM13 and IRX1 promoter. This data, together with other published or publically available human retinal (epigen)omics data, were integrated in a UCSC browser session to advance the interpretation of the WGS data.

RESULTS: First, a known noncoding single nucleotide variant (SNV) upstream of PRDM13 was found in one family, while a novel SNV at the same position was found in a second family. WGS revealed a novel tandem duplication, encompassing the same DNase I hypersensitive site upstream of PRDM13. Moreover, a novel SNV, located in an upstream noncoding region that physically interacts with the PRDM13
promoter, as shown by 4C-seq and UMI-4C, was found in a fourth family. Interestingly, this region is active at a specific developmental stage (D103) that is compatible with when retinal progenitor cells of the central retina exit mitosis. Several other noncoding interacting regions were identified by 4C-seq and UMI-4C, both for the PRDM13 (n=3) and the IRX1 (n=5) promoter.

CONCLUSIONS: The genetic architecture of NCMD was expanded with novel noncoding upstream variants with a likely effect on PRDM13 regulation. The retinal interaction profiles of PRDM13 and IRX1 advance the interpretation of novel noncoding variants identified using WGS and provide insight into the cis-regulatory mechanisms underlying NCMD, for the first time.
O4: Shallow whole-genome sequencing of cell-free DNA for B-cell lymphoma diagnosis and disease monitoring: a standardized approach with underappreciated potential

Lennart Raman¹, Malaika Van der Linden¹, Ciel De Vriendt², Bliede Van Den Broeck³, Kristoff Muylle⁴, Dries Deeren⁵, Kathleen Claes⁶, Björn Menten⁶, Fritz Offner² & Jo Van Dorpe¹

¹ Department of Pathology, Ghent University, Ghent University Hospital, Ghent, Belgium
² Department of Clinical Hematology, Ghent University, Ghent University Hospital, Ghent, Belgium
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⁶ Center for Medical Genetics Ghent, Ghent University, Ghent University Hospital, Ghent, Belgium

Background

Genetic material from B-cell lymphoma (BCL) is typified by recurrent multiple somatic alterations. Given the large cell turnover and its physical relation to the cardiovascular system, the disease presents itself as an excellent candidate for cell-free DNA (cfDNA) based research through liquid biopsies (LBs). Recent studies mainly focused on targeted sequencing techniques for single nucleotide variation and translocation detection. However, these methods remain expensive, require targeted panels and are yet to be clinically standardized. In contrast, the much cheaper shallow whole-genome sequencing (sWGS) for copy number alteration (CNA) detection, is operative at hospitals that offer noninvasive prenatal testing. Despite these benefits, a thorough evaluation of this approach for BCL diagnosis and disease monitoring was, until now, lacking.

Methods

Between May 2016 and September 2019, 126 BCL patients (39 Hodgkin [HL] and 87 non-Hodgkin [nHL] lymphoma) have been recruited for this study. In total, 257 case samples were analyzed, including 126 LBs at baseline; 98 longitudinal follow-up samples from 32 patients; and 33 paired solid biopsies (SBs), obtained as formalin-fixed paraffin-embedded (FFPE) samples. HL patients were initially treated with ABVD (Adriamycin, Bleomycin, Vinblastin, Dacarbazin), whereas nHL patients received R-CHOP(-like) (Rituximab, Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisone) therapy. The cohort was ultimately extended with 60 negative LB controls. DNA was sequenced by sWGS (0.5x coverage), where resulting reads...
were mapped to both human and lymphoma-associated viral reference genomes (e.g. Epstein-Barr virus [EBV]).

The copy number profile abnormality (CPA) score was developed to quantify the genome-wide deviation from the healthy diploid state. Likewise, the normalized viral read fraction (VRF) was defined to reflect the viral load. The 99th percentile of previous variables’ theoretical normal distributions, defined by the controls’ mean and standard deviation, was used as a 1% false discovery rate cutoff for abnormality calling. These limits were subsequently used to obtain tumor and virus detection sensitivity and specificity statistics.

Results

During tumor staging, 78.3% of HL and 73.5% of nHL LBs had detectable CNAs. Copy number profiles from solid-liquid pairs showed high concordance within nHL patients ($r=0.815\pm0.043$). The CPA score was higher in LBs for both HL ($P=.005$) and nHL ($P=.026$).

In 39/225 (17.3%) LBs, an elevated EBV VRF was noted. All but two EBV positive samples also had an abnormal CPA score, which significantly associates the latter categorical variables ($P=.005$). All samples that tested positive during routine chromogenic in situ hybridization (CISH) for EBV-encoded RNA detection on FFPE tissue had abnormally elevated EBV levels in plasma, whereas only two of the 34 CISH negative samples were retrieved as ‘false positives’, with subtle yet elevated amounts of plasmatic EBV titers.

Observations were consistent for refractory and relapsed patients: when detectable, CNA patterns were very similar across different longitudinal staging moments. Amongst the patients with follow-up, five were detected to be EBV positive (according to sWGS of cfDNA) at diagnosis. Of these, three went into complete remission and consequently no longer had abnormally elevated EBV levels; one refractory patient was recurrently noted with high EBV titers; and one relapsed patient was ultimately detected with an unusually high EBV VRF, after it earlier dropped to 0. Very similar conclusions could be made for the CPA score.

Following an extensive concordance analysis, the CPA score was found to be positively and significantly associated to tumor fraction; Ann Arbor stage; International Prognostic Index; lactate dehydrogenase and beta-2 microglobulin concentration; and, metabolic tumor volume, derived from PET/CT scans.
Finally, distinguishing HL from nHL, is characterized by an area under the curve of 0.968 during receiver operating characteristic analysis applied to an iterative out-of-bag random forest modeling procedure.

Conclusions

Both CNAs and viral fragments can be detected more frequently in LBs than SBs of BCL patients. CNA patterns remain more or less constant in refractory/relapsed patients across staging moments. There were no false positive LBs regarding tumor detection. Changes in copy number state fluently correlate to disease status. Finally, as a proof of principle, copy number profiles enable histological classification between, amongst others, HL and nHL.
O5: Non-invasive prenatal diagnosis by genome-wide haplotyping of cell-free plasma DNA

Huiwen Che1, Darine Villela2, Eftychia Dimitriadou3, Cindy Melotte1, Nathalie Brison1, Maria Neofytou1, Kris Van Den Bogaert1, Olga Tsuiko1, Koen Devriendt1, Eric Legius1, Masoud Z. Esteki3, Thierry Voet1 & Joris R. Vermeesch1

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Whereas non-invasive prenatal testing for aneuploidies (NIPT-A) is widely implemented, there is an increasing need for universal approaches for noninvasive prenatal testing for monogenic diseases (NIPT-M). Here, we present a cost-effective, generic cell-free fetal DNA (cffDNA) haplotyping approach to scan the fetal genome for the presence of inherited monogenic diseases. Families participating in the preimplantation genetic testing for monogenic disorders (PGT-M) program were recruited for this study. 250000 SNPs captured from maternal plasma DNA along with genomic DNAs from family members were massively parallel sequenced. Parental genotypes were phased via an available genotype from a close relative, and the fetal genome-wide haplotype and copy number were determined using cffDNA haplotyping analysis based on estimation and segmentation of fetal allele presence in the maternal plasma. In all families tested, mutational profiles from cffDNA haplotyping are consistent with embryo biopsy profiles. Genome-wide fetal haplotypes are on average 97% concordant with the newborn haplotypes and embryo haplotypes. We demonstrate that genome-wide targeted capture and sequencing of polymorphic SNPs from maternal plasma cell free DNA (cfDNA) allows haplotyping and copy number profiling of the fetal genome during pregnancy. The method enables the accurate reconstruction of the fetal haplotypes and can be easily implemented in clinical practice.
O6: The RNA Atlas, a single nucleotide resolution map of the human transcriptome

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The introduction of RNA-sequencing has enabled us to interrogate the human transcriptome at nucleotide resolution, revealing distinct RNA biotypes beyond protein-coding RNAs. Several consortium-based efforts have contributed to the discovery and quantification of these RNA biotypes in heterogeneous sample collections. However, these studies have mostly applied RNA-sequencing technologies dedicated to the small RNA and polyadenylated RNA fraction of the transcriptome. As a result, we still lack a systematic survey of the non-polyadenylated transcriptome and the circularized transcriptome and their relationship to other RNA biotypes. To capture the full diversity of the human transcriptome, we applied complementary RNA-seq methods on a heterogenous collection of 300 human samples including 45 tissues, 162 cell types and 93 cell lines. From these samples, strand-specific polyA, total RNA and small RNA libraries were generated and deeply sequenced to a total of 125 billion reads. By integrating these datasets, we assembled transcripts representing five major RNA biotypes, including...
mRNAs, lincRNAs, asRNAs, circRNAs, and miRNAs, culminating in a stringently selected transcriptome and its matching expression atlas. The majority of novel loci were non-coding in nature and highly enriched for non-polyadenylated single-exon lincRNAs. Additionally, we exploited broad intron-coverage from total RNA-sequencing to test and verify functional regulation by newly characterized ncRNAs, revealing 105 novel miRNAs and 1802 novel lncRNAs with clear evidence for target regulation. Our study represents a substantial expansion of the current catalogue of human ncRNAs and their regulatory interactions across a large set of cell types, tissues and cancer cell lines. All data, analyses and results are available for download and query in the R2 web portal and serve as a basis to further explore RNA biology, function and relation to disease.
O7: Deciphering oligogenic diseases with ORVAL

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The emergence of novel bioinformatics methods and resources able to analyse variant data from large cohorts of patients has revolutionised the field of medical genetics, allowing the identification of a large number of disease-related mutations relevant for diagnosis and therapy. Although these approaches have greatly improved our understanding of Mendelian cases, many difficulties remain to analyze more intricate genetic models involving variant combinations in different genes, an essential step for the discovery of the causes of oligogenic diseases. Digenic or bi-locus cases reported in scientific literature and compiled in the Digenic Diseases Database (DIDA), allowed for the development of VarCoPP, a machine learning method able to predict disease-causing bi-locus variant combinations. Furthermore, by using this data, a predictor that identifies the digenic effect class of a pathogenic bi-locus variant combination was created (i.e. the Digenic Effect predictor).

ORVAL (the Oligogenic Resource for Variant AnaLysis) integrates these new methods and generates networks of pathogenic variant combinations in gene pairs, as opposed to isolated variants in unique genes. It provides a novel user-friendly web platform that allows clinicians and researchers to predict the pathogenicity of an individual's variant combinations and examine potential oligogenic signatures within their biological context.

The ORVAL platform consists of a submission form, where users can submit genetic variant data with optional variant and gene filtering criteria. A variant processing pipeline generates variant combinations, annotates them and then predicts which of them may be disease-causing using VarCoPP.

The results are presented according to three levels of aggregation:
- At the variant combination level, each prediction is displayed along with their scores and can be further analysed with the Digenic Effect predictor by predicting whether a pathogenic combination is predicted as a True Digenic, a Monogenic + Modifier or
a Dual Molecular Diagnosis case. VarCoPP is also made interpretable by displaying the preference of each predictive feature for the neutral or disease-causing class.
- At the gene pair level, the disease-causing digenic predictions are used to rank gene pairs based on their predicted median pathogenicity.
- Finally, all gene pairs containing at least one predicted pathogenic variant combination, are displayed as an "oligogenic network". Users can interact with the network and filter the most relevant gene modules and explore them in a dedicated page that contains additional information and interactive visualisations, including the associated PPIs, protein cellular locations and pathway mappings of the involved genes.

ORVAL offers an innovative web-platform that integrates different resources to predict disease-causing oligogenic variant combinations and visualises the results within their biological context to help in understanding the underlying molecular effects and phenotypes associated. This tool provides a new essential step towards helping clinicians and researchers to improve their diagnosis for more complex genetic diseases.

ORVAL is freely available at https://orval.ibsquare.be.
O8: A Catalogue of the CGG Short-Tandem Repeats in the Human Genome

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Greater than 30 genetic disorders have been identified resulting from short-tandem repeat (STR) expansions. STRs are DNA motifs ranging from 1 to 6 nucleotides that are repeated in tandem, usually between 5 to 200 units, and they account for 3% of the total human genome. The CGG-repeat is a trinucleotide STR and is the genetic aetiology of the most common form of inherited intellectual disability and monogenic form of autism spectrum disorder, fragile X syndrome (FXS). In addition to FXS, the CGG-repeat is associated with several other neurological disorders including fragile X tremor ataxia syndrome (FXTAS), Jacobsen syndrome, neuronal intranuclear inclusion disease (NIID), Baratela-Scott syndrome, and DIP2B-associated and AFF3-associated intellectual disability. Highly repetitive genomic regions are a challenge to analyse, especially when GC-rich, and this has resulted in limited information on CGG-STR distribution in the human genome. Nonetheless, there is strong evidence linking expanded CGG-repeats to multiple rare disorders.

We hypothesize that multiple as yet undisclosed CGG-repeat expansions in the genome exist and contribute to human disease. Here, we aimed to catalogue the CGG-repeats within the human genome utilizing genome-wide STR genotyping tools, including Tandem Repeats Finder, GangSTR, and ExpansionHunter. A control cohort of 100 individuals with no known repeat-related disorders or repeat expansions were analyzed to detect and characterize CGG-repeats.

6110 unique CGG-repeats were detected in our control cohort. 76% of the CGG-repeat loci were polymorphic, while the remaining 24% of the CGG-repeat loci were stable. 341 genes that have been related to intellectual disability, contained or were within proximity of at least one polymorphic CGG-repeat. Different CGG-repeat distributions were observed across the chromosomes. While chromosome 1 contained the largest number of CGG-repeats, chromosome 22 showed the highest density of both total repeat and unstable repeat numbers with 5.7 repeats per Mbp and 4.5 polymorphic repeats per Mbp, respectively. We observed that 30% of all CGG-repeats were present within the 5’ untranslated region. Interestingly, CGG-repeats were near to absent in the 3’ untranslated and the immediate downstream gene regions. Every individual of our cohort displayed CGG-repeat polymorphisms and between 13% to 25% of the CGG-repeat loci in each individual were polymorphic, as they differed from the median population repeat length. Furthermore, it was observed that many of the polymorphic repeat loci could
undergo a significant expansion event, with several loci reaching a size equivalent to a premutation range as observed in other disorders. In general, the larger the median repeat length, the more polymorphic the CGG-repeat is, with repeats with a median size of 12 or higher always being polymorphic. However, smaller repeats also display considerable rates of polymorphism, for example, 55% of CGG-repeats with a median repeat size of 4 are polymorphic. While considerable variance in repeat length was expected in larger, polymorphic CGG-repeats, unexpectedly, we observed substantial expansions in CGG-repeat loci which normally exhibited exceedingly short median repeat lengths.

We have identified and categorised over 6000 novel CGG-repeat loci within the human genome. We have determined that at least three-quarters of the CGG-repeats are polymorphic, and identified many repeats that display characteristics similar to the known disease-causing CGG-repeats. Our research raises further questions about the biological role of CGG-repeats and what risk they may pose concerning inherited genetic disease.
O9: The role of FBN2 in Carpal Tunnel Syndrome: From Rare Disorder to Common Risk Factor?

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Carpal tunnel syndrome (CTS) is the most common form of peripheral entrapment neuropathy. Although CTS is quite common, its pathogenesis remains largely unknown. Recently, we have ascertained a family in which CTS occurred in subsequent generations at an usually young age. The earliest age of onset was 10 months. Additional clinical features in the affected individuals were a distinct face with short palpebral fissures, relatively short stature (height between -2 and -2.5 SD), and short Achilles tendons resulting in toe walking in childhood. Brachydactyly was a constant feature. Genome-wide linkage screen followed by whole exome sequencing (WES) revealed a heterozygous variant (c.5009T>G; p.Phe1670Cys, CADD=29.5, absent in Gnomad) in the fibrillin-2 (FBN2) gene that was subsequently shown to co-segregate with the abnormal phenotype in the family. The variant is located in the 23th calcium-binding EGF-like (cbEGF) motif of FBN2 and adds an extra cysteine residue, which are known to be important residues in intra-and intermolecular bridging. Size exclusion chromatography and multi-angle laser light scattering (MALLS) of recombinant mutant FBN2 fragments showed about 30% dimer formation of the mutant fragments, compared to almost no dimer formation of wild type FBN2 fragments. Since the variant is located close to a RGD sequence that interacts with cell surface receptors of the integrin family to mediate cell adhesion, we also performed cell adhesion assays. These experiments showed a decreased fibroblast adhesion to both mutant monomer and mutant dimer FBN2
fragments compared to wild type FBN2 fragments. Moreover, since a structural fibrillin-2 defect may affect normal TGF-β signaling and given the evidence for increased TGF-β signaling in carpal tunnel connective tissue of CTS patients, we have further investigated the effect of this variant on TGF-β signaling and histology in the carpal tissues of the family members. Immunohistochemical analysis of two family members revealed tissue fibrosis and an increased TGF-β signaling in the carpal tissues, especially in the transverse carpal ligament. In a further step, we wanted to investigate the role of FBN2 in the pathogenesis of CTS in a larger population. A variant burden test in a cohort of 216 CTS patients revealed a significantly (p<0.001) increased frequency of FBN2 variants in CTS patient alleles (13.7%) compared to controls (5.2%). These data strongly suggest a role for FBN2 in the pathogenesis of CTS.
O10: Unique trisaccharide proteoglycan linker region in b3galt6 knock-out zebrafish, recapitulating beta3GalT6-deficient disorders in human

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Galactosyltransferase II (β3GalT6), encoded by the B3GALT6 gene, is an essential enzyme for the biosynthesis of the tetrasaccharide linker region that attaches glycosaminoglycan (GAG) chains to proteoglycan core proteins. Biallelic loss-of-function variants in B3GALT6, cause two overlapping autosomal recessive disorders, spondylodysplastic Ehlers-Danlos syndrome (spEDS-B3GALT6) and spondyloepimetaphyseal dysplasia with joint laxity type I (SEMD-JL), affecting various connective tissues, including skin, bone, cartilage, muscle, tendon and ligaments. In this work, we established and characterized the first viable animal models for β3GalT6-deficiency through CRISPR/Cas9-mediated knock-out of the zebrafish b3galt6 gene. Anion-exchange HPLC demonstrated a strong decrease in chondroitin, dermatan and heparan sulfate dissacharide levels in bone, muscle and skin from b3galt6−/− zebrafish, indicating that, despite the absence of B3galt6 activity, small amounts of GAGs can still be produced. Subsequent LC-MS/MS analysis of the proteoglycan linker region of b3galt6−/− zebrafish revealed, for the first time, the presence of a unique trisaccharide linker region in an animal model, consisting of only one galactose instead of two. Furthermore, b3galt6−/− zebrafish recapitulate many aspects of spEDS/SEMD-JL including generalized skeletal dysplasia, craniofacial dysmorphologies, skin involvement and indications for muscle hypotonia. We found disturbed collagen fibril organization to be the most striking and consistent characteristic throughout different affected tissues. Taken together, we created a representative zebrafish model for spEDS/SEMD-JL and our findings unveil a new rescue mechanism for proteoglycan production in the absence of an essential galactosyltransferase, opening new avenues for therapeutic intervention.
Main techniques: CRISPR/Cas9 gene editing, sequencing, anion-exchange HPLC, LC-MS/MS, Alcian blue staining, Alizarin red staining, µCT scanning, transmission electron microscopy & swim tunnel experiments.
O11: Functional characterization of a Xenopus tropicalis knockout and a human cellular model of RCBTB1-associated inherited retinal disease shows involvement of RCBTB1 in the cellular response to oxidative stress

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Purpose: The function of RCBTB1, a gene that has recently been implicated in syndromic and non-syndromic inherited retinal disease (IRD), remains unknown so far. Patients with biallelic missense variants in RCBTB1 display diverse IRD phenotypes varying from retinitis pigmentosa and reticular dystrophy to chorioretinal atrophy. Here, we tested the hypothesis that RCBTB1 is involved in Nrf2-regulated protection against reactive oxygen species (ROS) in the eye, and more specifically in the retinal pigment epithelium (RPE).

Methods: A Xenopus tropicalis knock out (KO) animal model for rcbtb1 was generated using CRISPR/Cas9 genome editing. Histological examination and additional three-dimensional electron microscopy was performed on retinas of the rcbtb1 frogs. RNA-seq analysis was performed on RCBTB1-mutated patients' lymphocytes, treated with H2O2, as well as on embryos from the Xenopus tropicalis KO treated with CdCl2. An RCBTB1 knockdown (KD) cell line was generated in ARPE-19 cells and a variety of functional assays (e.g. flow cytometry, MTT-assay, cell death kinetics) was used to assess the consequences of RCBTB1 loss-of-function.

Results: Rcbtb1 animals showed dystrophic changes in the RPE, similar to observations in human cases, including loss of apical-basal cell polarity, loss of cuboidal cell morphology, spreading of the pigment granules and vacuolisation. Nrf2 downstream targets and several metallothioneins were differentially expressed in RNA-seq experiments, both in the KO animal and in human cellular models. The
functional assays in ARPE-19 cells revealed that RCBTB1 depletion affects cellular responses to external insults of oxidative stress.

Conclusions: We showed that the Xenopus tropicalis rcbtb1 KO recapitulates the human IRD phenotype, making it an excellent model to study RCBTB1-disease. Both in vivo results together with in vitro functional data generated on a human cellular model show involvement of RCBTB1 in the cellular response to oxidative stress. This provides insight into the mechanism underlying RCBTB1-associated IRD and uncovers potential therapeutic opportunities.
O12: Mutations in LTBP1 cause autosomal recessive cutis laxa syndrome

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Latent transforming growth factor ß (TGF ß) binding protein 1 (LTBP1) is an important extracellular matrix (ECM) component that interacts with fibrillin microfibrils and sequesters TGFß. Exome sequencing (ES) in two consanguineous families presenting with a variable constellation of mild cutis laxa (CL), facial dysmorphism, craniosynostosis, brachydactyly, syndactyly, cleft palate, and short-limbed stature identified homozygous premature truncating variants (PTV) in LTBP1, respectively a nonsense variant in exon 30 and a 1 bp deletion in exon 34. The former variant causes nonsense-mediated decay while the latter variant results in a stable mRNA as shown by RT-PCR with and without cycloheximide treatment. In electron microscopy on dermal tissue of patient PII:1, the elastic fibers appear mildly fragmented, with a ruffled microfibrillar network. Collagen is normal with regular diameters and tightly assembled collagen fibrils.

To investigate the disease mechanism underlying LTBP1-related CL, we used targeted CRISPR/Cas9-induced indel mutagenesis to disrupt ltbp1 in danio rerio. We generated two zebrafish lines with PTVs in ltbp1 regions homologous to the LTBP1 sites harboring the human variants, respectively a one base-pair deletion in exon 29, c.3271delG; p.(Gly1090Asp*28), and a ten base-pair deletion in exon 35, c.del4003_4012TGCCTGTGCT, predicted to result in p.C1334EfsX17, hereafter referred to as ltbp1cmg67/cm67 and ltbp1cmg76/cm76 mutant zebrafish. Mutant zebrafish show normal development and growth, but an increase of extra membranous bone on the haemal and neural arches of the caudal vertebrae at 4 and 11 months of age in both lines. At an early developmental stage (6 days post fertilization (dpf)), gene expression of ltbp1 is significantly downregulated in both
mutant lines, and fbn2b, efemp2a, fbln5 and tgfb2 were up. At the juvenile stage (15 dpf), gene expression of fbn1, fbn2a, fbn2b is significantly downregulated in ltbp1cmg76/cm76 mutant zebrafish and loxa is upregulated.

In conclusion, we identified pathogenic variants LTBP1 as a novel cause for cutis laxa with skeletal manifestations, which was partly recapitulated in a zebrafish model. These promising findings will enable us to study LTBP1 function in vivo and identify targets for therapeutic strategies in bone disorders.
Poster Presentations
P1: Initiative for the harmonization of the genetic proficiency testing performed by the Belgian Medical Centers for Human Genetics in the context of Rare Diseases

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Aims: The Belgian Plan for Rare Diseases¹ (RDs) has a willingness to support the Belgian Medical Centers of Human Genetics (BMCHGs) in the development of a Quality Management System and the participation to External Quality Assessment programs (EQAs). One of the objectives of the Action 2 of this plan is to develop a funding for the participation of the BMCHGs to EQAs focused on genetic tests performed in the context of hereditary rare diseases and hereditary rare cancers.

Method: At first instance, a screening of quality controls available in Belgium and abroad was performed in order to identify controls focusing on the diagnosis of hereditary rare diseases. Based on this preliminary screening step, all quality controls to which BMCHGs participate were inventoried by Sciensano (Belgian Institute for health).

Among them, financeable EQAs offered by accredited providers were analyzed by a working group (16 BMCHGs’ experts and 1 Sciensano quality officer) in order to select financeable ones.
Results: The working group has selected 75 financeable EQAs for which participation fees should be covered by the Belgian healthcare authorities. This selection was made on the basis of the clinical relevance and needs of available programs. Besides, the WG has proposed minimal frequencies of participation to those EQAs and general quality recommendations for dealing with poor performances and change management.

Indeed, no Belgian directives about the minimal frequency of participation to genetic EQAs exist and European recommendations in this field are numerous and heterogeneous between the different member states. In order to address this lack in consensus, the working group has developed national guidelines about the minimal frequency of participation to EQA schemes focused on hereditary rare diseases, with reference to international recommendations and national laboratory practice.

In these proposals, we recommend to annually assess all methods, technological platforms, (bio-informatic) pipelines, and performance of individual diagnostic tests, if possible through EQAs covering the genotyping and clinical interpretation, even if this latest is based on made-up clinical cases and reproduced images. In case of no available EQA focused on a specific method, an annual participation to disease-based EQAs involving the technique of interest is recommended. A few exceptions were included for particular circumstances or diseases. In case of poor performances impacting the diagnosis of the patient, centers should participate to an EQA the following year and implement actions to avoid future errors.

Conclusions: The guidelines will provide general recommendations to Belgian Medical Centers for Human Genetics on how often it is necessary to perform EQAs and thus, help them to improve their quality management. The guidelines will also help the Belgian Healthcare institution to estimate the budget needed to cover the participation fees of the BMCHGs.

The proposals of financeable EQAs and the necessary budget to cover participation fees have been submitted to the Belgian National Institute for Health and Disability Insurance (RIZIV-INAMI) for evaluation and approval in November 2019.

1. Plan Belge pour les Maladies rares, 2013
P2: Initial presentation of juvenile and adult patients with Autosomal Recessive Polycystic Kidney Disease (ARPKD)

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Introduction
Autosomal recessive polycystic kidney disease (ARPKD) is a rare form of polycystic kidney disease, usually presenting during early childhood. The clinical courses and complications in adulthood has been recently described in a large adult ARPKD cohort based on the international ARPKD registry and cohort study ARegPKD (Burgmaier et al, 2019).

Material & methods
Here we reviewed the initial presentation of 13 patients with ARPKD with a median age of 32.5 years at diagnosis ranging from 5 to 65 years. The cohort included 5 patients derived from pediatric nephrology division, 3 from adult nephrology division and 5 from adult gastroenterology and hepatology division. Clinical diagnosis is confirmed by molecular analysis of PKHD1 gene and most of patients were heterozygous for at least two class 4 variants (one is homozygous).

Results
Majority of patients (10/13) showed hypersplenism with thrombocytopenia as the consequence of progressive hepatic fibrosis (age ranging from 5 to 65y).
Liver cysts were described in 4/13 patients (by MRI in the 3 adults (3/8) at median age 50y (range 36-65y)).
Renal phenotype is highly variable with normal renal function in 9/13. Three showed chronic kidney disease while ESRD was the primary manifestation in an additional young girl. Interestingly, this patient presented normal blood pressure with normal kidney size but abnormal corticomedular differentiation. There were no renal or hepatic cysts found in this patient. This is not uncommon because only 7 patients exhibited multiple renal cysts (7/13). Finally, the presence of both liver and renal cysts leaded to clinical ADPKD diagnosis in 2 adult patients (2/8).

In one child (12 years old boy), the diagnosis was incidentally made by exome sequencing for neurodevelopmental delay. Renal ultrasound and biochemical data are both normal.

Conclusion
The present cohort illustrates different facets of ARPKD in patients who received their diagnosis later in life.
**P3: Paraoxonase 1 polymorphism L55M as a regulator of metabolic relevant pathways**

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Paraoxonase-1 (PON1) is a multifunctional protein primarily synthesized in the liver and secreted in the blood plasma where it associates with high density lipoprotein. The enzyme hydrolyses several organophosphorus pesticides, a number of exogenous and endogenous lactones, and metabolizes toxic oxidized lipids thereby exerting anti-oxidant and -inflammatory properties. PON1 activity varies widely among individuals and although nutritional, lifestyle and pharmaceutical factors are known, the biggest effect on PON1 levels is through genetic polymorphisms. Over 200 polymorphisms are already identified of which -108C/T, L55M and Q192R explain most of its variability. Several disease states (atherosclerosis, cancer, metabolic disease) have been associated with a decrease in PON1 expression. To further elucidate polymorphism-dependent changes in the PON1 phenotype in relation to metabolic disease, we genotyped the three common PON1 polymorphisms in a population of 146 obese patients with(out) non-alcoholic fatty liver disease. Transcriptome profiling with Affymetrix Genechip arrays was performed on liver biopsies. A weighted gene co-expression network analysis was carried out and illustrates that PON1 has a regulatory effect on genes of metabolic relevant pathways (p=0.001). In particular, PON1 L55M was strongly correlated with the expression of one specific gene. Further functional experiments in HepG2 cell lines are ongoing to validate our results.
P4: Delineating Ehlers-Danlos syndrome, the classical subtype: molecular and clinical characteristics of a large patient cohort.

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Introduction: The Ehlers-Danlos syndromes (EDS) are a clinically and molecularly heterogeneous group of rare heritable connective tissue disorders hallmarked by joint hypermobility, skin hyperextensibility and symptoms of general tissue fragility. The most recent classification distinguishes 13 different subtypes with pathogenic defects in 19 different genes. The severity of these disorders ranges from mild conditions to complex multisystemic disorders with disabling and sometimes life-threatening complications such as chronic widespread pain and vascular fragility. A disturbance of the collagen fibrillogenesis at one level or another is thought to be the central pathophysiological pathway of EDS.

The classical subtype of EDS (cEDS) is an autosomal dominant disorder characterized by the typical (combined) presence of joint hypermobility, skin hyperextensibility and wound healing problems with formation of atrophic widened scars. Pathogenic variants in either COL5A1 or COL5A2, encoding the α1 and α2 chains of type V collagen give rise to cEDS. Type V collagen is a minor fibrillar collagen with a broad tissue distribution. It is mainly found as \[\alpha_1(V)2\alpha_2(V)\] heterotrimers, which co-assemble with type I collagen into heterotypic fibrils. Reduced availability of type V collagen seems to be the major disease-causing mechanism of cEDS. Nevertheless, large gaps in our knowledge and understanding of this disease remain. We gathered and analyzed clinical and molecular data of 230 patients from 166 unrelated families with a clinical suspicion of cEDS.

Results: A causal molecular defect was identified in >90% of the families (n=143, 198 individuals). In 82,5% of the families (n=118, 171 individuals) a (likely) pathogenic in COL5A1 was found with a total of 100 unique (likely) pathogenic variants. 56% of these introduced (or were predicted to introduce) a premature termination codon, likely resulting in a null allele. Structural defects accounted for 28% of COL5A1 variants, including glycine substitutions (10%) mainly located in the helical region, in-frame indels (11%) mostly affecting the N-propeptide and (2%) genomic multi-exon deletions. Five (5%) non-glycine missense variants were found throughout the pro-a1 chain. Only 15,4% of the families (n=22, 23 individuals) harbored causal variants in COL5A2 (22 unique variants), mostly structural defects, including in-frame indels and glycine substitutions in the helical region. In addition, 4 individuals from 3
families harbored the unique COL1A1 variant (p(Arg312Cys)) which is associated with a cEDS-like phenotype. In 10 families (5%) with a cEDS phenotype, a variant of unclear significance (VUS) was found in either COL5A1 or COL5A2, and in about 10% of the families (n=13, 18 individuals) with a clinical diagnosis of cEDS, no causal defect was found. All individuals with a causal defect presented with a degree of skin hyperextensibility and atrophic scarring; even though this was sometimes mild/subtle. Generalized joint hypermobility (including historic joint hypermobility) was present in 92.5% of the individuals. Individuals with (likely) pathogenic in COL5A2 seem to be affected with a more severe disease and a significant higher prevalence of club feet, abdominal wall herniations, scoliosis and congenital hip dislocations. Within our cohort, 3 individuals with defects in COL5A1 suffered from vascular complications. The specific defect in COL1A1, p.(Arg312Cys), is associated with a higher prevalence of severe vascular complications. Together with the 4 patients in this report, 22 patients have been reported in literature with this specific defect and severe vascular complications have occurred in 4 (18%)

correction: causal defects could be identified in about 90% of the cEDS families. The majority of the cEDS patients harbor (likely) pathogenic variants in COL5A1; only 15% of all causal defects are located in COL5A2 but patients with causal defects in COL5A2 seem to present with a more severe disease. Patients with the specific COL1A1 c.934C>T, p.(Arg312Cys) mutation may present as cEDS but have a propensity to arterial rupture.
P5: RASA1 mosaic mutations in patients with capillary malformation – arteriovenous malformation

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Background
Capillary malformation – arteriovenous malformation is an autosomal dominant disorder, characterised by capillary malformations and increased risk of fast-flow vascular malformations, caused by loss-of-function mutations in the RASA1 or EPHB4 genes. Around 25% of the patients do not seem to carry germline mutation in either one of these two genes. While some of those 25% of patients may have mutations in as-yet-unidentified genes, mutations in RASA1 or EPHB4 that escape detection by less sensitive techniques, such as post-zygotic mosaic mutations, are also possible explanations.

Methods
DNA was extracted from peripheral blood lymphocytes, saliva or vascular malformation tissues from 4 patients. RASA1 and EPHB4 coding regions and exon/intron boundaries were analysed by targeted custom gene panel sequencing. A second panel and/or Sanger sequencing were used to confirm the mutations identified.
Results
Four distinct mosaic RASA1 mutations, with an allele frequency ranging from 3% to 25%, were identified in the 4 index patients with classical capillary malformation – arteriovenous malformation phenotype. Three mutations were known, one was novel. In one patient, a somatic second-hit was also identified. One index case had three affected children, illustrating germline mosaicism.

Conclusion
This study shows that RASA1 mosaic mutations can cause capillary malformation – arteriovenous malformation. Thus, highly sensitive sequencing techniques should be considered as diagnostic tools, especially for patients with no family history. Even low-level mosaicism can cause the classical phenotype and increased risk for offspring. In addition, our study further supports the second-hit pathophysiological mechanism to explain the multifocality of vascular lesions in this disorder.
P6: Novel DGAT1 gene mutation in infant with protein-losing enteropathy (PLE).

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Background: We present a case of a Belgian female infant of consanguineous parents of Turkish origin with prolonged diarrhea, feeding difficulties and recurrent infections since the age of 2.5 months. She was born on term and after 2.5 uneventful months she presented in Turkey with an acute gastro-enteritis complicated by Klebsiella sepsis (probably originating from a necrotizing arthritis of the right hip, confirmed several weeks later on MRI), severe metabolic derangement and hypoalbuminemia. After stabilization and treatment she was transferred to Belgium where vomiting and diarrhea recurred every time formula feeding was restarted or increased. The infant was on several different regimen, initially hydrolysed whey protein and later amino-acid based formula. PLE with associated hypoalbuminemia, hypogammaglobulinemia and failure to thrive was thought to be secondary to septic arthritis because of demonstrated albumin loss in the colon nearby the septic arthritis. Tufting enteropathy was suspected but not confirmed and a cow’s milk protein allergy was diagnosed but failed to sufficiently explain the clinical course. Genetic analysis showed a novel mutation in DGAT1 that supports a previously described aberrant gut epithelial lipid metabolism explaining all symptoms.

Methods: In UMC Utrecht a gene panel analysis throughout targeted massively parallel/next generation sequencing by the Illumina NGS sequencing system was performed. The congenital diarrhea gene panel contains 46 genes (www.umcutrecht.nl/NGS). This method shows a coverage of 98.5% and detects 95% of mutations in the panel.

Results: Genetic analysis showed a homozygous pathogenic mutation c.469-2A>G p.(?) in DGAT. Both parents showed a heterozygous state, confirming the result in their daughter. No other mutations were found in the examined genes.

Discussion: DGAT1 encodes for diacylglycerol acyltransferase type 1, a protein involved in triglyceride synthesis. Mutations in this gene are rare and most reported cases concern Turkish patients. Few known patients with a homozygous mutation present with severe congenital diarrhea and PLE. Although the mutation in our case was previously unknown, the phenotype is similar to other cases described. After enteral nutrition was replaced by parenteral lipid administration we saw a favorable evolution.
Conclusion: This rare case of feeding difficulties in an infant with a previously unknown DGAT1 mutation with favorable evolution after switch to parenteral lipids, supports the finding that DGAT1 deficiency causes PLE due to aberrant gut epithelial lipid metabolism.
P7: Is primary lymphedema in Phelan-McDermid syndrome caused by SHANK3 haploinsufficiency?

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We report on a 5 year-old boy who developed primary lymphedema in the left lower extremity at the age of 3 years. In addition, he presents significant cognitive delay, especially concerning language, behaviour problems with stereotypies and chewing non-food items and decreased perception of pain. Currently, 28 genes have been shown to cause different forms of primary lymphedema, explaining only about 30% of the cases. Different genetic tests have been performed for our patient: molecular karyotyping, fragile-X analysis, as well as the analysis of a large panel of genes involved in lymphedema. However, all were negative.

Whole-exome sequencing was subsequently used to search for the causative gene. It allowed to identify a nucleotide deletion in the SHANK3 gene (c.4191delC), leading to frameshift and premature truncation (p.Ser1398Profs*30). The deletion was confirmed by Sanger sequencing and was proven to have appeared de novo, as none of the parents has it.

Heterozygous deletions of 22q13.3, encompassing SHANK3, have been associated with Phelan-McDermid syndrome. Around 3% of the patients have SHANK3 point mutations. The main phenotypic features of the Phelan-McDermid syndrome are neonatal hypotonia, delayed or absent speech, developmental delay, intellectual disability and behaviour problems. Around 10-25% of patients with microdeletions develop lymphedema. The causal gene for lymphedema in that locus is still unknown.

As our patient has SHANK3 point mutation, we speculate that it could be the causal gene. If this is true, other genetic or non-genetic factors could be necessary for lymphedema to develop, explaining the reduced penetrance in the Phelan-McDermid syndrome.
P8: Isolation of primary endothelial cells from patient tissues to establish pre-clinical models

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Introduction: Vascular anomalies are treated by sclerotherapy, embolization and/or surgery. However, these treatments are often not curative. The majority of vascular malformation-causative mutations are thought to arise in the endothelium. To demonstrate this and to establish valid pre-clinical models, it has become important to isolate endothelial cells (ECs) from malformations in patients. They could be useful to identify novel molecular disease-specific therapeutic approaches.

The aims of this project are to (1) establish EC isolated from various vascular anomalies caused by mutations in different genes, (2) investigate the in-vitro and in-vivo functions of these ECs, and (3) test repurposing of drugs used for cancer treatment as the same signalling pathways are affected in both diseases.

Methods: Patient samples of different vascular malformations (n=61) were cut into 2 mm pieces and digested with a mixture of DNAse, collagenase and dispase to isolate single cells (mixes cell culture). Next, primary mixed cells were selected with CD31 using the MACS separation magnetic bead system (Miltenyi). To confirm enrichment of primary ECs, cell morphology was evaluated and immunofluorescence was performed using endothelial markers CD31 and von Willebrand factor. DNA of CD31+ as well as CD31- cells was prepared for deep-sequencing. Cell signalling was determined by Western blot analysis. To investigate sprouting behaviour of mutated ECs from patient tissues and human umbilical vein endothelial cells (HUVECs) spheroid assay was established.

Results: For all patient samples mixed cell cultures could be established. For 14 patient samples of different diseases, CD31+ cells that showed the typical cobblestone morphology and were positive for von Willebrand factor could be obtained. In contrast, CD31- cells showed an elongated mesenchymal-like shape and were negative for von Willebrand factor. Sequencing demonstrated that the mutation (mostly PIK3CA) occurred in CD31+ cells and not in CD31- cells and almost
all cells harboured the mutation. CD31+ cells harbouring a PIK3CA mutation had an increased p-AKT activation compared to HUVECs. Moreover, PIK3CA mutated ECs as well as HUVECs were able to build sprouts in spheroid assay although HUVECs sprouted less than the PIK3CA mutated ECs.

Summary and outlook: For several patient samples, CD31+ ECs could be enriched and these cells carried the mutation (mostly PIK3CA) demonstrating that this cell population is driving the disease. Moreover, intracellular signalling (PI3K/AKT/mTOR) was activated. CD31+ cells provide a perfect model to test different cancer drugs which interfere with this signalling pathway. Moreover, these cells will be injected into nude mice or zebrafish to see if they are able to build vascular malformations. These would be invaluable pre-clinical models to test repurposing of cancer drugs. Positive results with rapamycin could already be demonstrated in our previous VM mouse model.
P9: A novel genetic region prioritization tool to analyze oligogenic diseases

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Introduction

There are currently >7000 rare diseases, for over half of which the underlying genetic model is still unknown. Moreover, several common diseases previously thought to be uniform, complex genetic entities, are now, at least partially, considered to be genetically heterogeneous collections of rare, near-monogenic disorders for which, again, the causes are largely unknown. One major impediment to disease gene-discovery, even in this age of high-throughput, genome-wide sequencing, is the limited statistical power of most studies on rare diseases: e.g., small sample-sizes, or dilution of effect-sizes when the genetic cause is distributed over several changes, each of which does not account for a substantial number of patients. Nowadays, few bioinformatics tools are able to identify the genetic cause of oligogenic diseases.

Objective

To allow such analyses and make the best use of public and in-house available exome and whole genome data, our goal is to develop a new prioritization tool. The objective of this work is to build a powerful, flexible statistical software for association-testing of aggregated rare variants.

Materials and methods

Its function will be to discover the genetic underpinnings of human disease using 3 main components: (1) Identification of new regions of interest (beyond single variants). First, we will consider the entire gene to be the analyzed unit (aggregating all variants within a gene). In a second layer, we will group genes based on their involvement in the same molecular/biological pathways. Finally, for whole genome data, we will need to define completely new units of analysis. (2) Development of a new statistical framework for testing association of variants within the genetic regions of interest defined, comprised of tests representative of all main classes (Burden, Variance-Component and Omnibus tests). (3) A dedicated automated pipeline made of the statistical framework (as main component), along with other
modules that will provide information to weight the results (e.g. associated biological data like pathways, spatial distribution of variants within regions of interest). The pipeline will automatically generates hypotheses using heuristic methods and will be available as a free, stand-alone prioritization tool.

Results and Discussion
We showed that this framework improves results when compared to a "one shot" use of any single test, and greatly facilitates interpretation. We have rendered the best of the aggregation-based association tests accessible to biologists who might otherwise be reluctant to engage with data processing and programming (e.g. R, python, etc). This framework hasten the identification of new genetic region possibly associated with a particular phenotype. This approach is critical in the analysis of genetically heterogeneous and complex, multigenic disorders such as breast cancer, primary lymphedema, and cleft lip and/or palate, subjects of large-scale sequencing projects in our lab, which will require these tools to effectively exploit the large volumes of data being generated. We aim at proposing this tool as a clinically competent application, introducing a new step toward oligogenic disease understanding.
P10: In search of genetic modifiers that explain the phenotypic variability in SMAD3-related aortopathy

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Introduction: Thoracic aortic aneurysm and dissection (TAAD) is a frequent cause of morbidity and mortality in the Western world. Loeys-Dietz syndrome (LDS) is a monogenic connective tissue disorder, most typically presenting with early-onset TAAD. Remarkably, a fraction of patients remains cardiovascularly unaffected throughout life, suggesting that genetic modifiers for aortopathy exist. To date, however, no genetic modifiers for LDS have been described. This study is implementing a combination of genome-wide linkage analysis and whole-genome sequencing (WGS) in order to identify genetic modifiers that explain the extreme phenotypic variability in SMAD3-related aortopathy.

Material and Methods: Two large LDS families segregating a SMAD3 missense mutation at amino acid position p.Arg287 and presenting with significant variability in TAAD expressivity were selected. Mutation carriers included in the study were categorized as affected and unaffected, taking into account age and aortic Z-scores. Next, genome-wide SNP based linkage analysis was performed and LOD scores were obtained using MERLIN and SUPERLINK. Additionally, on gDNA from six affected and six unaffected mutation carriers whole-genome sequencing was performed to complement the linkage data.

Results: In the first (larger) family linkage analysis identified a chromosomal region (chr2:149,604,077-155,607,398) with a LOD score of 2.68, suggesting the presence of an aggravating modifier. Linkage analysis in the second family resulted in a linkage peak with a LOD score of 2.86 (chr18:43,013,157-57,021,550). Additionally, the WGS data analysis of the second family resulted in the discovery of a rare variant with a CADD score of 33 in TGFB2, i.e. a known TAAD causative gene, that is exclusively present in affected mutation carriers.

Conclusions and future work: The obtained data indicate that; (1) variants in known TAAD genes may play a role in modulating a primary causative mutation; (2) additional chromosomal regions segregate with the disease phenotype suggesting a presence of genetic modifiers and (3) distinct families may bear different genetic
modifiers as indicated by the linkage analysis. Next, we will use the WGS data in order to identify genetic modifiers in the linked regions and/or variants only present in affected/unaffected family members.
Objective
Cystic Fibrosis (CF) is an autosomal recessive multisystem disease and the result of bi-allelic pathogenic variants in the Cystic Fibrosis transmembrane conductance regulator (CFTR) gene. It is one of the most frequent human genetic disorders, predominantly affecting Caucasians, or individuals of Caucasian descent. Genotyping with identification of the CFTR mutations is essential to confirm a (clinical) diagnosis, for family planning, for cascade testing and, finally yet importantly, for active up to date patient management that is now available. The genotypic spectrum of CFTR mutations is wide, as more than 2000 sequence variants, scattered over all exons, are known. However, a very large majority of the pathogenic variants were seen in only few (or even one) families. To complement their present product range, Fujirebio Europe NV recently developed the INNO-LiPA CFTRiage assay.

Method
The INNO-LiPA CFTRiage is a line probe assay based on the reverse hybridization principle, and intended for the simultaneous in vitro detection and identification of 88 CFTR gene mutations and their wild type sequence. The test includes a direct analysis of the 11 most common mutations and 1 new mutation L927P, and 76 mutations in 10 pool probes that need to be further characterized in a second, confirmatory test. It was estimated that with the use of the INNO-LiPA CFTRiage Strip ~95% of the detectable mutations of our patient pool should already be visualized in the first step.

With the retrospective analysis of 100 previously characterized DNA samples, the Center for Medical Genetics has contributed to the validation of this new assay. This sample cohort included 20 normal samples (result of INNO-LiPA CFTR 19 + INNO-LiPA CFTR 17 + Tn Update) and 80 samples with at least one CFTR mutation (32 different mutations in total). Furthermore, a panel of 6 samples tested by the company and three international centers were compared in view of assessing the reproducibility and repeatability of the test protocol.

Results
All samples (100) gave a positive amplification, and blank samples were negative. All previously detected mutations that were included in this INNO-LiPA CFTRiage assay
could be traced back. One sample, with compound heterozygous alterations including a c.4243-1G>A variant, gave a positive test result for the c.4251delA (4382delA) variant included in the INNO-LiPA CFTRiage Strip and INNO-LiPA CFTR Italian Regional Strip, while this variant should not have been picked-up. This is a result of the rare combination of the position of both mutations and the probe design of M4382delA. Samples for reproducibility gave a 100% concordance result. We have also evaluated the additional mutation detection yield of this batch of samples as they were originally characterised with INNO-LiPA CFTR 19 + INNO-LiPA CFTR 17 + Tn Update.

Conclusion
The success rate, repeatability, intermediate precision and reproducibility were 100%. For the samples tested, an agreement of 99% was obtained with the reference test results. Simultaneously, in two other validation centers, similar results have been obtained. Consequently, this test is suitable for diagnostic CFTR genotyping. Furthermore, this kit has the advantage that only a single amplification reaction is necessary for the INNO-LiPA CFTRiage Strip and its confirmatory strips. Consequently, the use of the INNO-LiPA CFTRiage assay will save time in the lab, while the diagnostic yield is expected to increase.
P12: Targeted next-generation sequencing of the highly repetitive and purine-rich ORF15 region of RPGR, a hotspot for missing heritability in X-linked retinitis pigmentosa, a target for gene therapy

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Purpose: There has been a surge in clinical trials investigating gene therapy for inherited retinal diseases (IRD). An example is X-linked retinitis pigmentosa (XLRP), caused by mutations in RPGR in ~75% of cases. Currently, three clinical trials are investigating RPGR gene therapy. Establishing a molecular diagnosis of RPGR disease can be challenging as RPGR contains a large exon ORF15, harbouring 60% of the mutations but characterized by a highly repetitive and purine-rich sequence region (~75% of ORF). This complicates genetic testing of XLRP and is a cause of missing heritability in IRD. We aimed to accelerate the molecular diagnosis of XLRP by developing an NGS-based test targeting XLRP genes, including the mutation hotspot in ORF15. Methods: Primers were designed using in-house software to cover the entire coding region of RPGR exons 1-14 and ORF15. Amplification was performed using an optimized singleplex PCR for enrichment of exon ORF15. Six primer sets were designed to cover the hotspot region. Library preparation via a modified Nextera XT protocol was followed by NGS using short-read sequencing (MiSeq, Illumina). Data-analysis was performed using CLC Genomics Workbench, variant calling and annotation with VEP (Ensembl) and Alamut. Results: Our targeted NGS strategy was first validated in 13 patients with XLRP and allowed us to identify ORF15 variants located in and surrounding the highly repetitive region (chrX(GRCh38):38285535-38286845). All changes could be confirmed by Sanger sequencing. Sequencing quality of the targeted regions was evaluated by coverage-based analysis. Coverage of ORF15 and the repeat-rich region could be improved to an average coverage of 387x for the most repetitive region, compared to 0-37x in
whole exome sequencing data. We could obtain an average coverage of 2490x for the detected variants in the highly repetitive region. Conclusions: To cover the highly repetitive and purine-rich region of ORF15, we propose an optimized NGS-based test targeting low-depth genomic regions such as ORF15. As RPGR is a target for gene therapy, identification of all (likely) pathogenic RPGR variants is of utmost importance. Finally, our approach can be extrapolated to other repeat-rich regions of disease genes and improve the overall diagnostic yield of IRD.
P13: Compound heterozygous SCN5A mutations in a severe Brugada syndrome case

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Brugada syndrome (BrS) is an inherited cardiac arrhythmia, characterized by an increased risk for sudden cardiac death (SCD). Diagnosis of the syndrome is difficult, due to incomplete penetrance and variable expressivity. Until now, a spontaneous or ajmaline provoked specific ECG pattern (BrS type-1), is the only clinical diagnostic method. Next generation sequencing based molecular diagnostics provides a promising alternative. So far, over 20 genes have been associated with BrS. In most cases, patients carry mutations in the SCN5A gene encoding the Nav1.5 voltage-gated sodium channel. Here we present a severe case of Brugada syndrome caused by SCN5A compound mutations.

We performed genetic analysis of SCN5A in a male patient, who presented with collapse during cycling at the age of two. He was initially diagnosed with severe junctional bradycardia and hypotension. During follow-up, he experienced a new collapse, with left-sided brain stroke at the age of three, and repetitive collapses during fever. In the presented study we investigated the functional effect of the detected genetic variants.

Genetic testing of this patient showed two SCN5A variants in trans (c.4813+3_4813+6dup – Belgian founder mutation and c.4711 T>C, p.Phe1571Leu). Familial segregation analysis showed presence of the founder mutation in the proband’s affected father and paternal aunt, and de novo occurrence of the p.Phe1571Leu variant. Previously, the founder mutation was shown to cause a loss-of-function of the Nav1.5 sodium channel. Pathogenicity of the second variant was suspected by absence from the GnomAD database, high conservation of the phenylalanine at position 1571, and in silico classification as possibly damaging or damaging. Functional electrophysiological analysis of the p.Phe1571Leu variant,
performed in human embryonic kidney cells (HEK) with the variant alone and co-expressed with SCN1B wild type (WT) protein, suggested a loss-of-function of Nav1.5. Compared to the WT, the variant displayed a hyperpolarizing shift in the voltage dependence of inactivation while the activation parameters were unaffected. The effect of the mutation on the inactivation was rescued by addition of a peptide sodium channel toxin – nermetide α-1, further confirming the pathogenicity of the investigated mutation and proving that the mutation is not affecting protein permeability.

Based on the presented data, we provide support for the pathogenicity of the p.Phe1571Leu SCN5A variant, which together with the c.4813+3_4813+6dup founder mutation, explains the aggravation of the BrS phenotype in the presented case.
P14: Genetic causes of unexplained venous malformations

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Introduction
Venous malformations (VMs) are localized vascular anomalies that arise during foetal angiogenesis. They are commonly present at birth as compressible bluish lesions that grow proportionally with the child. The affected tissues are mainly skin and mucosa, however more severe forms can present further infiltration in the underlying structures, muscles and joints. VMs are due to alterations on different levels of the TIE2/PIK3/AKT/mTOR signaling pathway that is known to be involved in the angiogenesis process. Most VMs are sporadic (98%). Familial forms, termed VMCMs (cutaneomucosal venous malformations), are rare (2%) and known to be caused by inherited activating mutations in the TIE2/TEK gene.

Material and Methods
We have been able to collect frozen tissue samples from 427 patients and recorded their clinical features. So far, we have performed targeted NGS sequencing with IonTorrent or IonProton using panels containing genes involved in the TIE2/PIK3/AKT/mTOR pathway for 304/427 samples (at median 1000x coverage). In order to identify somatic mutations, Highlander, an in-house-developed bioinformatics tool for variant filtering, was used.

Results
Among the analysed tissues in our cohort, around 50% carry somatic mutations in the TIE2/TEK gene, the most frequent ones being c.2740C>T (L914F) and c.2690A>G (Y897C). 15% of the samples have mutations in the PIK3CA gene, with hotspots substitutions on c.3140A>G (H1047R) and c.1624G>A (E542K). Other changes have been found also in KRIT1, MAP3K3 and in three additional candidate genes, allowing
to explain another 10-15% of the lesions. Some of the latter may rather be VVMs. Anyhow, around 20-25% of the cases remain unsolved. These samples will undergo Whole Exome Sequencing (WES) at 200x coverage and RNASeq in order to identify novel genes and mutation that complete the picture, including for the first time the investigation of copy number variations (CNVs).

Conclusion
A high number of VMs carry mutations in TIE2/TEK or PIK3CA, while mutations in KRIT1 and MAP3K3 are less frequent in our cohort. Another 20% of the samples remain unsolved. Collecting and analyzing genetic data is fundamental for the ongoing and future clinical trials using repositioning of precision drugs.
**P15: Tyrosine kinase-inactivating mutations in EPHB4 cause severe forms of primary lymphedema and hydrops fetalis**

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**Introduction:**
Primary lymphedema (PLE) results from developmental and/or functional defects in the lymphatic system. It may affect any part of the body, with predominance for lower extremities. Twenty-eight genes have been linked to various types of primary lymphedema, either isolated, or as part of a syndrome. The proteins that they encode are involved in the VEGFC/VEGFR3 ligand-receptor signalling pathway. The mutations discovered so far account for about one third of all primary lymphedema cases, underscoring the existence of additional genetic factors. A few cases of hydrops fetalis, a feature frequently seen in patient/families with primary lymphedema, were reported to be caused by mutations in EPHB4. These mutations kill the tyrosine kinase activity of the EPHB4 receptor but receptor expression seems to remain stable. This leads to abnormal lympho-venous valve development. Interestingly, EPHB4 loss-of-function / haploinsufficiency mutations were previously discovered by our laboratory to cause capillary malformation-arteriovenous malformation 2 (CM-AVM2). The phenotypes are clearly distinct and exclusive, with global loss of protein in CM-AVM2, as opposed to inactive EPHB4 in PLE/Hydrops fetalis.

**Materials and Methods:**
We have used whole exome sequencing (WES) on a cohort of > 600 PLE patients. We filtered the identified variants with the Highlander software developed in our laboratory. In vitro mutagenesis and western blotting were used to study the effects of new variants identified in EPHB4. Fluorescence-activated cell sorting (FACS) was performed to study expression level of EPHB4 on cell surface.

**Results and Discussion:**
We identified a series of heterozygous missense variants in EPHB4. In vitro expression showed that two of these mutants found in severe PLE/hydrops patients caused non-phosphorylation of the receptor. The others had no impact on phosphorylation. FACS data demonstrated that all variants were well present on cell surface, similar to wild type EPHB4. These data underscore the importance of functional validation to distinguish mutations from very rare polymorphisms that are predicted to be pathogenic according to bioinformatic tools. This study allowed to identify EPHB4 variants as the disease cause in two PLE cases.
P16: The role of genetic variation in phenotypic variability and response to treatment in Marfan syndrome

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Purpose: Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder caused by pathogenic variants in FBN1. In a cohort of 302 MFS patients, we explored the proportion MFS patients that had a pathogenic variant in FBN1, and analyzed if the presence, type and location of FBN1 variants was associated with clinical phenotype, aortic root size and growth, and response to treatment.

Methods: Targeted resequencing of FBN1 and related thoracic aortic aneurysm and dissection genes was performed, followed by deletion/duplication testing.

Results: We identified a (likely) pathogenic FBN1 variant in 91% of patients and a variant of uncertain significance in 4% of patients. A minority of MFS patients had no variant identified (2%) or had non-FBN1 variants (3%). Dominant negative (DN) variants were significantly associated with lens dislocation. There were no differences in cardiovascular phenotype and treatment response between the haploinsufficient and DN groups. We did observe an association between pathogenic DN variants in the central region of FBN1 and cardiovascular severity. Importantly, at younger age the treatment effects regarding aortic root growth rate were larger.

Conclusion: Comprehensive molecular analysis identified a pathogenic FBN1 variant in the vast majority of clinically diagnosed MFS patients. Significant genotype-phenotype associations were established. These findings have implications for prognostic counseling of MFS families regarding cardiovascular and extra-cardiovascular phenotype.
P17: Analysis of patients with a personal and/or family history of pancreatic cancer with a custom designed broad cancer predisposition gene panel – a case-control study

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Pancreatic cancer is estimated to have a familial background in 5-10% of the cases. Although the underlying genetic basis for most of the familial clustering remains elusive, several familial cancer syndromes are associated with an increased risk of pancreatic cancer.

In this retrospective study, 414 individuals were selected because of a personal or a family history of pancreatic cancer in combination with breast and/or ovarian cancer, colon cancer or melanoma.

Germline DNA was analyzed using a custom designed SeqCAP Target panel of 66 (pancreatic) cancer predisposition genes (Roche) for both patients (n=414) and healthy controls (n=210) (i.e. employees of our hospital who do not have cancer, neither have a first-degree relative with cancer).

We subdivided our patient cohort in 2 major groups:
1) amongst 67 probands with pancreatic cancer, ATM and BRCA1/2 had the highest prevalence of mutations (6% and 7.5%, respectively); furthermore, mutations were observed in CHEK2 (4.5%) and PMS2 (3%).
2) in probands with cancer (different from pancreatic cancer) and a close relative with pancreatic cancer - we detected mutations in 24 different genes.

2a) In 271 probands with breast cancer and 24 probands with ovarian cancer with a close relative with pancreatic cancer, as expected, BRCA1/2 (14% & 12.5% resp.), and CHEK2 (1.8% & 8.3% resp.) are the most frequently mutated, next to ATM (2.2%, none in ovca group)

2b) In 23 probands with colorectal cancer and a relative with pancreatic cancer we detected 4 pathogenic mutations respectively in PMS2, MSH2, SLX4 and a homozygous mutation in MUTYH.

2c) No clear deleterious mutation was detected in the cohort of 13 melanoma probands with a relative with pancreatic cancer.
Interestingly, in 21 healthy controls we also detected (possibly) pathogenic variants in 13 cancer predisposition genes. Surprisingly, this included 3 deleterious variants in BRCA1/2 (1.4%, which is higher than generally reported in Caucasians (0.2%-0.5%) and warrants further investigations). Furthermore, we detected deleterious variants in genes with moderate penetrance: ATM (n=4, 1.90% - while in the literature ~1% carriersonship is described in the general population), CHEK2 (n=1, 0.48% where generally 1.5% is reported). Additionally, heterozygous deleterious variants in genes associated with autosomal recessive disorders were identified, but their prevalence is similar as in our patient cohort. For genes like RAD50 (1.43%, n=3) and RAD54L (0.95%, n=2) the number of truncating variants detected in the control population is higher than in the patient population; therefore, these genes are probably not associated with an increased cancer risk.

These preliminary results revealed inactivating mutations in cancer predisposition genes in a high proportion of the patients. Further segregation analysis in the families is indicated to evaluate their link with the different cancers and highlight the need for recommendations governing germline multi-gene panel testing of cancer patients with a personal or family history of pancreatic cancer.
P18: The involvement of MAN2C1 in the development of cortical malformations.

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Introduction:
The development of the cerebral cortex is a complex process, existing of three major steps: proliferation of neuronal progenitor cells, migration of neurons, and organization and maturation of the neurons. Interruption of one or more of these processes by genetic or environmental factors may result in malformations of the cortical development (MCD). Although pathogenic variants in >200 genes have been associated with MCD, etiology still remains to be elucidated in a large group of MCD patients. We here describe a patient with a homozygous variant in the mannosidase class 2C member 1 (MAN2C1) gene.

Patient description and methods:
The proband, a 17-year old boy presented with a complex brain malformation including severe cortical dysplasia, agenesis of the corpus callosum, interhemispheric cysts and pontocerebellar hypoplasia. Parents are non-consanguineous. The father of the proband has a simple arachnoid cyst.

Whole exome sequencing was performed and standard procedures were applied with an average coverage of 75X. A novel homozygous variant was detected in the MAN2C1 gene which was selected for further analysis. Real-time polymerase chain reactions (RT-PCR) and enzymatic activity assays for alpha-mannosidase (MAN2C1) were performed on mRNA and proteins of fibroblasts of the patient and controls. Subsequently, RNAseq was performed to study the effect of the MAN2C1 variant on the transcriptome of fibroblasts from the patient and controls. Finally, MAN2C1 expression was knocked down in the control fibroblasts, using siRNA.
Results:
A novel homozygous variant was detected in the MAN2C1 gene (c.607G>A, p.(Gly203Arg)) (NM_006715.3). This variant was not reported earlier and classified as variant of uncertain significance (VUS 3). Preliminary RT-PCR results showed that the expression level of MAN2C1 is downregulated compared to the expression in controls, although this downregulation was not significant. The variant in MAN2C1 causes a ± 50% decrease in cytosolic alpha-mannosidase enzymatic activity in the patient compared to controls. RNA sequencing data comparing patient and controls samples are inconclusive at this moment.

Discussion & conclusions:
We identified a novel homozygous variant in MAN2C1 as a potential cause of MCD. The MAN2C1 gene encodes a cytosolic mannosidase and is involved in the degradation of misfolded glycoproteins. Possibly, as a consequence of the accumulation of proteins, the final step in the development of the cortex (organization and maturation of neurons) is altered. Our preliminary analyses show that the expression level of the gene is not significantly altered in the patient compared to the controls, but that the enzymatic activity is decreased. Further functional analyses are ongoing in order to further elucidate the role of MAN2C1 in brain development.
P19: The association of CTDP1 mutation with malformations of cortical development

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Introduction

Malformations of cortical development (MCD) represent a major cause of developmental disabilities and severe epilepsy. Genes for MCD are mainly involved in proliferation and differentiation of neuronal stem cells, migration of neuronal precursors, and cortical organization. Some of the MCD causative genes have been identified, but part remain unknown. Here, we describe a patient with heterozygous variants in the C-terminal domain phosphatase 1 (CTDP1), a gene potentially involved in MCD.

Case report and methods

We describe a 2.5-year old girl presenting with congenital arthrogryposis, periventricular gray matter heterotopia, optic atrophy, absent optic chiasma and unilateral auditory neuropathy.

Whole exome sequencing was performed and variants in the CTDP1 gene were detected. To investigate the biological impact of pathogenic variants identified in CTDP1, in vitro cultured fibroblasts from skin biopsies of both the affected girl and controls were used to assess expression levels of the CTDP1 gene by real-time polymerase chain reaction (RT-PCR). Furthermore, the phosphatase activity of CTDP1 was assessed by performing western blotting of its target RNA polymerase II.

RNA-sequencing was accomplished to understand the transcriptome, and siRNA is used to achieve a knockdown of the CTDP1 gene.

Results

Exome analyses of the index and her parents revealed that the index is compound heterozygous for two novel variants in CTDP1: c.609G>C, p. (Gln203His) and c.2665C>T, p. (Arg889Trp). RT-PCR data showed a non-significant downregulation of CTDP1 expression in the patient versus controls. CTDP1 functions as a phosphatase which dephosphorylates the C-terminus of RNA polymerase II, making it available for
initiation of gene expression. In patient cells, RNA polymerase II was more phosphorylated compared to control cells.

Conclusion
The identified novel variants possibly have an association with MCD. The CTDP1 gene is involved in the regulation of RNA polymerase II. The INTS1 and INTS8 genes are also involved in this regulation and pathogenic variants in these genes give a phenotype similar to what has been detected in our index patient, supporting a role for CTDP1 in brain development. Our preliminary analyses show that the expression level of the gene is not significantly altered in the patient compared to controls, whereas the phosphatase activity is affected. More repeats of the experiments as well as further functional tests are needed in order to determine in what way the alteration in CTDP1 causes MCD.
P20: Tracking the origins of metastatic seeding in de novo metastatic prostate cancer

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Introduction & Objectives: Patients with metastatic prostate cancer (mPCa) at diagnosis have been shown to exhibit an aggressive clinical course, yet the how and when tumoral clones start spreading to surrounding tissues have been difficult to determine because of a lack of access to primary and metastatic tissues. To address this, we analyzed multiregional data from distinct primary and metastatic tumor sites.

Material & Methods: Samples for analysis were collected from 10 prostatectomy de novo mPCa patients from the Ghent University hospital and were taken prior to initiation of systemic therapy. From each prostatectomy specimen, spatially and pathologically distinct regions (n=89) were sampled and interrogated with custom targeted sequencing, along with matched synchronous metastatic lymph nodes (n=48) from 9 patients, prostate biopsy specimens (n=21) from 4 patients and one bone metastasis. Mutation profiles and similarity matrices were used to reconstruct the tumor subclonal architecture and their phylogenetic trees.

Results: The median follow-up was 25 months. All patients had an acinar subtype of prostate adenocarcinoma. 2 patients had visceral (M1c) metastatic disease, 3 patients had bone (M1b) metastases and in 5 patients the tumor has spread to distant lymph nodes (M1a). All patients showed somatic alterations in driver genes associated with advanced disease, such as TP53 and RB1. Phylogenetic reconstruction demonstrated branched rather than linear evolutionary tumor growth in most patients, with clonal diversity both within primary tumors and also between primary and metastatic sites. In 3 patients the primary tumor and metastatic samples were genetically homogeneous. Interestingly, in one case we found a high number of somatic mutations concomittent to mismatch repair defective etiology. This patient may benefit from immune checkpoint inhibitors such as pembrolizumab.
Discussion: Our findings elucidate the complex patterns of metastatic spread in de novo mPCa and warrant further investigations into the intra-patient clonal diversity at time of diagnosis.
P21: Role Of Genetic Determinants In Cleft Lip And Palate: A Whole Exome Sequencing Approach

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Background
Cleft lip and palate (CL/P) is a heterogeneous group of developmental disorders with heavy and chronic disease burden. The approximate incidence of CL/P is 1/700 newborns. Several genes carrying germline mutations have been identified underscoring genetic heterogeneity. Yet, for numerous patients and families the underlying causes are still unknown.

Physicians stratify patients with oral clefts into either syndromic CL/P or non-syndromic CL/P depending on whether the CL/P is associated with other anomalies or not. However, genes responsible for syndromes with CL/P can also be responsible for isolated cases of CL/P. Few genes, such as IRF6 have been implicated in both syndromic and non-syndromic forms of nsCL/P.

Methods
To unravel genetic bases behind syndromic and non-syndromic CL/Ps, we have collected DNA samples from CL/P patients over the last 25 years in collaboration with the multi-disciplinary team at the Centre Labio-Palatin (Cliniques universitaires Saint-Luc, UCL). In order to collect samples uniformly, a standardized questionnaire is used detailing family history and clinical phenotype. Altogether data and samples from >1200 index patients and their family members are currently included in this expanding cohort. To identify variants causing CL/P, we have performed whole exome sequencing (WES) on 191 CL/P patients from 133 families so far and look for rare possible damaging variants in candidate genes.

Results
Rare damaging variants were identified in the following genes: IRF6, GRHL3, TBX1, TP63, TBX22, FGFR1, DLG1 and LRP6. Five had a mutation in a gene identified to be mutated in non-syndromic CL/P: TP63 (one family), TBX1 (one family), LRP6 (one family) and GRHL3 (two families), and clinical reassessment verified the isolated
nature of their CL/P. Moreover, two canonical splice-site mutations were identified in TBX22. Furthermore, we discovered loss-of-function variants implicating FGFR1 and DLG1 genes in non-syndromic discontinues CLP patients. In addition, we recently found 2 different mutations in GRHL3 gene on 2 families with Van der Woude syndrome and 1 mutation in IRF6 on a non-syndromic CL patient.

Conclusion
The present study highlights the importance of WES analysis in CL/P patients. Out of 133 families, 13 variants were identified in candidate genes showing the potential for WES in CL/P. We will now focus at CNVs as well as non-candidate genes contributing to CL/P using our WES data.
P22: Partial deletion of TAB2 in a patient with major connective tissue involvement, broadening the phenotypic spectrum.

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Rare diseases are collectively common and it is estimated that about 80% of all rare diseases are of genetic origin, following a Mendelian or monogenic inheritance pattern. Usually, their underlying etiologies are rare variants in the coding regions of the genome but with the gradual implementation of whole genome sequencing (WGS), more and more causative non-coding and structural variants can be detected. Here we describe a female individual with a phenotype characterized by congenital heart disease, unilateral hearing loss, short stature, and several connective tissue deficits including an ultra-rare hypogenesis of the mesentery, hyperextension of articulations, slumped shoulders and facial dysmorphisms. Through the analysis of trio-based WGS data, we identified a 52 kb de novo deletion in chromosome 6 involving part of the TAB2 gene. This deletion causes a loss of the first two non-coding exons of TAB2 as well as of two of its enhancers. Gene expression assays confirmed reduced expression because of nonsense-mediated decay. In addition, we also review published clinical and molecular data of all previously reported patients with TAB2 haploinsufficiency, further delineating the phenotype and showing that connective tissue involvement is part of the clinical spectrum. This report further proves WGS to be a valuable diagnostic tool for the discovery of pathogenic variants, missed by exome analysis.
P23: Atm deficient zebrafish model reveals conservation of the tumour suppressor function

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Ataxia Telangiectasia (AT) is an autosomal recessive disease leading to a wide range of severe disabilities, including highly increased cancer risk, neurodegeneration and immune deficiency. AT is caused by inactivating mutations in the Ataxia Telangiectasia Mutated (ATM) gene. Several in vivo models for AT have been made, but are unable to fully encompass the neurodegenerative aspect. In addition, treatment of AT symptoms (oxidative stress, radiosensitivity) could benefit from having animal models suitable for large-scale compound screenings. Zebrafish is an increasingly popular model organism, but not much is known about the zebrafish atm gene. We developed zebrafish AT mutants and studied their phenotype in detail. We found that mutant atm zebrafish develop exclusively as males. Moreover, these males are all infertile, which is a clinical feature in some AT patients. Disabling the apoptosis pathway through tp53 mutation leads to rescue of the all-male phenotype, but not fertility. atm-tp53 double mutants display accelerated tumor formation, indicating that zebrafish atm plays a role as tumor suppressor and is involved in maintaining genomic stability. atm mutant embryos did not display radiosensitivity, possibly due to compensation mechanisms. The radiosensitivity phenotype could be observed by using a translation block morpholino. We could not establish a neurodegenerative effect, even in aged adults. In conclusion, zebrafish can be used to study the in vivo role of ATM in the DNA damage response pathway, but not its neurodegenerative aspect.
P24: CELSR1 variants in primary lymphedema

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Introduction: Developmental and/or functional defects in the lymphatic system are thought to be responsible for the occurrence of primary lymphedema (PLE). The disease may affect any part of the body in an isolated way or as a part of a syndrome. Using genetic approaches, including most recently whole-exome sequencing, 28 genes have been linked to PLE (Brouillard et al, 2014 & 2017). One of the mutated genes is a planar polarity gene, CELSR1, (Gonzalez-Garay et al, 2016). This has so far been shown in seven probands and their families. The reports suggest female-limited penetrance of the mutations (Erickson et al, 2019; Maltese et al, 2019).

Materials and Methods: We investigated more than 900 index patients from our cohort of PLE for CELSR1 mutations. We used whole-exome sequencing combined with Highlander-based filtering of damaging variants. Co-segregation studies are performed by Sanger sequencing for available family members.

Results: We have identified 7 most likely loss-of-function mutations (nonsense mutations or frameshifts) (0.8% of cohort), as well as 44 amino acid substitutions (4.74% of cohort). We are currently performing co-segregation analyses for all of them. 7/7 LOF index patients are female. They have PLE mostly on lower extremities. Among the known CELSR1 affected family members in our cohort, 58 are female and 16 are male.

Conclusions: CELSR1 variants can be found in about 5.5% of lymphedema patients. Even though we see female dominance among PLE patients with CELSR1 variants, we were not able to fully address the female-limited penetrance. We are collecting samples from additional affected and unaffected family members, with careful clinical examination, to strengthen the statistics.
P25: Compound heterozygosity of mutations located in the first and third B-propeller domain of LRP4 causes sclerosteosis in a Spanish patient

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Sclerosteosis is a rare autosomal recessive skeletal dysplasia that is characterized by progressive hyperostosis in both the axial and appendicular skeleton. In addition to the hyperostosis, patients with sclerosteosis often present with syndactyly of the fingers and tall stature. Initially, it was shown that loss-of-function mutations in SOST (encoding sclerostin) are the genetic cause of sclerosteosis. However, currently, it is known that hypomorphic mutations in the sclerostin binding partner LRP4 can also cause sclerosteosis. We have previously described three mutations in LRP4 which are all located in the cavity of the third β-propeller domain and result in an impaired LRP4-sclerostin binding.

With this report, we provide evidence that mutations outside the third β-propeller domain of LRP4 can also cause sclerosteosis. In a Spanish patient who demonstrates increased thickness of the skull and sclerosis of the axial skeleton, we’ve identified two compound heterozygous mutations in LRP4. One variant (p.R1170Q) is residing in the cavity of the third β-propeller domain and is known to be disease-causing. The other variant (p.R632H) is located in the first β-propeller domain of LRP4. In silico and segregation analyses support the pathogenicity of this variant. In addition, the high serum levels for sclerostin observed in this patient suggest that both mutations affect the LRP4-sclerostin binding. Furthermore, a Wnt luciferase reporter assay shows that the p.R632H variant interferes with the normal inhibition of the Wnt signaling pathway by sclerostin.

In conclusion, identification of a disease-causing mutation in the first β-propeller domain of LRP4 broadens the mutational spectrum of sclerosteosis-causing mutations. Additional studies are needed to further elucidate the mechanism whereby the p.R632H mutation affects the LRP4 function and to investigate the role of the first β-propeller domain in LRP4 during bone formation.
P26: A novel Belgian KCNQ1 founder mutation causes an overlap of Long QT syndrome and Brugada syndrome

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INTRODUCTION: KCNQ1, together with KCNE1, form the cardiac potassium ion channel responsible for the generation of the slowly activating delayed rectifier current (IKs), which is a major determinant of the action potential duration, and as such the length of the QTc interval on the electrocardiogram. Mutations in the KCNQ1 gene can lead to the development of Long QT Syndrome (LQTS). We have identified a novel founder mutation in exon 8 of KCNQ1, c.1124_1127DelTTCA or p.Ile375Argfs*43, in two separate families. This mutation leads to the loss of the C-terminus of the KCNQ1 channel.

METHODS AND RESULTS: We have been able to gather the clinical data of 21 of the mutation carriers who display a phenotypical spectrum ranging from asymptomatic carriership to Jervell-Lange-Nielsen syndrome, LQTS and Brugada (BrS) syndrome in a single patient. A haplotype analysis confirmed the founder status of the mutation. By sequencing cDNA from blood of two of the patients we have confirmed that the mutant mRNA escapes nonsense mediated decay. Electrophysiological experiments showed that the mutation leads to a decrease in current density when co-expressed with wild type KCNQ1 and wild type KCNE1. This effect appears to be dependent on the heart rate with a more pronounced decrease in the potassium current at higher frequencies. This phenomenon was further confirmed in the clinic, as the BrS patient, who had a normal resting QTc of 440msec, showed a prolongation of the QTc interval during and 2 minutes after an exercise stress test (respectively 468msec and 490msec). These findings are compatible with a loss of function phenotype, as expected for LQTS. However, we have also found that the mutation leads to an acceleration of the channel kinetics, which suggests a possible gain of function mechanism and might compensate for the loss in current expression. These findings could explain the variable phenotype with both LQTS and BrS diagnosed in a single family.
CONCLUSION: Although KCNQ1 is not an established BrS gene, a gain of function mutation has been previously described in a BrS patient. Our electrophysiological experiments support the clinical finding that this founder mutation in KCNQ1 causes an overlap syndrome of LQTS and BrS.

P27: Expanded carrier in screening in Flanders (Belgium): an online survey of knowledge, attitudes and preferences among non-pregnant reproductive-aged women

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Aims: To explore knowledge, attitudes and preferences regarding expanded carrier screening (ECS) among non-pregnant reproductive-aged women in Flanders (Belgium).

Materials and methods: Pharmacists of public pharmacies throughout Flanders (Belgium) were asked to distribute flyers to non-pregnant women who came in for a prescribed contraception between May 2019 and January 2020. The online survey could be administered through the link or QR-code mentioned on the flyer. Prior to completing the questionnaire, participants were informed about the research project and the concept of ECS. The questionnaire included questions that measured socio-demographic variables, knowledge, attitudes and preferences for the practical organization of a population-based ECS offer.

(Preliminary) Results: A total of 191 women used the link or QR-code to access the online survey. Data from 40 participants were excluded from further analysis due to incomplete completion. Our sample comprised of 151 non-pregnant reproductive-aged women, of which 80.1% were between the ages of 18 and 34. A majority of the participants expressed having a future childwish (69.5%). In total 96 (63.6%) of the women questioned indicated they would consider ECS for themselves in the future. Almost half (49%) of participants were willing to pay themselves for ECS. Majority of participants preferred ECS to be offered via the Gynecologist (82.8%), followed by the Centre for Human Genetics (61.6%) and the General Practitioner (50.3%).

Conclusion: to be determined (data-collection was closed on 12/01/2020; further data-analysis will be performed in the coming weeks).
P28: Unravelling collagenopathies using DEEP PHENOTYPING TOOLS on the skeleton of zebrafish models.

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Specific forms of Osteogenesis Imperfecta (OI) and Ehlers-Danlos Syndromes (EDS) caused by rare inheritable mutations in genes that encode type I collagen are grouped as type I collagenopathies. The human phenotypes of these rare heritable connective tissue diseases (HCTD) are well described and are mainly characterized by impaired bone quality, bone deformity and bone fragility. Recently, several publications have shown that zebrafish models for these specific forms of OI and EDS mimic the phenotype present in human patients. However, most of these studies employ a number of descriptive (qualitative) methods on connective tissues such as low-resolution micro-CT, whole mount staining and histology (paraffin sections and TEM) to superficially assess skeletal phenotypes that only allow to detect clear phenotypic differences with a matched WT control group. Here, we suggest deep phenotyping tools that can be used on the same individual zebrafish providing both qualitative and quantitative data that can be linked as to form a complete dataset of the phenotype of each individual animal and of the different animal models.

Classic X-ray images are a non-invasive, fast and easy method for procuring qualitative data of the zebrafish skeleton and can be used on life zebrafish. Micro-CT data, also procured non-invasively, can be 3D-rendered giving a more detailed view of the skeleton. This data is however more costly and time consuming compared to classic X-ray and cannot be obtained from live zebrafish. Whole mount clearing and staining with Alizarin red S for mineralised tissues is the golden standard to study the internal phenotype of the zebrafish skeleton. This ‘bone staining’ gives a detailed view of the skeleton, and the staining is quick and cheap. However, this is an invasive technique best used after classic X-ray and micro-CT. Histology provides the most detailed view of skeletal tissues, especially with serial sectioning, but is an invasive, low throughput and time-consuming technique.
Quantitative data can be procured from classical X-ray images, on which the skeleton can be scored to give a measure of phenotypic severity. The micro-CT data can be used for quantitative analysis via FishCuT software which gives data about mineralised tissue thickness, tissue volume and tissue mineral density. Quantitative data can also be procured from images of the external phenotype and the internal phenotype using the alizarin red stained skeleton. These images can be loaded into software that can calculate shape changes based on geomorphometric landmarks.

The tools above can be used to unravel the phenotype of type I collagenopathies in more detail, moreover the data will inform about inter- and intra-familial phenotypic variation in zebrafish. In addition, using an extreme phenotype approach, the data from the deep phenotyping tools can be placed in matrices and used to calculate mild and severe phenotypes, which can help to identify modifier genes of type I collagenopathies. The suggested methods can clearly be used in a robust workflow for deep phenotyping of skeletal tissues in zebrafish models.
P29: More than meets the eye in Brittle Cornea Syndrome: genotype-phenotype studies on novel and reported pathogenic variants in ZNF469 and PRDM5

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Brittle Cornea Syndrome (BCS) is a rare heritable connective tissue disorder characterized by blue sclerae and corneal fragility with a high risk of vision loss or blindness. Myopia, keratoglobus/keratoconus and even ruptures of the eye globes are often reported. In addition, hearing loss affects half of the patients and, combined with blindness, leads to severe sensory disability. The disorder is also associated with Ehlers-Danlos syndrome (EDS)-like symptoms such as joint hypermobility, muscle hypotonia and scoliosis and was therefore included in the 2017 international EDS classification.

BCS is inherited as an autosomal recessive trait and is caused by pathogenic variants in the genes ZNF469 and PRDM5, which encode (putative) transcription factors for extracellular matrix components. In total, 15 homozygous and 4 compound heterozygous ZNF469 variants are reported in 44 patients from 22 families and 12 homozygous PRDM5 variants in 25 patients from 13 families. The phenotype between patients with ZNF469 and PRDM5 pathogenic variants is reported to be indistinguishable.

At the Center for Medical Genetics in Ghent, we identified 14 patients from 10 families with BCS. Molecular analysis showed bi-allelic pathogenic variants in ZNF469 in five probands (one nonsense and four frameshift variants) and five in PRDM5 (two frameshift and three missense variants). Three of the PRDM5 variants were previously reported, whereas the other seven variants are novel. Only one patient had compound heterozygous variants, the other variants were homozygous. Clinically, these patients fit within the diagnosis of BCS. The most frequent ocular symptoms were blue sclerae (12/14) and thin cornea (6/14), with four patients having corneal ruptures upon minor trauma. Hearing loss was reported in 8/14 patients. EDS-associated symptoms such as joint hypermobility (11/14), feet deformities (7/14) and scoliosis (5/14) were also common. Furthermore, six patients had some degree of bone fragility and/or fractures. Three probands were referred to us for a suspicion of osteogenesis imperfecta, because of the combined presence of mild bone fragility, blue sclerae and hearing loss.

We reviewed all pathogenic variants associated with BCS in this and earlier reports. These include a total of 29 different ZNF469 variants in 51 patients from 27 families, of which compound heterozygous variants were present in five families and homozygous variants in the remaining 22 families, and 14 different PRDM5 variants...
in 32 patients of 18 families, all homozygous. The majority of molecular defects in the single-exon gene ZNF469 introduce a premature stop codon (90%), which include 20 frameshift variants and six nonsense variants. Also two missense variants and one whole gene deletion were detected in ZNF469. For PRDM5, four frameshift variants, two nonsense variants, four missense variants, three splice site variants and one in-frame deletion of multiple exons were identified. We performed genotype-phenotype analyses on all reported and novel patients of whom we had clinical data. We could detect that ocular ruptures occurred in significantly more patients with ZNF469 variants (33/45) than in patients with PRDM5 variants (12/31). No other significant differences were observed. Overall, our study adds to the current knowledge on BCS. We want to highlight the systemic character of the disorder and point out that osteogenesis imperfecta and the Ehlers-Danlos syndromes are important differential diagnoses. These can however be discerned from BCS by more pronounced generalized connective tissue symptoms and less ocular manifestations due to corneal fragility. Early diagnosis of BCS allows for preventive measures and correct follow-up, thereby decreasing the number of severe ocular events and improving the patients’ quality of life.
P30: Variant Record Fusion: a bioinformatics pipeline for Tumor-only NGS testing in clinical routine

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Here we present a novel approach for the detection and filtering of variants for NGS cancer diagnostic tests. This method allows us to detect low-frequency variants by using only tumor samples, circumventing the need for tumor matched normal samples, hence lowering costs and increasing sequencing capacity nearly two-fold.

To detect the wide range of different variants, a combination of different variant callers is frequently used. Current analysis approaches that make use of different variant callers merely rely on merging or concatenating individual sets of variant calls that have high confidence for a specific caller. This approach however, needs to tradeoff between low sensitivity or low specificity for variants that have lower confidence calls in individual variant callers, but are still true variants. Our approach obviates this problem by fusing normalized and decomposed variant-records of all different callers to allow filtering of individual variants based on the information from all different callers. Variants in the fused dataset are subsequently filtered using parameters specific to the individual caller, specific to a combination of different callers and specific to the combined variant calls of the entire run. The pooled likelihood matrixes of different callers in fused variant entries proved to be especially useful for categorizing low frequent variant calls (AF <=5%).

This pipeline was brought in clinical routine for two capture-based NGS test, namely one solid tumor gene panel (90 genes) and one hematological malignancy gene panel (75 genes). In this clinical pipeline 3 variant callers were used (unified genotyper (2x) and MuTect) and over 20 filter rules were defined. Only 2 from 25 cutoffs required a slight adaptation between the two different sample tests with different sample types, FF and FFPE, indicating a general applicability of this approach. Our approach is currently validated and in use at UZLeuven and the network of 14 other hospitals.
P31: Modeling PIK3CA-related overgrowth syndromes in Xenopus tropicalis.

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Introduction Xenopus tropicalis (X.tropicalis) and Xenopus laevis (X.laevis) have been widely used in disciplines of developmental biology and functional genomics. The importance of many signaling pathways has been evaluated by studies that were conducted in Xenopus embryos and tadpoles. Advances in genome editing techniques, especially CRISPR/Cas9, have facilitated the analysis of genes that are involved in human disease. The PI3K/AKT/mTOR pathway is crucial for cell proliferation, angiogenesis, apoptosis and other important biological processes. Aberrant activation of PI3K/AKT pathway leads to the development of overgrowth syndromes. Overgrowth syndromes (OS) are a composite group of conditions that display excessive growth of several body parts and affect a wide variety of tissues, like nerve, adipose tissue, skin, bone and muscle. PIK3CA activating mutations generate PIK3CA-related overgrowth syndromes (PROS), also known as CLOVES. Patients can display symmetric/asymmetric tissue growth in limbs as well as in body trunk, splayed feet, vascular malformations, scoliosis, neurological complications and large lipomas. Surgical resection of overgrowths, comforts patients short-term but does not provide a cure. Thus, it is important to identify compounds/drugs with prolonged efficiency. We generated an amphibian PROS/CLOVES model by targeting the pten gene in X. tropicalis embryos. We aspire to use this model as a platform for identification of new compounds with therapeutic efficacy for CLOVES. Materials and Methods X.tropicalis 4-cell stage embryos were injected with pten+myc+notch1* and pten+notch1* sgRNA complexed with Cas9. After 5 weeks, froglets' limbs and main body demonstrated overgrowths, whose volume was gradually increased. At critical point, animals were sacrificed and overgrowths as well as internal organs were histologically processed. Furthermore, the mutation pattern overgrowths was evaluated by targeted deep sequencing. Results Initially, Xenopus tropicalis embryos were injected with pten+myc+notch1* sgRNA and Cas9 recombinant protein. Several of the resulting mosaic mutant animals presented with excessive externally visible overgrowths. Dissected animals (5/9) presented, besides overgrowths, with muscle hyperthrophy and abdominal haemorrhage. Histological analysis characterized the overgrowths as lipomatous tumors with details of muscle tissue. Furthermore, immunohistochemical processing of lipomatous tumor demonstrated highly activated PI3K/AKT pathway. Several internal organs had
disorganized architecture and immunohistochemical analysis again revealed highly activated PI3K/AKT pathway. Samples from lipomatous tumors and limb overgrowths were used for targeted deep sequencing, revealing that bi-allelic inactivation of the pten gene is sufficient to drive CLOVES development, while notch1 and myc mutations were not consistently observed. Pten mutant animals will be used for validating our model by testing BYL719, a PIK3CA inhibitor that has shown efficacy in treating human CLOVES patients. Conclusion We generated the first amphibian model in CLOVES syndrome. This model is currently validated by further analysis. We believe that a Xenopus tropicalis CLOVES model could become a valid experimental platform for further compound testing or identification of new drug targets.
Human eye development is a strictly coordinated process requiring precise spatial and temporal gene regulation. Disruption of this process, either due to genetic or environmental factors, can result in major congenital ocular anomalies including an underdeveloped (microphthalmia) or even absent (anophthalmia) eye. Microphthalmia and anophthalmia are considered part of a phenotypic spectrum with ocular coloboma, a structural malformation resulting from incomplete fusion of the optic fissure, that likely have a shared, although highly heterogeneous, genetic basis. They are collectively named MAC (Microphthalmia-Anophthalmia-Coloboma), characterized by a tremendous clinical heterogeneity and often associated with other ocular abnormalities such as anterior segment dysgenesis (ASD). MAC-ASD is estimated to be responsible for approximately 15% to 20% of blindness and severe visual impairment in children worldwide. Identifying the underlying genetic cause is as such imperative for appropriate disease management and for guiding genetic counselling. However, due to its genetic heterogeneity, obtaining a molecular diagnosis is challenging. At the Center for Medical Genetics Ghent (CMGG) we have therefore set up an exome-based gene panel test for the molecular diagnosis of
MAC-ASD patients. In short, sample preparation was done with the SureSelectXT Low Input Human All Exon V7 kit using the Bravo platform (Agilent Technologies) and pooled libraries were sequenced on a HiSeq 3000 or NovaSeq 6000 platform (Illumina) aiming for a minimal coverage of 20x for at least 90% of the investigated genes. Data analysis was executed with a bcbio-based pipeline and variant filtering was performed with the in-house Seqplorer software. Besides single nucleotide variants (SNVs), we also analysed for copy number variants (CNVs) using the read depth-based algorithm ExomeDepth. As of May 2018 we have analyzed this MAC-ASD panel in 26 patients, 12 of which with MAC (46.2%), and 14 with ASD (53.8%). For nine of these, the molecular diagnosis could be confirmed resulting in an overall diagnostic yield of 34.6% which is in line with the range of 11-36% reported in literature. When looking at the disorder-specific yields, the highest yield is achieved for the ASD cases (50%), while only 16.7% of the MAC cases could be molecularly solved. In seven of the MAC-ASD patients (likely) pathogenic homozygous, compound heterozygous or heterozygous variants were identified in ALDH1A3, CPAMD8, FOXE3, MFRP, PAX6, PITX2 and PITX3. In two of the nine patients a CNV was found encompassing the single-exon gene FOXC1: a deletion in a patient with Axenfeld-Rieger syndrome and a duplication in a patient with iridogoniodysgenesis. These CNVs were confirmed with Multiplex Ligation-dependent Probe Amplification (MLPA). In conclusion, using exome-based MAC-ASD panel analysis we have established a molecular diagnosis in 34.6% of a MAC-ASD cohort, highlighting the efficacy of exome-based panel testing in heterogeneous conditions like MAC-ASD.
P33: Unraveling the molecular etiology of Pompe disease using RNA sequencing

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Pompe disease (glycogen storage disease II) is a rare, recessive lysosomal storage disease (LSD) caused by a deficiency in lysosomal acid alpha-glucosidase (GAA). It is characterized by progressive muscle weakness and, in the infantile form, cardiomyopathy. The pathophysiology is only partially understood.

In this study, RNASeq and miRNASeq were performed on fibroblasts from patients with Pompe disease and healthy controls. This allowed us to identify miRNA-dependent differential gene expression, providing a more complete picture of the molecular disease mechanism. The differentially expressed genes dataset was subjected to a gene ontology (GO) analysis and compared to known mRNA expression levels in different tissues affected in Pompe patients (e.g. cardiac and skeletal muscle).

In this dataset, 122 genes were identified as differentially expressed (DE) between both groups, of which 48 were downregulated and 74 upregulated. GO analysis revealed that a number of these genes are associated with or regulating muscle development and function, such as BEX1, PPARG and DES. Other DE genes are also involved in several signaling pathways, such as the ERK1/ERK2 cascade and Wnt signaling pathway.

miRNA sequencing revealed 12 differentially expressed miRNAs. Cross-referencing the predicted targets of these miRNAs with the mRNA DE genes list and performing GO analysis on them indicated a few pathways that are potentially affected in Pompe disease. Differential expression of genes such as UTRN and COL4A5, which are also targeted by a number of miRNAs in our results, suggests the involvement of the neuromuscular junction in the disease mechanism. Further studies on the role of DE genes and the associated pathways in Pompe patients can provide insight in the molecular mechanism for the muscular atrophy in Pompe patients.
P34: Identification and in silico evaluation of putative cis-regulatory variants within the 5’UTR of the ABCA4 gene in inherited retinal disease

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Purpose| Affecting 1 in 8,000-10,000 individuals worldwide, Stargardt disease (STGD1) is one of the most common autosomal recessive inherited retinal diseases (IRD). Although a plethora of pathogenic coding ABCA4 variants are already known, a significant fraction of cases still remains genetically unexplained. However, it is becoming increasingly clear that genomic variation in non-coding regions may be an unforeseen source of missing heritability. Here we explored one of such regions, more specifically its 5’UTR, in search of potentially pathogenic cis-regulatory variants with an impact on the expression of ABCA4.

Methods| In this study we analyzed data generated by targeted next-generation sequencing of ~1,600 exons and flanking intron boundaries of 108 IRD genes in ~3,200 IRD probands using molecular inversion probes, in the context of the ERDC4000 project conducted by the European Retinal Disease Consortium. Variants located within the ABCA4 5’UTR were firstly assessed based on population frequency and cross-species conservation. In silico evaluation of secondary structure formation and transcription factor binding activity was performed for the variants compared to the wild-type 5’UTR. Candidate variants were then investigated for potential effects on gene regulation by interrogating retina-derived epigenomic datasets and various computational tools for both variant annotation and prediction of pathogenicity.

Results| We identified seven rare (i.e. MAF < 0.001 in publicly available control datasets) potential cis-regulatory ABCA4 5’UTR variants, three of which are ultra-rare (i.e. absent from gnomAD and 1000G). All variants were heterozygous and
found uniquely in the cohort, mostly in unsolved IRD cases for which an ABCA4-associated disease could not be ruled out either on the basis of their phenotype or their mono-allelic genotype. Most variants were predicted to significantly disrupt secondary structure formation and/or affect motifs representing retina-specific transcription factor binding (e.g. CRX, NRL, OTX2). Furthermore, one variant was found to create a novel AUG start codon in a favorable Kozak sequence upstream of the canonical coding sequence, thereby introducing a 27 base pair-long upstream open reading frame out-of-frame with the downstream main open reading frame.

Conclusion| Although often overlooked in exome or genome approaches, 5’UTR variants are known to influence post-transcriptional and translational regulation. Thus far, no (likely) pathogenic cis-regulatory variants within the 5’UTR of ABCA4 have been reported. Here we present a comprehensive in silico analysis of ABCA4 5’UTR variants identified in IRD cases lacking a molecular diagnosis. Exploring the landscape and functional consequences of these and other putative regulatory noncoding ABCA4 variants will not only expand our understanding of its cis-regulation but also provide mechanistic insight into IRD and STDG1.

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P35: miRNA expression profiles in BRCA1-associated breast cancers reveal upregulation of specific miRNAs in tumors lacking a clear second hit in a large proportion of the tumor

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Background. MicroRNAs are small non-coding regulators of gene/protein expression and several were found to be associated with particular features of BRCA1-associated breast cancers. Several studies have concluded that not all BRCA1-associated breast cancers display loss of wild type BRCA1 allele in all tumor cells.

Aim. We hypothesize that overexpression of oncogenic microRNAs might impair the expression of the retained wild type BRCA1 allele.

Methods. Using small RNA sequencing we investigated miRNA expression in a well-characterized cohort of 51 BRCA1-associated breast cancers. We evaluated the association with molecular subtype, histopathological features and retention of functional BRCA1 on the DNA and protein level using differential expression analysis.

Results. We confirmed previously reported associations between microRNAs and specific histopathological features of breast tumors, including hormone receptor expression. Fourteen microRNAs were upregulated in tumors retaining the BRCA1 wild type allele in >50% of the tumor cells. In silico prediction showed complementary to interaction partners of BRCA1, which may impact BRCA1 functionality in an indirect manner.

Conclusions. This study revealed candidate microRNAs that are potentially active in BRCA1-associated breast tumorigenesis. These findings warrant further functional analyses and validation studies to evaluate their potential to act as a non-coding second hit.
P36: Chromosomal abnormalities found in miscarriage samples of spontaneous and pregnancies achieved via assisted reproductive technologies

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Introduction:
An estimated 15-20% of pregnancies end with spontaneous abortion. Chromosomal abnormalities are found in a significant part of these spontaneous abortions. The presence of a chromosomal abnormality, can help families in understanding and coping with this sudden loss and can be important for the management of further pregnancies.

Material and methods:
Over a period of 15 months (October 2018 – December 2019), 78 samples were collected from spontaneous abortions. In 56 cases a maternal blood sample was available and maternal contamination of the foetal sample was tested using QF-PCR (Devyser Complete v2). In total 18 samples were excluded from this study due to significant maternal contamination and a further 3 samples were failed due to low sample quality. The remaining 57 samples were tested for chromosomal abnormalities using QF-PCR (Devyser Complete v2) and array-CGH (CytoSure Constitutional v3 60k array).

Of the 57 samples included in this study 19 were procured from spontaneous abortions of pregnancies after assisted reproductive treatments and 38 were obtained from spontaneous pregnancies. The mean maternal age was 35,2 years. For the assisted pregnancy subgroup the mean maternal age was 36,5 years and for the spontaneous pregnancy subgroup the mean maternal age was 34,5 years.

Results:
Chromosomal aberrations were detected in 19 of 57 samples (33,3%): trisomy 21 (4), trisomy 22 (3), monosomy X (3), triploidy (3), trisomy 15 (2), trisomy 18 (1), trisomy 14 (1), trisomy 2 (1), and 1 unbalanced structural abnormality. A normal result was reported for 38 of 57 samples (66,6%). Of these, maternal contamination could not be tested for 11 female samples. Therefore, it cannot be excluded that some of these samples were of maternal origin.

Aneuploidy was found in 9 of the 19 samples from the assisted pregnancy subgroup (47,4%): trisomy 21 (3), trisomy 22 (2), monosomy X (2), trisomy 15 (1) and triploidy (1). Of the 10 samples (52,6%) with a normal result, maternal contamination could not be tested for 2 female samples.
For the miscarriage samples of the spontaneous pregnancy group an abnormal result was reported in 10 of 38 samples (26,3%): triploidy (2), trisomy 21 (1), trisomy 22 (1), trisomy 15 (1), trisomy 18 (1), trisomy 14 (1), trisomy 2 (1), monosomy X (1), and 1 unbalanced structural abnormality. For 28 cases (73,7%) no chromosomal abnormalities were reported. Maternal contamination could not be excluded in 9 female samples.

Conclusion:
Previous studies report chromosomal abnormalities in 23-61% of spontaneous abortion samples. This is comparable to the findings in the present series (33,3%). Remarkably, no case with trisomy 16, considered to be the most frequently reported trisomy in miscarriages with aneuploidy, was found in our cohort. This could be due to the small sample size. In contrast, trisomy 21 seems overrepresented when compared to previous reports. This might be explained by the relatively high maternal age in this study, mean maternal age in previous studies varied between 28 and 35 years. Interestingly, most trisomy 21 cases were found in the assisted pregnancy group (3/4).
Although the portion of cases with chromosomal abnormalities was higher in the group of assisted pregnancies, this result could be distorted by the higher number of normal female samples that could not be tested for maternal contamination in the spontaneous pregnancy group together with the small sample size of this study. These results confirm the major contribution of chromosomal abnormalities in spontaneous abortions. Therefore, cytogenetic testing should be considered after spontaneous abortion if miscarriage tissue is available. A blood sample from the mother should also be included to test for maternal contamination.

References
P37: Optimized workflow for sample tracking in a clinical NGS lab

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Next Generation Sequencing (NGS) assays provide a trove of personal information that aids diagnostics. However, the complexity of the wet lab workflow as well as the large number of processed samples increase the probability of sample swapping. This would result in wrong diagnosis and it is thus essential to ensure the traceability of the generated samples. To allow sample traceability, the sample is split into two upon reception and the diagnostic NGS test is run in parallel with an independent assay that genotypes a limited subset of Single Nucleotide Polymorphism (SNP) positions. The SNPs are targeted in all our custom panels that are used for diagnostics so that all samples can be tracked using the same assay. Sample identity is confirmed if both independent test identify the same SNPs.

Few years ago we developed a multiplex PCR-based SNP panel for sample tracking of 31 highly informative SNPs across European, Asian, and African populations. This panel had two drawbacks: it was labor intensive and the location of the SNPs necessitated a read length of at least 100 bp, both of which negatively impacted turn-around-time. We thus decided to use a new one-step PCR protocol from Swift, in which the SNPs are located at maximum 35 bp from one of the primers. From our initial 31 SNPs, 29 could be included in this new protocol. The one-step PCR protocol is automated on a Hamilton Star and can run on almost all Illumina sequencing runs. More than 5,500 and 1,000 samples have been successfully genotyped using our first and second protocol, respectively. The comparison of the SNP panel genotypes and SNPs from targeted and whole genome assays proved that both methods are reliable for the detection of sample swaps.
P38: Rare variants in Loeys-Dietz syndrome genes increase the risk for spontaneous coronary artery dissection but not for fibromuscular dysplasia

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Background: Spontaneous coronary artery dissection (SCAD) is pathologically characterized by separation of the inner intimal lining of the coronary artery from the outer vessel wall, leading to blood flow obstruction. It is the prime cause of acute myocardial infarction in women below the age of 50 as well as in pregnant or postpartum mothers. Fibromuscular dysplasia (FMD) presents with condensed areas of arterial stenosis, aneurysm, tortuosity and dissection. Over the past years, it has become clear that FMD is prevalent in SCAD cohorts and vice versa, yet this mutual relationship is poorly understood. Also the genetic etiology of both conditions remains largely elusive. In case reports and a small-scale exome sequencing-based cohort study (N=44), it was shown that SCAD can be caused by mutations in syndromic thoracic aortopathy genes.
Purpose: We aimed to investigate the contribution of rare genetic variants in vascular Ehlers-Danlos and the Marfan, Loeys-Dietz (LDS), Shprintzen-Goldberg syndrome genes to the etiology of SCAD and, because of the prominent clinical connection, FMD in a large multi-center patient cohort.

Methods: Haloplex-based gene panel sequencing of the coding regions and exon/intron boundaries of COL3A1, FBN1, TGFB2/3, SMAD2/3, TGFBR1/2 and SKI was performed in 191 SCAD patients with or without FMD and 111 isolated FMD patients on an Illumina NextSeq500 system. Subsequent variant filtering of the patient samples involved selection of heterozygous coding or splice site (±2 bp) variants of good quality (~visual inspection in Sequence Pilot) that are either absent in gnomAD v2.1.1, have a minor allele frequency (MAF) below 0.01%, or a MAF between 0.01% and 0.1% combined with a CADD score above 20. The gnomAD v2.1.1 database was used as an independent control dataset and filtered identically. Case-control burden analyses were performed using the Fisher’s Exact or Chi-Square Test.

Results: Rare variants in TGFB2 (1% vs 0.3%; p=0.02) and SMAD2 (0.8% vs 0.1%; p=0.003) are significantly enriched in SCAD patients compared to controls. Although not statistically significant, there is also a trend for more single gene rare variants in TGFB3, SMAD3, TGFBR1/2 and COL3A1 in SCAD patients. Clearly, the six LDS-related genes stand out of this single-gene analyses. Hence, a combined LDS-gene burden analysis was performed, revealing a highly significant association (3.9% vs 1.5%; p=0.00007). When performed for FMD, none of the previous analyses yielded a significant p-value or showed a trend.

Conclusion: We here provide evidence for an important contribution of rare genetic variants in LDS genes to the etiology of SCAD. Our findings suggest a strong role for the TGFbeta signaling in the pathogenesis of SCAD but not FMD. We also observed a tendency towards an increased burden of COL3A1 variants in SCAD patients. COL3A1 mutation analyses in even larger SCAD cohorts will be needed to shed a better light on the validity of this finding.
P39: First patient with Bloom syndrome caused by a deep intronic variant leading to pseudoexon activation

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Bloom Syndrome is a rare genetic autosomal recessive disorder characterized by genome instability, cancer susceptibility, immune abnormalities, severe growth delay, various skin lesions and dysmorphic facial features. It is caused by germline mutations in the BLM/RECQL3 gene, which encodes the RecQL3 helicase. This gene plays a role in the homologous recombination (HR) repair pathway. Mutations in this gene are associated with sensitivity to DNA damaging agents like MMC (mitomycin C).

We report a Belgian boy presenting with severe growth delay, microcephaly, facial dysmorphism and several immune defects. Increased sister chromatid exchanges (SCEs) were observed, indicating genomic instability, a pathognomonic sign for Bloom Syndrome. Molecular analysis had revealed heterozygosity for a nonsense variant (c.3379C>T, p.(Gln1127*)) , never described before, inherited from his father. Sequencing at the gDNA level had not revealed a second variant. Here, we present the identification of a deep-intronic variant by cDNA analysis.

From EDTA blood samples a phytohemagglutinin stimulated short-term lymphocyte culture was established. We established two cultures, allowing to treat one with puromycin prior to RNA extraction. Total RNA was extracted using the QIAamp® RNeasy Mini Kit. cDNA was synthesized using Superscript® III Reverse Transcriptase. Primers were developed to span the complete coding region of the BLM gene, followed by RT-PCR and Sanger sequencing.

cDNA analysis revealed an aberrant transcript encompassing a part of intron 15 of the BLM gene. Sequencing of the relevant part of genomic DNA revealed a deep intronic substitution (c.3032-258A>G, p.(?)) predicted to create a cryptic donor splice site. The patient inherited this variant from his mother. This deep intronic
substitution gives rise to two aberrant transcripts: r.[3019_3020ins3020-414_3020-259](in frame) and r.[3019_3020ins3020-424_3020-259](out-of-frame). We are currently evaluating the residual protein levels and if sensitivity to MMC can be established.

In summary, we report a Belgian boy compound heterozygous for a novel nonsense variant in exon 18 and a deep intronic variant in intron 15. This mechanism has never been described before in the BLM gene and further functional validations are ongoing. This case highlights the role of non-coding variations associated with genetic syndromes and the added-value of RNA-based approaches in patients with a clear phenotype lacking a molecular diagnosis.
P40: Integrated approach to dissect the cis-regulatory domain of the ABCA4 gene, implicated in a frequent hereditary blinding disorder, in human retina

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Purpose: Stargardt disease (STGD1) is a frequent inherited retinal disease (IRD) and one of the most common causes of blindness in the working age population in Europe and childhood (~1/8,000). Significant advances have been made over the recent years in identifying the mutations underlying STGD1, with over 600 mutations found in the coding region of the disease gene ABCA4, as well as a substantial amount of deep-intronic splicing mutations. Limited attempts however have been undertaken to decipher the regulation of ABCA4 and to explore its putative cis-regulatory elements (CREs). Genetic changes affecting CREs can lead to changes in transcription factor (TF) binding and/or induce changes in the chromosome topology and gene expression and ultimately have a pathogenic or modifying effect. By mapping and functionally validating putative CREs in human retina, we aimed to gain more insights into the cis-regulatory landscape of ABCA4 in human retina.

Methods:
For the mapping of possible CREs, we integrated publicly available and in-house human retinal epigenomics datasets. For CRE predictions, we used chromatin accessibility (ATAC-seq), topological data (4C-seq), histone modifications (ChIP-seq) and transcriptomics data (RNA-seq), all generated on human donor retina. To functionally validate in silico predicted CREs, luciferase assays were performed in RPE-1 cells, using pGL4.23 vectors. To generate a high-complexity quantitative chromosomal interaction profile for the ABCA4 gene, we performed Unique Molecular Identifiers 4C (UMI-4C) on human donor retina.

Results: Constructs were generated for a total of 22 predicted CREs, both in their native and reverse orientation, in order to assess their regulatory effect in vitro. Five constructs showed an increase of activity, with three of the cloned regions displaying markers of activation of gene expression (H3K4me1 and H3k4me1) and enrichment of OTX2 and CRX binding sites. Ten constructs showed a decrease in reporter activity, four of
which correspond with repressive markers. In a preliminary analysis of the retinal UMI-4C data, we could confirm a decrease in background noise compared to 4C-seq data and hence an improved sensitivity and resolution. Further optimization is ongoing. Conclusions: Using an integrated approach consisting of data-mining of retinal datasets, in vitro functional validation of putative retinal CREs and targeted chromosome conformation capture (UMI-4C), we have gained insight into the cis-regulatory domain of ABCA4. The CREs identified and validated in this study can represent targets of non-coding mutations in unsolved ABCA4-associated disease. More insight into tissue-specific cis-regulatory domains of disease genes such as IRD genes may advance the interpretation of non-coding variants located in CREs. Funding: This project has received funding from the European Union's Horizon 2020 Marie Sklodowska-Curie Innovative Training Networks (ITN) under grant No. 813490.
P41: Greig cephalopolysyndactyly syndrome: Two cases report

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The Greig cephalopolysyndactyly syndrome (GCPS) is a pleiotropic, multiple congenital anomaly syndrome. Clinical diagnosis is challenging because the findings of GCPS are relatively non-specific, and no specific and sensitive clinical have been delineated. We tried to investigate two patients who consulted for craniofacial dysmorphism in the laboratory of Cytogenetics, Molecular Genetics and Reproduction Biology of Farhat Hached Hospital, Sousse, Tunisia and who displayed abnormalities in the chromosome 7. Our aim was to understand the different mechanisms underlying these chromosomal defects and establish a relevant genotype-phenotype correlation.

On examination, the two patients displayed dysmorphic traits including microcephalia, scaphocephaly, frontal bossing, hypertelorism, microphthalmia, anteverted nostrils. We noticed for both patients syndactyly of the 2nd and the 3rd fingers. Furthermore, the psychomotor development was severely delayed for the first patient.

An R-banding Karyotype was firstly realized for the two patients showing for the first case a derivative chromosome 7 evoking a deletion on the 7p region. A whole-genome array-CGH using Agilent Human Genome CGH array kit 44K (Feature Extraction9.1, CGH Analytics 4.5, Santa Clara, California, USA) confirmed an interstitial deletion of 11 Mb in the short arm of chromosome 7 from bands 7p14.1 to 7p12.3 for the first patient and discovered a 75 kb interstitial deletion in the short arm of chromosome 7 of the band 7p14.1 for the second patient. A Fluorescence in situ hybridization (FISH) using probes mapping to 7p14.1 (RP11-259N24) confirmed the GLI3 gene deletion for the two patients.

GLI3 encodes a zinc finger transcription factor that functions in the hedgehog signal transduction pathway. GLI3 haploinsufficiency is related to GCPS characterized by frontal bossing, scaphocephaly, and hypertelorism associated with pre- and postaxial polydactyly and variable syndactyly. Traits that were effectively described in our two patients. However, the first patient displayed severe mental retardation and speech delay, features generally absent in GCPS. Other genes included in the deletion boundaries could explain this variant of GCPS.
P42: Targeted deletion of a cis-regulatory element of abca4 using a paired guide RNA CRISPR/Cas9 system in Xenopus tropicalis

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Stargardt disease (STGD1) is one of the most frequent inherited retinal diseases (IRD) affecting 1/8,000-10,000 people worldwide. Mutations in the ATP-binding cassette subfamily A member 4 gene (ABCA4) have been associated with STGD1. It has been shown that 25-35% of the STGD1 cases have only one or no coding variant in the suspected disease gene ABCA4, suggesting missing heritability in the non-coding genome. Cis-regulatory elements (CREs) are regulatory DNA sequences that contain transcription factor binding sites (TFBSs), enabling to regulate transcription of its target genes. Previous studies have shown that the CRISPR/Cas9 system can be used to disrupt CREs in model organisms. Xenopus (X.) tropicalis is an interesting model organism for IRD, having the major cell types of the human eye, thousands of eggs that are easy to manipulate for CRISPR/Cas9 injections, and it has a true diploid genome. Here, we aimed to map and functionally study CREs of the abca4 region in X. tropicalis. Moreover, we aimed to generate and characterize a stable knock-out of a CRE of abca4 in X. tropicalis using CRISPR/Cas9 editing. Putative CREs of abca4 were determined according to epigenetic markers H3K4me1 and Pol II in X. tropicalis whole embryo. Regulatory activity of putative CREs was tested using in vitro luciferase assays. A putative CRE of abca4, showing around 2-fold increase in luciferase activity compared to empty vector, pointing to an enhancing transcriptional effect, was selected as target. Next, this CRE was targeted using paired guide RNAs (gRNA) and Cas9 in X. tropicalis embryos to create a deletion. Two gRNAs were designed as flanking the target CRE of abca4. The genomic region flanking the CRISPR target site was amplified and sequenced. Genome editing experiments using paired gRNA CRISPR/Cas9 system showed the deletion of the target CRE compared to using one gRNA and/or non-injected embryos. Further assessments including histology, immunohistochemistry, TUNEL assays and electroretinography to characterize the disease phenotype are ongoing. In conclusion, regulatory elements can be disrupted in model organisms using paired
gRNAs. Regulatory animal models may contribute to the annotation of the non-coding genome and provide insights into the regulation of IRD genes such as abca4.

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P43: Optimisation of bacterial WGS workflow to implement into clinical use

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Background: Whole genome sequencing (WGS) of bacterial pathogens has several advantages over current diagnostic methods. WGS can provide clinically and epidemiologically useful information related to pathogen identification, antimicrobial resistance and typing. Further, the exhaustiveness and the digitalization of the results are major advantages compared to conventional analysis which have to be repeated when new parameters need to be assessed. Large scale adoption WGS in the clinical environment is lagging however due to cost and complexity of data analysis which are mainly driven by the cost of reagents, multiple manual steps and finally the time required by bio-informaticians to process the data. Here we present a novel integrated, high-throughput and highly automated workflow that significantly decreases, cost, turn-around-time and data analysis complexity of bacterial WGS.

Materials/methods: We optimized several aspects of the workflow. First, we validated the Nextera XT (Illumina) library preparation on the Echo (Labcyte) acoustic liquid handling system. This system allows to work with minimal amounts of reagents, as small as nanoliters, and to work on large series of samples with minimal hands-on time. Second, we allowed automated transfer of sequencing data to a cloud-based repository. Third, we developed and automated two pathogen-specific cloud-based pipelines. To improve functionality and compatibility, we integrated these modules as Docker images to make the entire system modular and easy to share. Such system could easily be transferred to partner laboratories willing to perform bacterial WGS without investing in laborious bioinformatical development, validation and maintenance.

Results: In this proof-of-principle study, we successfully performed 454 WGS analysis. The optimized workflow compared to the conventional workflow resulted in significant decrease in reagents cost (40%) and hands-on time in the lab (90%). Automated data processing after demultiplexing was typically finalized after 49 minutes (Streptococcus pneumoniae) and 55 minutes (Mycobacterium tuberculosis).
Conclusions: Considering the higher automation and the better traceability of this new approach together the decreased cost and time-to-result, this optimized bacterial WGS workflow meets our quality standards and cost constraints. This evaluation allows to reconsider the place of bacterial WGS for clinical diagnostic and surveillance purposes in our University Hospital.
P44: The zebrafish as a model for reverse genetic screening of osteoporosis candidate genes

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In recent years, genome-wide association studies (GWAS) have revolutionized the understanding of the genetic architecture of common, complex diseases such as osteoporosis. The genes identified through this approach often need a functional confirmation, which is a laborious task in traditional (rodent) animal models. The zebrafish has the potential for medium-throughput functional analysis of candidate genes for skeletal disorders. Nevertheless, a zebrafish model for osteoporosis has not yet been described.

Osteoporosis-pseudoglioma syndrome (OPPG) is an autosomal recessive disorder characterized by reduced bone mass and strength, caused by loss-of-function mutations in LRP5 (low-density lipoprotein receptor-related protein 5), a co-receptor in the WNT signaling pathway. Here we describe the first reliable genetically induced form of osteoporosis in the zebrafish in a lrp5 knockout (KO). Molecular analysis confirmed reduced lrp5 transcripts and absence of Lpr5 protein. Reduced survival was observed in lrp5 KO larvae after the onset of mineralization. The fraction of larvae that did survive, displayed delayed ossification as was shown via mineral staining. Subsequently, these fish were grown to adulthood and analyzed via μCT. This revealed decreased bone volume and decreased bone mineralization in the vertebral column, which are hallmark features of osteoporosis. Regeneration of skeletal tissue was studied by finclipping the caudal fin. Westernblotting and qPCR revealed a decreased response of Lrp5 and downstream targets, validating the importance of lrp5 during wnt-mediated osteogenesis in zebrafish.

Next, we performed CRISPR/Cas9 mediated mutagenesis targeted at lrp5 followed by F0 phenotype analysis (crispant screening). Miseq sequencing of the mosaic larvae revealed out-of-frame mutations in on average 90% of the reads. Consequently, we showed a similar skeletal phenotype in the F0 ‘crispants’ to that of the stable lrp5-/- larvae, showing that crispant screening in zebrafish is a promising approach to functionally screen a large set of osteoporosis candidate genes.

In summary, we present lrp5-/- zebrafish as the first genetic osteoporosis zebrafish model that can be used as a screening platform for potential therapeutics.
Additionally we show that F0 crispant screening can be used for functional validation of candidate genes for osteoporosis as high rates of out-of-frame mutations in the lrp5 gene are sufficient to induce phenotypes.
P45: Microcephaly, epilepsy and permanent neonatal diabetes syndrome (MEDS): Clinical and molecular delineation of a new Tunisian case

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An autosomal recessive disorder [OMIM # 614231] including the triad of microcephaly, generalized seizures and permanent neonatal diabetes (MEDS) was reported as the best of our knowledge only eight cases in association with two mutations in IER3IP1 gene (immediate early response-3 interacting protein-1). Here, we report on the clinical and molecular data of an additional case of, pathologically proven, IER3IP1-associated MEDS in order to widen the phenotypic spectrum caused by the IER3IP1 gene mutations.

The proband was a boy of 3 years of a healthy consanguineous Tunisian couple with unremarkable family history. He presented an insulin-requiring permanent neonatal diabetes, microcephaly, and generalized seizures. The clinical examination exposed numerous dysmorphic features and disorder of sex development including micropenis and unilateral testicular ectopy. Brain MRI showed atrophy of the supratentorial level. Both R-Banded karyotype and Whole exome sequencing were performed.

The chromosomal analysis indicated a normal male karyotype 46,XY in all metaphases. Whole exome sequencing displayed a homozygous missense change, c.62T>G (p.Val21Gly) found in exon 1, predicting a change in protein structure within the hydrophobic/transmembrane. Interestingly, this change seems to reduce the ability of cells to respond to endoplasmic reticulum stress and leads to an increase in cell death and decrease in cell proliferation.

Collectively, IER3IP1 is a gene involved in the development of microcephaly and diabetes via dysregulation of apoptosis. However, abnormal genital have been described in only two other cases were reported with retractile testes. It remains unclear whether hypogonadism might be a feature of the syndrome. Interestingly, only two cases are caused by homozygous missense change c.62T>G found in exon 1 while other cases described (6/8) are caused by homozygous missense change c.233T>C found in exon 3. Our findings shed light on the genetic heterogeneity of MEDS and contribute to understand the pivotal role of IER3IP1.
gene on the mechanisms of brain development and on the pathogenesis of infantile epilepsy and early-onset permanent diabetes and why not hypogonadism. In addition carriers of IER3IP1 mutation are from Arab countries (most cases reported in the Mediterranean area) which suggest a founder effect.

Supplementary studies are required in order to determine if this is a mutational hotspot region or a common origin for mutations in the IER3IP1 gene, additional clinical features as hypogonadism and particularly in view of its occurrence in Arab countries.
P46: Cytogenetic characterization of Small Supernumerary Marker Chromosomes (sSMC) and genotype-phenotype correlation

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sSMC are chromosomes of unknown and non-recognizable structure and origin that can take many shapes and are additional to normal chromosomes. The associated phenotypes are variable and the risk depends on several factors, including the inherited or de novo character, the shape, as well as the euchromatin content and the structure of the marker. In the present study, we explored 49 SMC by conventional cytogenetics, FISH and array CGH. This series includes 9 fetuses, 33 children with different indications, 7 couples with hypofertility. Combining all techniques, we identified and characterized eight types of SMC in postnatal, three different types in the infertile population and four in the prenatal period. In the first group of children, almost 52% of SMC derived from chromosomes 15 and 18. The remaining 48% were der (22) (6 cases), der (21) (3 cases), der (9) and the der (13/21).

In the prenatal period, we identified two derivatives of chromosome 20, two derivatives 22 and two derivatives of the pericentromeric region of chromosomes 13 or 21. In the infertile group, we identified an SMC(15), a derivative of chromosome X, two CMS (22) and a ring 4 accompanied by a deletion 4 p14p16.1. About 16% of cases still of unknown origin. Through this study, we emphasize the importance of choice as well as the combination of cytogenetic techniques for a better characterization of SMC. All molecular cytogenetics techniques combined will allow to elucidate the role of this material in the determination of phenotypes, facilitating the search for candidate genes in these unexplored regions and to deduce a chromosomal mechanics, which have been at the origin of this type of rearrangement, sometimes very complex and poorly known such as Chromothripsis. Finally, precise genetic counseling results.
P47: A wide range of protective and disease causing variants in Aggrecan influence the susceptibility for otosclerosis

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Otosclerosis is a frequent cause of hearing loss among young adults in several populations, including European, northern African and Indian populations. In white populations of European origin, it has a frequency of 0.3% to 0.4%. It is a multifactorial disorder involving bony remodeling in the otic capsule, resulting in stapes fixation and conductive or mixed hearing loss. Genetically, otosclerosis is considered a complex disease with both sporadic as well as familial cases. In large families, the disease presents with an autosomal dominant inheritance pattern with reduced penetrance. Linkage analysis has identified eight different loci (OTSC1-5, OTSC7-8 and OTSC10), but no causative genes have been found. Until now, association studies investigating otosclerosis as a complex disease have shown a limited number of associated variants with low odds ratio’s only. Despite the evidence for a strong genetic etiology, little is known today about the genes responsible for otosclerosis.

A particularly interesting candidate gene within the OTSC1 locus is ACAN. Although ACAN has an attractive function, no disease causing mutation was found in the
(single) linked family. The ACAN gene codes for the aggregan protein that has an important role in the extracellular matrix of cartilage and in skeletal growth. In order to investigate a potential role of ACAN in otosclerosis, we resequenced a large group of 1642 sporadic otosclerosis cases, 97 members from 47 otosclerosis families and 1484 unscreened ethnically matched controls. Single-SNP association analysis showed 14 variants significantly associated with the phenotype after multiple testing correction. Conditional analysis using logistic regression accounting for linkage disequilibrium indicated the presence of at least 10 independent association signals. The effect size of the associated variants ranges from very strong (in the range of a monogenic disease) with very low frequency to low effect size (with an odds ratio below 3) with high frequency, which is typical for common complex traits. The allelic odds ratios of the variants identified four damaging and ten protective variants.

We feel that the current results represent an important breakthrough in the genetics of otosclerosis, illustrating an important role for ACAN, and nicely explaining the genetic characteristics of the disease. Moreover, the results are quite extraordinary, with a single gene containing multiple variants of both protective and disease causing nature, ranging from high frequency with low effect size, to very high effect size with very low frequency. To our knowledge, these findings are unique in the field of complex genetics.
P48: Development of an Extended Carrier Screening in Belgium

BeGECS working group

Belgian Centers of Medical Genetics

The eight Belgian Centers of Medical Genetics, see https://www.college-genetics.be, developed a new preconceptional test called BeGECS - Belgian Genetic Extended Carrier Screening. This new, large scale genetic test is available at own cost for all willing couples before pregnancy.

The BeGECS test consists in:
- the analysis of 1200+ autosomal recessive genes including 100+ X-inked recessive genes,
- the reporting of class V (pathogenic) and class IV (probably pathogenic) variants if both partners are carrier of a mutation in the same gene, or individually if on the X-chromosome,
- the reporting of individual carriership for 7 frequent autosomal recessive diseases showing a carrier frequency >1/50 (ACADM, CFTR, DHCR7, GJB2, HBB, PAH, SMN1).

Ethical and clinical considerations were used as guidance during the design of the BeGECS test.

The main anticipated difficulties are variant data nursing, interpretation of variants and their clinical penetrance, and the possible increase of genetic counselling and pre-implantation testing requests as well as accelerated cascade screening.

A first feasibility work-up confirmed that consanguineous couples are rare, but at a high risk for recessive diseases in their progeny. Unrelated couples are a low risk population, but could lead to more diagnosis in absolute number. Thanks to couple analysis, bioanalysis time is kept reasonable.

The running set-up phase does suggest several barriers to implementation:
- Doctors and patients’ perception of the cost/benefit ratio of the test
- Doctors and patients’ perception of BeGECS premium price (discrimination of low income couples)
- Couples’ reluctance to/lack of familiarity with (paying) preconceptional tests
- Doctors’ reluctance to/lack of familiarity with proposing (premium) paying tests
- Doctors and patients’ request for a shorter TAT
- Possible need for confirmation of difficult variants, influencing TAT
Melorheostosis is a very rare sclerosing bone dysplasia characterized by asymmetrical progressive cortical hyperostosis. In addition, surrounding soft tissues are usually also affected. The localized lesions and the usual sporadic occurrence suggest that melorheostosis is caused by a somatic mutation in early development. Recently, somatic mosaicism was identified for mutations (p.Q56P, p.K57N, or p.K57E) in the negative regulatory domain of MAP2K1, resulting in increased signalling in affected tissues. In this study, we screened for MAP2K1 mutations in both affected and unaffected tissues from four sporadic melorheostosis patients. For three patients, we performed whole exome sequencing on DNA extracted from affected bone and unaffected tissues. In two patients, we identified mutations in affected tissues (p.K57N and p.K57E), previously described by Kang et al.. For a fourth patient, DNA and RNA extracted from both an affected and an unaffected skin biopsy was available, on which whole genome sequencing (WGS) and RNA sequencing was performed. WGS did not reveal a pathogenic mutation in MAP2K1. However, gene set enrichment analysis of the transcriptome data demonstrated upregulation of proliferative pathways. Interestingly, increased proliferation of MAP2K1p.K57N-positive osteoblasts has been reported by Kang et al. In-depth analysis of the RNA-seq data revealed a novel variant (p.C121S) in the catalytic domain of MAP2K1, only present in the affected skin. Using allele-specific PCR, we confirmed the presence of this variant in both DNA and RNA from the affected skin. Affected bone tissue was unfortunately not available for further confirmation. Interestingly, both the p.K57N and the p.C121S mutations have been reported before in melanoma patients. Here, in vitro characterisation has shown that both mutations increase phosphorylation of the MAP2K1 downstream effector Erk. In conclusion, our study strongly suggests that not only variants (p.K57N and p.K57E) in the regulatory domain of MAP2K1 but also in the catalytic domain (p.C121S) may cause melorheostosis.
P50: The Gasdermin E Gene Has Potential as a Pan-Cancer Biomarker, While Discriminating between Different Tumor Types

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Due to the elevated rates of incidence and mortality of cancer, early and accurate detection is crucial for achieving optimal treatment. Molecular biomarkers remain important screening and detection tools, especially in light of novel blood-based assays. DNA methylation in cancer has been linked to tumorigenesis, but its value as a biomarker has not been fully explored. In this study, we have investigated the methylation patterns of the Gasdermin E gene across 14 different tumor types using The Cancer Genome Atlas (TCGA) methylation data (N = 6502). We were able to identify six CpG sites that could effectively distinguish tumors from normal samples in a pan-cancer setting (AUC = 0.86). This combination of pan-cancer biomarkers was validated in six independent datasets (AUC = 0.84–0.97). Moreover, we tested 74,613 different combinations of six CpG probes, where we identified tumor-specific signatures that could differentiate one tumor type versus all the others (AUC = 0.79–0.98). In all, methylation patterns exhibited great variation between cancer and normal tissues, but were also tumor specific. Our analyses highlight that a Gasdermin E methylation biomarker assay, not only has the potential for being a methylation-specific pan-cancer detection marker, but it also possesses the capacity to discriminate between different types of tumors.
P51: mSINGSmetal: an improved MSI calling algorithm on targeted somatic NGS panels

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Microsatellite instability (MSI) arises through dysregulation of the mismatch repair (MMR) pathway, through germline mutations in key MMR proteins leading to Lynch syndrome, or through acquired events such as MLH1 promotor hypermethylation, giving rise to sporadic MSI. MSI occurs frequently in colorectal (15% of CRC) and endometrial carcinoma. MSI is routinely detected by immunohistochemical detection of key MMR proteins (MSI-IHC) or fragment length analysis of consensus repeat loci (Bethesda panel-based MSI-PCR and Biocartis Idylla MSI test). MSI detection by NGS (MSI-NGS) is possible, as illustrated by the mSINGS python script on 15 marker regions (Salipante S, 2014), and this offers advantageous in cost efficiency, turnaround time and development of tumor-specific marker panels.

Our study compares the analytical performance of MSI-NGS using mSINGS with immunohistochemistry (MSI-IHC), PCR (MSI-PCR) and Biocartis Idylla MSI on colon cancer and other frequently microsatellite-instable tumor types such as endometrium or stomach cancer. Default mSINGS shows a high correlation with the other techniques for colon cancer, but not for other tumour types. The latter is likely explained by suboptimal biomarker loci for non-colon tumours and intrinsic limitations in the mSINGS script. Here we present mSINGSmetal, a reimplemention of the original mSINGS script, with an optimized MSI region length calling and MSI profile visualization and definition of new parameters to construct an Artificial Intelligence Model. The newly trained model resulted in improved performance of NGS-based MSI detection in non-colon tumours while preserving performance in colon tumours.
P52: Detection of HLA-B27 presence by LAMP PCR directly from patients' whole blood samples

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LaCAR MDx Technologies

Ankylosing spondylitis (AS) is a common, highly heritable inflammatory arthritis affecting primarily the spine and pelvis and affects approximately 0.5% of the population.

Accumulating evidence has demonstrated that human leukocyte antigen (HLA) class I molecule B27 (HLA-B27) is strongly associated to AS: >90% of AS patients are HLA-B27 positive. Nowadays, detecting presence of HLA-B27 alleles in patients suspected for AS is recommended as an additional tool for diagnosis. More than 150 different allelic variants of B27 have been recognized (HLA-B*27:01 to HLA-B27161) in AS patients. HLA-B27*05 and B27*02 are widely prevalent in European populations, and B27*05 and B27*04 predominate in Asian populations. We developed a LAMP-based method for the detection of all HLA-B27 alleles, directly from whole blood: The Lamp Human HLA-B27 direct detection KIT (LC-HLAB27Direct-LP).

The LC-HLAB27Direct-LP kit works directly on whole blood samples. EDTA-Whole blood is lysed in Lysis Buffer in a 1/200 ratio for 1-10 minutes. Afterwards, 5 µl of lysed sample is added to 20 µl of a ready to use Reaction Buffer for analysis. A positive and negative control are included in the kit and should be included in each run.

The Lamp Human HLA-B27 direct detection KIT contains specific primers allowing the loop-mediated amplification (LAMP) of the HLA-B27 alleles specific region (Exon 3) and an internal control (IC). Detection is done using an intercalating dye specific for double stranded DNA. After amplification, the temperature is increased to 99°C, during the melting curve analysis, the temperature is gradually decreased to 80°C while the change in fluorescence emission due to the annealing of fragments is measured.

HLAB27-positive samples will show a HLAB27 specific melting peak, but might also show a melting peak for the internal control. HLAB27-negative samples will show only an IC specific melting peak. Each run contains a positive (HLA-B27 positive peak) and a negative control (no amplification).

Clinical validation of the kit was done by comparing results obtained with our kit to an accredited method. 204 samples from anonymized patients were tested, including 96 patients known to be positive for HLA-B27. All samples were analyzed...
with 100% accuracy compared to reference methods. The kit was tested for its repeatability and reproducibility by performing the same tests with two operators, two sample types, on two non-consecutive days and in duplicates. All results were as expected. Stability studies revealed that the kit can sustain 8 freeze thaw cycles and should be stored at -20°C.

In conclusion, our technique allows for a rapid detection of HLA-B27 presence in whole blood from patients and is CE-marked on the LightCycler 480 (I, II &Z) (Roche), CFX96 (Bio-Rad), LightCycler 96 (Roche) and Rotor-Gene Q (QIAGEN) instruments.
P53: Diagnostic yield of Next Generation Sequencing in patients with neuromuscular disorders in UZ Leuven.

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INTRODUCTION: Neuromuscular disorders (NMD) is a wide term covering different genetic disorders affecting muscles, nerves and neuromuscular junctions. This makes it a heterogeneous category of neurologic disorders. In this study the neuromuscular disorders include muscular dystrophy, congenital myopathy, ion channel muscle disease, metabolic myopathy and congenital myasthenia. The clinical presentations of NMD are heterogeneous, with an overlap between different subcategories. Besides the clinical heterogeneity, NMD are also genetically heterogeneous. One gene can cause a wide variety of clinical and/or pathological features, while similar clinical features can be caused by mutations in different genes.

Since next generation sequencing (NGS) is an effective diagnostic tool for the parallel investigation of a large number of genes, it is well suited to diagnose NMD. We will present the results of 4 years of panel based NGS of 160 patients with neuromuscular disorders.

METHODS: From 2016 to 2019, an in-house developed disease-associated gene panel (clinical exome consisting of 6210 genes (Roche Nimblegen; v4)) was performed on 160 undiagnosed patients with NMD. These 160 patients were divided into two cohorts, children (54 patients) and adults (106 patients).

Data from single case patients were obtained by next generation sequencing on an Illumina Hiseq 2500. A disorder specific subpanel was extracted from the clinical exome. The content of the panel was updated every year and currently the NMD panel contains 197 genes. Annotation and classification of the variants was performed using Alissa Interpret software (Agilent; v5.2). The analysis was carried out for 3 modes of inheritance (autosomal dominant, autosomal recessive and X-linked) with a population frequency cut-off of 2%. Variants were classified using ACMG criteria. Likely pathogenic and pathogenic variants were confirmed by Sanger sequencing and were reported.

RESULTS: Initially, the NMD gene panel was divided in 3 subpanels: metabolic myopathy, channelopathy and the subpanel including muscular dystrophy, congenital myopathy and congenital myasthenia. The subpanel according to the phenotype of the patient was analysed. In 2019, due to the clinical heterogeneity
and the genetic overlap, it was decided to analyse all three subpanels panel for each patient.

The overall diagnostic yield in our cohort of 160 patients was 25.6%. These 41 patients were identified with pathogenic or likely pathogenic variants in a causative gene associated with the patient's disorder. Variants of unknown significance were identified in 19 patients (11.9%). Analysis of the cohort of children (54 patients) allowed the detection of disease-causing variants in 22 patients, resulting in a diagnostic yield of 40.7%. Analysis of the cohort of adults (106 patients) allowed the detection of disease-causing variants in 19 patients, resulting in a diagnostic yield of 17.9%.

CONCLUSION: We aimed to evaluate the diagnostic yield of our current NGS approach on patients with NMD. Our study showed that NGS analysis on patients with a neuromuscular phenotype achieved a diagnostic yield of 25.6%. Interestingly, the diagnostic yield depended on the onset of the disorder. Noteworthy, we could not detect copy number variations (CNV), nucleotide repeat expansions and mitochondrial DNA via our NGS approach.
P54: Reanalysis of clinical exome data from patients with rare developmental disorders results in new diagnoses

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Background
The use of trio whole-exome sequencing for rare developmental disorders currently has a diagnostic yield of 25-30% [Gilissen et al, Nature 2014; Wright et al, Lancet 2015; Fitzgerald et al, Nature 2015]. At the Center for Human Genetics in Leuven, the use of massive parallel sequencing in the diagnosis of patients with intellectual disability and multiple congenital anomalies (ID/MCA) has hitherto been limited to the analysis of a custom designed clinical exome or mendeliome consisting of 6210 genes known to be associated with disease. Trio-based analysis of this clinical exome results in a (molecular) diagnosis in 30% of the patients. In 85% of the patients a de novo pathogenic variant was found. However, the majority of patients with ID/MCA for whom the clinical exome has been analyzed still has no diagnosis. New gene-disease associations are being reported at rapid pace and literature reports a 10-36% increase in diagnostic rate upon reanalysis of whole-exome sequencing data [Wright et al, Genet Med. 2018]. Therefore, we sought to systematically reanalyze clinical exome data in order to improve the diagnostic yield.

Methods
A cohort of 42 patients with ID/MCA was selected, for which no molecular diagnosis was found upon initial trio-based analysis of the clinical exome in 2017. Reanalysis aimed at (1) assessing the impact of new knowledge established since first analysis of the clinical exome, (2) excluding the X chromosome as a diagnosis and (3) looking for diagnoses in autosomal recessive (AR) genes with only one pathogenic SNV variant. We then reanalyzed the existing clinical exome data in light of newly discovered disease-associated genes and using different variant filtering strategies. We also extended the analysis by looking at CNVs called from clinical exome data using the CoNIFER algorithm.

Results
Reanalysis of clinical exome data from patients with ID/MCA in whom the first analysis did not identify a molecular diagnosis resulted in a 3% increase in diagnostic yield. One new diagnosis was made because of a novel gene-disease association
since initial analysis of the clinical exome and consisted of a de novo frameshift mutation in PBX1 (OMIM 617641). Analysis of the CoNIFER CNV data resulted in another genetic diagnosis identifying a multi-exonic deletion in GDI1 (OMIM 300849) on chromosome X. Reanalysis did not result in any additional AR diagnoses.

Conclusion
The results will be further discussed with respect to what is described in literature.
P55: SLC35A1 and SLC35A4 both transport CDP-ribitol from the cytosol to the Golgi apparatus.

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Mutations in genes required for the glycosylation of α-dystroglycan lead to muscle and brain diseases known as dystroglycanopathies. However, the precise structure and biogenesis of the assembled glycan are not completely understood. Likewise, the function of several genes involved in the assembly of this glycan are still unknown.

We have recently discovered the function of three genes mutated in dystroglycanopathies called ISPD, FKTN and FKRP. Our work demonstrates that these three genes collaborate to introduce a unique modification (i.e. a modification with ribitolphosphate) into the glycan of α-dystroglycan. Ribitol-phosphate groups are known components of bacterial capsules. Yet, for the first time, we were able to show that this modification is also present mammalian cells and plays an important role in the glycosylation of α-dystroglycan.

However, some questions still remain unresolved. It has been shown that FKTN and FKRP are Golgi resident proteins. But the CDP-ribitol is formed by the enzyme ISPD in the cytoplasm. Yet, the transporter required for the entry of CDP-ribitol into the Golgi apparatus remained elusive. Interestingly, two studies indicated that the Golgi apparatus CMP-sialic acid transporter SLC35A1 could be implicated. This transporter has previously been shown to be the dedicated CMP-sialic transporter. Our data strongly suggests that this transporter is indeed involved in the transport of CDP-ribitol. Moreover, we also uncovered another transporter: SLC35A4. Taken together, our data points towards a redundant function of two independent transporters for CDP-ribitol.

Of note, these observations might lead to the development of novel therapeutic approaches, since the supplementation of a metabolic precursor (i.e. ribitol) to ISPD-mutant fibroblast cell lines partially restores α-dystroglycan glycosylation. Hence, dietary supplementation with ribitol needs to be considered as a novel therapeutic approach for a subset of dystroglycanopathy patients.
P56: Diagnostic yield of panel-based next generation sequencing in patients with inherited peripheral neuropathies in Belgium

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Background: Inherited peripheral neuropathies (IPN) represent a clinically and genetically heterogeneous group of neurological disorders. With a prevalence of 1/2500, Charcot-Marie-Tooth (CMT) disease is one of the most common forms of IPN. CMT is typically subdivided in three main types: the demyelinating form (CMT1), the axonal form (CMT2) and the intermediate form (CMTI). Other common hereditary neuropathies are distal hereditary motor neuropathy (dHMN) and hereditary sensory neuropathy (HSN). The introduction of next generation sequencing (NGS) made it possible to analyse all IPN-related genes simultaneously, thereby greatly improving the molecular diagnostics of IPN.

Methods: From 2016 until 2019, an in-house developed disease-associated gene panel (clinical exome consisting of 6210 genes (Roche Nimblegen v4)) was performed in 203 undiagnosed index patients with IPN. Data from single case patients were obtained by massive parallel sequencing on an Illumina Hiseq 2500. A disorder specific subpanel was extracted from the clinical exome. The content of the IPN panel was updated every year and currently contains 106 genes. Annotation and classification of the variants was performed using Alissa Interpret (Agilent; v5.2). The analysis was carried out for the three modes of inheritance (autosomal dominant, autosomal recessive and X-linked) with a population frequency cut-off of 2%. Variants were classified using ACMG criteria. Likely pathogenic and pathogenic mutations were confirmed by Sanger sequencing and reported.

Results: Causative mutations were identified in 29 (14.3%) patients and variants of unknown significance (VOUS) were found in another 33 (16.3%) patients. In the remaining 141 (69.5%) patients, no causative variant was detected. The most frequently mutated genes in this cohort were: MPZ, GJB1, MFN2, SH3TC2, GDAP1 and NEFL. Additionally, we found 7 novel mutations that have not been previously described in literature.

Conclusion: Our diagnostic yield is in agreement with the literature data. For variants of unknown significance, further segregation analysis is planned in order to potentially confirm the molecular diagnosis of these patients. Variant interpretation remains challenging and a good collaboration between the genetic laboratory and
the clinicians is crucial to improve the turnaround time of the test. In the near future, moving from clinical exome to whole exome sequencing (WES) will facilitate our diagnostic panel updates and open new opportunities such as the detection of copy number variations (CNV).
P57: An intronic AAGGG repeat expansion in RFC1 causes CANVAS: an UZ Leuven cohort study

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Background: Cerebellar Ataxia with Neuropathy and Vestibular Areflexion Syndrome (CANVAS) is an adult-onset slowly progressive neurodegenerative condition characterized by the impairment of three neurological pathways responsible for balance: the cerebellar, sensory and vestibular system. In April 2019, a biallelic expansion of an intronic AAGGG repeat in RFC1 was identified as the genetic cause of CANVAS by Cortese et al. The expansion differs from the reference allele (AAAAG)11 in both size and nucleotide sequence. They described three possible conformations: (AAAAG)exp, (AAAGG)exp and (AAGGG)exp. Only the (AAGGG)exp is thought to be pathogenic and causes CANVAS or late-onset ataxia. Recently, Akçimen et al identified two additional conformations, (AAGAG)exp and (AGAGG)exp, reflecting the dynamic nature of the RFC1 repeat.

Methods: In a first instance, two brothers and an isolated female clinically diagnosed with CANVAS were tested for the RFC1 repeat expansion. In a second part, we assembled a cohort of 93 adult patients with progressive late-onset ataxia. All patients had no family history of the disease and CAG repeat expansions for SCA1, SCA2, SCA3, SCA6 and SCA7 were already excluded. Screening of the RFC1 repeat expansion was performed on genomic DNA by short-range flanking PCR and repeat-primed polymerase chain reaction (RP-PCR) using primers targeting the three conformations separately ((AAAAG)exp, (AAAGG)exp and (AAGGG)exp).

Results: All three CANVAS patients showed no PCR-amplifiable product on flanking PCR, indicating the presence of an expansion on both alleles. RP-PCR with primers targeting the pathogenic AAGGG repeat unit confirmed the presence of an AAGGG repeat expansion in these patients. Secondly, screening of our cohort of 93 late-onset ataxia patients revealed seven (7.5%) additional sporadic cases carrying the recessive AAGGG repeat expansion. All cases presented with cerebellar ataxia and polyneuropathy. Unfortunately, clinical information about vestibular function was insufficient or absent in most cases, making it impossible to confirm the diagnosis of CANVAS at this point.

Conclusion: We have shown that the genetic test to identify the presence of a recessive AAGGG repeat works well in our laboratory. Further analysis via Southern
blot is required to determine the exact size of the expansion and to confirm the diagnosis in these patients (data in progress). Further investigation of the new conformations (AAGAG and AGAGG) is also planned.

Literature describes a higher yield (22%) in similar cohort studies compared to the 7.5% that we obtained. Future studies will be necessary to determine the diagnostic yield of RFC1 and it might be necessary to define stronger selection criteria for RFC1 genetic testing. It is therefore of utmost importance to perform extensive neurological exams on patients with late-onset ataxia, which in turn is complicated due to the slowly progressive course of this complex disorder.
P58: LTBP3 mutation identified in a patient with a severe valvular disease

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A fifty four year old male was referred to genetics consultation for presenting a severe mitral valve insufficiency for which he underwent a valvular replacement. Heart ultrasound showed a severe excentric mitral valve insufficiency with broken ropes and partial eversion of anterior leaflet at A2. He had a personal history of ischemic heart disease and high blood pressure. Supra-aortic ultrasound and abdominal CT scan did not detect any aneurysm. Kidney kysts were detected. One of his four brothers died after a stroke. There was a family history of ischemic heart disease on the paternal side (father and paternal uncles) but no known history of valvular disease. A maternal uncle had arrythmia (peacemaker). At clinical examination the patient showed a height of 1.6 m with dolichostenomelia, pectus carinatum, thickened skin and lobeless ears.

Array CGH was normal. Genetic testing of thoracic aortic aneurysm panel (UZ Antwerpen) identified a LTBP3 heterozygous c.341delC p.(Arg1281Alafs*38) variant in exon 28, confirmed by Sanger sequencing. It was not found in SNP and 1000 Genomes nor in the Exome Variant Server or the GnomAS databases. This variant is present in the last calcium-binding EGF-like domain of the LTBP3 protein and is expected to create an aberrant tail of 38 aminoacids. Homozygous and heterozygous LTBP3 loss-of-function variants have been described to cause thoracic aortic aneurysms and dissections. Heterozygous carriers have later onset of the aortopathy as well as dental abnormalities. At this moment this variant is classified as probably pathogenic variant (class 4).

Geleophysic dysplasia, a progressive condition resembling a lysosomal storage disorder, is characterized by short stature, short hands and feet, progressive joint limitation and contractures, distinctive facial features, progressive cardiac valvular disease, and thickened skin. The molecular diagnosis is established in a proband who carries biallelic pathogenic variants in ADAMTSL2 or a heterozygous pathogenic variant in either FBN1 or LTBP3. LTBP3 mutations can be found in patients with arterial aneurysms and only one patient with valvular disease has been described. Segregation analysis of the mutation is currently ongoing.
P59: VarCoPP: a machine learning method to predict bilocus pathogenic variant combinations

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Although several machine-learning methods have been developed to assess single variant pathogenicity in the development of disease, these were not created to explicitly deal with situations where multiple variants are involved or cases of multiple clinical diagnoses in a single individual. As data on bilocus variant combinations (i.e. combinations of variants in gene pairs) leading to disease constantly accumulate in the scientific literature, we developed VarCoPP, the Variant Combination Pathogenicity Predictor, which is an innovative tool for the pathogenicity assessment of such variant combinations.

We trained VarCoPP on pathogenic bilocus variant combinations from the Digenic Diseases Database (DIDA) and neutral bilocus combinations from the 1000 Genomes Project (1KGP). For this, we kept exonic variants with a MAF frequency below 3% on confirmed protein-coding genes. We annotated all combinations with variant, gene and gene pair level features leading (after a recursive feature elimination procedure) to 11 useful features that could be used for predictions. Finally, we trained 500 different Random Forests (RFs) - each one using a different subset of the control dataset - to create an ensemble predictor (VarCoPP) that predicts the pathogenicity of bilocus variant combinations using a majority vote among the different RFs.

With VarCoPP we were able to differentiate pathogenic from neutral bilocus variant combinations of the training set using cross-validation, reaching a Matthews Correlation Coefficient of 0.74. VarCoPP ranks the results and provides evaluation scores for each prediction: a Classification Score (CS), that denotes the likelihood of
a bilocus combination being pathogenic and a Support Score (SS) that shows how many RFs agree with that decision. By testing on unknown neutral data from 1KGP we used these scores to enhance the clinical applicability of our method, as we defined confidence zones, delimited by minimum CS and SS scores, which guarantee with 95% or 99% probability that a prediction is indeed a true positive, guiding clinicians towards the most relevant results. Validation with 23 new bilocus disease cases, as well as 76 Dual Molecular Diagnosis cases, shows that the method has high sensitivity as well, reaching performance metrics similar to our cross-validation procedure on the training data.

Our results show that the first steps to multivariant pathogenicity prediction can be taken, expanding our capability to assist clinicians and geneticists in unraveling the causes of multilocus diseases.
P60: Identification and characterization of a novel and unusual myopathy-causing 11bp deletion mutation within the TARDBP gene

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Neurodegenerative diseases are often characterized by the presence of specific protein aggregates and as such are classified as proteinopathies. Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are two such proteinopathies. Proteinopathies are further subclassified according to the major aggregating protein found within the aggregates. In ALS and FTD, the TDP-43 proteinopathy is a major subgroup. In 97% of ALS cases and 45% of FTD patients, TDP-43 positive inclusions are found. Furthermore, missense mutations in the C-terminal region of TDP-43 are a genetic cause of ALS, emphasizing the crucial role of this protein in the pathogenesis of this disease.

TDP-43, is a predominantly nuclear RNA/DNA-binding protein which is tightly regulated and widely expressed across a variety of tissues. Structurally, TDP-43 contains two RNA recognition motifs (RRM1 and RRM2), a nuclear localisation signal (NLS) and nuclear export signal (NES), and a low complexity domain (LCD) in the C-terminus. The RRM mediates the binding to certain RNA/ssDNA whilst the LCD ensures the correct microenvironment needed for its proper functioning, through a process of liquid-liquid phase transition. TDP-43 shuttles form the nucleus to the cytoplasm, but has higher nuclear concentrations. In pathological conditions however, the protein is depleted from the nucleus and becomes insoluble, mainly forming cytoplasmic aggregates. Yet, the specific molecular mechanisms responsible for the pathogenesis remain unknown. Thus, unravelling the biophysical properties of TDP-43 aggregation and gaining insight into the pathological disease mechanism of TDP-43 aggregates is of utmost importance to get a deeper understanding of the overall pathogenic mechanisms of TDP-43 proteinopathies.

Recently, our lab performed whole exome sequencing in a large 3-generation family with an autosomal dominant slowly progressive neurogenic myopathy and discovered a never-before-reported and uncommon causal 11bp deletion mutation
in the TARDBP gene (c.1152_1162del) segregating with the disease. Whereas the
great majority of the known ALS-causing mutations are missense point-mutations in
the LCD of the C-terminal region, this mutant variant causes a frameshift that leads
to an entirely novel C-terminal domain (CTD). Since the LCD region is known to play
a key role in mediating the formation of pathological TDP-43 aggregates, our novel
mutation provides a unique opportunity to dissect the pathogenic role of TDP-43,
more specifically the role of its LCD by comparing the different pathological
conditions.
More precisely, we will first investigate the influence of the different mutations on
the physical properties (LLPS, aggregation and solubility) of the different proteins
using in silico and in vitro assays. In a next step, we want to shed light on the different
pathogenic pathways induced by the different mutant forms of the protein. To that
end, differences in binding partners will be mapped using pull-down assays in
combination with proteomic analysis. Subsequently, the impact of the identified
binding partners on the pathogenic outcome will be investigated. To do so, the
different mutations will be modelled and characterized in two model systems:
Drosophila melanogaster and iPSC-derived neurons.
Through the study of this unique variant it is our aim to further deepen our
understanding of the molecular and biophysical mechanisms that are the cause of
pathological TDP-43 aggregation, with the hope of coming a step closer to fully
unravelling the complex pathways underlying ALS and other TDP-43-related
proteinopathies.
P61: Genetic, proteomic, neuropathological and cellular studies to unravel RNF216-mediated neuronal degeneration

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Background: Mutations in genes controlling ubiquitin signaling pathways are found to be associated with mutant protein aggregation and mis-folding which are hallmarks of neurodegenerative diseases. Our group was among the first to report bi-allelic mutations in RNF216, an E3 ubiquitin ligase, as the genetic cause of Gordon Holmes syndrome (GHS), an adult-onset rare neurodegenerative disorder characterized by ataxia, dementia and hypogonadotropic hypogonadism (Margolin et al, 2013) or Huntington-like disorder (HDL) (Santens et al, 2015). Neuropathological analysis of one patient revealed ubiquitin-positive intranuclear inclusions (Margolin et al, 2013). To date, > 10 mutations in RNF216 have been reported. They are not only found within the catalytic active site of the protein but also in the more N terminal part of the protein. Mutations within the catalytic site of the protein are shown to abolish K63 ubiquitination whereas other mutations preserve this activity.

Research questions: The pathogenic mechanisms by which RNF216 mutations lead to GHS and HDL are not understood. Our efforts are therefore directed towards (1) the identification of novel RNF216 mutations for genotype-phenotype correlation studies, (2) the identification of the protein composition of the pathological intranuclear inclusions and (3) the cellular and biochemical function of the RNF216 protein.
Results: We describe a Spanish patient with HDL caused by 2 novel compound heterozygous missense mutations in RNF216. In addition, a detailed neuropathological study in brain material of a patient of one of the two originally reported Belgian families confirmed the presence the ubiquitin- and p62-positive intranuclear inclusion that were negative for Tau, α-synuclein and TDP-43. Frozen brain material was used to perform a more generalized proteomics approach, which yielded a list of interesting candidate genes. Histopathological examination of these candidate genes is currently ongoing. Finally, we are using several cellular model systems including patient fibroblast and Hela cells. Hela cells were transfected with wildtype RNF216 (isoform A and B separately), with mutant RNF216 constructs. The transfected cells were used for immunostaining using RNF216, Ubiquitin and p62 antibodies. Interestingly the transfected cells revealed RNF216 and ubiquitin positive intranuclear speckle-like bodies. The identity of these intranuclear bodies are being analyzed through use of specific antibodies for speckle and paraspeckles (NONO and PSPC1 antibodies).
P62: Implementation of WES-based gene panel testing in patients with neurodegenerative disorders at the University Hospital of Ghent: a retrospective observational analysis of 176 patients

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Introduction Neurodegenerative diseases are an important cause of morbidity and mortality and are associated with tremendous costs for health care systems. A significant part of these disorders is hereditary. A prompt and correct diagnosis not only has beneficial psychological consequences for patients and families but is also elementary for genetic counseling and potential therapeutic strategies. Neurodegenerative diseases are characterized by clinical and molecular heterogeneity, thus challenging diagnostics. Sequential targeted single gene testing has become obsolete and has been replaced by gene panel testing, enabling the analysis of multiple candidate genes at once. In January 2019 6 new WES-based gene panels have been introduced at the University Hospital of Ghent to facilitate diagnostics in patients with neurodegenerative diseases. In this study we want to establish the added value of these panels and define patient- and disease-specific characteristics associated with a high diagnostic yield.

Methods 6 novel gene panels were introduced in routine diagnostic setting at the CMGG (Centre for Medical Genetics Ghent): 1. Ataxia/spasticity (260 genes) 2. Leukodystrophy (179 genes) 3. Paroxysmal/episodic disorders (41 genes) 4. Movement disorders (127 genes) 5. Neurodegeneration with brain iron accumulation (16 genes) 6. Progressive myoclonic epilepsy (34 genes). Sequencing is performed on a Novaseq 6000 Illumina platform and data-analysis is done making use of Seqplorer, an in house developed tool for the analysis of WES-data. Statistical analysis is done using SPSS26 software.

Results From January the 1st up till December 31st 2019 176 cases have been analyzed; 48% are male, 52% female. The mean age of the patients is 50 years with a majority of the patients being adults and only 2% under 18 years of age. The leukodystrophy panel is most popular (43%), followed by the ataxia/spasticity panel (26%) and the movement disorders panel (20%). The overall diagnostic yield was 15%. In 70% of the cases no potential causal variants could be identified. In another
15% variants of unknown significance were found, whose further classification depends on family segregation data, additional paraclinical investigations, functional assays or data sharing efforts. The diagnostic yield was the highest among the patients presenting with cerebellar ataxia and/or spastic paraplegia: in 50% of these patients a definite or potential causative variant was identified. The diagnostic yield and the age of the patient were not significantly correlated. The causal mutations were missense (62%), nonsense (25%) and splice-site (10%) mutations. In 1 patient a larger exonic deletion was found via Exome Depth, an algorithm to detect relative read depth differences.

Conclusion We conclude that WES based gene panel testing is a successful tool to diagnose rare inherited neurodegenerative diseases. Multi-gene panel testing has the potential to avoid long and expensive diagnostic odysseys often including multiple sequential genetic tests and additional unnecessary (para)clinical investigations, though the cost-effectiveness was not touched upon in this study. The overall diagnostic yield after analysis of 176 cases was 15%, with another 15% of potential but unsure diagnoses. Patients suffering from ataxia and/or spastic paraplegia were most likely to obtain a molecular diagnosis. Age is not a drawback to initiate genetic testing.
P63: Sample identity confirmation in an exome sequencing workflow using pxlence’s Human Sample ID Kit

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Background
In both molecular diagnostic and research setting, gene panel, exome, and whole genome sequencing are routinely performed. Consequent to the custody transfers and complex library preparations that these samples undergo, they are prone to sample mix-ups and contaminations. It has been estimated that sample mix-ups occur in up to 1% of cases, showing the need for an independent and accurate method for sample identity confirmation. Here, we developed and validated an easy, cost-effective and flexible sample identity test. As proof-of-concept, we applied our Human Sample ID Kit a samples from a diagnostic exome sequencing workflow.

Methods
Target SNPs for the Human Sample ID multiplex PCR were selected based on their minor allele frequency, exonic location and overlap with the capture region of established exome enrichment kits. For compatibility with fragmented DNA from FFPE tissues or liquid biopsies, short amplicons of 130-170 bp around the SNPs were designed. By incorporation of universal tails in the locus specific primers, sequencing library preparation of the multiplex product is achieved by performing a limited-cycle indexing PCR step. Performance of our DNA sample identity method was evaluated by 150 bp paired-end sequencing on a MiSeq instrument (Illumina), after standard pooling and clean-up of the libraries.

Results
An excellent homogeneous coverage and on-target specificity was achieved using the Human Sample ID Kit consisting of 44 polymorphic SNPs and 6 gender markers. For all tested DNA samples, all polymorphic SNPs displayed a coverage higher than 20% of the mean coverage. More impressive, 92.6% of the SNPs had a uniform coverage within 2-fold of the mean. The mean on-target rate of the Human Sample ID Kit was 94%. Based on the resulting genotypes, all DNA samples could be unambiguously discriminated and linked to the corresponding exomes.

Conclusion
We developed and validated a novel sample tracking test for exome or whole genome sequencing, involving a straightforward ready-to-sequence 2-step multiplex
PCR reaction. The Human Sample ID Kit has a discrimination power of more than 1:85,000 and was shown to unambiguously discriminate DNA samples. In principle, our know-how could also be used to design customized gene panel-specific sample tracking solutions. The Human Sample ID Kit is available from www.pxlence.com.
**P64: WiNGS: A widely integrated platform for federated interpretation of whole genome sequencing data**

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Genomic medicine is driving the field of precision medicine, but its extensive use of genomic information is highly disruptive to current medical procedures, IT infrastructure and towards the role of clinical geneticists. We explored the feasibility to share large scale genomic data while preserving privacy, in combination with extensive clinical interpretation resources. Here, we present the resulting platform, WiNGS, enabling broad implementation of genomic medicine.

WiNGS is aimed at breaking down the complexity of analyzing genome sequencing data. It uses a federated data model to optimize ICT requirements of Whole Genome Sequencing (WGS) interpretation. Both genotype and phenotype data of individuals are kept locally, at the geographically distributed genomic centers, to ensure data protection. To facilitate setup, locale data stores are provided as a containerized module including the noSQL database and all required communication routines. Whereas sensitive data is kept at local data stores, sample-independent information is managed centrally. We provide variant annotation as a centralized service, together with the phenotype and disease knowledge base used to classify variants. This further reduces infrastructural requirements of individual centers, whilst synchronizing information across centers.

We believe that our platform is a major step for the diagnosis and treatment of rare diseases. First, the phenotyping module allows clinicians to already initiate a case before the sequencing data is available. Second, cross-center collaboration through the online and centralized WiNGS interface is provided based on fine-grained access control lists. Third, WiNGS offers near real-time analysis of WGS data, presenting family based variant selection results in mere seconds. And finally, the WiNGS platform has the power to compare data from patients from different hospitals, based on both genotype and phenotype, without revealing the patient’s identity. For rare diseases, this type of meta-analysis is the key to a successful diagnosis.
Preimplantation Genetic testing (PGT) for chromosomal abnormalities is performed for patients carrying chromosome abnormalities (PGT for structural rearrangements or PGT-SR; e.g. balanced reciprocal translocations or inversions) or for patients who experience fertility problems (PGT for aneuploidy or PGT-A; e.g. repeated implantation failure, recurrent miscarriages, severe male factor). Selecting and transferring embryos without an aneuploidy or structural rearrangements, could improve ongoing pregnancy rates and live birth rates while reducing miscarriage rates or avoid the birth of an affected child.

The PGT (and the prior IVF procedure) is a very extended, stressful and complex trajectory while precise outcome predictions are still hard to grasp. Counseling PGT couples remains a delicate matter, due to numerous parameters (anamnesis, indication, parental age...) influencing patients' clinical outcome. Comparing the success rates (e.g. fertilization, pregnancy and live birth rate) from the Ghent University Hospital to (inter)national available reference data (e.g. the European Society of Human Reproduction and Embryology or ESHRE), is part of good clinical practice. However, the evolution of PGT technology shifts in high speed, going from different timepoints of biopsy to altered genetic screening methodologies, with worldwide different strategies and guidelines. A straightforward reliably comparison of the clinical outcomes across a range of IVF centers and genetics labs is therefore difficult. However, there is important work in progress on determining structured and clear (inter)national guidelines. By detailed evaluation of our in house data, it will provide us a better insight into the complete PGT pathway, which can be used to evaluate and compare our outcomes with other IVF clinics and the (inter)national guidelines, to internally monitor the IVF and PGT procedure and to counsel patients with more individual and patient-focused information.

Therefore, we performed a retrospective observational cohort study at the Department for Reproductive Medicine and the Center for Medical Genetics of the Ghent University Hospital, Belgium. Patients who underwent an ICSI treatment during January 2016 to April 2019 and had undergone PGT-SR (39 couples, 68 oocyte retrieval cycles, 280 embryos) or PGT-A (77 couples, 90 oocyte retrieval cycles, 303
embryos) were included. PGT-SR and PGT-A was performed by microarray-based comparative genomic hybridization (arrayCGH) or shallow whole genome sequencing (sWGS), followed by copy number variation (CNV) analysis, with a genome wide resolution of ~5 Mb. Live birth rate per transfer was the primary clinical outcome measure of this study. Secondary outcomes measures were pregnancy rate per embryo transfer and pregnancy loss rate per pregnancy.

This extended study provided us valuable information for adequate monitoring the quality of the complete PGT process within the Ghent University Hospital and to properly inform couples starting a PGT trajectory.
P66: PhenBook: federated data structure model to collect and analyse phenotype

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The main bottleneck for the biomedical use of next-generation sequencing is the medical interpretation of large-scale genomic data. Currently, the use of only genomic data with limited or low-quality access to the curated clinical and phenotypic information is a major obstacle to accurately diagnosing disease mutations. This implies a need for a secure system that collects and stores phenotypic information of the individuals and links them to genotype.

We propose a bioinformatics interface called PhenBook under WiNGS (Widely integrated NGS platform) project. This interface stores the clinical observations of patients in a structural way using Human Phenotype Ontology (HPO) and associates them with OMIM and Genes. This information is being used in the variant filtering and prioritization. By this way, we increase the accuracy of the results.

Since WiNGS is based on the federated data model, users are able to securely and with respects to the privacy of the individuals, query the data across different centers and check the frequency of occurrence of a certain mutation in combination with the phenotype of interest. We hope that our model helps researchers toward the discovery of rare diseases.
P67: Advanced variant filtering for interpretation of WGS data

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Today’s clinical sequencing covers only a tiny portion of the entire genome. The reason: storage and computing requirements for full-scale analysis are nearly impossible to meet. Therefore, the goal of this project has been to develop a bioinformatics workflow (pipeline) for multi-step processing of human full genome sequencing data, from the data generated by the sequencing instrument located in different locations and making the data ready for interpretation by a genomics specialist. To address all these challenges, we developed WiNGS (Widely integrated NGS) platform to break down the complexity of analyzing genome sequencing data. WiNGS using a distributed data model to optimize the ICT infrastructure required to support and enable Whole Genome Sequencing (WGS). Analyzing the huge amount of complex data needs an advanced filtering option to reduce the number of potential mutations from thousands of cases to few. Therefore, in the WiNGS platform, we developed an advanced, user-friendly and optimized filtering module. Filters are constructed based on the tree structure and users are able to add different kind of comparisons such as AND, OR, NOT, and etc., with few clicks. We showed that by this filtering module how much time the researchers can save to accomplish their goals.