T01 - Mapping the 3D genome of the human retina and its role in retinal disease

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Genome-wide cis-regulatory elements (CREs) coordinate retinal development by meticulously controlling gene expression. Yet a map of the retinal 3D genome, required to link CREs to their target genes, is still missing. Therefore, we mapped the 3D genome of the adult human retina to (1) assess the structure and conservation of topologically associating domains (TADs) at retinal disease loci; (2) determine how these are affected by pathogenic structural variants.

We used in-situ Hi-C to map genome-wide interactions in neural retina and retinal pigment epithelium from adult donor eyes (n=3), as well as patient fibroblasts with dominant cone dystrophy due to a duplication at the IRXB cluster (n=2). Comparing TAD structure in Hi-C maps from retina versus clinically accessible tissues (e.g. fibroblasts, lymphoblastoid cells; public Hi-C data), we found that retinal tissues displayed distinct TAD structures at several retinal disease loci. This has important implications for the usability of Hi-C on accessible patient material in a clinical context. For example, using Hi-C on patient fibroblasts, we determined that a duplication at the ultraconserved IRXB locus resulted in the formation of a neoTAD containing IRX5. However, comparison to retinal TAD boundaries implied that a different neoTAD would be formed in the retina, possibly with distinct effects on gene expression.

In summary, we have generated the first 3D genome maps of the human retina and identified both conserved and retina-specific 3D topologies. The latter potentially limit the usability of Hi-C on clinically accessible tissues for the interpretation of structural variation in (retinal) disease.

T02 - Single-cell evaluation of DNA damage in offspring after prenatal exposure to chemotherapy

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Introduction
Chemotherapy during pregnancy is considered relatively safe. However, given that chemotherapeutics are genotoxic and can cross the placenta, it might be hypothesized that prenatal exposure associates with a genetic signature in newborn DNA.
Methods
Cord blood mononuclear cells (CBMCs) of (i) pregnant breast cancer patients treated with epirubicin, cyclophosphamide and/or paclitaxel (BrCa+EC/T, n=3), (ii) non-treated control pregnant breast cancer cases (BrCaCo, n=4), (iii) pregnant Hodgkin lymphoma patients treated with doxorubicin, bleomycin, vinblastine and dacarbazine (HL+ABVD, n=4) and (iv) healthy pregnant women (HPr; n=10) were subjected to (a) cytokinesis-block micronucleus analysis and (b) whole-genome sequencing (WGS) of clonally expanded hematopoietic stem cells in order to map chromosomal breakage and/or loss via micronucleus frequency (MNF), and the presence of single nucleotide variants (SNVs) and/or small indels respectively.
Results
We observed a significant 3-4-fold increase in MNF in CBMCs from BrCa+EC/T (27.50MN/1000cells), and HL+ABVD patients (33.57MN/1000cells) compared to HPr cases (7.27MN/1000cells; $p<0.01$), pointing to chromosomal instability being linked to prenatal chemotherapy exposure. Remarkably, CBMCs from BrCaCo patients also showed a significant 2-fold increased MNF (18.76MN/1000cells) compared to HPr cases ($p<0.01$), suggesting a genetic and/or oncological factor contribution. WGS revealed a significant increase in somatic SNVs (2-3-fold; $p=0.04$) and indels (5-7-fold; $p=0.0001$) in cord blood DNA from all treated cases compared to HPr (n= 3). Both EC/T and ABVD treatment associated with elevated C>T and C>A substitutions, whereas ABVD treatment was also linked to T>C conversions. Using the SBS COSMIC catalogue, the EC/T-linked mutation pattern showed similarities with the reported platinum signature, whereas the ABVD-linked mutation pattern showed analogy with DNA mismatch repair signatures.

Conclusions

These primary findings indicate that prenatal chemotherapy exposure is linked with an increased MNF, SNVs and indels in cord blood cells. Further investigations in larger cohorts are ongoing to confirm these findings and define the exact mutational signature.

T03 - Hybrid Autoencoder with Orthogonal Latent Space for Robust Population Structure Inference

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Analysis of population structure and genomic ancestry remains an important topic in human genetics and bioinformatics. Commonly used methods require high-quality genotype data to ensure accurate inference. However, in practice, laboratory artifacts and outliers are often present in the data. Moreover, existing methods are typically affected by the presence of related individuals in the dataset.

In this work, we propose a novel hybrid method that combines the strengths of traditional matrix decomposition-based (e.g., principal component analysis, i.e., PCA) and more recent neural network-based (e.g., autoencoders, i.e., AE) solutions. Performances were evaluated using both simulated data and real genotype data from the 1,000 Genome project, the Human Genome Diversity project, and the Adolescent Brain Cognitive Development project.

Based on the incorporation of Identity-by-State information, our method achieves the best robustness in simulations projecting poor quality target samples onto a reference ancestry space. Interestingly, like PCA and in contrast to AE, the stability, and therefore repeatability, of the ancestry inference is very acceptable and due to the presence of orthogonality in the latent representation dimensionality selection is done more readily. Furthermore, like AE and in contrast to PCA, our approach can construct a stable ancestry space in the presence of relatedness. Finally, the learned latent representations reflect various properties of the data such as cluster identities, such that our method improves both super-population clustering but also data visualization using a lower number of dimensions.

T04 - Mutational processes in a Dutch cohort of children with Constitutional Mismatch Repair Deficiency

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Constitutional Mismatch Repair Deficiency (CMMRD) is a high-risk childhood cancer predisposition syndrome. Most patients develop multiple tumors during childhood and decease at young age. Patients with CMMRD have bi-allelic germline pathogenic variants in one of the mismatch repair (MMR) genes PMS2, MSH6, MSH2 or MLH1, impairing repair of single base substitutions (SBS) and small insertions and deletions (indels). Tumors of CMMRD patients are characterized by a high tumor mutational burden (TMB) and specific mutational signatures, but the tissue-specific effects of MMR deficiency and the impact of therapy are not fully understood. We aim to study these mutational processes by analyzing the mutational patterns in relation to the MMR gene involved, tissue type and prior treatment.
We sequenced tumor and normal DNA from 17 Dutch CMMRD patients. Together, these patients developed 68 (pre)malignant conditions, of which we were able to include 15 GI-tract tumors, 13 brain tumors and 10 hematological tumors. In line with previous studies, we found that tumors of patients with PMS2 deficiency have markedly different SBS mutational patterns, strongly resembling MMR-associated reference signature SBS26 in hematological tumors and a combination of SBS26 and SBS15 in GI-tract and brain tumors. Nearly all brain tumors and a subset of GI-tract tumors were ultra-hypermutated (SBS TMB above 100 mut/MB) due to a somatic POLE mutation, suggesting that POLE-mediated ultra-hypermutation is required for brain tumor development. The mutational patterns in these tumors also reflect a POLE defect (combined presence of SBS14, SBS15 and indel signature ID1). Subsequent hematological malignancies in two patients revealed a significant contribution of temozolomide-associated signature SBS11, which is in line with prior treatment with this agent in an MMR-deficient context. In conclusion, we have shown that tumors in CMMRD patients show gene-specific and tissue-specific mutational patterns and can display therapy-associated patterns in subsequent tumors.

T05 -

T06 - An unexpected moonlighting function of GTF3A in anti-herpesviral immunity: a new monogenic cause of herpes simplex encephalitis?

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Herpes simplex virus type 1 (HSV-1) is one of the most common human pathogens affecting several billion people worldwide. In rare cases, the virus causes herpes simplex encephalitis (HSE). In approximately 5% of patients with HSE monogenic defects of TLR3/IFN-I signaling have been described, emphasising the role of primary immunodeficiencies underlying HSE pathogenesis. Whole exome sequencing in a patient with HSE revealed compound heterozygous loss-of function mutations in GTF3A, located in one of the conserved cysteine residues of the C2H2 zinc finger. The identified GTF3A mutants demonstrated impaired 5S rDNA-binding ability. HSV-1 infection of primary patient fibroblasts and HEK293T GTF3A knock-in clones showed increased viral replication. ChIP-Seq analysis in search of alternative targets of GTF3A identified the recently described RIG-I ligand RNA5SP141. We showed that GTF3A mutations abrogate RNA5SP141 expression with decreased RIG-I activation which ultimately resulted in an impaired type I IFN defense against HSV-1 infection. Our work illustrates the role for host RNA as immunostimulatory ligands in viral infections and identifies for the first time an impairment of non-coding RNA metabolism as a monogenic cause of impaired anti-herpesviral immunity.

T07 - Loss of adipocyte phospholipase gene PLAAT3 causes lipodystrophy with neurological features due to inactivated arachidonic acid-mediated PPARγ signaling

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PLAAT3 is a phospholipid modifying enzyme predominantly expressed in white adipose tissue (WAT). It is a candidate drug target as Plaat3 deficiency in mice protects against picornavirus infection and diet-induced obesity. We identified five patients from three independent consanguineous families, with homozygous loss-of-function mutations in PLAAT3, presenting with severe lipodystrophy and neurological features including intellectual disability and a demyelinating peripheral neuropathy. PLAAT3-deficient WAT showed a failure to liberate arachidonic acid (AA) from membrane phospholipids resulting in an inactive gene network downstream of adipogenesis master regulator and anti-diabetic drug target PPARG. CRISPR/Cas9-mediated PLAAT3-/- human adipose stem cells (ASC) displayed insulin resistance and showed a disturbed differentiation characterized by a significant decrease in lipid droplet formation and a downregulation of PPARγ and perilipin. These findings establish PLAAT3 deficiency in humans as a novel type of hereditary lipodystrophy due to an AA- and PPARG-dependent defect in WAT differentiation and function.

T08 - Somatic activating PIK3R1 and non-hotspot PIK3CA mutations associated with a newly identified clinical phenotype: Capillary Malformation with Dilated Veins (CMDV)

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Introduction:
Capillary malformations (CMs) are vascular anomalies usually due to activating mutations in the GNAQ or GNA11 gene, that occur in endothelial cells (ECs). In this study, we focused on CMs that did not have such a mutation.
Materials and Methods:
We used deep-targeted next-generation sequencing (NGS) to study DNAs extracted from CM lesions that did not carry a GNAQ or GNA11 mutation. We identified three somatic variants in the PIK3CA gene and four in the PIK3R1 gene, encoding respectively for the catalytic and the regulatory subunit of the PI3K kinase, in 11 samples belonging to 9 patients. The identified variants are all known somatic mutations in cancer. Yet, the variants were all variable non-hotspot mutations. We also isolated primary endothelial cells from n=2 lesions, and we characterized these cells with 2D and 3D in vitro studies.
Results and Discussion:
A posteriori characterization of the patients' lesions unravelled a unreported vascular phenotype: a pale capillary malformation associated with visible dilated veins. Thus, we named these newly-identified lesions as capillary malformation with dilated veins (CMDV). Two lines of patient-derived endothelial cells (ECs) harbouring two distinct identified PIK3R1 variants showed increased sprouting ability compared to wild type HUVEC cells. In addition, activation of the PI3K/AKT/mTOR pathway in CMDV-isolated ECs was indicated by increased AKT phosphorylation. This activation was partially inhibited following treatment with the mTOR inhibitor rapamycin, the AKT inhibitor MK2206 and the PIK3CA inhibitor BYL719, opening patient-tailored treatments by repurposing cancer drugs for CMDV. This underscores the importance of genetics studies to better stratify distinct capillary phenotypes and distinguish them from GNAQ and GNA11 mutated common CMs.

T09 - MAN2C1, a new gene associated with the development of cortical malformations

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The development of the cerebral cortex is a complex process in three major steps: (1) proliferation of the neuronal progenitor cells, (2) migration, and (3) organization and maturation of the neurons. Defects in one step may result in malformations of the cortical development (MCD). MCD can be caused by environmental factors or genetic alterations, although the cause is often unclear, and many genes remain to be discovered.
We report the clinical, biochemical, and molecular features of six individuals from four different families with bi-allelic pathogenic variants in the mannosidase class 2C member 1 (MAN2C1) gene identified through exome sequencing. MAN2C1 encodes a cytosolic mannosidase, involved in the catalyzation of oligosaccharides resulting from both the endoplasmic reticulum-associated degradation pathway (ERAD) and the hydrolysis of release of free oligosaccharides from lipid-linked oligosaccharide by OST (LLO pathway) in the cytosol. The clinical phenotype was characterized by dysmorphic facial features, congenital anomalies such as tongue hamartoma, variable degrees of

intellectual disability, and brain anomalies including polymicrogyria, interhemispheric cysts, hypothalamic hamartoma, callosal anomalies, hypoplasia of brainstem and cerebellar vermis. We confirmed the pathogenicity of three of the identified missense MAN2C1 variants by complementation experiments with isogenic MAN2C1-KO HAP1 cells. We further demonstrate that MAN2C1 variants lead to accumulation and delay in the processing of free oligosaccharides in proband-derived cells. These results highlight the involvement of MAN2C1 in human neurodevelopmental disease and the importance of free oligosaccharide catabolism.

T10 - CAMLG-CDG: a novel Congenital Disorder of Glycosylation linked to defective membrane trafficking

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The transmembrane domain recognition complex (TRC) pathway is required for the insertion of C-terminal tail-anchored (TA) proteins into the lipid bilayer of specific intracellular organelles such as the endoplasmic reticulum (ER) membrane. In order to facilitate correct insertion, the recognition complex (consisting of BAG6, GET4 and UBL4A) must first bind to TA proteins and then to GET3 (TRC40, ASNA1) which chaperones the protein to the ER membrane. Subsequently, GET1 (WRB) and CAML form a receptor which enables integration of the TA protein within the lipid bilayer. We report an individual with the homozygous c.633+4A>G splice variant in CAMLG, encoding CAML. This variant leads to aberrant splicing and lack of functional protein in patient-derived fibroblasts. The patient displays a predominantly neurological phenotype with psychomotor disability, hypotonia, epilepsy and structural brain abnormalities. Biochemically, a combined O-linked and type II N-linked glycosylation defect was found. Mislocalization of syntaxin-5 in patient fibroblasts and in siCAMLG deleted Hela cells confirms this as a consistent cellular marker of TRC dysfunction. Interestingly, the level of the v-SNARE Bet1L is also drastically reduced in both of these models, indicating a fundamental role of the TRC complex in the assembly of Golgi SNARE complexes. It also points towards a possible mechanism behind the hyposialylation of N and O-glycans. This is the first reported patient with pathogenic variants in CAMLG. CAMLG-CDG is the third disorder, after GET4 and GET3 deficiencies, caused by pathogenic variants in a member of the TRC pathway, further expanding this novel group of disorders.

T11 - A cross-country comparison of women's perspectives on non-invasive prenatal testing in Belgium and the Netherlands

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Background/Objectives:
Belgium and the Netherlands are among the few countries in the world to offer non-invasive prenatal testing as a first-tier screening test to all pregnant women. An informed and autonomous decision is considered important when deciding to participate in prenatal screening for aneuploidy. Despite the similarities, counselling modalities and uptake rates between both countries differ. We assessed the differences in perspectives of pregnant women who opted for prenatal screening with NIPT.

Methods:
A cross-country comparison study between the Netherlands and Belgium, using a questionnaire. The questionnaire was developed for the TRIDENT-2 study and assessed informed choice (MMIC), and personal and societal perspectives on Down syndrome.

Results:
A total of 1031 women having NIPT participated in the survey study; 444 women from Belgium (B) and 587 women from the Netherlands (NL). Differences between Belgian and Dutch women were shown for the level of informed choice (58.8%(B) and 82.6%(NL)), intention to terminate in case of confirmed Down syndrome (61.9%(B) and 50.5%(NL)) and how the disorder was perceived in terms of severity (80.9%(B) and 64.3% (NL)). More Belgian women indicated that they believed parents are judged for having a child with Down syndrome, compared to the Dutch women (42.3% vs. 16.3%). Also, Belgian women were less positive about the care and support for children with Down syndrome, as compared to their Dutch counterparts (22.5% vs. 62%).

Conclusion:
This study indicates that counseling modalities and societal and cultural aspects may impact women's perspectives.

T12 - The presence of viral DNA in a cohort of 108,349 Dutch NIPT samples and its relation to characteristics in pregnancy and cell-free DNA biology

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Viral infections during pregnancy are a major health concern to mother and fetus. By repurposing cell-free Non Invasive Prenatal Testing (NIPT) sequencing data, we investigated the prevalence and abundance of viral DNA in a cohort of 108,349 pregnant women.
We detect viral DNA in approximately 40% of the NIPT samples across a wide range of viral species: Herpes, Adeno, Papilloma, Parvo and Polyomaviruses. Several are known to be potentially harmful during pregnancy and/or childbirth, including Cytomegalovirus, Parvovirus B19 and Hepatitis B. Viral DNA was mostly detected at very low abundance. However, several cases had exceptionally high viral loads for Parvovirus B19, Hepatitis B and others.
The presence of viral DNA, specifically that of Cytomegalovirus, was significantly associated with various pregnancy related characteristics, such as gestational age, maternal age, fraction of fetal cfDNA and the total cfDNA concentration.
We demonstrate the feasibility to detect viral DNA from typical genome-wide NIPT cfDNA sequencing and describe the main characteristics of the viral DNA in our cohort. Our dataset of detected viral sequence reads is made publicly available to guide future clinical implementations.

T13 - Multicentric longitudinal performance monitoring of different non-invasive prenatal screening technologies used in Belgium

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Background/Objectives :

Belgium was the first country to fully reimburse the noninvasive prenatal screening (NIPS) as a nationwide first-tier screening test to all pregnant women. Different commercial and in-house developed NIPS technologies are being used. Although the accuracies (sensitivity, specificity, positive predictive value, negative predictive value) of the commercial tests are provided by the companies, multicentric longitudinal studies to monitor and compare performance of those methods are lacking. Since all invasive prenatal genetic testing following positive NIPS are analyzed at the Belgian genetic centers, we are uniquely positioned to determine the performance of different NIPS technologies.

Method/Results:

From all invasive genetic tests performed from 01/01/2020 to 01/05/2021, 303 were done following a positive NIPS in a clinical laboratory with respectively 134, 37 and 24 indicative of trisomy 21, 18 and 13. For trisomy 21, the actual PPVs for VeriSeq®(Illumina), Harmony®(Roche) and Vanadis®(Perkin-Elmer) were respectively 69%, 91% and 65%, significantly lower than the 95%, 98% and 94% advertised. The PPV from the 8 genetic centers using a Laboratory Developed Test (LDT) was 92% (Van Den Bogaert K et al Genet Med. 2021).

Conclusion:

This difference in PPV has a significant impact on both pregnant women and the health care system. In Belgium there are about 120000 pregnancies per year. For example, with a population incidence for trisomy 21 of 0.3%, a PPV of 69% versus 92% corresponds to a yearly increase of unnecessary invasive tests from 28 to 112. Our study underscores the value of laboratory developed testing to improve prenatal – and by extension – overall health care.

T14 - Noninvasive Prenatal Test results indicative of maternal malignancies: a nationwide genetic and clinical follow-up study

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Objectives
Noninvasive Prenatal Testing (NIPT) for fetal aneuploidy screening using cell-free DNA derived from maternal plasma can incidentally raise suspicion for cancer. Diagnostic routing after a malignancy suspicious-NIPT faces many challenges. We detail malignancy suspicious-NIPT cases, describe the clinical characteristics, chromosomal aberrations and diagnostic routing of the patients with a confirmed malignancy. Clinical lessons can be learned from our experience.
Methods
Patients with NIPT results indicative of a malignancy referred for tumor screening between April 2017 and April 2020, were retrospectively included from a Dutch nationwide NIPT implementation study, TRIDENT-2, in which both genome-wide NIPT and targeted NIPT were performed. NIPT profiles from patients with confirmed malignancies were reviewed and the pattern of chromosomal aberrations related to tumor type was analyzed. We evaluated the diagnostic contribution of clinical and genetic examinations.
Results
Malignancy suspicious-NIPT results were reported in 0.03% after genome-wide NIPT, and malignancies confirmed in 16 patients (16/48, 33.3%). Multiple chromosomal aberrations were seen in 23 of 48 patients with genome-wide NIPT, and a malignancy was confirmed in 16 patients (16/23, 69.6%). After targeted NIPT, 0.005% malignancy suspicious-NIPT results were reported, in 2/3 patients a malignancy was confirmed. Different tumor types and stages were diagnosed, predominantly hematological malignancies (12/18). NIPT data showed recurrent gains and losses in primary mediastinal B-cell lymphomas and classic Hodgkin lymphomas. Magnetic resonance imaging and computed tomography were most informative in diagnosing the malignancy.
Conclusion
In 231,896 pregnant women, a low percentage (0.02%) of NIPT results were assessed as indicative of a maternal malignancy. However, when multiple chromosomal aberrations were found, the risk of a confirmed malignancy was considerably high. Referral for extensive oncological examination is recommended, and may be guided by tumor-specific hallmarks in the NIPT profile. The incidence of a confirmed malignancy following a malignancy suspicious-NIPT can be directly used in clinical practice, enabling malignancy focused counseling and prompting an efficient diagnostic work-up.

T15 – NICUSeq: A Trial to Evaluate the Clinicak Utility of Human Whole-Genome Sequencing (WGS) Compared to Standard of Care in Acute Care Neonates and Infants (NICU-Seq).

The presentation highlights the results of the recently published NICUSeq clinical trial, which investigated the impact of Whole-Genome Sequencing (WGS) on the management of acute care neonates. The study's findings show that both focused clinical management and diagnostic efficacy are significantly increased when patients have access to WGS testing. The study's findings show that both diagnostic efficacy and change of management are significantly improved when patients have access to WGS testing.

T16 - Generic genome sequencing: one lab flow for all

Gaby Schobers[1], Ronny Derks[2], Ermanno Bosgoed[2], Debby Hellebrekers[3], Nicole de Leeuw[2], Alexander P. Stegmann[3], Erik-Jan Kamsteeg[2], Aimee Paulussen[3], Marjolijn Ligtenberg[2], Arthur van den Wijngaard[3], Christian Gilissen[4], Marinus J. Blok[3], Han G. Brunner[5], Helger G. IJntema[1], Marcel R. Nelen[2] & Lisenka E.L.M. Vissers[1]

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Genetic laboratories maintain numerous workflows to diagnose the full spectrum of hereditary and congenital diseases, including traditional approaches and advanced technologies. A single generic workflow would increase efficiency quite dramatically. We therefore assessed whether genome sequencing (GS) can replace all existing workflows supporting germline genetic diagnoses.

We performed GS (NovaSeq6000TM; 37x mean coverage) on 1,000 cases with 1,271 clinically relevant variants, selected from one year's diagnostic yield in a tertiary referral center, identified through 15 different workflows. Variants were binned by size and type: small variants (SNVs/indels <50 bp), large variants (CNVs and repeat expansions) and other variants (SVs and aneuploidies). VCFs were queried per variant and assessed in Trusight Software Suite (DRAGEN Germline Pipeline, TSS, Illumina).

Overall, 93.9% (1,194/1,271) of variants were detected with GS. Detection rates differed per type, with small variants detected in 95.2% (825/867), large variants in 91.9% (328/357), and other variants in 87.2% (41/47). Importantly, variants were identifiable through routine clinical interpretation strategies, including disease-based clinical filters or gene-specific searches in TSS. Variants that remained undetected were mosaic or located in homologous/repetitive regions.

GS is an efficient generic workflow to capture clinically relevant germline variants in a 'one-test-fits-all-strategy'. Besides those already known for short-read sequencing, no new challenges in variant detection were identified. GS can therefore not only replace exome sequencing, but also >99% of Sanger sequencing, allele specific PCRs, smMIP, MLPA, array, and cytogenetic analyses including karyotyping and FISH. These results provide perspective on how genetic laboratories will evolve in the near future.

T17 - GENType: all-in-one preimplantation genetic testing by pedigree haplotyping and copy number profiling suitable for third-party reproduction

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Objective:
Preimplantation genetic testing (PGT) is performed in an assisted reproductive technology setting and was designed to prevent transfer of embryos affected by a genetic disorder. As of today, PGT is performed in three different contexts: testing for recognized heritable monogenic disorders (PGT-M), screening for aneuploidy (PGT-A), and screening for structural chromosomal rearrangements (PGT-SR). Given the varying natures of these investigations (e.g., targeted versus genome-wide, etc.), a cost-effective and automatable 'all-in-one' approach is necessary. We aimed to develop a workflow and user-friendly visualization platform for all-in-one PGT, suitable for parents-only haplotyping (absence of affected family member) and, for the first time, third-party reproduction.

Design and Methods:
257 samples biopsied from cell lines and human blastocysts were whole genome amplified and processed by our newly developed reduced representation sequencing-based technology 'GENType'. Quality metrics, genome-wide haplotypes, b-allele frequencies and copy number profiles were generated by our novel visualization tool 'Hopla'. PGT-M results were deduced from relative haplotypes, while PGT-SR/PGT-A results were inferred from read-count analysis and BAF profiles. Suitability for parents-only haplotyping or third-party reproduction by single-parent haplotyping was assessed by excluding additional family members or one biological parent from analysis, respectively. Results were validated against reference PGT methods.

Results:
Genome-wide haplotypes of single cells were highly accurate (mean>99%) compared to bulk DNA. Unbalanced chromosomal abnormalities (>5Mb) were detected by GENType. For both PGT-M as well as PGT-SR/PGT-A, our technology demonstrated 100% concordance with reference PGT methods for diverse WGA methods. Equally, for parents-only haplotyping and single-parent haplotyping (of autosomal dominant disorders and X-linked disorders), PGT-M results were 100% concordant. Furthermore, the origin of trisomies in PGT-M embryos was correctly deciphered by Hopla.

Conclusion:
GENType together with Hopla offers an all-round PGT solution for diverse families without the need for personalized assays, microarray technology or whole genome sequencing.

T18 - The Dutch Center for RNA Therapeutics: a center to develop antisense oligonucleotide therapies for patients with nano-rare mutations

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Antisense oligonucleotides (AONs) offer the potential to treat patients with genetic diseases. Notably, for tissues allowing local injection, such as the brain and eye where high local exposure can be achieved with 3-4 infusions of low amounts of AONs annually. Proof-of-concept has been shown for example in spinal muscular atrophy and Leber congenital amaurosis. This approach can also benefit patients with private mutations, as was recently evidenced by the development of the custom-made AON milasen for a patient with Batten's disease. This underlines the potential of AONs as personalized medicines, specifically for patients with private mutations that are associated with brain or eye phenotypes. However, pharmaceutical companies are usually not interested in the development of such approaches, due to the extreme rarity of these variants.

The Dutch Center of RNA Therapeutics (DCRT) is a collaboration of Dutch academic centers with a track record in AON development that aims to develop therapies for patients with nano-rare variants and to offer these therapies in a not-for-profit manner. The DCRT works in alignment with the N-of-1 collaborative (global) and the 1 mutation 1 medicine (1M1M, European) initiatives. In the first two years, we have identified several patients with mutations that are suitable for splice modulation by AONs. Here, we outline the pre-clinical development of AON-based splice correction for a cryptic splicing mutation underlying Stargardt disease affecting the eye, and Beta-propeller Protein-Associated Neurodegeneration (BPAN) affecting the brain. We describe the Dutch roadmap towards clinical implementation, highlighting also the efforts to align developments internationally.

T19 - Analysis of the genomewide BAF profiles of selected SNPs allows reliable aneuploidy detection in preimplantation embryos, independent of haplotyping.

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We developed a new method for aneuploidy detection in biopsies obtained from preimplantation embryos. First, the raw B-allele frequency (rBAF) values of SNPs are obtained from a sample of interest with SNP array or NGS. Next, the BAF values for specific categories of SNPs (cBAF) are visualized, categories which are based on the parental genotype combination. Together with the analysis of the Log2R profile, this allows discrimination of all common types of chromosomal anomalies without haplotype information, as shown by reanalyzing data from 8376 chromosomes in 349 embryos which had previously been analyzed with Karyomapping. Without haplotype information, we identified all chromosomes with both parental homolog (BPH) anomalies (n=70) and chromosomes with a non-mosaic copy number loss larger than 5Mb (n=93) that had been detected with Karyomapping. We propose to use the cBAF profiles for an improved aneuploidy detection in MDA amplified trophectoderm samples.

T20 - Speaker:

Rajendra Kumar Chauhan

Fluidigm

Abstract:
The advent of next generation sequencing (NGS), together with efficient PCR assays have accelerated our understanding of a myriad of different research applications – from gene expression studies, to viral epidemiology, microbiome research, all the way down to biomarker discovery and validation.

In this presentation we will highlight how Fluidigm microfluidic based library preparation methods have allowed for the identification of germline pathogenic variants and tumor genes with targeted NGS assays in multi-site studies of more than 120,000 samples. We will also highlight how targets discovered with NGS methods can be efficiently validated using high-throughput gene expression qPCR assays on hundreds to thousands of samples at a time.

T21 - A tapt1 knockout zebrafish line with aberrant lens development and impaired vision models human pediatric cataract

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Mutations in the gene coding for human trans-membrane anterior-posterior transformation protein 1 (TAPT1) were previously reported to cause a complex lethal Osteochondrodysplasia characterized by undermineralization of the skeleton, several fractures and congenital anomalies. In another report, a TAPT1 mutation was identified in a patient with pediatric cataract (posterior lenticonus cataract), although without any evidence of skeletal involvement. This indicates a broad phenotypic spectrum for TAPT1 mutations, where pleiotropic and severity differences most likely depend on the type of mutation.

In this study, we report a patient with Osteogenesis imperfecta (osteopenia, multiple fractures, bowing of long bones), several dysmorphic features and bilateral cataract. The patient carries a homozygous 2-bp deletion (c.185_186del, p.(Arg62ProfsTer15)) in exon 1 of the TAPT1 gene, resulting in the first frameshift mutation reported to date. To gain insights into the pathogenetic mechanisms caused by loss of TAPT1 and to investigate the resulting phenotypic spectrum, a CRISPR/Cas9 knock-out (KO) zebrafish model was created. The zebrafish tapt1a-/-;tapt1b-/- mutant has an aberrant eye phenotype, with a small and fibrotic lens, dysregulated retinal layers and severely impaired vision. A marked increase in pigmentation of the eye and skin was noted. Zebrafish KO mutants showed a major increase in the locomotor activity during light-dark transmission in a visual motor response test (VMR). The cartilaginous and mineralised structures did not show any differences between mutants and wild type siblings. Finally, RNAseq analysis revealed a significant downregulation of crystallin gene expression and the phototransduction pathway, and increased inflammation and extracellular matrix production, corresponding to the observed lens abnormalities.

In conclusion, our study reports on the first patient and corresponding zebrafish model with complete loss of TAPT1. Morphological and functional phenotyping showed that this zebrafish model recapitulates human pediatric cataract, while transcriptomic analysis revealed the underlying pathogenetic mechanisms induced by loss of TAPT1.

T22 - Single-cell transcriptional dynamics and in vivo enhancer assays provide insight into gene regulatory networks of PRDM13 and IRX1 implicated in North Carolina macular dystrophy

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Purpose: North Carolina macular dystrophy (NCMD) is an autosomal dominant developmental disease, hypothesized to be a retinal enhanceropathy with noncoding variants and duplications overlapping with cis-regulatory elements (CREs) near PRDM13 or IRX1. We aimed to provide insight into the disease by single-cell(sc) transcriptional dynamics and in vivo enhancer assays. Methods: Single-nucleus RNA-seq (snRNA-seq) data of embryonic and adult (n=3) human retina was mined for sc expression of PRDM13 and IRX1. Five previously identified candidate CREs were assessed using enhancer assays in Xenopus (X.) tropicalis and/or albino X. laevis using a enhancer detection vector. Result: Transcriptional profiling during retinal development showed predominant expression of PRDM13 in amacrine cells, with low expression in retinal progenitor cells (RPCs), horizontal, and retinal ganglion cells (RGCs). IRX1 showed low expression during development, with highest expression in RGCs and weakest in RPCs. One of the hotspot regions for PRDM13 drove EGFP expression in eye and brain. IRX1 cCRE drove EGFP expression in the neural plate and tube at NF stage 15 and 20. At NF stage 42 and 45 EGFP expression was seen in neural crest derivatives and the eyes, and at stage 55 in the eye. The cCRE containing the shared duplicated region of IRX1 showed lower EGFP expression. Conclusion: A shared developmental expression in amacrine cells and RGCs and to a lesser extent in RPCs has been shown for PRDM13 and IRX1. Genetic defects in both loci may affect CREs that are active in early RGCs. Due to the dominant nature of NCMD a gain-of-function impairing PRDM13 or IRX1 expression can be suspected, perturbing macula-specific synaptic interactions between amacrines and RGCs during retinogenesis.

T23 - SRSF1 haploinsufficiency is responsible for a new syndromic form of developmental delay including marfanoid habitus with intellectual disability

Elke Bogaert[1], Aurore Garde[2], Thierry Gautier[3], Pontus LeBlanc[1], Fatima El It[4], Yannis Dufourd[4], Frederic Tran Mau-Them[5], Hana Safraou[5], Emma Van Reempts[1], Sylvie Nguyen[4], Nika Schuermans[1], Dimitri Hemelsoet[6], Christophe Philippe[5], SRSF1 Consortium[7], Anne-Sophie Dennomé-Pichon[5], Ange-Line Bruel[5], Christel Thauvin-Robinet[5], Laurence Faivre[2], Bekim Sadikovic[8], Jérôme Govin[9], Bart Dermaut[1] & Antonio Vitobello[5]

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SRSF1 (also known as ASF/SF2) is an evolutionary highly conserved protein know to be a master regulator of constitutive and alternative splicing. Through international data sharing, we gathered 16 patients (9 females and 7 males) carrying germline 14 different SRSF1 variants including four frameshift, two nonsense, seven missense variants, and one microdeletion of the region 17q22 including SRSF1. Variants occurred mostly de novo, however in one family a germline mosaicism was suspected to explain the recurrence in two siblings, while in another case the inheritance was unknown. Main clinical features included developmental delay, intellectual disability, hypotonia, behavioral disorders, marfanoid habitus and cardiac anomalies. To prove pathogenicity of the missense variants and one frameshift variant, we exploited a previously established in vivo splicing assay in Drosophila. Eye-specific overexpression of SF2, the Drosophila ortholog of SRSF1 is described to induce a severe developmental phenotype due to missplicing of key genes involved in normal eye development. In the current study, we replicated this finding and found that overexpression of the human SRSF1 was a phenocopy pointing towards functional conservation of SF2 and SRSF1. In addition, previously characterized splicing-deficient forms of SRSF1 lost the capacity to cause an eye phenotype, indicative for the crucial involvement of the endogenous splicing activity of SRSF1 in the phenotype. Furthermore, whole transcriptome sequencing uncovered splicing defects in key genes involved in neuron development as the primary molecular explanation of these eye phenotypes. Using this functional assay, we found that 6 out of 7 clinical missense variants lost their ability to disturb normal eye development upon overexpression and were therefore classified as splicing-deficient loss-of-function variants. Overall, these results indicate that haploinsufficiency of SRSF1 is responsible of a new syndromic neurodevelopmental disorder with recurrent marfanoid habitus and intellectual disability.

T24 - Live mouse tracker reveals autistic symptoms in the Fmr1 KO mouse model

Mathijs van der Lei, Dale Annear & Frank Kooy

University of Antwerp

The Fmr1 KO mouse is a valuable model for fragile X syndrome, paralleling the human disorder in many of its aspects. It has been extensively used in preclinical studies. In practice, the approach for testing the effectiveness of a novel compound is based on a battery of behavioral tests. This is labor-intensive, time-consuming, costly, and therefore not fitted to screen for multiple drugs and certainly not for potential combination therapies.

We introduce the live mouse tracker (LMT) for behavioral analysis of Fmr1 KO mice. The LMT system combines computer vision through a depth-sensing infrared camera, machine learning, and radio-frequency identification. The system is able to extract 35 basic behavioral traits of up to 4 mice from a single 24h recording. This is a major advantage because there is now no need to start with costly and time-consuming individual tests for each compound. Thus, the system can determine at a first glance the effectiveness of novel compounds and/or combination therapies.

Preliminary data (n= 10 Fmr1 KO and n=10 WT littermates) showed a significantly further distance traveled by Fmr1 KO mice compared to WT ($p=0.006$). In addition, more isolated behavior was recorded in Fmr1 KO mice, as measured by move alone, stop alone, and rear isolated ($p< 0.05$). Furthermore, social-behavioral traits like the grouping of three animals, social approach, make contact, side to side contact, oral-oral and oral-genital contact were significantly decreased in Fmr1 KO mice ($p<0.01$). Therefore, we conclude that baseline measurement of Fmr1 KO mice showed hyperactivity and abnormalities in social interactions in our LMT system, potentially compatible with autism observed in patients. These remarkably robust behavioral abnormalities will be used to detect the efficacy of novel compounds.

In conclusion, our LMT system is capable of characterizing behavioral abnormalities of Fmr1 KO mice in just 24h, and is thus a promising tool for future preclinical studies of drug development.

T25 - A novel neurodevelopmental syndrome caused by loss-of-function of the Zinc Finger Homeobox 3 (ZFHX3) gene

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Neurodevelopmental disorders result from impaired development and functioning of the brain. Here, we identify loss-of-function variation in ZFHX3 as a novel cause for syndromic intellectual disability (ID). ZFHX3, previously known as ATBF1, is a zinc-finger homeodomain transcription factor involved in multiple biological processes including cell differentiation and tumorigenesis.
Through international collaboration, we collected clinical data of 34 individuals with premature truncating variants or (partial) deletions of ZFHX3. Loss-of-function variation of ZFHX3 consistently associates with (mild) ID, postnatal growth retardation, feeding difficulties, and recognizable facial characteristics as supported by artificial intelligence (Face2Gene).
Data-mining and in-house generated expression data show increased nuclear expression of ZFHX3 during human brain development and neuronal differentiation. To identify the direct binding partners of ZFHX3, we performed immunoprecipitation followed by mass spectrometry in neural stem cells and SH-SY5Y. We show that ZFHX3 interacts with the chromatin remodelling BRG1/Brm-associated factor complex and the cleavage and polyadenylation complex. In addition, we identified a specific DNA methylation signature in leukocyte-derived DNA.
In Drosophila melanogaster, ZFH2 is considered the ZFHX3 orthologue. We used a reverse genetic approach to characterize ZFH2 deficiency in Drosophila melanogaster. ZFH2 is expressed in the third instar larval brain and its knockdown results in an adult lethal phenotype, suggestive for a key role in development.
In conclusion, loss-of-function variants in ZFHX3 are a novel cause for syndromic ID and are associated with a specific DNA methylation episignature. Our results indicate a role for ZFHX3 in chromatin remodelling and mRNA processing.

T26 - Routine transcriptome sequencing improves diagnosis for neurodevelopmental disorders by identifying pathogenic effects of non-coding, putatively benign and missed variants

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A molecular diagnosis is key for predicting outcome, treatment and genetic counseling options in neurodevelopmental disorders (NDD). However, in about half of NDD cases routine DNA-based diagnostics fail to establish a molecular diagnosis, due to insufficient coverage or lack of predictive value of non-coding variants. Transcriptome analysis (RNA-seq) improves the diagnostic yield for some groups of diseases, but has not, to our knowledge, been applied to NDD and in a routine diagnostic setting.
Here, we explored the diagnostic potential of RNA-seq in a cohort of 96 undiagnosed individuals including 67 undiagnosed NDD subjects. We created a user-friendly web-application to analyze RNA-seq data from single individuals' cultured skin fibroblasts for gene, exonic and intronic expression outliers, based on modified OUTRIDER Z-scores. Candidate pathogenic events were complemented/matched with genomic data and, if necessary, confirmed with additional functional assays.
We identified pathogenic small genomic deletions, mono-allelic expression, deep intronic variants resulting in pseudo-exon insertion, but also exonic synonymous variants or predicted "benign" nonsynonymous variants with deleterious effects on transcription. This approach increased the diagnostic yield for NDD by 12%. Identified pitfalls during transcriptome analysis include splice abnormalities in putative disease genes caused by benign polymorphisms and/or absence of expression of the responsible gene in the tissue of choice. This was misleading in one case and could have led to the wrong diagnosis in the absence of appropriate phenotyping.
Nonetheless, our results demonstrate the utility of RNA-seq in molecular diagnostics and stress the importance of multidisciplinary consultation and clinical phenotyping. In particular, RNA-seq is useful for the identification and interpretation of unexpected pathogenic changes in mRNA processing and expression in NDD.

T27 - Overview of cancer predisposition syndromes in a national, unselected cohort of 836 children with a neoplasm

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Background/Objectives: The diagnostic approach of cancer predisposition syndromes (CPSs) in children with cancer is shifting from a phenotype-driven approach towards a genotype-first approach. To decide on best practice in CPS diagnostics, it is essential to evaluate the yield of universal germline sequencing and to compare it with targeted genetic testing based on clinical selection. However, a reliable comparison is difficult since recent reports on a phenotype-driven approach in large, unselected childhood cancer cohorts are lacking.

Methods: Medical records of newly diagnosed children with cancer in the Netherlands between 01/06/2018 and 31/12/2019 were screened for medical history and clinical genetic assessment. In this period, it was standard practice that pediatric oncologists checked for characteristics of CPSs and selected children for referral to clinical geneticists.

Results: In 72/836 patients (8.6%) a CPS was identified (26 different conditions), of which the majority (96%) was identified by a phenotype-driven approach. Down syndrome and NF1 were the most common CPSs diagnosed. In 42/72 patients (58%) a CPS was identified after these children had developed a neoplasm. The specific type of neoplasm was the most frequent indicator for referral to a clinical geneticist and targeted genetic testing, whereas family history played a small role.

Conclusion: The mostly phenotype-driven diagnosis of CPSs in our unselected cohort revealed a CPS prevalence similar to that in earlier genotype-based studies, but the spectrum of CPS diagnosis is clearly different. This study can be used as a reference cohort for future genotype-driven studies.

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T28 - Polygenic risk scores predict overweight and obesity in the Dutch population

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Background: Obesity, the fifth leading risk of global deaths, has grown to epidemic proportions. Its etiology is largely unknown but it has a substantial hereditary component. Global GWAS have identified 941 genetic variants influencing body-mass index explaining 6% of its heritability in Caucasian subjects. In this study, we combined these variants into a polygenic risk score (PRS) to assess obesity risk in an adult Dutch Caucasian population cohort.
Methods: A BMI-PRS was calculated using 941 previously identified variants, and the PRS was tested in 11,209 participants of the Rotterdam Study (mean±SD age=65.7±10.2 years) to predict BMI as a continuous and categorical outcome (under-weight, normal-weight, overweight, obese, and morbid-obese). We evaluated the risk conveyed by PRS as a linear instrument (per 1 SD) and categorical (highest 10% population-based on PRS value vs. middle 50% of the PRS distribution as a reference group representing the "average" population)
Results: The PRS was associated with BMI (beta-estimate=0.9 [95% confidence interval 0.7-1.1];$p<1\times10-16$). One SD increase of the PRS significantly increased the risk of being underweight, over-weight, obese and morbid-obese by 0.8, 1.3, 1.8 and 2.2 fold times, respectively. Similarly, the top 10% of the population with the highest BMI-PRS showed increased risks of 1.1, 1.5, 2.6 and 4.5 for these BMI categories, respectively. The risk increased exponentially in the PRS distribution tails, up to 8.2 of the top 1% PRS for morbid-obese vs. normal-weight.
Conclusions: These results confirm that the global PRS for BMI has a significant impact on BMI in a Dutch population of Caucasian elderly. Identifying the biological pathways affected by an individuals' genetic background could aid in targeted and personalized intervention strategies long before the onset of obesity. These and other utilities of the BMI-PRS are being investigated as part of the Genotyping on all patients (GOALL) project.

T29 - The potential of 3D facial analysis to recognize monogenic autism in the spectrum

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Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that are typically characterized by social and communication deficits, as well as restricted interests and repetitive behaviors. With the advent of next generation sequencing, many monogenic causes for ASD have been identified. However, the large variability in the number and severity of clinical features in patients with monogenic causes makes it difficult to distinguish them from ASD patients with multifactorial causes. Since it is known that monogenic ASD is clinically associated with facial dysmorphism, an objective analysis of facial shape might help to stratify ASD patients by underlying genetic causes. In this work, we explore the utility of objective facial phenotyping to improve the recognition of patients with monogenic ASD. Three-dimensional (3D) facial images were collected from 152 ASD patients who presented in the outpatient-department for a genetic diagnostic workup. We investigated the 3D facial shape of ASD patients in relation to the presence of intellectual disability on the one hand and to the outcome of a molecular diagnostic workup on the other hand. The assessment of facial gestalt by ten experienced dysmorphologists was used to validate the possible contribution of 3D facial shape analysis in genetic diagnostics. 3D facial shape analysis revealed that the overall magnitude of facial dysmorphism and facial asymmetry was associated with monogenic ASD. Combining 3D facial shape analysis with the opinion of individual dysmorphologists improved the capacity to discriminate between ASD patients with monogenic causes and patients with unknown causes. These data suggest that 3D facial shape analysis may contribute to the recognition of patients with monogenic ASD in genetic diagnostics.

T30 -

P01 - Primary mediastinal large B-cell lymphoma is hallmarked by large-scale copy-neutral loss of heterozygosity

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The development of primary mediastinal B-cell lymphoma (PMBL), an aggressive subtype of non-Hodgkin lymphoma, is driven by cumulative genomic aberrations. To identify the driver mutational events, we screened PMBL cases by SNP arrays (Illumina HumanCytoSNP-12v2.1 BeadChip), fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), whole-exome sequencing (WES) and whole-genome sequencing (WGS) [NovaSeq 6000 (Illumina), Oxford Nanopore Technology sequencing]. The screen uncovered an extreme burden of copy-neutral loss of heterozygosity (CN-LOH) in PMBL which distinguishes this tumour from other B-cell malignancies, including the biologically related diffuse large B-cell lymphoma (respectively on average per patient 4.04 and 1.8). We identified large-scale CN-LOH lesions in 90.9% (30/33) of diagnostic PMBLs and both investigated PMBL-derived cell lines. The cohort showed 133 extra-large (25.3-248.4 Mb) CN-LOH lesions affecting up to 14 chromosomes per case. Notably, CN-LOH stretches non-randomly clustered on chromosome 6p (60%), 15 (37.2%) and 17q (40%), and frequently co-occurred with homozygous mutations in MHC I (6p21), B2M (15q15) and GNA13 (17q23) genes, as yielded by preliminary whole-exome/genome sequencing data. Altogether, our findings implicate large-scale CN-LOH as a novel mutational process contributing to the molecular pathogenesis of PMBL. The prevalent occurrence of segmental CN-LOH in a heterozygous diploid context, alongside the lack of common CNVs and/or recurrent scars in regions flanking CN-LOH regions revealed by long-read sequencing, points to a key role of mitotic homologous recombination. This mechanism usually follows double-strand breaks and likely acts as an errant DNA repair mechanism leading to CN-LOH.

P02 - Missense variants in ANKRD11 cause KBG syndrome by impairment of stability or transcriptional activity of the encoded protein

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Purpose:
Although haploinsufficiency of ANKRD11 is among the most common genetic causes of neurodevelopmental disorders, the role of rare ANKRD11 missense variation remains unclear. We characterized the clinical, molecular and functional spectra of ANKRD11 missense variants.

Methods:
We collected clinical information of individuals with ANKRD11 missense variants and evaluated phenotypic fit to KBG syndrome. We assessed pathogenicity of variants by in silico analyses and cell-based experiments.

Results:
We identified 29 individuals with (mostly de novo) ANKRD11 missense variants, who presented with syndromic neurodevelopmental disorders and were phenotypically similar to individuals with KBG syndrome caused by ANKRD11 protein truncating variants or 16q24.3 microdeletions. Missense variants significantly clustered in Repression Domain 2. Cellularly, most variants caused reduced ANKRD11 stability. One variant resulted in decreased proteasome degradation and loss of ANKRD11 transcriptional activity.

Conclusion:
Our study indicates that pathogenic heterozygous missense variants in ANKRD11 cause the clinically recognizable KBG syndrome. Disrupted transrepression capacity and reduced protein stability each independently lead to ANKRD11 loss-of-function, consistent with haploinsufficiency. This highlights the diagnostic relevance of ANKRD11 missense variants, but also poses diagnostic challenges, as the KBG-associated phenotype may be mild and inherited pathogenic ANKRD11 (missense) variants are increasingly observed, warranting stringent variant classification and careful phenotyping.

P03 - Following therapy response through liquid biopsies in metastatic colorectal cancer patients: the lead-in FOLICOLOR trial

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Introduction
Detection of methylation markers in liquid biopsies hold great potential for the follow-up of metastatic colorectal cancer patients (mCRC). However, trials that study the patients' benefit of using these markers to detect progressive disease (PD) are still lacking. Previous studies demonstrated great potential for NPY methylation in follow-up of mCRC but did not collect sufficient data to determine a cut-off value to predict PD. Therefore, this study was designed to determine the optimal cut-off value of NPY methylation to detect PD.
Methods
In this prospective study, patients with RAS and BRAF wild-type mCRC, starting first-line treatment with FOLFOX/FOLFIRI + panitumumab received follow-up with biweekly liquid biopsies and CT-imaging every 8 weeks. Patients were followed for at least 9 months or until PD or metastasectomy. NPY methylation was measured in three LB samples collected at each timepoint using the QX200 droplet digital PCR system (Bio-Rad).
Results
At baseline 14/15 patients had detectable NPY methylation (median 22.22%). The median methylation ratio decreased to 0.21% after 2 cycles of therapy (p=0.001). A decrease in NPY methylation ratio after 2 cycles of therapy corresponded to response on first CT-imaging (after 4 cycles of therapy).
In a first analysis, the median follow-up was 227 days and four patients reached end of study. One patient developed PD during a therapy break of 49 days. At that time, NPY methylation ratio increased from undetectable to 1.89% while there was no increase in CEA.
Conclusions
The first results of the lead-in FOLICOLOR trial confirm that a decrease in NPY methylation ratio after two cycles of therapy predicts response on first evaluation. However, since only one patient has developed PD additional data is required to draw a conclusion regarding the optimal cut-off value. Therefore, further follow-up and liquid biopsy collection are ongoing.

P04 - Isolated aneurysmal disease as an underestimated finding in individuals with JAG1 pathogenic variants.

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Pathogenic variants in JAG1 are known to cause Alagille syndrome (ALGS), a disorder that primarily affects the liver, lung, kidney and skeleton. Whereas cardiac symptoms are also frequently observed in ALGS, thoracic aortic aneurysms have only been reported sporadically in post-mortem autopsies. We here report two families with segregating JAG1 variants that present with isolated aneurysmal disease, as well as the first histological evaluation of aortic aneurysm tissue of a JAG1 variant carrier. Our observations shed more light on the pathomechanisms behind aneurysm formation in JAG1 variant carriers and underline the importance of cardiovascular imaging in the clinical follow-up of JAG1 variant carrying individuals.

P05 - Replacing the current techniques for hematological tumor-diagnostics with Optical Genome Mapping.

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Introduction
In our current haematological tumour diagnostics we use time-consuming and expensive techniques like karyotyping, SNP-arrays and FISH to resolve complex rearrangements in leukaemia. We want to investigate whether Bionano Optical Genome Mapping (OGM) has the potential to replace the current methods in a single automated assay.

Method
We re-investigated 10 bone marrow aspirates with OGM. The lab-procedure was performed at the Services Lab (Clermont-Ferrand). Ultra-high molecular weight DNA was purified, DNA molecules were labelled and the labelled samples were scanned on a Saphyr instrument. The de novo assembly and rare variant annotation pipeline were executed on Bionano Solve software V3.6. Reporting and direct visualization was done on Bionano Access V1.6.

Results
We were able to identify all the already known aberrations. Besides this, we found a putative fusion with the MECOM gene in a AML patient that had been karyotyped only. Further, we saw that a presumptuous balanced t(9;22) that we previously detected with karyotyping in a CML patient was actually unbalanced. The 6 MB deletion on chromosome 22 was below the threshold of karyotyping (5-10 MB). We need to confirm these additional findings.

Discussion
In our experience OGM is fast, less expensive and of higher resolution than current methods. OGM combines the advantages of karyotyping, SNP-array and FISH and has the potential to replace these techniques. This is outlined above, the 6 MB deletion in the assumed balanced t(9;22) is missed with karyotyping but will probably be detected with SNP-arrays that cannot detect balanced translocations while OGM combines the advantages of both techniques. Additional benefits are the avoidance of culturing and the low input amount of 1.5 million cells. Before introducing OGM in our tumour standard of care workflow, we want to confirm our potentially clinically relevant extra findings and map 10 additional bone marrow aspirates.

P06 - Establishing the neurodevelopmental phenotype and genotype-phenotype correlations in individuals with a TRIP12 mutation

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Background/Objectives:
Haploinsufficiency of TRIP12 causes a neurodevelopmental disorder characterized by intellectual disability associated with epilepsy, autism and dysmorphism, also named Clark-Baraitser syndrome. Less than 25 individuals harboring pathogenic TRIP12 variants have been reported. We aim to further delineate the TRIP12-associated phenotype and review genotype-phenotype correlations. In addition, characteristic facial traits are objectified through image analysis based on deep-learning algorithms.
Methods:
37 individuals were recruited through a collaborative call via ERN-ITHACA. Clinical data was collected and the pictures of 21 individuals were uploaded into the GestaltMatcher database for analysis of facial morphology.
Results:
One inherited and 35 de novo TRIP12 variants were identified, including frameshift (n=16), nonsense (n=6), missense (n=5) and splice (n=3) variants as well as intragenic deletions (n=5) and a multigene deletion disrupting TRIP12.
Though variable in severity, global developmental delay was noted in all individuals, with language deficit most pronounced. Half of the individuals showed autistic features, but there was no clear correlation with the mutation type. Susceptibility to obesity seemed to be a recurrent feature in older individuals. Seizures were reported in a minority and proved to be refractory in individuals with a missense variant.
Facial analysis shows a clear gestalt including deep-set eyes with narrow palpebral fissures, downturned corners of the mouth and large, low-set ears with prominent earlobes.
Conclusion:
We report the largest cohort to date of individuals with pathogenic TRIP12 variants, further delineating the associated phenotype including introduction of a facial gestalt and expanding genotype-phenotype correlations. These findings will improve future counseling and patient guidance.

P07 - Casq2 deletion: a zebrafish model of CPVT

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Introduction
Autosomal recessive mutations in CASQ2 are the second most common genetic defect identified in catecholaminergic polymorphic ventricular tachycardia (CPVT), one of the most lethal forms of inherited cardiac arrhythmia. Recent findings from our center as well as literature(1) suggest an autosomal dominant inheritance is also possible for CASQ2 mutations.

Objectives
Due to the optical translucency of zebrafish larvae, it is feasible to visualize the cardiac calcium and voltage dynamics in vivo, providing a sensitive method for studying the mechanisms of calcium-mediated cardiac arrhythmia. We developed a zebrafish model of CASQ2-related CPVT, which can be used to further confirm the autosomal dominant hypothesis.

Methods
The zebrafish casq2 knockout model was developed with CRISPR-Cas9, on the background of a transgenic zebrafish line expressing cardiac dual voltage and calcium reporters (Ace2N-mNeon and R-GECO). The voltage and calcium signals were measured with a Leica SP8 light sheet microscope at 3 days post-fertilization, after overnight exposure to the adenylate cyclase activator forskolin.

Results
Casq2-/- embryos showed a decreased heart rate compared to wildtype embryos at baseline (median of 121 and 158 beats per minute and n of 37 and 15, respectively; $p < 0.001$ with Wilcoxon test). Delayed afterdepolarizations induced by forskolin exposure were observed significantly more often in casq2 -/- embryos compared to controls (8/14 and 2/17, respectively; $p = 0.018$ with Fisher's exact test).

Conclusion
The casq2 knockout is the first zebrafish model of the classical CPVT genes. Similar to the human and murine phenotypes, we observe bradycardia at rest and a sensitivity to DADs upon stimulation.(2-3) This model will provide a promising opportunity for further testing of CASQ2 inheritance patterns.

References
(1) Ng K, et al. Circulation, 2020. 142(10):932. PMID:32693635
(2) Postma AV, et al. Circulation Research, 2002. 91:e21–e26. PMID:12386154
(3) Knollman BC, et al. J Clin Invest, 2006. 116(9):2510-20. PMID:16932808

P08 - Novel mRNA therapy restores GALT protein and enzyme activity in young classic galactosemia zebrafish

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Messenger RNA (mRNA) has emerged as a novel therapeutic approach for inborn errors of metabolism. Classic galactosemia (CG) is an inborn error of galactose metabolism caused by a severe deficiency of galactose-1-phosphate uridylyltransferase (GALT) activity leading to neonatal illness and chronic impairments affecting the brain and female gonads. In this proof of concept study, we used our zebrafish model for CG to evaluate the potential of human GALT mRNA (hGALT mRNA) packaged in two different lipid nanoparticles to restore GALT expression and activity at early stages of develop-ment. Both, one cell-stage and intravenous single-dose injections resulted in hGALT protein expression and enzyme activity in the CG zebrafish (galt knockout) at 5 days post fertilization (dpf). Moreover, the levels of galactose-1-phosphate (Gal-1-P) and galactonate, metabolites that accumulate because of the deficiency, showed a decreasing trend. LNP-packaged mRNA was effectively translated and processed in the CG zebrafish without signs of toxicity. This study shows that mRNA therapy restores GALT protein and enzyme activity in the CG zebrafish model, and that the zebrafish is a suitable system to test this approach. Further studies are warranted to assess whether repeated injections safely mitigate the chronic impairments of this disease.

P09 - Generation and validation of human iPSC model from a severely affected Loeys-Dietz Syndrome type 3 patient with a SMAD3 p.Arg287Gln mutation

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Introduction:
Loeys-Dietz Syndrome (LDS) is an autosomal dominant connective tissue disorder presenting with cardiovascular, skeletal, craniofacial, and cutaneous abnormalities. The major cause of death in LDS patients is thoracic aortic aneurysm and dissection. Vascular smooth muscle cells (VSMCs) play a prime role in the development of aortopathy. An important limitation as regards the study of the cellular mechanism in LDS is the inaccessibility to VSMCs in an early disease stage. Therefore, alternative cellular disease models are highly needed. Stem cell models are promising novel tools to study genetic disorders such as LDS.
Results:
We generated the first induced pluripotent stem cell (iPSC) model of a severely affected LDS patient (aortic surgery at age 32 years) carrying a SMAD3 p.Arg287Gln mutation. Peripheral blood mononuclear cells were reprogrammed using a non-integrating Sendai viral vector. The autonomous pluripotency state of the resulting iPSC model was proven by the presence of the pluripotency markers (Oct3/4, Sox2, Nanog, Tra 1-60, and Tra 1-81) in absence of the viral vector. Trilineage differentiation potential was assessed via directed differentiation and subsequent expression analysis of the different germ layer markers at the mRNA level. Genome stability of the resulting iPSCs was assessed with a genome-wide SNP array, proving the absence of copy number variants in known aortopathy genes as well as genes with a key role in the cardiovascular system. The presence of the SMAD3 p.Arg287Gln mutation was demonstrated in the generated iPSCs by Sanger sequencing.
Utility:
The resulting iPSCs can be further differentiated into disease-specific cell types, such as iPSC-VSMCs. This iPSC model presents an excellent platform to study “aortic aneurysm in a dish” and will enable the identification of the cellular pathomechanisms of LDS. Furthermore, this model can be used in large-scale drug screenings, facilitating novel drug discovery.

P10 - Variant effect prediction based on custom long-read transcriptomes

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Our knowledge of transcript annotations is still incomplete and may result in a failure to detect disease-causing variants. For example, in patients with primary immunodeficiencies it could be valuable to annotate transcripts that are only expressed under certain conditions such as host-pathogen interactions. Current variant annotation software uses only precomputed pathogenicity prediction scores based on reference transcripts. The pipeline presented here is designed to annotate variants with custom transcript annotations for downstream prioritization.
The input of the pipeline is a sample-specific/non-reference long-read transcriptome in fasta format obtained from peripheral blood nuclear cells of a healthy individual that was stimulated with various pathogens, variant file(s) derived from unsolved exome data of patients with suspected inborn errors of immunity (IEI) in VCF format, and a reference genome build. The Ensembl Variant Effect Predictor is used in conjunction with Polyphen-2 to provide custom variant annotations. Our pipeline is available at https://github.com/cmbi/VEP_custom_annotations.
The input long-read transcriptome contained 37,434 novel transcripts detected through PacBio IsoSeq on peripheral blood cells exposed to various immune stimuli. The re-annotation pipeline was tested on 148 undiagnosed IEI patient's exomes. Out of a total of 802,352 variants, 6.2% had a more severe effect in the novel transcript annotation than in the reference.
Genetic variant annotation may benefit from long-read sequencing approaches that discover novel transcripts. This benefit can be reaped without extensive bioinformatic knowledge using this pipeline. Our pipeline outputs crucial information for further prioritization of potentially disease-causing variants, and will become increasingly useful due to the rising number of long-read RNAseq datasets.

P11 - Feasibility of follow-up studies and reclassification in Spinocerebellar Ataxia gene variants of unknown significance

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Spinocerebellar ataxia (SCA) is a heterogeneous group of neurodegenerative disorders with autosomal dominant inheritance. Genetic testing for SCA leads to diagnosis, prognosis and risk assessment for patients and their family members. While advances in sequencing and computing technologies have provided researchers with a rapid expansion in the genetic test content that can be used to unravel the genetic causes that underlie diseases, the large number of variants with unknown significance (VUSes) detected represent challenges. To minimise the proportion of VUSes, follow-up studies are needed to aid in their reclassification as either (likely) pathogenic or (likely) benign variants.
In this study, we addressed the challenge of prioritizing VUSes for follow-up using (a combination of) variant segregation studies, 3D protein modeling, in vitro splicing assays and functional assays. Of the 39 VUSes prioritized for further analysis, 13 were eligible for follow up. We were able to reclassify 4 of these VUSes to LP, increasing the molecular diagnostic yield by 1.1%. Reclassification of VUSes remains difficult due to limited possibilities for performing variant segregation studies in the classification process and the limited availability of routine functional tests.

P12 - What does a Genetic Counsellor do in the University Hospital of Liege?

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CHU de Liège

Introduction :
Lately, the University Hospital of Liège has faced a growing demand for genetic consultations. In addition to increasing the number of clinical geneticists, the University Hospital opened a first position of Genetic Counsellor (GC) in 2013, followed by a second one in 2017 and a third one in 2020. The GC profession, although recognized in many countries around the world, is not official yet in Belgium. Since 2015, a national working group has been set up and a procedure is underway to create an MSc in genetic counselling. The aim of this study was to characterize and evaluate the activities of GCs in our hospital.

Methods :
We performed a retrospective observational study of the patients from the University Hospital of Liège who had consulted with a GC between 2016 and 2021. We also analyzed the other interventions/tasks of the GCs. We performed a descriptive statistical analysis.

Results :
The main task of the GCs is to receive patients in consultation either in pair with a geneticist or in "genetic counselling" consultation under the supervision of a geneticist. Other tasks are mainly administrative. The number of consultations has increased by 707.5% between 2016 and 2021 and the number of administrative interventions has also increased by 5740% between 2013 and 2020.

Conclusion :
The number of specific "genetic counselling" consultations and the administrative interventions have highly increased between 2016 and 2021. The tasks of the GCs are varied and essential for the overall care of the patient, in collaboration with the rest of the (para)medical team.

P13 - History of a diagnostic errancy : how new technologies may allow for a diagnosis after 40 years

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Introduction :

This clinical case demonstrates that subtle metabolic impairments may not be detected by basic screening performed in routine diagnostics. In addition to metabolic diagnostics, it's imperative to use more precise molecular biology techniques.

Case description :

46 year old woman, born at full term after a pregnancy and normal delivery, of non-consanguineous parents presented with mild developmental delay since infancy. A waddling walk was noted at the age of 5.
Legge-Calvé-Perthes disease diagnosed at the age of 6, necessitating a bilateral hip prosthesis. She underwent surgery for severe scoliosis in childhood.
In her twenties, she was diagnosed with a demyelinating sensory-motor polyneuropathy leading to amyotrophy of the 4 limbs with predominance in the lower limbs.
At age 45, she presented cardiac arrhythmias diagnosed as probable arythmogenic right ventricular dysplasia.
Clinical and genetics investigations over a period of over 40 years had not revealed an explanation for the developmental delay, neuropathy and cardiac condition (Normal EEG and brain MRI).

Results :

Normal caryotype and CHG-array, normal metabolic analysis including mucopolysaccharides and oligosaccharides, GJP1 gene analysis.
Genetic analysis of a cardiac arrhythmia panel was normal.
Analysis of a neurodevelopmental panel (859 genes) revealed a compound heterozygous GNPTAB mutation, confirming a diagnosis of mucolipidosis type III. Re-evaluation of skeletal X-rays confirmed associated skeletal changes.

Conclusion :

Due to her mild phenotype, a clinical diagnosis of classic mucolipidosis was never evoked.
Routine metabolic diagnostic testing may not be sensitive enough to diagnose mild forms of metabolic conditions, gene panels must absolutely cover the genes for storage diseases.

P14 - Striking phenotypical differences between Ipo8 knock-out mouse models on different genetic backgrounds

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IPO8 encodes importin-8, a ubiquitously expressed nuclear transport receptor of the importin-β family. Importin-8 translocates cargoes such as proteins, RNAs and ribonucleoproteins from the cytosol to the nucleus in a Ran-GTP dependent manner. We recently found bi-allelic loss-of-function variants in IPO8 causing a syndromic form of thoracic aortic aneurysm. Also, an Ipo8 knock-out (Ipo8-/-) mouse on a C57Bl/6N genetic background displayed root and ascending aortic aneurysms from 8weeks of age onwards as well as an aortic expression signature compatible with dysregulation of the TGFβ signaling pathway. In this study, we examined the influence of backcrossing the Ipo8-/- to Sv129 background.

Echocardiographic screening of Ipo8-/- mice on Sv129 genetic background did not reveal aortic aneurysms, which is in sharp contrast to the pronounced aortic phenotype of the C57Bl/6N Ipo8-/- mice. 33% of C57Bl/6N Ipo8-/- male mice died from thoracic aortic rupture around 36weeks, a finding never observed in the Sv129 Ipo8-/- mice up to 52weeks. Histological characterization, pSmad2 immunohistochemistry and RNA-sequencing of the aortic wall of the Sv129 Ipo8-/- mouse strain is currently ongoing. Embryonic lethality of 50% was observed in C57Bl/6N Ipo8-/- mice, whereas no embryonic lethality was observed in Sv129 Ipo8-/- mice. Timed matings are being conducted to investigate this lethality in C57Bl/6N Ipo8-/- mice. Based on the striking divergent cardiovascular phenotype of the C57Bl/6N and Sv129 Ipo8-/- strains and the embryonic lethality only observed in the C57Bl/6N mice, we hypothesize that aberrant cardiovascular development might be involved. Pregnant C57Bl/6N mice will hence be dissected at E13,5, i.e. an important gestational phase for cardiovascular development. Besides a lower weight of Sv129 Ipo8-/- mice, no other striking phenotypical abnormalities could be observed.
In conclusion, we describe striking differences in the (cardiovascular) phenotype of Sv129 versus C57Bl/6N Ipo8-/- mice, emphasizing the importance of mouse genetic backgrounds in disease modelling.

P15 - The landscape of the clinical genetics specialty training in Belgium: an online survey

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Medical genetics has been a recognized clinical speciality in Belgium since June 2017. To obtain the recognition, the training consists of 4 years in clinical genetics combined with 2 years of another general clinical speciality. At least 1 year and a maximum of 2 years of the clinical genetics training should be in a recognized genetics laboratory.
An online questionnaire was sent to the current trainees in clinical genetics in Belgium. The questions concerned demographic, and clinical and scientific experience.
The response rate was 94% (n=30/32). The mean age of the trainees was 29 years. 73% (n=22) of them identified themselves as female and 77% (n=23) were training at a Flemish university.
53% (n=16) of the trainees switched to clinical genetics from another discipline, paediatrics being the most popular previous training (n=7). 23% (n=7) of the current trainees were fully recognized in their previous discipline before starting a training in clinical genetics. For all the trainees, previous experience in another speciality was approved or presumed to be approved by the genetics commission to be recognized for the general clinical training.
73 % plan to do (n=10) or are currently doing (n=12) a PhD. 13% of the trainees have already finished their PhD.
Most of the trainees aim at training outside of their alma mater, in Belgium or abroad, for 52% and 71% of them, respectively. Because of the often complex trajectories, the estimated graduation dates lie between 2022 and 2029 for the current trainees.
Medical genetics is a 'young' speciality, therefore a lot of trainees switched to this training from another clinical speciality, even after obtaining their recognition in their respective field. There is a large scientific interest from the trainees as most of them have done a PhD or are planning to do one.

P16 - Perlman Syndrome : A prenatal and genetic diagnostic challenge

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Background/Objectives:
Perlman syndrome (PS) is a very rare congenital overgrowth syndrome, with a prevalence estimated to be less than 1/1.000.000. Generally the diagnosis is based on neonatal clinical appearance and histologic findings. For a long time the genetic basis of PS was unknown with an assumed autosomal recessive inheritance. Since 2012 mutations in DIS3L2 gene have been found to be associated to PS. Some cases have been described prenatally, but precise diagnosis in a prenatal setting remains difficult to obtain due to overlap with other overgrowth syndromes. Both prenatal imaging and genetic diagnostic technologies have enormously evolved over the past decade and are being implemented in prenatal diagnosis today, enhancing diagnostic yield.
Methods:
Here, we a present prenatal case of PS, with phenotyping by subsequent ultrasound and MRI examinations and molecular diagnosis by NGS, with an overview of existing literature. Accurate prenatal phenotyping of PS and comparison with Beckwith-Wiedemann and Simpson-Golabi-Behmel sydrome was made.
Results:
Confirmed prenatal diagnosis of PS was obtained by subsequent high resolution imaging with next generation sequencing (NGS) on amniotic fluid sample, showing 2 pathogenic variants in the DIS3L2 gene, not yet described in existing literature.
Conclusion:
Since knowledge of the concerned gene and use of new technologies, for imaging as well as genetic analysis, the diagnosis of PS can be made prenatally. This is of great value during prenatal counseling of pregnant couples, as PS is known to have a poor prognosis.

P17 - Exome sequencing as a first-tier genetic test for male infertility: a validation study

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According to current guidelines, patients with severe oligozoospermia or azoospermia are offered karyotyping, azoospermia factor (AZF) deletion screening and/or CFTR mutation testing. In this study, we set out to test whether whole exome sequencing (WES) with combined CNV and SNV analysis is a reliable first-tier method to (partially) replace the currently used tests, with the option to expand the testing strategy with newly discovered monogenic causes presently left undiagnosed.

WES was performed on coded DNA samples of patients with an AZF deletion (n=21), mosaic/non-mosaic sex chromosomal anomaly (n=32), CFTR variant (n=23), or other variants in known infertility genes (n=3), using the Twist Human Core Exome + RefSeq Panel Kit (Twist Bioscience) for enrichment. The data were analyzed using our standard diagnostic pipeline, including CNV callers ConiFER and ExomeDepth.

WES performed equally well or superior in the detection of AZF deletions and (mosaic) aneuploidies, such as 47,XXY. Structural rearrangements involving the X and Y chromosome, including 46,XX(SRY+), were reliably detected. Although none of the r(Y), i(Y) or idic(Y) anomalies were missed, karyotyping remains necessary for further characterization as this was hindered by coverage gaps. All previously reported SNVs and CNVs, including 14 mutations detected by the Elucigene CF-EU2v1 kit currently used for CFTR mutation screening, were correctly identified, except for one deep intronic cystic fibrosis-causing variant that was not covered.

Our study shows that WES-CNV and SNV analysis is a reliable method to detect the most common causes of male infertility, even though karyotyping will still remain necessary. Aside from simplification of the testing strategy, our WES strategy allows for the possibility to expand the testing by including analysis of at least 92 recently discovered genes involved in male infertility. Further studies are ongoing by running our current and new diagnostic workflows in parallel to further validate this novel strategy.

P18 - Deciphering the genetic architecture of inherited retinal diseases (IRD) in the Iranian population by integrated exome sequencing

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Purpose: To uncover the underlying molecular causes of inherited retinal disease (IRD) in 105 unrelated families of Iranian descent, an integrated approach consisting of whole exome sequencing (WES) and autozygosity mapping was used.

Methods: WES was performed in 105 Iranian IRD families, predominantly originating from a consanguineous background (77%). Data-analysis was performed using the in-house Seqplorer tool. Copy number variants (CNVs) were assessed via the ExomeDepth algorithm and validated using qPCR. Variants were validated, classified (ACMG/ACGS guidelines) and segregation analysis was performed. The AutoMap tool was used to determine runs of homozygosity (ROHs) in unsolved patients, which were then inspected for variants in novel candidate genes using Seqplorer and QCI Interpret Translational.

Results: By interrogating known IRD genes (n=290) using a WES-based analysis, we were able to obtain a molecular diagnosis for 85% of the IRD cohort. In total, 103 (likely) disease-associated variants were identified in 42 genes, 58 of which are novel variants (56%). ABCA4, EYS, AIPL1 and CRB1 were the four most implicated genes. In addition, the importance of structural variation (SV) in IRD was demonstrated, with CNVs identified in 8% of the cohort, including novel CNVs in CDHR1, CHM and RD3. Homozygous nonsense and missense deleterious variants were found in novel retina-expressed candidate IRD genes, specifically OGDHL, PFKFB2 and QRFPR.

Conclusions: This integrated study using WES and an in-depth analysis of the variants provided insight into the genetic architecture of IRD in Iran, an understudied population. We provided 85% of patients with a molecular diagnosis and expand the molecular spectrum of IRD in Iran by the identification of novel variants in known IRD genes in the majority of patients, emphasizing the power of WES as a first-tier genetic test in consanguineous IRD cohorts. Finally, autozygome-guided exome sequencing revealed several novel candidate genes for IRD in unsolved cases.

P19 - Joint effects of CHEK2 c.1100delC mutation and treatment on contralateral breast cancer risk

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Breast cancer (BC) patients with a CHEK2 c.1100delC germline mutation have an increased risk of contralateral breast cancer (CBC). Studies in general population-based BC cohorts showed that radiation treatment for the first BC increases CBC risk, while systemic therapy decreases CBC risk. We aimed to assess, in the largest dataset available to date, the joint effects of CHEK2 c.1100delC status and adjuvant therapy on CBC risk.
The study dataset derived from the international Breast Cancer Association Consortium consisted of 69,345 women (including 748 CHEK2 c.1100delC carriers) from European ancestry diagnosed with invasive, stage I-III BC between 1980 and 2018. Delayed entry Cox regression models, stratified by country, were used to estimate the association of adjuvant therapy with CHEK2 mutation status and time to CBC. Analyses were adjusted for age at diagnosis, ER-status, nodal status, size and grade of first BC. Potential differential effects of adjuvant therapy by CHEK2 c.1100delC status were tested by including interaction terms in the multivariable model. Multiple imputation was used to handle missing values.
Within this dataset, chemotherapy (HR=0.81; 95%CI=0.68-0.95) and endocrine therapy (HR=0.71; 95%CI=0.59-0.84) reduce CBC risk, while there was no effect of radiation therapy on CBC risk found (HR=1.02; 95%CI=0.87-1.18). Furthermore, there was no evidence of differential effects of chemotherapy (Pinteraction =0.45), endocrine therapy (Pinteraction =0.91) or radiation (Pinteraction=0.20) by CHEK2 c.1100delC status on CBC risk.
Preliminary results showed no strong evidence that CHEK2 c.1100delC carriers may respond differently to any adjuvant treatment than non-carriers.

P20 - Inactivating PTH/PTHrP Signaling Disorder type 1 caused by homozygous variant in PTH1R gene presenting as pseudohypoparathyroidism type 1: about a second case

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Parathyroid hormone (PTH) elevation, hypocalcemia, and hyperphosphatemia are caracteristic of pseudohypoparathyroidisms (PHP). The term inactivating PTH/PTHrP signalling disorders (iPPSD) was proposed to classify PHP based on the molecular defect. iPPSD1 encompasses 5 phenotypes associated with PTH1R variants: (1) Blomstrand chondrodysplasia (BCD), a lethal hydrops fetalis, (2) Eiken syndrome (ES), associating delayed ossification, Arnold-Chiari type 1 malformation (CM1) and short stature, (3) isolated primary failure of tooth eruption (PFTE), (4) Jansen metaphyseal chondrodysplasia, consisting of severe short stature with osteopenia, and (5) isolated PHP type 1b, reported in one adult patient. Herein, we describe a 10 month-old child presenting characteristics associated with various iPPSD1 phenotypes. He experienced 3 episodes of acute generalized hypertonia with loss of consciousness, all resolved spontaneously. He exhibited multiple agenesis of primary teeth. Clinical examination showed arched eyebrows, telecanthus, and mild retrognathia, and features compatible with Albright hereditary osteodystrophy. The biological work-up lead to PHP diagnosis. CM1 was detected on brain magnetic resonance imaging. Hands X-rays underlined delayed bone age, osteopenia, short metacarpal bones, and absence of the medial phalange ossification. Comparative genomic hybridization array, GNAS gene (iPPSD2) analysis and epilepsy targeted gene panel detected no pathological variation. Trio-based whole exome sequencing unveiled an homozygous substitution c.723C>G p.(Asp241Glu) in PTH1R gene inherited from heterozygous parents. The variant was not found in large databases. The affected amino-acid residue is highly-conserved (between species and among H. sapiens orthologs). It is located in close contact with the receptor ligand (I.e. PTH). The vast majority of bioinformatic tools (10/12) recognizes the variant as deleterious. Under treatment with calcium carbonate and alfacalcidol, biological parameters improved and there was no recurrence of acute episode. Based on this second report of PHP in a patient carrying PTH1R variants, we propose that the iPPSD1 phenotypes form a spectrum extending between chondrodysplasia and PHP.

P21 - Genetic basis of cleft lip and palate

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Introduction: Cleft lip and/or palate (CL/P) is the most common cranio-facial malformation often divided into syndromic CL/P (syCL/P) and non-syndromic CL/P (nsCL/P). In general, patients with syCL/P follow Mendelian inheritance, whilst those with nsCL/P are thought to have a complex etiology.
Materials and Methods: We analyzed 81 a priori non-syndromic index CL/P patients from a continuously growing cohort of 1400 CL/P patients by whole exome sequencing (WES). We looked for Mendelian mutations using Highlander as well as copy number variations using ExomeDepth.
Results: We unraveled pathogenic or likely pathogenic variants in 12 families in COL2A1, CTNND1, TP63, CHD7, PHF8, IRF6, and GHRL3. We also identified, and validated by molecular karyotyping, a deletion in TP63 in 2 siblings.
Conclusion and perspectives: We identified mutations in 16 % of index cases by WES, providing an accurate diagnosis as well as the possibility of genetic counseling. In some cases, we identified pathogenic variants in syCL/P genes in a priori nsCL/P cases, demonstrating that patients with CL/P without cardinal signs or familial history of a syndrome may still carry a mutation in a gene linked to syCL/P. We also identified a new phenotype in blepharocheilodontic syndrome 2: imperforate anus. These results show that WES is an important tool for identifying the genetic cause of CL/P. For the remaining patients for which we have not identified pathogenic variants in candidate genes, we will enlarge our research towards the rest of the genes in the human genome to identify new genes for clefts.
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P22 - Are CEBPA-associated familial acute myeloid leukemia predisposing to solid tumors ? About a family

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CEBPA-associated familial acute myeloid leukemia (AML) is defined by mono-allelic germline CEBPA pathogenic variant in an individual or a family with AML. The pathophysiology is thought to rely on the Knudson's two-hit hypothesis. Penetrance is nearly complete (> 80%), the median age at AML diagnosis is 24 years and the relapse rate is around 50% at 10 years. Patients' surveillance consists of complete blood count every 6-12 months. We report a 41-year-old male patient presenting with colonic adenoma in high-grade dysplasia, right kidney tumor and a suspect thyroid nodule. All three were diagnosed during work-up for a profound anemia. His personal medical history was marked by AML at age 12 which was successfully treated by hematopoietic stem cell transplantation (HSCT) from his sister, bilateral cataract treated at age 26 and hypergonadotropic hypogonadism. AML developed in two first-degree (sister & brother, both died of AML) and two second-degree (2 nieces) relatives of the patient (age: 18-38 years). The patient had a progeroid-like androgynous appearance with a high-pitched voice. Whole exome sequencing highlighted a CEBPA gene class 4 variant (c.350del p.(Gly117Alafs*43)) in patient's leukocytes (derived from his affected sister) and in DNA extracted from urine sample (patient's own genetic material). Family study is ongoing. Solid tumor were reported only twice in patients with CEBPA-associated familial AML (colon cancer at age 43 and renal cancer). In addition to our patient, these cases raise the question: do mono-allelic germline CEBPA pathogenic variants predispose to solid tumor and should the follow-up of these patients be adapted accordingly ? Tumor DNA sequencing looking for acquired somatic CEBPA pathogenic variant is ongoing. CEBPA-associated familial acute myeloid leukemia (AML) arises in the context of germline mono-allelic variant followed by a second hit. The literature and our experience may suggest predisposition to CEBPA-associated solid tumors.

P23 - Two years follow up of a patient with a de novo RAC1 gene syndrome

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RAC1 is a widely studied Rho GTPase, a class of molecules that modulate numerous cellular functions essential for normal development. RAC1 gene encoded GTPases belong to the RAS superfamily of small GTP-binding proteins. Members of this superfamily appear to regulate a diverse array of cellular events, including the control of cell growth, cytoskeletal reorganization, and the activation of protein kinases. De novo missense RAC1 variants cause a newly recognized genetic syndrome with variable degree of neurodevelopmental delay, brain malformations, and additional dysmorphic features.

1-year-old girl with congenital cataracts, mild motor developmental delay and failure to thrive was seen in the Genetics Department. No clinical diagnosis was considered at her first visit. MR brain imaging later showed polymicrogryria. Extended genetic testing was discussed with the parents and clinical exome genetic test detected a de novo heterozygous c.191A>G, p.(Tyr64Cys) likely pathogenic variant in RAC1 gene. Two-year clinical follow up showed prominent glabella, mild hyperthelorism, epicanthus left, short neck, pectus excavatum, posture in kyphosis, long fingers and toes, overlapping toes, fetal finger pads and sacral dimples. These clinical data provide further information for the phenotype/ genotype correlation and naturel evolution of this rare genetic condition.

P24 - CLEC16A mislocalization and impaired interaction with the retromer underly a recessive severe neurodevelopmental disorder.

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Background: CLEC16A is a C-type lectin (CLEC) transmembrane protein that recognizes and guides antigens to the cell surface and has been localized to the late endosomes of antigen presenting cells. CLEC16A functions as E3 ubiquitin ligase which prevents autophagy and promotes mitophagy. GWAS studies have associated CLEC16A SNVs to autoimmune disorders like multiple sclerosis and type-1 diabetes. However its role in physiological development is unexplored.

Methods: We identified bi-allelic loss-of-function variants in CLEC16A, in siblings from unrelated families, with a severe neurodevelopmental disorder, progressive microcephaly, brain atrophy, corpus callosum dysgenesis, growth delay, hypotonia and early demise. We studied the cellular CLEC16A properties in vitro and in zebrafish embryos.
Results: Exogenous expression in HEK293T cells shows that CLEC16A prominently localizes to early endosomes, while the protein bearing a human C-terminal deletion loses it physiological localization. Proteomics of CLEC16A interactome shows binding to the retromer heterotrimer components VPS35, VPS26 , and to TRIM27, an interaction which is partially lost for the C-terminal truncated protein. Targeted knock-down of Clec16a by CRISPR-Cas9 in zebrafish embryos resulted in the accumulation of acidic/phagolysosome compartments and abnormal staining of mitochondria, both in neuronal and microglial lineages. This phenotype could be rescued with WT but not with mutant mRNA.
Conclusion: This study reveals a constitutional function of CLEC16A during human brain development. Retromer is a crucial component of the endosomal network, mediates retrograde transport to the trans-Golgi network and plasma membrane, and regulates autophagy and mitophagy. The discovery of interactions between CLEC16A and retromer show the importance of (retromer dependent) endosomal trafficking during brain development.

P25 - Development of an optimised CRISPR pipeline for knock-in zebrafish models: reducing animal numbers

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Background/objective:

As CRISPR-Cas9 establishes itself as an easy-to-use and reliable gene-editing tool in zebrafish, the rearing and selection process of correctly CRISPRed fish remains time-consuming. In general, the average knock-in (KI) efficiency lies between 1-5%. This indicates that only a small percentage of the injected eggs contains the desired KI. Hence, many fish need to be reared at random while only a fraction will potentially pass it on to their offspring. Therefore, we developed a selection pipeline which reduces the number of fish to be reared and saves time overall.

Methods:

For the creation of a Brugada syndrome KI zebrafish line, multiple guide RNAs and donor single stranded oligodeoxynucleotides (ssODNs) were screened for their KI efficiency by injecting them in fertilized zebrafish eggs at the one cell stage. At three days post fertilisation (dpf), DNA was extracted from live embryos with the Zebrafish Embryo Genotyper (ZEG) device followed by downstream next generation sequencing on a Miseq instrument (Illumina). At 4 dpf, we had an estimate of KI reads for each screened larva. Below 5 dpf, the zebrafish larvae are not considered laboratory animals.

Results:

With the best combination of guide RNA and ssODN an average KI percentage of 2.99 (± 5.09, N=122) was obtained. Only the larvae that contained more than 2% KI reads were reared. Using the ZEG as a preselection tool, a specificity of 93% was achieved with a sensitivity of 41% for this specific locus (N=59).

Conclusions:

Using this methodology, only fish with a 2% or higher number of KI reads were selected, resulting in a substantial fewer number of fish that needs to be reared.

P26 - Validation of new gene variant classification methods: a field-test in diagnostic cardiogenetics

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Background
In the molecular genetic diagnostics of Mendelian disorders, solutions are needed for the major challenge of dealing with the large number of variants of uncertain significance (VUS) identified using next-generation sequencing (NGS). Recently, promising approaches to calculate case excess scores (CE) and etiological fractions (EF) and gnomAD-derived constraint metrics scores have been reported that estimate the likelihood that rare variants in specific genes or regions are pathogenic. Our objective was to study the usability of these scores into diagnostic variant interpretation, using our clinical cardiomyopathy cohort.
Methods and Results
Patients (N=2002) referred for clinical genetic diagnostics underwent NGS testing of 55-61 genes associated with cardiomyopathies. Previously classified likely pathogenic (LP) and pathogenic (P) variants were used to validate the use of data from CE, EF and gnomAD constraint analyses for (re)classification of associated variant types in specific cardiomyopathy subtype-related genes. The classifications corroborated in 94% (354/378) of cases. Next, we applied these constraint data to interpret and (re)classify 1229 variants (identified in 812 patients) previously classified as VUS. This led to the reclassification of 23 unique VUSs to LP, increasing the diagnostic yield with 1.2%. In addition, 106 unique VUSs (5.3% of patients) were prioritized for co-segregation or functional analyses.
Conclusion
Our analysis confirms that the use of constraint metrics data can improve cardiogenetic variant interpretation and classification. We therefore recommend the inclusion of constraint scores in variant interpretation protocols and to also apply these in other cohorts and disorders.

P27 - Disease-associated familial balanced chromosome translocation detection and breakpoints characterization by Oxford Nanopore technology long-read sequencing

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IPG

Precise localization of breakpoints is crucial for exploring genetic causes in patients with disease-associated balanced rearrangements.

We report on a patient referred for genetic analyses for hypotonia, motor delay, relative macrocephaly and absence in the context of a paternal family history of intellectual disability, behavioral disorders and epilepsy. Previous genetic analyses, including molecular karyotyping and whole exome sequencing, have been inconclusive. However, the proband, her father and her half-brother carry the same balanced translocation between the long arms of a chromosome 7 and the long arms of a chromosome 11, with breakage and reunion estimated at q11.2 and q13.

The long-read Oxford Nanopore technology was used to define the exact breakpoints of the translocation and to determine whether or not some genes were impacted by it. This technology allowed us to redefine the breakpoints as follow : chr7:82433004 - chr11:88570476: t(7;11)(q21.11;q14.2) and to highlight that 2 genes, GRM5 and CACNA2D1 were impacted, which could explain the epilepsy and intellectual disability phenotypes.

P28 - Indication and incidence of sperm donor restrictions, the decision of impacted recipients concerning future donor treatments and their outcome

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Objective: To study the incidence and indication for donor restriction and how it affects treatment choices for women undergoing or planning medically-assisted reproduction (MAR).
Design, duration: Single centre retrospective study. Restricted sperm donors were identified from imported sperm from January 2010 to December 2019 at the University Hospital of Ghent sperm bank facility.
Subjects, setting, methods: The population at study were both the restricted donors due to inheritable disease risk concerns as well as their recipients. A subgroup of these recipients were confronted with the decision about treatment continuation with the restricted donors (‘decision cohort’). Information on the indications for restriction and recipient’s characteristics were collected and analysed.
Results: Two-hundred out of 1,124 (17.8%) sperm donors were restricted during the study period. Multifactorial and autosomal recessive disorders were the most common ones (27.5 and 17.5%, respectively). Autosomal dominant, de novo, infectious, and X-linked conditions accounted for 8.5%, 7.5%, 1.0% and 0.5%, respectively. The way of transmission was unknown in 75 cases (37.5%). The sperm had been used in 798 recipients, of which 172, receiving sperm from 100 donors, constituted the ‘decision cohort'. Seventy-one (41.3%) recipients agreed to continue MAR. The age of the recipient at the time of donor restriction (OR 0.857 [95% CI 0.800-0.918]; P <0.001) and the time between first treatment and donor restriction (OR 0.806 [95% CI 0.713-0.911]; P <0.001) were inversely related to the odds of accepting the restricted donors’ specimens.
Conclusions: Our findings further support the need to identify genetically transmitted disorders, with a special emphasis on autosomal recessive conditions. Multifactorial diseases and de novo mutations still account for a large number of the reported disorders, adding to the complexity of screening donors and educating recipients about disease risks in the offspring. Genetic counselling of women is fundamental to the decision-making process in these cases.

P29 - Diagnostic yield of a NGS panel in a Brugada syndrome cohort

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Background:
Brugada syndrome (BrS) is a rare inherited cardiac arrhythmia disorder affecting 1/2000 individuals. Its diagnosis requires presence of a spontaneous or sodium channel blocker induced ST-segment elevation on an electrocardiogram (ECG). BrS patients are at risk for ventricular fibrillations which could lead to sudden cardiac death. In general, only for 25-30% of the patients a genetic diagnosis can be established in one of the BrS associated genes, of which 20-25% carry a variant in the SCN5A gene.

Methods:
We collected clinical history, ECG parameters and genetic results of 294 BrS patients (61% male) screened with a diagnostic panel for inherited primary electrical disorders covering initially 51 and in a later version 60 genes.

Results:
In total, 43.5% of patients carried a variant of uncertain significance (VUS, class 3; n=102) or (likely) pathogenic variant (class 4 and 5; n=26) following the ACMG guidelines. Most of the class 4/5 variants are found in the SCN5A gene (23/26), whereas the remainder were identified in KCNE1/LMNA/SCN2B. 43.9% of patients had a Shangai score above 3.5 (definite BrS) of which 14.7% carried a class 4/5 variant. Only 4.2% of patients with a Shangai score between 2 and 3 carried a (likely) pathogenic variant. Of the 22.4% of patients with a familial history, 20% carried a class 4/5 variant.

Conclusion:
The overall diagnostic yield in our cohort is 8.8%, increasing to 15% in BrS patients with a definite diagnosis, or 20% in clear familial patients, which is slightly lower than reported in literature.

P30 - Optimization of seeding conditions of induced pluripotent stem cell-derived cardiomyocytes on multi electrode array (MEA) devices

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Background:
Induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) are an innovative useful tool for the study of inherited cardiac disorders. They recapitulate the disease phenotype of the patient in vitro allowing the study of disease mechanisms through the use of multielectrode arrays (MEA). As MEA offers high-throughput and repetitive analysis of electrically active cells, it is an interesting substitute for the patch-clamp technique. However, as this technology is rather new, there are still some challenges in its routine application for electrophysiological analysis of iPSC-CM. An important hurdle is the passaging of iPSC-CM onto the MEA surface.

Methodology:
To find the most optimal conditions to passage in-house created iPSC-CM grown on Matrigel they were passaged on different timepoints (day 20 or day 30 of differentiation), density (30 000 cells/6µL or 60 000 cells/6µL) and on different surface coatings (Matrigel, Fibronectin or Gelatin). These cells were passaged on dummy single-well MEAs and onto glass coverslips, which form a substitute to the MEA surface due to the likeliness between both surfaces.

Results:
The success of passaging and attachment was evaluated based on (1) the number of cells that survived the passaging and their capacity to form a monolayer consisting of beating iPSC-CM, (2) cell morphology parameters (cell circularity and aspect ratio) and (3) cytoskeletal structures such as sarcomeric alpha actinin (SAA), cardiac troponin I (TNNI), connexin 43 (Cx43) and myosin light chain 2 (MYL2) by immunocytochemistry.

Conclusion:
Our experiments showed that in-house differentiated iPSC-CM are better passaged at a later timepoint of differentiation and need to be seeded in a high density on a Matrigel coated surface. These parameters delivered a more mature cell morphology. We found an optimal timeframe for subsequent electrophysiological analysis of five days (between day 5 and day 10 after passaging).

P31 - A different light on COL4A2 variant interpretation

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Background/Objectives:
Pathogenic variants in COL4A1 and COL4A2 are known to cause a phenotype with a broad spectrum of neurological findings. COL4A2 variants are less frequent compared to COL4A1 variants. This has caused a biased representation, as COL4A2 is rarely considered separate from COL4A1 variants. In this study we re-assessed the current knowledge on COL4A2 variants.

Methods:
After PubMed search, we collected all COL4A2 variants published until February 2022 and reviewed the LOVD and Clinvar database. All variants were reclassified using current ACMG guidelines.

Results:
Twenty-three articles described 97 cases with 49 different COL4A2 variants. Remarkable was the discordance between published class and the classification using ACMG guidelines in more than 30% of the variant descriptions . In some cases, a strong discordance was noted between pathogenicity based on computational results, functional testing or population frequencies. A striking example is the COL4A2 variant, p.Glu1123Gly. Functional testing in 2012 showed a cellular phenotype, but current population frequency suggests this variant to be incompatible with monogenic disease.
Interestingly, the prevalence of confirmed de novo cases (12%) was distinctly lower compared to the previously reported 40% in COL4A1 variants. Screening of family members carrying the familial variant detected brain MRI abnormalities in 59% of the cases, even in clinically unaffected cases.

Conclusion:
These results show that COL4A2 variant interpretation is complicated due to phenotypic variability, and conflicting predictions of pathogenicity. Additional research about the pathogenic mechanism of COL4A2 variants is imperative for correct interpretation of COL4A2 variants.

P32 - The performance of GS as a first-tier test for neurodevelopmental disorders

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Background: Genome sequencing (GS) can identify novel diagnoses for patients who have exhausted routine diagnostic procedures. We tested whether GS is a better first-tier genetic diagnostic test than current exome-based standard of care (SOC) by assessing the technical and clinical validity of GS for patients with neurodevelopmental disorders (NDD).
Methods: Using a prospective parallel design, we performed both GS and exome sequencing in 150 consecutive NDD patient-parent trios. We developed a strategy for the filtering, prioritization, and interpretation of genomic variants, based on variant type and mode of inheritance. Diagnostic yield was calculated from disease-causing variants affecting exonic sequence of known NDD genes as primary outcome measure.
Results: GS (30%, n=45) and SOC (28.7%, n=43) had similar diagnostic yield. All 43 conclusive diagnoses obtained in SOC were also identified by GS. These 43 conclusive diagnoses included a mixture of single nucleotide variants (SNVs, n=26) , insertion-deletion variants (InDels, n=13), copy number variants (CNVs, n=3), and a repeat expansion (n=1). In addition, all 31 possible diagnoses obtained in SOC were also identified by GS. SOC, however, required integration of multiple test results (average 1.5; range 1-6) to obtain these conclusive and possible diagnoses. GS yielded two more conclusive diagnoses, and four more possible diagnoses than ES-based SOC (35 vs 31). Interestingly, all six likely pathogenic variants detected only by GS were CNVs. These CNVs were not identified in ES due to limitations in CNV calling or the absence of targets in the ES enrichment procedure.
Conclusion: Our data provide the technical and clinical validity of GS to serve as routine first-tier genetic test for patients with NDD. The additional diagnostic yield from GS is limited. Still, GS comprehensively identified all variants in a single experiment, suggesting that GS could constitute a more efficient genetic diagnostic workflow for patients with NDD.

P33 - Predicting biology instead of disease risk by overlapping oncological polygenic risk scores.

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Introduction: Polygenic risk scores (PRS) have been constructed and validated for many cancer types. The most advanced, such as breast, lung or colon cancer PRS, are making its way into clinic and society. For example, through the “Genotyping on all patients” (GOALL) and “Societal impact of genetic science” (SENSE) personalized medicine and prevention programs in Rotterdam and the Netherlands, respectively. In this project, we combine PRS for different cancers and perform biological annotation and clustering to partition genetic risk into cancer-related biological pathways.

Methods: we extract PRS from literature for the most common cancers, and construct a network of disease-variant (GWAS results) and variant-variant (based on LD) associations. The network is soft clustered and visualized using R. All variants are annotated to genes using Ensemble’s variant effect predictor and FUMA. Gene enrichment analyses are performed using GSEA in R.

Results: First results indicate between 5-10% overlap in genetic variants between cancer types, which varies per type. Tissue development, cell cycle, DNA metabolic process and immune response terms are enriched across all cancer types, while some biological processes appear specific to one cancer type, such as response to estradiol to breast cancer variants.

Discussion: Preliminary results indicate shared biology between different cancer types, which can be ascertained by gene annotation and enrichment analysis. Identifying through which biological pathways an individual’s risk is cause might have diagnostic and prognostic implications, e.g., altered treatment options for breast cancer caused primarily through DNA metabolism or hormone response variants.

P34 - CDH1 pathogenic variants – Future for risk estimation based on protein location?

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Background:
CDH1 pathogenic variants are associated with hereditary diffuse gastric cancer (DGC) and/or cleft lip and palate (CLCP). However, which specific variants cause an increased risk of DGC, CLCP or both and with which penetrance remains to be elucidated.
Case report:
A 31-year-old female, known with a unilateral CLCP, was referred to the clinical genetics' consultation after a severe fetal bilateral CLCP was detected at 21 weeks of pregnancy. Based on the severity of the CLCP the couple decided to terminate the pregnancy. Standard genetic testing for CLCP, microarray and IRF6 analysis, revealed no pathogenic variant. In the subsequent pregnancy, again a severe bilateral CLCP was detected and the pregnancy was terminated. Shared whole exome sequencing revealed a heterozygous probably pathogenic c.774_776del p.(Asn258del) variant in the CDH1 gene in the patient and the affected foetuses. This CDH1 variant has not been reported before and was predicted to result in the in-frame deletion of the last amino acid of the first extracellular domain of the extracellular region of mature E-cadherin.
According to the most recent guidelines (Blair et al. 2020), annual gastric surveillance is indicated in patients with a CDH1 variant without family history of DGC. However, for our patient with a de novo variant located close to a linker region between the extracellular domains the optimal management was unclear: mutations in this region are reported to be associated with CLCP but a lower risk for DGC (Selvanathan et al. 2020). The ERN GENTURIS expert panel confirmed that based on this data, the variant should not be classified according to the CDH1 guidelines for DGC (variant of unknown significance), but rather according to the ACMG guidelines (probably pathogenic variant for CLCP). However, based on the lack of evidence on this variant, annual gastric and breast surveillance remains indicated in this patient.

P35 - Benchmarking of long-read structural variant callers using Oxford Nanopore data=

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As long-read sequencing (LRS) technologies mature, several bioinformatics tools designed to identify structural variants (SVs) have been developed. To allow validation of these tools, Zook et al. published a highly curated SV truth set of Genome in a Bottle sample NA24385, consisting of deletions and insertions. We performed a benchmarking analysis with five LRS SV callers against this set utilising in-house generated Oxford Nanopore reads.

SVs are called with cuteSV, SVIM, sniffles, pbsv, and nanovar. The callers are assessed in terms of resource usage, reproducibility, and calling performance. The latter is evaluated with Truvari giving recall and precision statistics on SV detection. We further investigate the influence of read support, sequencing coverage, SV type and length, and integration of call sets with Jasmine.

CuteSV achieves overall best performance, while nanovar lags behind in both resource usage and calling statistics. A coverage greater than 20x offers no additional advantage for reliable SV detection, while the recommended read support of one third of the coverage proves to be too stringent. Integration of call sets with Jasmine should include three callers to compete with stand-alone call sets.

We propose a minimum coverage of at least 15x for optimal sensitivity and specificity. Read support should be set at one fifth of the coverage to obtain optimised calling performance. CuteSV performs best in both sensitivity and specificity, and resource usage. Further work is however needed to assess results for different SV types and more complex regions.

P36 - Extending the molecular and phenotypic spectrum of Bainbridge-Ropers syndrome.

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Background: We report on a 16-year-old girl with developmental delay, first child of non-consanguineous couple of northern European origin. She was born at 37 weeks of gestation following an uncomplicated pregnancy. Neonatal hypotonia, feeding difficulties and dysmorphic features such as microcephaly, facial asymmetry and absent fifth toenails were present at birth. Over the years, she has been diagnosed with severe developmental delay, seizures and absent speech. Genetic testing repeatedly failed to confirm several possible clinical diagnoses. Clinical assessment at 16 years showed sparse hair, long face, high forehead, hypertelorism, tubular nose, low-hanging columella, prominent glabella, bushy arched eyebrow with medial flaring, mild synophrys, high narrow palate, crowded teeth, thick and everted lower lip, elbow and lumbar hypertrichosis, kyphoscoliosis, and small and dysplastic fifth toenails. Some of her features were suggestive of Coffin-Siris syndrome and further extended genetic testing was discussed with the parents.
Methods: Gene panel test for neurodevelopmental disorders was performed on Illumina NGS sequencing system and likely pathogenic or pathogenic variants were reported and subsequently confirmed by Sanger sequencing.
Results: A de novo heterozygous pathogenic variant NM_030632.3(ASXL3):c.1346dup, p.(Ile450Asnfs*12) in ASXL3 gene was detected.
Discussion: Bainbridge-Ropers syndrome (BRPS) is a rare genetic condition, caused by de novo ASXL3 gene pathogenic variants. Affected children have neurodevelopmental delay with no or limited speech, hypotonia and feeding difficulties. Behavioural problems, seizures, palatal, dental and skeletal abnormalities are common. Dysmorphic facial features, such as long face, hypertelorism, tubular nose with prominent nasal bridge, down slanting palpebral fissures, low-hanging columella, high-arched and narrow palate, micrognathia and synophrys are reported by majority of affected individuals. However, hypertrichosis and absent/small toenails are not yet mentioned in the literature. In the present patient, the detected ASXL3 mutation is a novel pathogenic frame shift variant. Other ASXL3 pathogenic frame shift variants have previously been reported in BRPS patients.
Conclusion: We present a BRPS patient with new clinical features that extend further the molecular and phenotypic spectrum of this rare genetic condition.

P37 - Parental experiences of recontacting for extended genetic testing after a terminated pregnancy for fetal malformations

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Objectives
Genome-wide analysis approaches have recently been introduced in clinical genetics since they increase the possibility of finding a genetic diagnosis. In the case of couples who had a termination of pregnancy (TOP) due to foetal congenital malformations, these techniques might provide an answer to the question why this happened and meet the parent's need to know. The aim of this qualitative study is to explore the experiences of couples who were recontacted for additional diagnostic genetic testing after having a TOP for foetal malformations. In addition, why are these couples still opting for genetic testing several years after the termination?

Methods
This study included parents of a retrospective cohort of 85 foetuses that underwent a TOP for congenital malformations between January 2015 and December 2018 in UZ Brussel. After selection, 31 participants were eligible for recontacting, given that a clinical diagnosis is suspected based on the observed congenital malformations, however a molecular diagnosis remains unclear. After receiving a standardized letter, 14 couples agreed to come to the Genetics department to participate in the study. All interviews were transcribed verbatim and anonymized, imported in NVIVO 12 and coded by the thematic analysis of Braun & Clarke (2006).

Results
Despite the years that passed since the TOP, these participants were still motivated to perform new genetic testing. Both intrinsic (searching for answers for themselves and their children) and external motivators (contributing to science and helping parents) played an important role as a driver to come in the Genetic clinic again. All participants were pleased that the medical team took initiative with this sensitive approach, since they would not have taken the initiative themselves.

Conclusion
These results show that there is an interest from these participants in being recontacted which has important implications for the clinical practice. We discerned both personal and altruistic contemplations as motivators.

P38 - Diagnostic yield of partial exome sequencing in 872 children with neurodevelopmental disorders

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Neurodevelopmental disorders (NDD) are genetically and phenotypically heterogenous conditions, currently best investigated by agnostic sequencing of a large number of genes(1). Partial exome sequencing, focusing only on disease-associated genes, is less costly, while also enabling a higher sequencing depth than WES or WGS(2).
We evaluated the diagnostic yield of an in-house designed, regularly updated, partial exome sequencing panel (Mendeliome), containing 4867 disease-associated genes in its most recent iteration. We retrospectively reviewed 872 patients that underwent Mendeliome sequencing for NDD, done with at least one parent, between 2016 and 2021 in our departments. We investigated diagnostic yield based on phenotypic sub-groups, defined by the presence or absence of a given set of HPO terms, and on phenotypic complexity.
Overall yield of Mendeliome-sequencing was 26%. Best results were observed in syndromic (37%) and epilepsy- associated intellectual disability (ID) (39%). Yield for Autism spectrum disorders (ASD) was 19% (ASD without ID: 15%, ASD with ID: 26%). Yield was 23% in severe- and 36% in mild to moderate ID. In epileptic patients, overall yield was 27% (focal epilepsy: 19%, non-focal epilepsy: 28%). Concerning phenotype complexity, we observed yields as low as 14% for patients with only 1 associated HPO term, and up to 42% for patients with over 10 terms. A decrease in overall diagnostic yield from 31% to 17% was observed between 2016 and 2021, probably due to the gradual inclusion of less syndromic patients. Boys were overrepresented in our cohort (60% vs 40%). Diagnostic yield in boys was 22%, while being 32% in girls. For duo- and trio-sequencing we observed a diagnostic yield of 23% and 27%, respectively. Observed yields were comparable to those described in a recent meta-analysis(3).

Conclusion: Diagnostic yield of Mendeliome sequencing is highly dependent on the patient's phenotype and phenotype-complexity, with syndromic NDD ranking highest and isolated ASD lowest.

References:

1. The NDD Exome Scoping Review Work Group, Srivastava S, Love-Nichols JA, Dies KA, Ledbetter DH, Martin CL, et al. Meta-analysis and multidisciplinary consensus statement: exomesequencing is a first-tier clinical diagnostic test for individuals withneurodevelopmental disorders. Genet Med. 2019 Nov;21(11):2413–21.
2. Saudi Mendeliome Group. Comprehensive gene panels provide advantages over clinical exome sequencing for Mendelian diseases. Genome Biol. 2015 Dec;16(1):134.
3. Stefanski A, Calle-López Y, Leu C, Pérez-Palma E, Pestana-Knight E, Lal D. Clinical sequencing yield in epilepsy, autism spectrum disorder, and intellectual disability: A systematic review and meta-analysis. Epilepsia. 2021 Jan;62(1):143–51.

P39 - Objective 3D facial phenotyping in Cri-du-Chat Syndrome

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Background: Cri-du-Chat Syndrome (CdCS) is a genetic disorder caused by deletions on the short arm of chromosome 5. The phenotypic spectrum is broad and multiple critical regions have been defined. We report five patients from three families with a small 2.7Mb 5p15.33-15.32 deletion overlapping with a proposed critical region for facial dysmorphism. We objectively assess the facial phenotype of these individuals by 3D imaging and we use 3D facial images of a large cohort of individuals with CdCS as a reference. We aim to study facial genotype-phenotype correlations in CdCS and to evaluate the involvement of the 5p15.33-15.32 region in facial dysmorphism in CdCS.

Methods: We modelled the 3D facial shape of our patients (n=5), a large cohort of individuals with CdCS (n=97) from a dataset with various genetic disorders (n=3313) and controls (n=40) using dense surface registration. To objectively model shape variation, we calculated facial signatures using craniofacial growth curves. We applied principal component analysis and cosine-based distance metrics to these signatures to compare the facial phenotype of our patients with the reference CdCS group, with other genetic disorders and with controls.

Results: All 5p15.33-15.32 deletion carriers clustered away from CdCS patients. The cosine distances to the average CdCS phenotype independently indicated that facial features in both groups are distinct (range: 0.77-1.30). The within-group variance for our patients was high, objectively supporting the clinical observation of a heterogeneous facial phenotype in these patients.

Conclusion: Facial dysmorphism in patients with a small 5p15.33-15.32 deletion is distinct from the facial dysmorphism in a large cohort of CdCS patients. We present 3D facial phenotyping as a tool to objectively study genotype-phenotype correlations.

P40 - Unraveling complex structural variants by long read sequencing and adaptive sampling

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In recent years, Oxford Nanopore Technology (ONT) has emerged as a promising actor for structural variants identification and repetitive regions analysis by long read sequencing. Nevertheless, as its first targeted sequencing solution requires a lot of preliminary developments, its use in human genetics was mainly restricted to whole genome sequencing with low depth coverage. Recently, ONT has added the adaptive sampling option on its sequencing devices, i.e. a software-controlled enrichment method allowing targeting of genomic regions of interest without any specific wet lab enrichment procedure.

We selected patients with known or strongly suspected disease locus : patients in whom only one pathogenic mutation was identified in a gene associated with a recessive disorder or in whom a structural variant is suspected following short read sequencing analysis. Adaptive sampling was then performed on the basis of a personalized bed file. The effect of region of interest size on the relative enrichment was also evaluated.

Our preliminary results appear promising, as we reached a mean fold-enrichment of 4.5 on targeted regions, along with depth coverage around 15-20X. Although still limited, this enrichment was sufficient to identify various structural variants (such as inversion, Alu insertion, complex indel) and allowed us to reach a diagnostic for several patients.

The adaptive sampling option proposed by ONT appears extremely flexible and allows easy case-by-case adjustment and analysis. While still perfectible, we believe that this approach could be a valuable help in the daily practice of genetics experts, in addition to classic genetic testing.

P41 - Parents' experiences with whole-exome sequencing in pediatric renal cancer

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Rationale
In pediatric cancer, germline whole-exome sequencing (WES) contributes to the identification of predisposing factors, which in turn can facilitate surveillance and family counseling. However, studies on the impact of sequencing studies on families are scarce in pediatric cancer. Our qualitative study explores families' experiences to improve counseling and support.

Methods
33 interviews were conducted with parents after recruitment for a germline WES study in children with renal tumors, comprising a renal cancer predisposition gene panel analysis and optional exome wide trio-analysis. The interviews were analyzed using an inductive thematic approach.

Results
Parents were positive about participating in genetic sequencing and reported both individual and altruistic motives. Altruistic motives e.g., helping future patients, seemed to be relatively more important after the child's treatment had finished compared to families recruited during treatment. Families recruited shortly after diagnosis felt overwhelmed. Parents often preferred exome-wide (trio-)analysis over cancer panel analysis, although afterwards many had difficulties distinguishing these two. Families in which a predisposition was not identified felt relieved, but some worried about yet undiscovered genes or felt disappointed. In most families with a predisposition no significant distress was observed, although in some the predisposition added up to the already existing psychosocial burden.

Discussion
Families are positive about participating in the genetic study; however, we identified several challenges pertaining to timing, consent, and post-test support. We suggest counseling families during a relatively stable phase in their child's treatment trajectory. Separating consent for panel and exome-wide analysis could possibly help parents to make a more deliberate decision. Attention is necessary for families who receive negative test results and for those who already have a high burden.

P42 - Atlas of retina cell-specific transcript and protein isoforms

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The retina is the light-sensitive tissue at the back of the eye. It consists of numerous cell types, including rod and cone photoreceptors. Our knowledge about the transcript and protein isoforms expressed in the retina is not yet complete. Genetic defects in genes that code for proteins involved in normal retina function can cause inherited retinal degeneration (IRD). IRDs affect about 1 in 2,000 individuals worldwide. It is estimated that approximately 1/3 of causative variants in IRD-associated genes disrupt splicing. Several previous studies revealed retina-specific splicing factors and isoforms. However, we need understanding about the retina transcriptome and proteome to better understand the underlying mechanism of IRDs and how to possibly treat them. Published studies are based on short-read RNA-sequencing and can therefore only provide limited information on retinal isoforms. In this study, we analysed three human post-mortem neural retina samples with PacBio long read RNA-sequencing to create an atlas of retina transcript isoforms. We identified more than 100,000 unique isoforms in more than 15,000 genes. More than 40 percent of the isoforms were classified as novel based on the GRCh38 reference annotation. Compared to all genes, IRD-associated genes show a high proportion of novel isoforms. Both novel exons and exon skipping events were found in IRD-associated genes. Moreover, 24 out of 75 previously identified retina specific micro-exons could be identified. In a next step, we will use mass spectrometry to confirm retina transcript isoforms on protein level. To conclude, the retina cell atlas provides novel insights into the retina transcriptome and can be used as reference for future IRD research.

P43 - Tandem repeat variability in frontotemporal dementia: a population scale assessment

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Tandem repeats are associated with approximately 60 human disorders, primarily neurological, but remain among the most complicated elements in the genome for accurate detection and sizing. In addition, standard short-read sequencing analysis methods are inadequate, especially if repeats expand beyond the read length. However, current methods mitigate this limitation and enable the investigation of tandem repeat lengths across populations. Simultaneously, long-read nanopore sequencing is becoming a routine method for comprehensive genome analysis and dramatically improves the resolution for repeat analysis.

We combine modern analysis methods in large-scale short-read population sequencing with long-read population sequencing for a complete assessment of tandem repeat variability and their role in frontotemporal dementia.

P44 - Pilot study demonstrates the feasibility, the diagnostic power and the utility of rapid whole genome sequencing for critically ill pediatric patients in Belgium

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Purpose
Genetic diseases are an important causes of admission and death in pediatric intensive care units. Early diagnostic orients the care and prevents irreversible harms. Whole Genome Sequencing in short turnaround time (TAT) or rWGS, has proven to be a very valuable exploration approach in critically ill pediatrics patients. This pilot study aims at evaluating the feasibility, the efficiency and the utility of the rWGS in Belgium.
Methods
We leveraged the collaboration between two pediatric institutions and the center for human genetics of the University of Liège, a series of Cloud-based computing data analysis services and a high-throughput NGS platform to develop a rWGS workflow intended to deliver diagnostics to critically ill pediatric patients before hospital discharge. WGS was performed on NovaSeq PE300cy in trio for 9 and in duo for 1 proband. The study was approved by the institutional review boards.
Results
Ten unrelated critically ill patients without any clear diagnostic, including five girls and five boys, were recruited from the Neonatal (NICU) and Pediatric Intensive Care Units (PICU) for 4 each, and the neuropediatric unit for the remaining 2. A definite diagnostic was reached in 6 out of 10 patients, including a dual diagnostic, in 39.29 hours (95% CI 38.3 - 40.3). Out of six diagnosis, five were six clinically unsuspected. The molecular diagnostic guided multidisciplinary care in 5 patients and disease specific care in 1 patient. The time constraints and the cost of the process were identified as the current main limitations to the broad introduction of rWGS for critically ill newborns and infants.
Conclusion
We have successfully implemented the fastest rWGS platform in Europe. Our workflow has one of the highest rWGS yields. This study establishes the usefulness of rWGS in a Belgian setting and defines the path for a nation-wide semi-center implementation of rWGS.

P45 - Deep phenotyping of patients with developmental disorders and their relatives to support interpretation of rare inherited copy number variants

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Introduction:
In patients with developmental disorders (DD), copy number variants (CNV) inherited from seemingly unaffected parents are typically disregarded in variant interpretation. However, understanding the phenotypic contribution of inherited rare variants is crucial for genetic counselling. Therefore, an extended family-oriented approach is proposed, including deep familial phenotyping and multi-omics in carriers and non-carriers.
Methods:
Deep phenotyping (medical, developmental and behavioral, using standardized instruments) of carriers and non-carriers within the nuclear family, enables familial segregation analysis of a CNV with DD (sub)phenotypes. Trio whole genome sequencing (WGS) is used to identify additional pathogenic variants causing or contributing to the phenotype. RNA and capture Hi-C sequencing on EBV-immortalized cell lines is used to examine the CNV regulatory effect. This combined approach was applied to interpret a rare paternally inherited deletion ([GRCh37] 4:141693186-142147039x1) in a 15-year-old boy with moderate intellectual disability, autism spectrum disorder, developmental coordination disorder, hypotonia and facial dysmorphic features.
Results:
Standardized IQ-assessment (Wechsler Scales) of all nuclear family members revealed that CNV carriers (FSIQ; index 40, father 71) scored significantly lower on cognitive abilities than non-carriers FSIQ; mother 100, sibling 92). Within the domain of behavior and emotional problems (CBCL-scores) and adaptive functioning (ABAS-III-NI) the behavioral profiles of the index patient and his father were more overlapping than the profiles of the other family members. De novo, recessive, X-linked and paternally inherited variant analyses of SNV, indels and additional CNVs were negative. RNA-seq shows four differentially expressed genes in the CNV or flanking regions (INPP4B, SETD7, MAML3, ZNF330). Their contribution to DD is subject to further study.
Conclusion:
This multi-omics approach and correlation through deep familial phenotyping is suggestive of a contributory effect of this CNV to DD in this family.

P46 - A complex structural variation in the EYA4 gene implicated in a non syndromic autosomal dominant hearing loss (DFNA10).

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We report on a four-generation Belgian family with non syndromic sensorineural hearing loss (SNHL). The family includes a 10-year-old boy patient, his mother, two maternal uncles, his maternal grand mother and the great-grandmother. Hearing impairment is found to be mid-frequency, symmetric, and progressive, ranging from early childhood to 30 years.
Targeted exome sequencing (mendeliome) analysis conducted using DNA of the young proband and his mother, revealed a structural variation at the 3' end of the exon 11 of the EYA4 gene (EYA transcriptional coactivator and phosphatase 4). Pathogenic variants in EYA4 are responsible for postlingual, progressive and autosomal dominant hearing loss (DFNA10). Less than ten families are reported in the literature with pathogenic variants in this gene. We fully characterized this variation using Sanger sequencing, Nanopore long read sequencing and splicing analysis. The variant, c.974_988+1143delins226, implied the deletion the 3' canonical splicing site of the exon 11 and was predicted to impact splicing of the EYA4 transcript. As EYA4 is weakly expressed in blood, RNA extracted from proband's peripheral blood cells culture was used to perform the splicing analysis. We showed a loss of exon 11 leading to degradation of the mutated transcript by RNA decay. Finally, we confirmed the segregation of the variation with the phenotype in the family (5 affected patients and 2 unaffected).

Our results highlight the need to analyse structural variation and the importance of blood cells culture analysis with puromycine treatment to characterize splicing variants, even in genes poorly expressed in blood. We broad also the complexity of the genotype and the phenotype associated with pathogenic variants in EYA4.

P48 - Trio-based sequencing in patients with sporadic inborn errors of immunity: a retrospective cohort study

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De novo variants (DNVs) are currently not routinely evaluated as part of diagnostic whole exome sequencing (WES) in patients with inborn errors of immunity (IEI). This study explored the additional value of systematic DNV assessment in a retrospective cohort of 123 patients with sporadic PIDs. Patient-parent trios sequenced at the Radboud University Medical Center were eligible for inclusion when the IEI in-silico gene panel was analysed and the phenotype of the index patient suggested sporadic disease. Exome-wide analysis was performed to retain rare, coding, non-synonymous de novo SNVs. Variants were further prioritized based on gene and variant level metrics. In immune cells from a selected patient, functional validation experiments were performed at the level of RNA splicing, NF-kB signalling and cytokine production. Candidate DNVs were identified in 15 (12.2%) trios, in addition to 12 (9.8%) inherited (likely) pathogenic mutations. These potentially disease-causing DNVs were identified in known IEI genes NLRP3 and RELA, and novel candidate genes including PSMB10, DDX1, KMT2C and FBXW11. Furthermore, the FBXW11 canonical splice site DNV, carried by a patient with autoinflammatory disease, was shown to cause defective RNA splicing, increased NF-κB signalling and elevated IL-1β production. This retrospective cohort study advocates the implementation of trio-based sequencing in routine diagnostics of patients with sporadic IEI to improve the solve rate. Furthermore, we have provided functional evidence supporting a causal role for FBXW11 loss-of-function mutations in autoinflammatory disease.

P47 - Methylome analysis of cfDNA to predict preeclampsia presymptomatically

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Background/Objectives: Preeclampsia is a leading cause of intra-uterine growth retardation, premature birth, and low birth weight. Early preeclampsia risk assessment is of the utmost importance for pregnant women, as it can minimize adverse perinatal events by enabling both closer monitoring and early pharmacological intervention. However, first trimester screening models that are solely based on maternal demographic characteristics and medical history typically have a low detection rate and are inadequate for effective prediction.

Methods: Here, we assessed if methylation differences in the plasma-derived cell-free DNA (cfDNA) between case and control groups are indicative of a risk to develop preeclampsia. We developed a method to measure cfDNA methylation at 34,735 selected regions of interest using target-enrichment bisulfite sequencing. We next compared cfDNA from expectant mothers around 12 weeks of gestation that will go on to develop preeclampsia with matching controls, as well as cfDNA obtained at the moment of preeclampsia diagnosis.

Results: In both sample sets, differences in DNA methylation changes between control and preeclamptic pregnancies were detected. These changes enable classification of patients (n=44) and controls (n=27) at time of diagnosis (area under the receiver operating characteristic (AUROC) curve of 0.95), and crucially, also early in pregnancy (< 15 weeks), when patients (n=96) could be differentiated from controls (n=92) (AUROC of 0.83).

Conclusion: cfDNA methylome analysis can contribute to early preeclampsia risk assessment, in a time window where prophylactic therapy can still be initiated, which can result in better pregnancy management.

P49 - Identification and characterization of a novel retina-specific lncRNA upstream ABCA4 with a potential role in ABCA4-associated inherited retinal disease

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Purpose| Inherited retinal diseases (IRDs) are a major cause of blindness. While mutations in coding regions account for 60% of IRDs, non-coding variants can explain missing heritability. A major knowledge-gap lies in the role of long non-coding RNAs (lncRNAs), highly tissue-specific molecules that regulate gene expression at the right time and place. However, little is known about their function in the retina. Here we identified a novel retina-specific lncRNA located upstream ABCA4, the gene implicated in Stargardt disease.

Methods| Expression specificity was determined by re-analysis of short-read (GTEx, adult human retina, retinal organoids) and single-cell RNAseq data. Nanopore long-read sequencing and single-molecule RNA in situ hybridization (RNAScope/BaseScope) were performed on adult human retina. Chromatin interaction profiles (UMI-4C) were generated to evaluate interaction with the ABCA4 promoter. Genomic variation was evaluated in smMIPs data of the ABCA4 locus in 1,054 Stargardt cases.

Results| Short-read RNAseq analysis of ~7,400 transcriptomes of 54 tissues (GTEx) and 152 transcriptomes of adult human retinas revealed a novel retina-specific lncRNA upstream ABCA4. Long-read sequencing of donor retina identified two isoforms, for which expression was demonstrated in the outer nuclear layer of adult human retina. The lncRNA is transcribed from an active retinal enhancer interacting with the ABCA4 promoter, suggesting a cis-acting effect on ABCA4. We identified 4 heterozygous novel copy number variants overlapping the lncRNA, representing likely pathogenic/modifying alleles. Perturbation knockdown studies in human retinal explants are ongoing to further elucidate its function.

Conclusion| We identified and characterized a novel retina-specific lncRNA, potentially implicated in ABCA4-associated IRD. This study provides novel insight into the role of this lncRNA - an unexplored class of molecules in the retina field - in gene regulation and IRD pathogenesis, which may ultimately entail therapeutic perspectives.

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P50 - Ultra-efficient and non-translation dependent nonsense mediated RNA decay due to a COL3A1 splice site variant in a patient with mild vascular Ehlers-Danlos phenotype

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A 54-years female proband presented with a mild vascular Ehlers-Danlos syndrome phenotype. The cardiac outflow tract appeared normal, however easy bruising was seen as well as increased visibility of facial veins. Upon genetic screening, a heterozygous splice site variant (c.3256-1G>A) was identified in the COL3A1 gene. Skipping of exon 45 was predicted as the pathogenic mechanism, however, this did not fit with the mild phenotype of the patient. Moreover, the variant was also present in the unaffected mother of the proband.
Patient dermal fibroblasts were cultured with and without a nonsense mediated RNA decay (NMD) inhibitor, puromycin as well as cycloheximide. COL3A1 Sanger sequencing was carried out at gDNA and cDNA level. Fractionation of the nucleus and cytoplasm was performed to distinguish nuclear and cytosolic transcripts.
The heterozygous splice site variant was indeed confirmed by Sanger sequencing on gDNA level. On cDNA level, inhibition of NMD did not result in an observed differentially spliced transcript, despite the good quality of the sequences. Based on the study of other heterozygous exonic SNPs, we were able to confirm that only one COL3A1 allele was amplified at cDNA level. Therefore, NMD appeared to occur in an ultra-efficient manner and was not translation dependent. Fractionation of nuclear and cytoplasmic transcripts confirmed that the mutant transcripts undergo NMD in the cytoplasm but are still detectable in the nucleus. These transcripts show the shift of the used splice-site with one nucleotide.
Based on our nuclear fractioning as well as SNP detection experiments, we can conclude that the COL3A1 variant c.3256-1G>A leads to haplo-insufficiency by ultra-efficient non-translation dependent NMD. This is in line with prior observations that COL3A1 haploinsufficiency correlates with a mild vascular EDS phenotype. Our findings also underline another important molecular lesson: if at first glance no effect on splicing is observed after cDNA screening, it must be confirmed (e.g. with the presence of heterozygous SNPs) that both alleles are expressed.

P51 - Unraveling the genetic basis of early-onset inherited retinal disease in a Saudi Arabian cohort reveals a novel RIMS2-related family

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Background/Objectives:
In this study, we aimed to uncover the molecular causes of Leber Congenital Amaurosis (LCA) and earlyonset retinal dystrophy (EORD) in 15 families of Saudi Arabian descent, using an integrated approach consisting of autozygosity mapping and targeted resequencing or whole exome sequencing (WES).
Methods:
A total of 18 patients (7 females and 11 males ranging between 2-9 years old) from 15 Saudi consanguineous families underwent ophthalmological examinations to establish a clinical diagnosis. They underwent autozygosity mapping, targeted gene testing combined or not with whole exome sequencing (WES). Variants were validated, classified according to ACMG/ACGS guidelines, and subjected to segregation analysis if family members were available.
Results:
Patients displayed decreased best corrected visual acuity, photophobia, amaurotic pupils, congenital nystagmus, and oculo-digital sign. Likely pathogenic variants were found in 13/15 studied families in genes previously implicated in LCA/EORD, including 6 novel variants and a putative founder RPGRIP1 variant (c.1107del;p.(Glu370Asnfs*5)) for the Saudi Arabia population. Interestingly, a novel homozygous RIMS2 splice variant c.1751+1G>T was identified in an EORD patient without any signs of systemic involvement or neurodevelopmental symptoms. All variants were found in runs of homozygosity (ROH), apart from two heterozygous GUCY2D variants.
Conclusion:
Our approach uncovered 13 distinct likely pathogenic variants in 13/15 studied families (86%), demonstrating the power of autozygosity-guided WES in a genetically heterogeneous consanguineous cohort with LCA/EORD. Finally, we report a biallelic RIMS2 variant in a seemingly non-syndromic patient and corroborate the previous role of RIMS2 in EORD pathogenesis.

P52 - NGS as a screening method for Epilepsy, progressive myoclonic 1A suspected patients.

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Epilepsy, progressive myoclonic 1A (Unverricht and Lundborg disease or EPM1), with autosomal recessive inheritance is mostly caused by expansion of the dodecamer repeat CCCCGCCCCGCG in the 5' non coding domain of CSTB gene. Up to date, this diagnosis is performed in routine using Southern Blotting to detect the characteristic 60 to 80 repeats expansion.
Short read sequencing by NGS is not the best choice to analyse repeat expansions. However, we added this specific region of CSTB gene in our custom panel and analysed several patients previously tested by Southern Blotting (and known to carry an expansion of this repeat).
When estimating the number of copies in the region of interest via a classical CNV analysis approach, we observed that these control patients all had similar depth of coverage profiles in this specific region, with a depth ~33% percent lower than expected for heterozygous expansion and ~66% lower for homozygous expansions.
These results allow us to use NGS as a screening method for EPM1 suspected patients instead of dealing with Southern Blotting. In addition, this screening takes place together with our routine gene panel CNV analysis, which makes it more time and cost effective than when combined to the Southern Blotting approach.

P53 - MOLGENIS Variant Interpretation Pipeline: integrating best practice and new methods to prioritize genetic variants causal for a patient's phenotype

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Thus far, almost 7000 diseases with a molecular basis are defined, of which almost 6000 single gene disorders, and around 4000 genes are known to harbor the pathogenic variants causal for the patient's phenotypes. Still, the diagnostic yield of genetic testing varies between 24-68%, depending on patient inclusion criteria, whether trio's are studied, patient's phenotype(s) and analysis strategies. Fortunately new methods are published frequently, however their timely implementation necessitates a flexible analysis workflow. Therefore we present MOLGENIS Variant Interpretation Pipeline (VIP), a flexible system to prioritize genetic variants in a VCF on the likelihood to be causal for a patient's phenotype. VIP integrates best in class software, such ENSEMBL Variant Effect Predictor, SpliceAI, gnoMAD and CAPICE, with validated decision protocols from routine diagnostics practice and integrating inputs from experts from EU Solve-RD, EJP-RD and CINECA projects. VIP provides annotation, prioritization and filtering of variants through GENMOD-based inheritance matching and HPO-based phenotype matching, including SV, low-penetrance and GRCh38 pilots. Currently it is investigated if non-coding variant annotation sources, such as JARVIS and NCer can help prioritize pathogenic variants outside the coding region, bringing interpretation of complete whole genome sequencing data a step closer.
Using these options and annotations users can create custom filter trees to classify variants. Moreover, interactive HTML reports can be generated to filter and select variants of interest. We believe VIP helps get the best analysis methods to patients, faster, but also to help solve unsolved cases by allowing integration of newly available tools. VIP is open source, and we welcome community contributions to add novel tools and create new pipeline configurations suited to different use-cases. Find the latest release here: https://github.com/molgenis/vip/releases and https://github.com/molgenis/vip-report/releases.
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P54 - FiberFISH mapping of 22q11.2 rearrangements shows locus heterogeneity

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Background: The 22q11.2 deletion syndrome (22q11.2DS) is with an estimated incidence of 1 in 1500 to 3000 live births about ten-fold more frequent than any other genomic disorder. The driver of the rearrangements is thought to be non-allelic homologous recombination (NAHR) between two of several low copy repeats (LCRs) on chromosome 22 (LCR22-A until LCR22-H). The full sequence nor the breakpoints of these LCR22s have been charted due to the genetically complex nature of the LCR22s. Another layer of complicity lies within the huge inter-individual variability of LCR22A.

Methods: Cell lines from patients and parents were established with an LCR22-ADdel (n=12), LCR22-ABdel (n=2), LCR22-ACdel (n=2), LCR22-BDdel (n=1), LCR22-CDdel (n=1). The fiber-FISH technique was used to map the LCR22s and rearrangements at subunit level and provided a tool to determine the parent of origin.

Results: Multiple specific haplotypes could be discovered for all duos. In patients a rearranged locus ranging from 20 kb to 160 kb could be detected. We identify rearrangements in different subunits.

Conclusion: Fiber-FISH confirms and provided an excellent tool to detect the variation of different haplotypes for LCR22s in the human population. We demonstrate variability in the position of the NAHR subunits, suggesting that rearrangements within the LCRs occur at different positions. In addition, we enlarge the spectrum of LCRA variation in the human population and, for the first time, identify the rearrangements in LCRs-B and C.

P55 - Embryo Tracking System for efficient and scalable diagnostic massively parallel sequencing

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Preimplantation genetic testing (PGT) is offered for three indications, namely monogenic disorders (PGT-M), structural rearrangements (PGT-SR) and aneuploidies (PGT-A). Although state-of-the-art sequencing-based methods that assay hundreds of thousands to millions of single nucleotide polymorphisms made a paradigm shift in preimplantation genetic testing (PGT), from locus- and family-specific to genome-wide and generic, they are still laborious, making sequencing-based PGT costly and posing risks for human errors. Here, we report an easy-to-use embryo tracking system (ETS) that notably increases efficacy and scalability of PGT. We validated this chemistry using single- and few-cell (n=2-10) DNA samples derived from 330 in vitro fertilized preimplantation embryos. Our ETS-PGT is unique in its incorporation of the ETS fragments, with (i) an extra 20-nucleotide sequence that allows the restriction enzyme to bind, (ii) an adjacent restriction site that is specific for the sequencing-based PGT procedure, (iii) an extra primer binding site that makes sample tracking universal for any sequencing-based wet-lab protocol, as well as (iv) a complementary, integrative computational pipeline that automatically traces the embryos. We demonstrate that ETS eliminates 6 crucial control steps in the sequencing-based PGT, enabling not only higher quality assurance (reducing human error), but also increasing the scalability of the process by enabling a fully robotized comprehensive PGT. ETS has broad applicative value in reproductive genetics and beyond, as it can easily be adapted to other massively parallel sequencing workflows, such as single-molecule molecular inversion probes, whole genome sequencing and non-invasive prenatal testing.

P56 - Two types of variants pointing out a crucial role for RNF216 in healthy and diseased brain

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Mutations in RNF216, an E3-ubiquitin ligase, have been identified as the genetic cause of Gordon Holmes syndrome (GHS), a rare recessive neurodegenerative disease. RNF216 mutations can be roughly classified into two groups based on their location within the protein. The first group of mutations cluster within the catalytic domain and lead to a loss of ubiquitin ligase activity. In the brain of one patient with a homozygous R751C variant in the catalytic domain, intranuclear ubiquitin-positive inclusions were found (Margolin et al, 2013. The second group of mutations reside in the N-terminal region, outside the catalytic domain with preserved ligase activity. Still, the mechanism by which this second group of mutations is leading to loss-of-function is elusive. Interestingly, in a previously reported patient with a homozygous N-terminal G456E mutation outside the catalytic domain (Santens et al, 2015) we replicated the neuropathological findings observed in the R751C patient, pointing towards a similar loss-of-function mechanism at play. Immunohistochemical analysis shows ubiquitin and p62 immunoreactive intranuclear inclusions which are negative for hyperphosphorylated tau, ß-amyloid and TDP-43. Intriguingly, we identified a patient combining variants from both groups: one variant, C752Y, in the catalytic domain and one, W449R, in the N-terminus, suggesting that indeed both variants convergence into a loss-of-function pathway.
As the endogenous function of RNF216 is crucial to understand RNF216-mediated neurodegeneration, we want to investigate beyond the catalytic activity, a novel and additional nuclear function for RNF216. This will be enabled by in-vitro experiments and proteomics on human GHS-patient brain tissue and fibroblasts, hiPSC-derived neuronal cultures expressing RNF216 disease-variants, and BioID.
The physiological and pathological mechanisms explored in this project hold the potential to greatly improve our understandings of GHS, shed light on novel key players in neurodegeneration, and open new avenues for a novel therapeutic target for this rare neurodegenerative disease.

P57 - Barrett's esophagus after esophageal atresia corrective surgery

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The surgical correction of Esophageal Atresia (EA) can impact the natural reflux barrier many patients suffer from motility problems, chronic gastroesophageal reflux and reflux esophagitis. These are risk factors for the development of Barrett's Esophagus (BE), a metaplastic lesion in which esophageal squamous epithelium is replaced with gastric columnar epithelium. EA patients have an increased population risk and earlier age of onset of BE. Recent advances have identified specific BE patient subclusters associations to the risk of developing esophageal adenocarcinoma (EAC). Interestingly, BE in EA patients seems to progress into EAC as well as esophageal squamous cell carcinoma and could represent a distinct subpopulation. We compared the transcriptomes of mucosal esophageal biopsies of adults born with EA who developed BE to those of BE patients who did not have EA/TEF in their medical history. Differential expression analysis was done using DESeq2 after deconvolution using Granulator. To evaluate differences in isoforms-exon usage we used DEXSeq, aberrant splicing was evaluated using FRASER (Find Rare Splicing Events in RNA-seq), Chromosomal stability using SuperFreq and mutational signatures and driver gene variation were determined with VarScan and FreeBayes. Using this experimental set-up, we created genomic signatures. Initial analysis revealed differences in some of these signatures as well as increased inflammatory, stress response and activation of oncological processes. Results hint at differences in tissue homeostasis between BE patients with and without EA in their medical history. It is worthwhile to investigate these differences more systematically in a larger study population.

P58 - An integrated approach using multi-omics data to dissect cis-regulatory role of ultraconserved non-coding elements (UCNEs) in human retina

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Background/Objectives:
Whole genome sequencing (WGS) has revealed an increasing number of pathogenic non-coding variants in inherited retinal diseases (IRD), with a majority of deep-intronic variants. We focus on the role of ultraconserved non-coding elements (UCNEs) defined as genomic regions >200 bp characterized by at least 95% human-chicken conservation, and their association with gene regulation and disease. Using an integrated multi-omics approach, we set out to assess a potential cis-regulatory role for active UCNEs in human retina.

Methods:
To predict UCNEs with active enhancer-like marks, we integrated publicly available transcriptomic (bulk and scRNA-seq) and epigenomic (ATAC-seq, DNase and histone modifications for active enhancers) datasets derived from human retinal material. WGS data from ~3,331 probands of the ophthalmological disease cohort of the 100,000 Genome Project Genomics England was mined to retrieve sequence variants (SNVs) and structural variants (SVs) within UCNEs.

Results:
Interrogation of multi-omics data derived from human retina revealed a total of 1,349 retina-active UCNEs. Interestingly, 40 genes under putative UCNE control are linked to an eye-disease phenotype. A total of 79 rare SNVs and 10 SVs related to genes implicated in IRD or other eye phenotypes were identified.

Conclusion:
We identified 1,349 retina-active UCNEs potentially acting as CREs and representing an understudied target of non-coding variants that may explain missing heritability in IRD. Deciphering retinal UCNEs and their cis-regulatory landscapes will contribute to functional genome annotations in the retina and to the non-coding morbid genome of IRD. Ultimately, they may represent novel targets for treatment.

P59 - Towards a federated infrastructure for human data across Europe

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Access to data is recognised as a crucial aspect to e.g. deliver personalized medicine and to decide on new policies in health care and public health. ELIXIR coordinates the efforts at European level to develop an infrastructure to share human molecular data, across borders compliant to local regulations and with respect for privacy. Ultimately, the goal is to create a federated ecosystem of interoperable services that enables population scale genomic and biomolecular data to be accessible across international borders, accelerating research, and improving the health of individuals across Europe.

Establishing this infrastructure requires close collaboration between representatives of all the countries involved to ensure engagement and subsequent adoption of the proposed technologies. These discussions range from legal and policy matters to technical discussions e.g. on security aspects. Therefore, a large number of stakeholders are involved in building this ecosystem, across multiple large-scale projects. Accordingly, it is often hard for individuals to get the bigger picture on how this infrastructure is being established.

In this poster we will provide an overview of the state-of-the-art of the infrastructure for sharing human genomics data in Europe, highlight the different projects that are shaping this and how they relate to each other to build the entire ecosystem of the 1+MG initiative. We will touch upon the Beyond 1+MG and HealthyCloud coordinating projects as well as how these are brought together with the Genome of Europe into the Genomic Data Initiative.

P60 - Different IVF culture media do not affect the methylome of IVF children

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Background/objectives: A growing number of children born are conceived through in vitro fertilization (IVF) procedures that have been linked to increased risks of adverse perinatal outcomes and altered growth profiles in the resultant children. These outcomes are also influenced by the media used for embryo culture and this effect is hypothesized to be mediated epigenetically, e.g. through DNA methylation. Therefore, we investigated the methylome in IVF offspring who underwent embryo culture in different media.

Methods: We profiled the umbilical cord blood (UCB) methylome of IVF-neonates cultured in G5 or HTF (n=106), and the saliva methylome of 9-year-old IVF children (n=120), cultured in G3 or K-SICM, using the Infinium Human Methylation EPIC BeadChip. Analyses were carried out separately on UCB and saliva samples using empirical Bayes moderated mixed effects linear models adjusted for potential confounders. Methylation outliers represent values more than three interquartile ranges from the upper or lower quartiles.

Results: In both comparisons (UCB and saliva) we identified no significant methylation differences between the culture medium groups in terms of: (i) systematic differences at single CpG sites or regions, (ii) imprinted sites/genes or birth weight associated sites, (iii) stochastic differences presenting as DNA methylation outliers or differentially variable sites, and (iv) epigenetic gestational age acceleration (UCB samples only).

Conclusion: The IVF culture media investigated did not lead to methylome differences in the resultant neonates/children, suggesting that culture medium induced epigenetic alterations resolve prenatally. To investigate environmental-epigenetic interactions occurring earlier during human development and their impact on development, (epi)genetic and transcriptomic profiling of single cells from preimplantation embryos is required.

P61 - Deciphering the genetic cause of recurrent and spontaneous pregnancy loss

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Introduction
The prevalence of miscarriage is approximately 15.3% of all recognized pregnancies. 80% of pregnancy losses occur in the first trimester. In 60% of cases chromosomal abnormalities are found, whereas in livebirths this number is less than 1% when no prenatal diagnosis was used. No study previously performed a SNP-based haplotyping analysis on miscarried products of conception biopsies from distinct locations, comparing early (~7 weeks gestational age) SPL and RPL in a moderately large (n=86) study population. In this study we investigated the prevalence and nature of (mosaic) de novo genomic aberrations in RPL and SPL.

Materials and Methods
Samples from 86 families with pregnancy loss (n=42 RPL cohort, n=44 SPL cohort) with normal karyotyping results in both parents were analyzed in this study. DNA was isolated from blood of both parents and placental tissues from the miscarried products of conception. The placenta tissues were sampled from two distinct extraembryonic and embryonic germ layers, i.e., the extraembryonic mesoderm and the chorionic villi cytotrophoblast, respectively. We performed SNP-genotyping and applied haplarithmisis to delineate allelic architecture of fetal tissues.

Results
We analyzed 175 fetal DNA samples. Within the RPL cohort, we found aberrations in 24 tissues (14 families), including genome-wide and chromosomal abnormalities. Within the SPL group, we found aberrations in 16 tissues (8 families), including genome-wide, chromosomal, and segmental abnormalities, suggesting that mosaic alterations may cause pregnancy loss.

Conclusions
Multiple genome wide aberrations are found in the RPL group but only one in the SPL group, indicating an association between genome wide mosaic aberrations and RPL. While aberrations in the RPL group were more maternal in origin, in the SPL group they were more paternal in origin. Our findings can lead to a better understanding of causative factors for SA and RPL and the need for a SNP-based non-invasive prenatal testing.

P62 - Identification of potential core functions in human immune responses of genes with previously unknown function

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Inborn errors of immunity (IEI) is a group of genetic disorders with both heterogeneous genetic causes and phenotypic manifestations, complicating its diagnosis. Many genes known to cause IEIs have a function in the innate immune response, also known as the “first line of defence”. Exome and genome sequencing leads to a diagnosis in 15-30% of IEI cases, indicating a potential for improvement, in part through the identification of novel genes with a role in the immune system and, in turn, in IEIs. In order to identify core genes in the human innate immune response, we applied QuantSeq 3’ mRNA sequencing in in vitro pathogen-stimulated peripheral blood mononuclear cells (PBMCs) from 5 healthy donors. PBMCs were stimulated with LPS, S. Aureus, PolyIC and C. Albicans for 4 and 24 hours to mimic early and late innate immune responses to gram-negative and gram-positive bacteria, viruses and fungi, respectively. Overlapping genes differentially expressed in stimulated PBMCs compared to unstimulated cells allows for the identification of genes that likely have core functions in the immune response. As a proxy for undescribed genes, we extracted “Corf” and “KIAA” genes, of which four are differentially expressed in all stimuli - strongly implicating a role in human host-pathogen responses; KIAA0040, C11orf21, C1orf122 and C15orf48, of which only the latter has recently been found to be implicated in the regulation of inflammation and immunity. These results indicate the potential for characterising the innate immune response, which provides candidate genes that may play a role in the pathogenesis of IEIs. We will reanalyse the genetic variation in these uncharacterised genes with a potential role in the immune response in exomes of IEI patients in our in-house database, potentially contributing to the growing number of genes implicated in IEIs and for the diagnosis of patients using previously missed disease-causing variation.

P63 - BeSolveRD: The Belgian Genome Resource to Resolve Rare Diseases

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Background/Objectives: Despite the diagnostic implementation of chromosomal microarrays/shallow whole genome sequencing and whole exome sequencing (WES) for patients with intellectual disability/developmental disorders (ID/DD), approximately half remain undiagnosed using this standard of care (SoC). To (1) technically validate whole genome sequencing (WGS) at different genetic centers in Belgium, (2) investigate the clinical utility of WGS for ID/DD diagnosis and (3) to assess the health economic impact, the Belgian genetic centers engaged in a multicentric prospective randomized control trial, called BeSolveRD.

Methods/Results: A total of 800 patients and both parents will be recruited of which half will be processed by SoC and half by WGS. We have performed ring trials to compare different methods to generate WGS libraries, allowing the optimization and validation of WGS in all centers. The WGS pipelines for SNV detection of all centers and their performance have also been assessed. Most pipelines use the same tools and perform very similarly despite the different implementation. Finally, we have created a federated database to share variant and sequence information.

Conclusion : We will present the process of collaboration, the challenges and the intermediate output data.

P64 - ATRIP-deficient patient expands molecular and clinical spectrum of Seckel syndrome

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Background/Objectives:
We report the second ATRIP-deficient patient clinically diagnosed with Seckel Syndrome (SS). The ATRIP protein is required for ATR stabilization by complex formation and is crucial for localization of the ATR-ATRIP complex to regions of DNA damage and ATR activation. Besides the typical clinical SS characteristics (primary dwarfism, facial dysmorphia, skeletal abnormalities, microcephaly and mental retardation), our patient suffers from an immunodeficiency. However a link between ATRIP and the immune system was not previously reported.
Methods:
Whole exome sequencing (WES), transcriptomics, western blot, micronucleus assays, flow cytometry and single cell RNA-Sequencing.
Results:
The patient is homozygous for a splice variant (c.829+5G>T) in ATRIP leading to out-of-frame exon 5 skipping. Western blot showed absence of ATRIP protein and analysis of micronuclei in response to DNA damage by mitomycin C and ionizing radiation revealed defective DNA repair. Downstream substrates of the ATR-ATRIP complex are currently investigated.
WES ruled out a pathogenic variant in 460 genes linked to inborn errors of the immune system. Immunophenotyping reveals low absolute B cell numbers, aberrant T cell subsets, decreased plasmacytoid dendritic cells, low CD56dimCD16+ natural killer cells and increased low density neutrophils. Additionally, a first look at the single cell RNA-Seq data suggests a recombination deficiency during B and T cell development, as was published for ATR-deficient SS patients (1), and allows to further elaborate the immune phenotype.
Conclusion:
We expanded the molecular and clinical spectrum of SS and further validations will provide insights into the link with the immune system and will contribute to the disease mechanism.
References:
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P65 - Genetic anomalies and diagnostic yield in an 11-year birth cohort of craniosynostosis patients

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Craniosynostosis is a rare congenital anomaly, defined by premature fusion of one or more cranial sutures. Craniosynostosis can occur in isolation or as part of a clinical syndrome, in which case a genetic cause is more likely to be identified. The genetic cause of craniosynostosis may impact the clinical course and treatment of patients with craniosynostosis. Knowledge on the genetic etiology therefore is key to ensure adequate counseling and to improve clinical management of craniosynostosis patients. In line with this, the Dutch craniosynostosis guideline recommends genetic diagnostic testing in patients with craniosynostosis. This study aims to assess both the prevalence of the different subtypes of craniosynostosis in an 11-year birth cohort of craniosynostosis patients as well as the diagnostic yield of genetic testing. We conducted a retrospective cohort study among patients who presented at the outpatient clinic of the Erasmus University Medical Center, The Netherlands. We included all patients, born between 2010-2021, with radiologically confirmed craniosynostosis. We included 993 patients (n= 334 female/659 male), of whom 856 presented with single-suture craniosynostosis (449 sagittal, 268 metopic, 116 unicoronal, 14 unilambdoid, 9 frontosphenoidal) and 136 patients presented with multisutural craniosynostosis, and one unknown (preliminary results). This study will discuss the prevalence of chromosomal and monogenic (likely) pathogenic variants and provide an update on the diagnostic yield of genetic testing in our 11-year birth cohort craniosynostosis patients. Finally, we will compare the diagnostic yield for different types of craniosynostosis with the long term aim of improving genetic testing strategies and counseling.

P66 - Using zebrafish to study the cardiovascular effects caused by fibrillin defects

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Defects in the fibrillin-1 gene (FBN1) can lead to the development of Marfan syndrome (MFS), with potentially severe cardiovascular manifestations. MFS patients are particularly susceptible to a progressive aortic dilation leading to potential dissection and wall rupture. Current treatment is aimed at minimizing the risk of severe complications through preventive pharmacological treatment and surgical aortic repair when indicated. Although these strategies have led to improved survival, some patients still present with fatal complications. Partly because the pathophysiological mechanisms remain incompletely understood, no causal treatment for the disease is available.
We aimed to generate a zebrafish model to study the mechanisms relating fibrillin defects to cardiovascular manifestations, and used CRISPR/Cas9 to disrupt the 3 fibrillin genes in zebrafish (fbn1, fbn2a, and fbn2b).
We found that zebrafish lacking fbn1 and/or fbn2a do not show any detectable phenotype during development. On the other hand, approximately 50% of homozygous fbn2b mutant (fbn2b-/-) zebrafish embryos show endocardial detachment, leading to vascular embolism and premature mortality at 7-9 dpf. Interestingly, the remaining fbn2b-/- zebrafish survive normally, but during larval stages develop a dilation of the bulbus arteriosus. This structure is strongly related to the aortic root in humans, which is the predominant location of aortic dilation in MFS.
In addition, the caudal vein of all fbn2b-/- embryos develops abnormally as a cavernous structure lacking vessel integrity. This phenotype resolves in embryos retaining normal blood flow. We found that pharmacological inhibition of blood flow led to a more severe caudal vein phenotype in fbn2b-/- embryos than in wild-type controls.
These data indicate that fbn2b-/- zebrafish can be a relevant model to explore the mechanisms leading from fibrillin deficiency to the cardiovascular symptoms observed in MFS. Our preliminary results suggest that there is an interplay between fibrillin deficiency and biomechanical signaling.

P67 - Is targeting the epigenome the key to reducing therapy resistance in lung tumours?

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A major problem in lung cancer treatment is resistance acquisition. Patients initially respond well to therapy but have a dismal 1-year survival rate. A key driver of resistance is intratumoral cell heterogeneity. Variability within cell populations is integral to biology, a certain plasticity allows cells to rapidly respond to external cues. In cancer however elevated transcriptional heterogeneity increases the likelihood for cells to survive the selective pressures of therapy. Nevertheless, drivers of cancer cell heterogeneity are poorly understood.
We believe that this heterogeneity is epigenetically regulated, and can control acquisition of drug resistance. Therefore, we are first studying how the epigenome controls transcriptional heterogeneity. Using CRISPR droplet (CROP) sequencing, we investigate how blocking epigenetic enzyme activity affects cell-intrinsic heterogeneity. CROP-sequencing is a state-of-the-art method combining single cell RNA sequencing with CRISPR screening. In a proof-of-concept screen targeting 20 epigenetic enzymes, we identified two interesting hits. DNMT1 loss, increased heterogeneity, while loss of the histone reader, ATAD2 resulted in a decrease in heterogeneity. Both have known cancer associations, for instance higher levels of ATAD2 are correlated with disease recurrence and poor survival in breast cancer. We have now expanded our screen to include all 267 enzymes known to catalyze epigenetic modifications and are working on including an additional 5 lung cancer cell lines.
Secondly, we are assessing how epigenetically induced heterogeneity affects resistance acquisition, and whether reducing heterogeneity can curb therapy resistance. To this end, we are performing a single-cell small molecule inhibitor screen targeting a number of epigenetic enzymes. By subsequently exposing cells to chemotherapies or therapies targeting EGFR or KRAS-driven cancers, we will assess if cell populations with an epigenetically altered heterogeneity show differences in resistance acquisition.
Ultimately, this project aims to improve patient outcome by understanding the mechanisms that drive intratumor heterogeneity and their influence on resistance acquisition.

P68 - Two siblings with congenital heart disease, postaxial polydactyly and sparse hair caused by bi-allelic variants in DPH1

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Germline bi-allelic loss-of-function variants in the gene for diphthamide biosynthesis protein 1 (DPH1) were linked in 2015 to an ultra-rare and severe cause of neurodevelopmental delay with short stature, dysmorphic facial features and sparse hair or Loucks-Innes syndrome. Additional features include limb and central nervous anomalies. To date, 18 patients have been described in literature with predominantly homozygous mutations. Here we describe a 19-year-old female who was the first child of non-consanguineous parents. She was born with intra-uterine growth retardation, hypotonia, failure to thrive, perimembranous ventricle septum defect, bilateral postaxial polydactyly, oxycephaly, nasal hypoplasia, microphthalmia, cutaneous hemangioma and absence of eyelashes. Imaging by MRI showed an ectopic neurohypophysis and atlantooccipital fusion. Her younger sister showed similar features, including ectopic neurohypophysis and a more severe cardiac phenotype, featuring ventricular septal defect, pulmonary stenosis and atrial septal defect secundum type. She died at 4 months due to postoperative complications. A younger brother is in good health. No (likely) pathogenic variants were found by conventional and molecular karyotyping, nor by clinical exome analysis in 2015. Subsequently, quad whole genome sequencing (WGS) analysis was performed including WGS analysis from the healthy brother. WGS showed compound heterozygosity for a predicted splice variant and missense variant in NM_001383 DPH1 (c.574-2A>G and c.G688C:p.A230P) in the index patient. These variants haven't been described in literature, are absent in reference databases, are predicted to be damaging by in silico prediction tools and are located in the diphthamide synthase domain. Parents and healthy sibling were heterozygous carriers for one DPH1 variant. The phenotype of the affected siblings corresponds well to that of DPH1 syndrome, although the association of ectopic neurohypophysis and DPH1 is unprecedented, warranting neuroimaging and endocrine follow-up in all patients diagnosed with DPH1 syndrome.

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P69 - Tumor spectrum in two large Belgian families with POT1-tumor predisposition syndrome

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Introduction:

POT1 gene, or Protection of Telomeres protein 1 gene, encodes a nuclear protein involved in telomere maintenance [PMID 15964812]. Disease-associated POT1 variants, causing POT1 tumor predisposition (POT1-TPD), were initially described in families with cutaneous melanoma, chronic lymphocytic leukemia, sarcoma (particularly cardiac angiosarcoma), and gliomas. Fewer than 100 families have been published so far [PMID 33119245].

We report two families with a history of multiple neoplasms due to POT1 germline mutation.

Observation:

Family 1: the pathogenic variant of POT1 gene c.233T>C (p.Ile78Thr) had been identified in patient who had his first tumor (liposarcoma of the leg) at the age of 73, a second sarcoma of the arm and a papillary thyroid cancer at the age of 74. His eldest daughter had a melanoma at the age of 30, and his youngest daughter presented low-grade appendiceal mucinous neoplasm.

Family 2: the pathogenic variant of POT1 gene c.349C>T p.(Arg117Cys) has been identified in a family with several cases of melanomas, soft tissue sarcomas, heart angiosarcoma, thyroid cancer, leukemia, lung cancer and glioblastoma. The youngest age of cancer diagnostic was 28.

Discussion:

The p.(Arg117Cys) variant has already been identified in 3 Li-Fraumeni-like families with multiple cancers including cardiosarcomas, melanomas, sarcomas, or lung cancers [PMID 26403419].

The p.(Ile78Thr) has been reported in an individual with multiple melanomas and in three families of Ashkenazi origin. This variant was also detected in an individual with two melanomas and thyroid cancer [PMID 30586141].

POT1-TPD is inherited in an autosomal dominant manner. The types of POT1-related tumors can vary among different members of the same family. The penetrance of cancers is unknown but based on those two families and cases reported in literature, it seems elevated with variable age of onset for the first neoplasia. Describing these two new families further increases the spectrum of cancers detected in POT1-TPD.

P70 - Local TGF-beta sequestration by fibrillin-1 regulates vascular wall homeostasis in the thoracic aorta

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Background: Aortic dissection and rupture is the main cause of early cardiovascular mortality in patients with Marfan syndrome (MFS). MFS is caused by a fibrillin-1 deficiency, which binds transforming growth factor beta (TGF-beta) via interaction with latent TGF-beta binding proteins (LTBPs). The role of TGF-beta in MFS has been controversial, with earlier studies suggesting that excess release of TGF-beta due to decreased interaction with dysfunctional fibrillin-1 leads to aortic dilation and vascular damage, while other studies have shown an important protective effect for TGF-beta. To further elucidate the role of TGF-beta, we studied the in vivo effects of disrupted sequestration of TGF-beta to fibrillin-1 in mouse models of MFS.
Methods: Mice lacking the fibrillin-1 binding site for LTBPs (Fbn1H1D/+), mice with a truncated fibrillin-1 (Fbn1GT-8/+), and mice with a combination of both alleles (Fbn1GT-8/H1D) were subjected to in vivo cardiac ultrasound analysis. Ex vivo phase-contrast synchrotron X-ray imaging of the entire excised thoracic aorta was performed at the Paul Scherrer Institute.
Results: While Fbn1GT-8/+ and Fbn1H1D/+ mice had a normal life span, Fbn1GT-8/H1D mice showed increased mortality due to aortic rupture starting at 4-5 months of age. The Sinuses of Valsalva was dilated both in Fbn1GT-8/+ and Fbn1GT-8/H1D mice at 6 months of age, but not in Fbn1H1D/+ mice. Significant elastic lamellae fragmentation was observed in the thoracic aortic wall of Fbn1GT-8/+ mice, and to a larger extent in Fbn1GT-8/H1D mice. Surprisingly, localized elastin fragmentation was also found in the ascending thoracic aorta of Fbn1H1D/+ mice despite a lack of aneurysm development.
Conclusions: Our data indicate that loss of LTBP binding to fibrillin-1 leads to the development of localized microdissections in the absence of aortic aneurysm, and exacerbates the aortic wall morphology in mice with truncated fibrillin-1. We therefore hypothesize that local TGF-beta sequestration is required to maintain aortic homeostasis.

P71 - A structural variant of the C-terminal prion-like domain of TDP-43 causes vacuolar muscle degeneration

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Neuronal TDP-43-positive inclusions are a hallmark lesion found in frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), present in 45% and 97% of cases, respectively. Missense mutations in the C-terminal prion-like domain (PrLD) of TDP-43 are the main type of disease-causing mutations reported for ALS. This domain mediates self-interaction, and mutations of this region are thought to lead to aggregate formation. However, the scope of TDP-43 proteinopathies goes beyond neural tissues, with reports showing TDP-43 pathology in vacuolar myopathies. Nevertheless, genetic evidence for a primary role for TDP-43 in myopathies is still amiss. Here we identified a multigenerational family with an autosomal dominant rimmed vacuole myopathy. Whole exome sequencing and genome-wide linkage analysis mapped the disease to an 11bp deletion in TARDBP (LOD-score of 3.6), causing a frameshift mutation in the C-terminal domain (CTD) of TDP-43 (TDP-43p.Trp385IlefsTer10). This constitutes a novel type among described TDP-43 mutations, which are predominantly missense mutations. Patient-derived muscle biopsies revealed the presence of p62/TDP-43-positive sarcoplasmic inclusions and nuclear depletion of TDP-43. Additionally, we verified higher numbers of autophagosomes and a transcriptomic signature indicative of reduced mitochondrial and lipid metabolism, alongside a switch in sarcomeric protein isoforms suggesting increased muscle regeneration. Together with these observations, functional assays in D. melanogaster showed that TDP-43p.Trp385IlefsTer10 retains normal function but has reduced toxic gain-of-function properties. By studying this unique variant of TDP-43 it is our goal to clarify the importance of the CTD of TDP-43 and how its remodelling can affect the formation of aggregates. Furthermore, these results genetically link TDP-43 to vacuolar muscle degeneration for the first time. This not only highlights the importance of the PrLD in pathological conditions in a tissue-specific manner, but it also expands the implications of TDP-43 proteinopathies, from a nearly neuronal-exclusive context into a broader spectrum encompassing myopathies as well.

P72 - Barriers and Facilitators for implementation of patient-centered care in Cardiogenetics: a Delphi study

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Background Inherited cardiovascular disease is rare and requires counselling and follow-up at the genetic and the cardiology department, and patients may need psychological and social guidance as well. Complex phenomena like genomics and cardiovascular health require a multidisciplinary and multifaceted approach. In current clinical practice, a biomedical focus is dominant and expertise from psychological sciences and patients is often lacking. The current study aims to explore key barriers and facilitators of implementing patient-centered care in cardiogenetics.
Methods We performed a three-round modified Delphi study, using the input of a virtual panel of experts, comprising of 25 medical professionals, 9 psychosocial professionals and 6 patient representatives working in cardiogenetics In the brainstorming phase, round 1 of the study: workshop breakout sessions were transcribed at verbatim, coded and processed into unique statements listed as barriers and facilitators. In round two, the expert-panel received via mail long-lists and were asked to validate, add or revise the list of barriers and facilitators. In round 3 the most relevant barriers and facilitators were ranked on importance.
Results Overall, the experts identified 6 barriers, dispersed across various levels of implementation. Having a blind spot for the patient perspective was ranked of the highest importance, while the lack of communication between the various involved departments was ranked the lowest. We selected 9 facilitators, 2 were workflow related, 5 advocated various aspects of increased multidisciplinarity, and two concerned suggested improvements in patient communication.
Conclusions This study revealed important differences between the perspectives of the various stakeholders. Medical professionals often have a biomedical orientation, which patients and psychosocial professionals found to be a barrier for patient-centered care. To improve clinical care in cardiogenetics and move the field towards patient-centered care we should focus on patients' health goals and guide therapeutic decisions by integrating the different perspectives in treatment modules.

P73 - FOXL2 mutation update for BPES: in silico assessment and ACMG classification of 234 unique sequence variants, and review of structural variants

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Background/Objectives:
BPES (OMIM 10100) is an ultra-rare autosomal dominant developmental disorder, characterized by an eyelid malformation associated (type I) or not (type II) with primary ovarian insufficiency (POI). It is a genetically homogeneous condition with only one disease locus, implicating the single-exon gene FOXL2 (OMIM 605597). In 88% of typical BPES cases, a genetic defect of the FOXL2 region is identified, varying from coding sequence and copy number variants (CNVs) to reciprocal translocations and non-coding CNVs disrupting cis-regulatory elements. Here, the aim was to collect all reported and in-house FOXL2 variants, classify them and submit them to LOVD and ClinVar databases.

Methods:
Variant collection was performed through a literature search on FOXL2 and BPES between 2001-2021. This was completed with in-house variants, identified via clinical genetic testing and downstream research testing in the Center for Medical Genetics Ghent (CMGG). All sequence variants were subsequently classified according to the ACMG standards.

Results:
In total, 711 genetic defects of the FOXL2 region were found. Of these, 88% are intragenic FOXL2 variants, 234 are unique and 44 are novel. The polyalanine tract is a known mutational hotspot of FOXL2, illustrated by the high percentage of pathogenic polyalanine expansions (27%). Reclassification of disease-causing sequence variants revealed 75% class 5, 24% class 4 and 1% class 3 variants. Furthermore, the mutation spectrum is characterized by 9% coding CNVs and 2% non-coding CNVs, all but one located upstream of FOXL2. The remaining 1% are translocations along with chromosomal rearrangements of 3q23.

Conclusion:
This study led to a comprehensive overview of the entire mutational landscape of the FOXL2 region in BPES, curated following the most recent standards. A complete database of previously published and novel FOXL2 variants, including ACMG classification, will facilitate the interpretation of FOXL2 variants identified in BPES patients which is of clinical use.

P74 - Development of a non-mammalian model of ectodermal dysplasia 9 of the hair/nail type

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Introduction: Mutations in HOXC13 cause ectodermal dysplasia 9 of the hair/nail type (ECTD9). The disease is characterized by postnatal hypotrichosis (sparse hair growth) and nail dystrophy. Hoxc13 knockout pigs, mice and rabbits partially recapitulate the human symptoms. We here aimed to test whether ECTD9 can be modeled in the Western clawed frog (Xenopus tropicalis), one of few amphibians that develop claws.
Materials & Methods: By use of CRISPR/Cas9 and subsequent breeding, a biallelic knockout of hoxc13 was established in Xenopus tropicalis. Careful selection and design of a sgRNA was performed using CRISPRscan and Indelphi in order to obtain highly efficient genome editing, favouring the generation of frameshifting mutations. Mosaic F0 animals were intercrossed to obtain compound heterozygous (hoxc13-/-) F1 offspring.
Results: Mosaic F0 offspring was generated by microinjection of CRISPR reagents into two-cell stage Xenopus tropicalis embryos. Up to 70% genome editing efficiency was obtained. The presence of hoxc13 frameshift indels led to the absence of one or multiple claws on one or both hind limbs in mosaic F0 animals. Compound heterozygous animals harbouring biallelic frameshift mutations completely lacked claws, whereas compound heterozygous animals harbouring monoallelic in-frame mutations in combination with frameshift mutations or biallelic in-frame mutations alone all had normal claws.
Conclusion: Our study reveals a critical role of Hoxc13 in non-mammalian skin appendages and expands the repertoire of animal models for ECTD. The Xenopus model will allow to further elucidate the evolutionarily conserved genetic regulation of nail and skin development.

P75 - Benefits of whole exome sequencing to advance the genetic diagnosis in patients with differences (disorders) of sex development

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Background/Objectives:
Differences of sex development (DSD) are heterogeneous conditions affecting the development of chromosomal, gonadal or anatomical sex. Although over 75 genes have been associated with DSD, the diagnostic yield of whole exome sequencing (WES) studies is typically not higher than 35% in a clinical setting. Here, we investigated the benefits of WES for the genetic diagnosis in patients with DSD.
Methods:
Between 2016 and 2022, 144 unrelated index patients with a clinical diagnosis of DSD or the broader DSD umbrella underwent WES-based panel testing interrogating the coding regions of 130 genes implicated in DSD, primary ovarian insufficiency and hypogonadotropic hypogonadism. Variants were extracted and classified according to the ACMG guidelines. Copy number variant (CNV) analysis was performed using the ExomeDepth algorithm.
Results:
In 13% of patients, we identified a likely pathogenic (LP) or pathogenic (P) rare variant in 12 distinct DSD genes, including AR (6), NR5A1 (2), WT1 (2), ATRX, CYP21A2, DHX37, HSD3B2, HSD17B3, RXFP2, SRD5A2, SRY, and TXNRD2. The majority are sequence variants; four defects are CNVs identified using ExomeDepth. Interestingly, in two brothers displaying bilateral cryptorchidism and infertility an intragenic RXFP2 deletion was found to occur in trans with a heterozygous missense variant, corroborating its role in familial bilateral cryptorchidism.
Conclusion:
We demonstrate the benefit of WES-based genetic testing of DSD in a clinical context. The low detection rate emphasizes the need for more stringent inclusion criteria on the one hand and for advanced genome analysis to solve missing heritability in this condition.

P76 - Referral by the ophthalmologist leads to the diagnosis of a severe neurological disorder

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We report on a 7-year old boy, third child of a double consanguineous couple, with signs of visual impairment since the age of 4, with an initial diagnosis of hypermetropic astigmatism. As his vision worsened over time, he was diagnosed with cone-rod dystrophy. The boy had epileptic seizures since the age of five years. At that time, speech impairment was noted by the neurologist. The following years, his developmental delay became more pronounced, necessitating special-needs education. His parents recently observed a decline in his capabilities. Brain MRI showed cerebellar atrophy.

Exome-based targeted testing of the RetNet Panel, interrogating 290 genes implicated in inherited retinal diseases, was performed in the proband. Seggregation testing in the parents was performed by Sanger sequencing. Variant classification was performed using ACMG criteria.

Gene panel testing showed a novel, pathogenic homozygous variant in the MFSD8 gene: GRCh38 (hg38): NM_152778.2: c.77_79delinsAA; p.(Leu26Ter). Segregation analysis confirmed that both parents were heterozygous carriers. The variant found in MFSD8 in this patient is a frame shift variant, resulting in a premature stop codon. The variant is located in a region where nonsense mediated decay can be expected. The variant is not previously described in literature. However, another homozygous variant leading to the same amino acid change was reported in two siblings with a neurodegenerative disorder.

Biallelic loss-of-function variants in MFSD8 cause autosomal recessive neuronal ceroid lipofuscinosis type 7, characterized by neurodegeneration, ophthalmological symptoms, seizures and cerebellar atrophy. The onset of symptoms is usually in childhood and the disorder is rapidly progressive. The ophthalmological symptoms may appear first, as shown in this child.

The ophthalmological referral of this boy lead to the diagnosis of neuronal ceroid lipofuscinosis type 7. The case illustrates that relatively benign and frequently occurring clinical symptoms may sometimes be the first sign of severe and rare syndromes.

P77 - Multivariate genetic mapping of 3D cranial vault shape in multi-ethnic and admixed children

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Evidence from human dysmorphology and knock-out animal models has led to the identification of causal genetic variants in inherited human disorders that are associated with abnormal craniofacial characteristics. However, these studies have focused on abnormal craniofacial phenotypes, and hence, little is known about the genetics underlying normal craniofacial variation. In addition, the lack of ethnic diversity in genetic studies, especially with rapidly increasing global admixture, raises concerns that require addressing. Here, we performed a multivariate genome-wide association study of 3D cranial vault shape extracted from magnetic resonance images in 4198 European and 2570 non-European, mostly admixed children in the Adolescent Brain Cognitive Development cohort. By adjusting 3D cranial vault shape for both global and local genetic ancestry components, individuals of up to a 3-way admixture (having European, African, and Native American ancestry) could be included in a single genome-wide scan with boosted sample size and power while keeping the type I error under control. This yielded 30 genetic loci, including transcription factors and growth factors known to play a role in the skeletal and craniofacial development through WNT, FGF, and TGF-β signaling. Most of these loci are also implicated in disorders that result in abnormal craniofacial development. Specifically, we found associations with BMP2 and BBS9, which have been associated with craniosynostosis in a previous genome-wide association study. These results suggest that common variation near genes implicated in abnormal craniofacial development contribute to normal variation in cranial shape and that of the craniofacial complex in general.

P78 - Efficient exon 53 skipping of the human dystrophin transcript in a mouse model for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the DMD gene encoding for dystrophin. The absence of dystrophin results in continuous contraction-induced damage in skeletal muscle. One way of restoring dystrophin expression is by using antisense oligonucleotides (AONs) to reframe the disrupted open reading frame of the transcript. AONs bind to the target exon in the pre-mRNA dystrophin transcript, thereby hiding it from the splicing machinery, which results in skipping of the target exon. This leads to production of a shorter but semi-functional dystrophin protein. Although some AONs are already conditionally approved for DMD, this is based on restoration of very low levels of dystrophin, because delivery of AONs to muscle and efficiency of exon skipping is still a challenge.
In an effort to optimize AON efficiency, we assessed exon 53 skipping in the DMD gene with AONs of the following chemical modifications, all with a phosphorothioate (PS) backbone: FANA, FRNA, LNA-2'OMe, LNA-FRNA and αLNA-FRNA. First, we determined exon 53 skipping in immortalized human control myoblast cultures. Here we found efficient exon 53 skipping was induced whether the AONs were delivered via transfection or gymnosis. The FRNA and LNA-FRNA modifications were most efficient. Pronounced exon 53 skipping levels were also observed in the skeletal muscles and heart of hDMDdel52/mdx mice receiving weekly subcutaneous injections of 50 mg/kg for six weeks starting at 4 weeks of age. In the gastrocnemius of these mice, the LNA-2'OMe and LNA-FRNA modified AONs were most efficient (70.9 ±10.7% and 86.5 ± 7.3% skip, respectively) with comparable levels in triceps, diaphragm and heart. All AONs were well tolerated based on plasma markers for liver and kidney function. We are currently evaluating dystrophin protein levels in skeletal muscles and heart.

P79 - Mutational processes in childhood acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is marked by low mutational burden. Nevertheless, a subset of children with ALL has a substantially elevated number of mutations, a phenomenon known as hypermutation. We previously found that hypermutation occurs more frequently in ALL relapses, suggesting an effect of therapy, but a small subset of cases is already hypermutated from initial diagnosis. To study the causes and consequences of hypermutation in childhood ALL, we performed whole exome/whole genome sequencing (WES/WGS) on normal and leukemic DNA. We included 26 patients with multiple relapses and 48 patients with single relapse, identifying a mutational process in 11 (42%) and 10 (21%) patients, respectively. We discerned two mutational signatures exclusively present in relapse, one of which is known to result from thiopurine treatment. Remarkably, in six relapsed ALL patients, we observed a mutational signature strongly resembling a pattern of UV-induced mutations typically found in melanomas. This pattern was always detected from the earliest stages of the disease, but the etiology and consequences for outcome are still unknown. Finally, we observed aberrant APOBEC mutagenesis, an antiviral defence system targeting single-stranded DNA, in patients at diagnosis and (continuing in) relapse. To reveal the frequency of APOBEC-associated hypermutation at diagnosis, we subsequently filtered an RNA sequencing dataset of 214 ALL samples at diagnosis for potential somatic mutations that resembled the APOBEC-associated signatures. WGS-based validation eventually confirmed APOBEC mutagenesis in six patients, of which three were ETV6-RUNX1-fusion positive, a good-prognosis ALL subtype. In conclusion, we have shown that multiple distinct mutational mechanisms contribute to hypermutation in ALL. Two of these mechanisms occur already at diagnosis, suggesting a role in the earliest stages of ALL development. Whether these mechanisms can cause ALL, and what the consequences are for therapy and outcome requires further studies.

P80 - Connection between glucose metabolism and gene expression of critical neuromuscular components in Pompe's disease: new hints explainig the hypotonic phenotye?

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Pompe's disease is caused by the presence of bi-allelic pathogenic alterations in the GAA gene, resulting in the inability of patients to degrade glycogen. This translates to a hypotonic phenotype in combination with hypertrophic cardiomyopathy in severe cases. We performed genome wide mRNAseq on fibroblasts of Pompe patients and observed a marked increase of the transcription factor PPARgamma, together with an mRNA decrease of the neuromuscular junction component COL13A1 and the neuronally expressed KCNMA1 potassium ion channel. We have treated patients fibroblasts with the PPARgamma inhibitor G3335 and could observe a significant upregulation of both COL13A1 and KCNMA1 mRNA, indicating that PPARgamma functionality indeed impacts on expression of both genes. However, we could only show this effect in a subset of patients. As PPARgamma is a known important regulator of the energy metabolism and glucose is the buidling block of glycogen, we speculated that altering the glucose content of the culture medium could potentially impact on the expression of COL13A1 and KCNMA1 as well. Growing Pompe fibroblasts in glucose free medium indeed resulted in a significant mRNA upregulation of COL13A1 and KCNMA1 mRNA respectively. This is a important new finding which directly links the cellular glucose metabolism or uptake to the expression of two important mediators of neuromuscular junction and neuronal functionality. Furthermore, this finding could imply that a treatment regime of Pompe patients with a glucose restricted diet could be beneficial.

P81 - Identification of a causal mutation in DLG3 in the MRX20 family

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Here, we describe one of the 105 historical families that were assigned MRX numbers. MRX20 is one of the larger pedigrees which presents with non-syndromic intellectual disability. In 1995 Lazzarini et al. (AJMG 57: 552-7) could map the causal region to the centromeric region on the X-chromosome. In this study we identified the causal gene and investigated its possible effects with contemporary techniques.

Using WES, a causal c.194del mutation in the DLG3 gene, an established ID gene, was identified leading to a frameshift mutation. Aside, we found two variants of unknown significance that segregated in the family as well; in SSX1 (c.358G>T) and USP27x (c.56A>G). Differential expression analysis led to the identification of 14 differentially expressed genes with an adjusted p-value lower than 0.1. Pathway analysis was performed using the ingenuity pathway analysis (IPA) tool, which resulted in the identification of four related networks, which are at the moment further looked into at our lab. Apart from this, it revealed a link with intellectual developmental disorder with persistence of fetal hemoglobine and a connection with several hepatotoxicity phenotypes. In the future, an additional clinical examination will be executed in this family to investigate the relevance of these findings. So far, we were able to confirm seven differentially expressed genes using real-time PCR (with a p-value < 0.01); WWTR1, LDHA, CDCA4, PPP1R16B were found to be upregulated and BCL11A, NMT2 and PEX26 were found to be downregulated. All statistics were performed using a two-tailed Mann Withney-U test.

In short, we identified a causal mutation in DLG3 in the MRX20 family apart from two additional variants of unknown significance. Fourteen genes were significantly differentially expressed, of which 7 are confirmed at this moment. Further research is ongoing.

P82 - IPSC reprogramming to create SEMD (biglycan type) iPSC-derived chondrocyte models

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Loss-of-function pathogenic variants in the X-linked biglycan gene (BGN), encoding the extracellular matrix protein biglycan, have been linked to an aortopathy syndrome called Meester-Loeys Syndrome, while specific missense variants cause spondyloepimetaphyseal dysplasia (SEMD, biglycan type). Little is known about the pathomechanisms underlying SEMD, biglycan type. To improve the current understanding, it is key to develop a representative human disease model. Induced pluripotent stem cell (iPSC)-derived chondrocyte disease models have been shown to exhibit several key aspects of known disease mechanisms of skeletal dysplasia and are therefore considered highly suitable. Prior to creating iPSC-derived chondrocyte disease models, iPSCs need to be generated. To do so, dermal fibroblasts of two male patients with SEMD, biglycan type (both carrying p.Gly259Val), were reprogrammed into iPSCs using the CytoTuneTM-iPS 2.0 Sendai Kit (Invitrogen). This kit contains three reprogramming vectors delivering and expressing the key genetic factors (i.e. OCT3/4, SOX2, KLF4 and c-MYC) necessary for iPSC generation from somatic cells. From these patient iPSCs, isogenic controls were created using CRISPR/Cas9-based correction (outsourced to Synthego) of the p.Gly259Val BGN variant. Pluripotency of the created iPSCs was confirmed using immunocytochemistry (ICC) for OCT4, SOX2, NANOG, TRA-1-60 and TRA-1-81 and by demonstrating their ability to differentiate into the three germ layers, i.e. ectoderm, mesoderm and endoderm, using real-time quantitative polymerase chain reaction for appropriate markers of the respective germ layers. Genomic integrity and identity of the iPSC lines were verified using the HumanCytoSNP-12 BeadChip (Illumina) and, importantly, all the created iPSC clones (n=6) were shown to be free of Sendai virus. In conclusion, we have successfully established two patient iPSC lines as a first step in the creation of iPSC-chondrocyte models to study the mechanisms underlying SEMD, biglycan type.

P83 - Genome-wide and pathway-specific polygenic risk scores in an Alzheimer's disease case-control cohort

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Genome-wide association studies identified over 100 loci significantly associated with AD, with APOE the strongest. Polygenic risk scores (PRS) combining the effect of AD-associated variants offer great possibilities for risk prediction. However, there is no consensus on the optimal p-value threshold (pT) for SNP inclusion, the role of APOE in these scores, nor whether pathway-specific scores (pPRS) would be better than genome-wide scores.

Using PRSice and the summary statistics from Kunkle et al.(1), we calculated PRS for different pTs (PRS-AD) in a well-characterized AD case-control cohort (N-case=179, N-control=98). We also built PRS excluding the APOE region (PRS-noAPOE), including only the APOE region (PRS-APOE) or the two main APOE variants (rs429358-rs7412, APOEε2+ε4), and the weighted sum of PRS-noAPOE + APOEε2+ε4. pPRS were built for pathways defined by Kunkle et al.(1) and Tesi et al.(2)

We found the best model fit with APOEε2+ ε4 with an R^2 of 37.5% for discriminating cases from controls. For PRS-AD, relaxing the pT results in a huge drop of R2: from 29.3% at pT=5E-8 (N-SNP=65, p=1.82E-11) to 1.4% at pT=0.05 (N-SNP=45,125, p=9.24E-02). A similar trend is observed for the other scores, except for the PRS-APOE score where also the number of SNPs is relatively stable when relaxing pT (N-SNP=35 at pT=5E-08, N-SNP=67 at pT=0.05). Removing APOE from PRS-AD resulted in a drop in R^2 from 29.3% to 5.0% (pT=5E-8). All pPRS except for the angiogenesis pathway are significantly associated with AD, mainly because of APOE being included.

Our analyses show that AD PRS are largely driven by APOE, and especially the two main APOE variants. Our results further are in line with recent research suggesting that AD has an oligogenic architecture rather than a polygenic. The exact role of AD-associated variants beyond APOE, but also of other variants within the APOE region, needs to be further explored.

1. Kunkle et al [Nat Genet. 2019;51(3):414–30]
2. Tesi et al [Transl Psychiatry 10, 332 (2020)]

P84 - Confirmation of a role for pathogenic THSD4 variants in the etiology of thoracic aortic aneurysm and dissection

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Background
Loss-of-function variants in THSD4, encoding ADAMTSL6, which is a member of the A disintegrin and metalloproteinase with thrombospondin motifs super family, were recently reported to cause familial thoracic aortic aneurysm and dissection in five probands.

Methods
Using whole exome and genome sequencing, we identified three additional probands carrying likely pathogenic THSD4 missense variants.

Results
The first male proband presented at age 32 and 49 years with a type A dissection and arteria lienalis aneurysm surgery, respectively. Familial segregation demonstrated that the c.1448C>T; p.(Thr483Ile) variant was present in his father with a borderline aortic root aneurysm (40 mm) and absent in an unaffected child. In the second family, the c.2575G>A; p.(Gly859Arg) variant was found in a male who underwent a David procedure at age 53 years because of an aortic sinus diameter of 52 mm as well as in his 23-year-old daughter with borderline aortic sinus measurements. Both presented with Marfanoid features including mitral valve prolapse, tall stature, scoliosis, arachnodactyly and pes plani. Finally, in a male proband who underwent aortic replacement surgery at age 52 years because of an aortic sinus of 48 mm, the c.2948A>G p.(Asn983Ser) variant was detected. His family history is significant for aortic aneurysm in his brother and early death in their father. The first variant is located in the ADAM-TS spacer domain whereas the two other variants are in the functionally important TSP1 and PLAC domains, respectively. All affect highly conserved amino acids with CADD scores ranging between 24 and 31 and are rare in gnomAD (each occurring only twice).

Conclusion
Our data confirm that likely pathogenic THSD4 variants cause a spectrum of thoracic aortic and arterial aneurysmal disease (including arterial lienalis aneurysm), combined with connective tissue disease findings.

P85 - DeltaMSI: AI-based modeling of microsatellite instability scoring by next-generation sequencing

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Microsatellite instability (MSI) arises through loss-of-function of DNA Mismatch Repair (MMR) enzymes. MSI occurs in 15% of colorectal and endometrial cancers and in 3%-7% of tumors in other organs. Its detection is important since MSI cancers respond well to immune therapy. MSI results in shortening or extension of microsatellite markers and thus shifts in the indel distribution of aligned sequencing reads amenable for fast and automated analysis by bioinformatic scripts on routinely captures NGS data. A widely used script, mSINGS, simply counts the number of indel distribution peaks in 10 informative marker loci and compares these to a baseline microsatellite stable reference set for binary scoring of loci as stable/unstable. We previously found that mSINGS correlates well with other methods such PCR-fragment analysis or immunohistochemistry for MMR proteins, but fails to detect minimal MSI shifts.
Here we present an improved script, DeltaMSI, that includes extra parameters for indel distribution scoring. To process the added data complexity, we used AI: on a training set with curated clinical truth we applied machine learning. The model with the highest AUC was validated on an independent cohort of 1000 consecutive tumor samples in comparison to reference techniques.
The integration of DeltaMSI into routine NGS pipelines is expected to reveal more therapeutically actionable MSI tumors and increase our understanding of its prognostic and predictive relevance.

P86 - The prevalence and clinical consequences of mosaic genome wide paternal uniparental disomy (MGWpatUPD) in Beckwith Wiedemann Syndrome patients

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Background:
Mosaic paternal uniparental disomy of a part of chromosome 11 (patUPD11) is detected in approximately 20% of patients with Beckwith-Wiedemann syndrome (BWS). Patients with pUPD11 are known to have an increased risk of specific pediatric tumors as part of the clinical spectrum of BWS. More recently a number of cases have been described in which the disomy was extended to the entire genome: mosaic genome wide paternal uniparental disomy (MGWpUPD). Although most cases described were of young age at the time of publication, some cases indicate that MGWpatUPD might be at increased risk of developing additional tumors later in life and suggested life-long vigilance for tumor development. However, this is based on a limited number of patients reported to date and patients with benign tumors have also been included in the analyses. The prevalence of MGWpatUPD and its clinical relevance remains unknown.

Aims:
To determine the prevalence of MGWpatUPD in patients with patUPD11.
To determine the clinical consequences of MGWpatUPD.
To suggest recommendations for tumour screening for patients with MGWpatUPD.
To decide whether testing for MGWpatUPD should be implemented in routine diagnostics of patients with patUPD11.

Methods:
This study is approved by our local medical ethical commission (MEC). A Multi Locus Imprinting Disorders analysis (MLID MS-MLPA ME-034) is performed to detect MGWpatUPD in patUPD11 patients. Additionally, all patUPD11 patients will be briefly interviewed about their medical history in general, and specifically occurrence of tumors is asked.

Results:
Approximately 90-100 patients with patUPD11 will be approached by their own counselor to be asked to participate in our study.

Conclusion:
Our study provides an overview of the prevalence of MGWpatUPD in patients with patUPD11 and describes a comparison of clinical features between patUPD11 and MGWpatUPD patients. If necessary, we will suggest recommendations for future diagnostics of patients with of MGWpatUPD patients.

P87 – WAITING FOR APPROVAL TO PUBLISH

P88 - Two years' experience of BeGECS at the Center for Medical Genetics Ghent: results from 200 couples.

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Since the end of 2019, the Center for Medical Genetics Ghent has introduced preconception expanded carrier screening (ECS). The ECS test, called BeGECS, includes over 1200 autosomal recessive and X-linked genes and informs couples planning a pregnancy on a possible increased couple risk for an affected child, as well their individual carrier status for seven autosomal recessive conditions with a carrier frequency of 1/50 or higher (cystic fibrosis (CFTR), spinal muscular atrophy (SMN1), non-syndromic sensorineural hearing loss (GJB2-GJB6), Smith-Lemli-Opitz syndrome (DHCR7), sickle cell anemia and beta hemoglobinopathies (HBB), Medium-Chain Acyl-CoA Dehydrogenase deficiency (ACADM), phenylketonuria (PAH)).

Over the last two years we have performed BeGECS in 200 couples with different indications for consultation: the initiation of a preimplantation genetic testing (PGT) procedure, preconceptional advice, the specific request for BeGECS , consanguinity and the initiation of a known donor trajectory. In short, DNA was enriched with a custom SureSelectXT Low Input kit (Agilent Technologies) and sequenced on a NovaSeq 6000 (Illumina). Data analysis was executed with a bcbio-based pipeline and variants were filtered with our in-house developed Seqplorer platform. Complementary to this NGS-based analysis, assays were performed for FMR1, SMN1, DMD and GJB6.

The observed individual carrier rates for the seven most-frequent conditions are in line with the known carrier rates except for CFTR (1/15), GJB2 (1/22) and DHCR7 (1/30) which are higher than expected. Regarding the couple risk for an affected child, BeGECS revealed an increased couple risk for an additional disorder for six of the PGT couples, while twelve couples were found to have a previously unknown increased couple risk (autosomal recessive disorders: ABCC6, MUTYH, TYR, CFTR (2x), CYP21A2 (2x), FV (3x), SLC12A3, STX11 and PRF1; X-linked disorders: G6PD (3x), FRM1 and GLA). This result now enables these couples to make an informed and autonomous decision about their reproductive options.

P89 - Two Candidate Genes for a Spectrum of Neurological Diseases

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Many inherited neurological disorders have overlapping clinical and genetic features that complicate studying pathomechanisms. Next-generation sequencing technologies may facilitate genetic diagnosis in these patients, as well as help identify novel candidate genes.
We analyzed genetic causes of pathogenicity in a cohort of 56 consanguineous patients primarily presenting with Charcot-Marie-Tooth disease using whole-exome sequencing. We identified two candidate causative genes and performed in vitro analyses to study disease mechanisms. Patient-I presented with a CMT-like phenotype with unusual features including visual impairment, optic nerve atrophy, and dysarthria. A homozygous FXN missense mutation was identified in three affected individuals in the family which did not alter FXN mRNA or protein levels in patient fibroblasts. Patient-II presented with truncal and gait ataxia, dysdiadochokinesia, intention tremor, dysmetria, and axonal neuropathy. This patient carried a homozygous frameshift mutation in the SEPT11 gene which causes decreased mRNA expression and lack of Sept11 protein in patient fibroblasts.
The patients analyzed in this study were initially diagnosed with CMT; however, the genetic findings are suggestive of a spectrum of neurological disorders highlighting the clinical and genetic heterogeneity of these diseases. We conclude that analyzing WES data based on initial clinical diagnosis might not always be sufficient, especially in neurological disorders with overlapping features. In addition, mutations in FXN and SEPT11 should also be screened for the genetic diagnosis of patients with relevant clinical features.

P90 - Switches in SOX gene expression during cancer development in the uterine cervix.

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Cervical cancer is the fourth most frequent cancer in women, with human papillomavirus (HPV) infection being the major cause for cervical cancer development.
SOX genes, involved in sex determination and the development of different organs, are candidate regulators of stem cells during embryogenesis and adult stem cell maintenance. Most recent studies of SOX genes focused on their involvement in cancer.

We studied the mutually exclusive SOX2 and SOX17 expression patterns in normal squamous (SOX2+) and glandular (SOX17+) epithelium (including SOX17+ reserve cells) and their switches in premalignant lesions, including cervical intraepithelial neoplasia (CIN) and adenocarcinoma in situ (AIS), by using immunohistochemistry, in situ hybridization and SOX17 promoter methylation studies.

The strong immunohistochemical association between SOX17 positive reserve cells and the switch from SOX17 to SOX2 expression in areas of squamous metaplasia, suggests that reserve cells, next to basal cells in the squamous epithelium, are potential targets for the formation of squamous lesions upon viral infection.
SOX2 expression is increased with progression of CIN lesions, with a specific histo-morphological distribution of SOX2 being observed in CIN3, and SOX2 copy number and viral load of HPV having an impact on SOX2 expression levels and distribution patterns during progression. These data support the view that CIN3 originates from infected immature metaplasia as compared to CIN1/2 where basal cells are infected.
For AIS we show a direct correlation between the topographical distribution of SOX17 expression and the methylation status of its gene promoter. This explains the heterogeneity of SOX17 expression in these glandular lesions

P91 - Homologous Recombination Deficiency in Ovarian Cancer: can the academically developed Leuven HRD test compete with Myriad myChoice PLUS in predicting response to PARP inhibitors?

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We describe the predictive value of the Leuven HRD test compared with Myriad myChoice PLUS (Myriad test) on 468 ovarian cancer samples from the PAOLA-1/ENGOT-ov25 trial. This phase III trial evaluated first-line standard therapy including bevacizumab in advanced ovarian cancer with the addition of maintenance olaparib or placebo. Significant improved progression-free survival (PFS) was observed in homologous recombination deficient (HRD) tumors, with or without BRCA mutation (BRCAm), tested with Myriad test in contradiction to HRD negative tumors, revealing the need for HRD testing in first line. Here we present the results of an alternative academic laboratory-developed HRD test compared with the Myriad test. The BRCAm status, genomic instability score (GIS) and HRD status were compared between both tests. The main objective was to compare the predictive value of both tests for predicting PFS in the olaparib versus placebo arm. All samples were tested by the Myriad test first within the scope of the PAOLA-1 trial; remaining extracted DNA was used for the Leuven HRD testing. Prevalence of pathogenic BRCA variants was 147 (31%) with the Leuven HRR gene panel versus 151 (32%) with Myriad test. Tumors with a pathogenic BRCAm and/or GIS≥56 were considered Leuven HRD positive (Myriad cutoff GIS≥42). With the Leuven and Myriad test, patients were considered HRD positive in 54% (n=254) and 52% (n=242), respectively. In Leuven HRD status positive tumors, the median PFS was 44.8 months in the olaparib group and 20.7 months in the placebo group (HR 0.386; 95% CI 0.271–0.548). The HR for Myriad test in this group was 0.373. 35% (n=164) of the samples were HRD negative with the Leuven test compared to 39% (n=182) with Myriad test. A robust correlation was observed, the Leuven HRD test showed a similar impact on PFS as the Myriad test in the PAOLA-1 samples.

P92 - Homozygous NDUFS6 splice mutation highlights the importance of peripheral neuropathy in the clinical spectrum of primary mitochondrial disorders

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Introduction: NDUFS6 is a nuclear-encoded mitochondrial gene mitochondrial that encodes an accessory subunit of Complex I (NADH-ubiquinone oxidoreductase). It has been associated with Leigh syndrome, an early-onset neurodegenerative mitochondrial disease. Until now, biallelic mutations in NDUFS6 have been reported in severely affected individuals that do not live beyond the first year of life. Here we report two patients with axonal Charcot-Marie-Tooth (CMT) neuropathy harboring a homozygous splice variant in NDUFS6.

Methods: Whole exome sequencing data coupled with homozygosity mapping was performed in two siblings from a consanguineous family and a diagnosis of axonal CMT. After the exclusion of mutations in known CMT genes, we conducted variant filtering and prioritization within shared regions of homozygosity. The resulting candidate variants were segregated in available family members. The functional effect of the final candidate variant was characterized in patient-derived EBV-transformed lymphoblasts at the RNA and protein level.

Results: We have found a homozygous splice-site variant c.309+5G>A in NDUFS6 in two adult siblings with early-onset CMT. The splice variant leads to loss of the canonical NDUFS6 transcript and expression of an alternative transcript that lacks exon 3. Immunoblotting showed reduced levels of NDUFS6 protein and the presence of an unidentified larger isoform. We hypothesize that this larger isoform corresponds to a precursor protein that is not properly cleaved upon entry to the mitochondria. Further functional experiments to establish the disease relevance of the larger isoform and screening of additional unrelated CMT patients with mutations in NDUFS6 are currently ongoing.

Conclusions: This work reports NDUFS6 as a novel autosomal recessive CMT gene and is able to expand the phenotypic spectrum of NDUFS6-related mitochondrial disease.

P93 - A multi-disciplinary approach to solving undiagnosed patients - Unsolved Cases Unit Groningen.

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After solving 30-40% of all patients submitted for clinical genetic testing, the possible avenues for follow-up testing are diagnostically limited. Experimental diagnostics can help to bridge the gap between the clinic and research, providing the next step after diagnostic genetics has been exhausted. At the UMCG, we have implemented a team focused on solving well-phenotyped unsolved cases using an experimental diagnostic approach.
With input from a patient representative, a multi-disciplinary team of clinicians, researchers, laboratory geneticists was established. Progress is tightly monitored with biweekly meetings to avoid endless experimental diagnostic tracks and clinicians are provided with regular updates. To date, twenty-two patients have been registered and chosen for inclusion. The phenotypic spectrum includes developmental delay, MCA and skin ailments. Seven patients require further functional testing to enable re-classification for VUS. The remainder are undiagnosed and chosen either for whole genome sequencing, RNAsequencing or still to be determined.
To date, a homozygous missense variant in PLAA was found to initiate alternative splicing using mRNA techniques, resulting in truncation of the gene. With this result the phenotype of the patient, who has brain anomalies, hypotonia, edema of the hands, episodes with bradycardia and saturation loss, epilepsy and feeding difficulties, is explained. Based on recent publications, a likely pathogenic in-frame deletion in the CHD3 gene was found to explain the phenotype of a patient presenting with hypertelorism, macrocephaly and global developmental delay. In a third patient, a VUS missense variant in EDA might explain a family with ectodermal dysplasia. Using RT-PCR and Western blot techniques we are comparing EDA function in healthy vs. affected family members. Testing for the remaining patients is ongoing.
Our results demonstrate the added value of incorporating an experimental diagnostic approach in genome diagnostics to assist in solving the unsolved cases. Our experience shows that this approach is supported by patients and clinicians.

P94 - Discordant fetal sex with cfDNA indicating monosomy x but male phenotype on ultrasound: a case report

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Fetal sex discordance between noninvasive prenatal cfDNA analysis and ultrasound is encountered in approximately 1 in 1.500-2.000 pregnancies. Specifically, monosomy X on noninvasive prenatal screening (NIPS) with male fetal sex by ultrasound has been reported in the context of idic(Y)/45,X, iso(Y)/45,X or 46,XY/45,X mosaicism. The frequency of confined placental mosaicism showing 45,X in a male pregnancy is estimated to be 1 in 24.000. Here, we report on the first spontaneous pregnancy of a healthy non-consanguineous couple. During prenatal assessment, NIPS and ultrasound follow-up revealed discordant findings. Two repeated NIPS samples were indicative for monosomy X (45,X). However, ultrasound suggested a normal male phenotype. Direct fluorescence in situ hybridization analysis on uncultured amniocytes detected the presence of an SRY specific signal. Further investigation using chromosomal microarray on amniotic fluid showed two heterozygous terminal deletions, with breakpoints within each of the pseudo-autosomal regions (PAR). There are no protein-coding genes involved in the PAR1 deletion, while the deletion in the PAR2 region encompasses the SPRY3, VAMP7 and IL9R genes. We hypothesize that these findings might be explained by a 45,X,r(Y) fetal and 45,X placental genotype, with loss of the ring Y chromosome in placental tissue due to mitotic instability. Additional conventional karyotyping is needed to confirm this hypothesis. We expect no phenotypic consequences for the fetus, however the possible presence of a ring Y chromosome may affect fertility due to impaired crossing-over and segregation during meiosis. Natural transmission of an r(Y) chromosome has been reported from a 46,X,r(Y) father to his 47,XX,r(Y) son. Since the presence of an r(Y) in germ cells seems to be associated with a higher risk for (sex) chromosomal aneuploidies in offspring, sperm analysis at adolescent age could have an added value.

P95 - Diagnostic yield of exome based panel sequencing in a CHD patient cohort

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Congenital heart disease (CHD) is the most frequent birth defect, affecting more than 8 in 1000 live born babies. CHD may occur in the context of a syndromic constellation or isolated (non-syndromic CHD). Since both genetic and environmental factors have been implicated, the pathophysiology of CHD is complex and the molecular etiology remains unknown in the majority of the patients. Here we present the diagnostic yield of exome based panel sequencing of 471 genes associated with CHD in a patient cohort of 304 CHD probands, including 82 syndromic and 222 non-syndromic cases who had a normal CNV sequencing. Overall, a class 4 or 5 variant was identified in 6,6% (20/304) of the CHD probands, comprising 15 single nucleotide variants (SNVs) and 5 indels. In the syndromic CHD, the yield of genetic testing was 16,0% (13/81) (10 SNVs, 3 indels). In non-syndromic CHD, a (likely-) pathogenic variant was identified in 3,1% (7/223) (5 SNVs, 2 Indels) of the probands. Of note, two (likely) pathogenic variants identified in non-syndromic CHD, affect genes associated with syndromic CHD, although the probands have no extracardiac anomalies and a normal neuromotor development at last evaluation. In conclusion, extensive genetic testing for CHD genes is critical in the diagnostic approach, both in syndromic and non-syndromic CHD. Identifying the molecular etiology can provide us insight into the unanticipated extracardiac involvement, long-term outcomes, and recurrence risk in future pregnancies.

P96 - Challenges in the validation of small and large CNVs from WES data

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Whole Exome Sequencing (WES) is commonly used to diagnose rare hereditary diseases. Most laboratories using WES in a diagnostic setting validate the detection of Single Nucleotide Variants (SNVs) using Genome In A Bottle (GIAB) cell lines for which both confident variant and reference calls are available. The detection of Copy Number Variants (CNVs) from WES is however not always validated. The gold standard dataset provided by GIAB includes CNVs that cannot be detected using short read sequencing. Also, CNV detection from WES is often based on read counts and comparison to a reference set and will thus not necessarily detect common variants. The resolution of CNV calls is highly dependent on the capture probe design, with most CNV tools being unable to detect small CNVs. Finally, there are no guidelines describing how CNVs should be validated from WES data, i.e. how many CNVs should be included in the validation set, which CNV sizes should be considered and how to interpret CNVs detected by one technology only. WES is thus often complemented with other diagnostic tests such as arrayCGH (aCGH) and shallow Whole Genome Sequencing (WGS) and/or Multiplex Ligation-dependent Probe Amplification (MLPA) for the detection of large and small CNVs, respectively. We compared CNV calls from aCGH and MLPA to WES CNVs detected by CoNIFER and ExomeDepth with and without filtering common variants and defined rules on how CNVs should be prioritized in order to maximise both sensitivity and precision.

P97 - Understanding inherited genetic variation in Parkinson's disease through long-read and single-cell multi-omics analyses.

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Introduction: To date, the functional contributions of inherited and acquired genetic variation to the pathogenesis of Parkinson's disease (PD) remain largely unknown. Given that gastrointestinal dysfunction often precedes the onset of motor symptoms, understanding the role of gut-brain axis in PD pathogenesis is highly relevant.

Methods: Here, using brain and gut tissue samples from both PD patients and controls across the age spectrum from 35 to 95, we first leverage long-read sequencing to comprehensively chart the genetic variation at PD risk loci, which have been identified in large-scale genome-wide association studies (GWAS). We next probe how this variation perturbs the expression of genes in specific cell (sub)populations of the substantia nigra and cingulate cortex in the brain and the right colon. To this end, we perform single-cell gene expression and chromatin accessibility profiling of both the same and different single-cells using 10X Genomics as well as the in-house open source HyDrop platform.

Results: We have so far sequenced 50 whole-genomes with long native Nanopore reads, a subset of which leveraging Fiber-Seq, which additionally encodes long-range single-molecule chromatin accessibility status. We are exploring both reference-based alignment and de novo diploid assembly approaches to capture and phase complex variants on the disease-associated allele. We also generated 10X Genomics multi-ome data on brain and gut tissues of these 50 individuals, which we are currently mapping onto the personalised diploid genomes to discern allele- and cell-type specific signals. As we scale up our efforts, we will perform cohort-level quantitative trait locus analyses, enabling identification of further PD-relevant genes and cell (sub)types. Over the coming years, we hence aim to deliver the mechanisms of PD-candidate gene expression (dys)regulation in the normal condition, with ageing, and in PD, as well as generate a unique genomic and single-cell multi-omic resource for the community.

P98 - Effects of autozygosity on common diseases

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Increased autozygosity, measured by the proportion of the genome that is in runs of homozygosity (FROH), is associated with various traits, such as height and cognition. Much less is known of the association of autozygosity with human disease. In this study we quantify the association of autozygosity and the full spectrum of human disease in ~400.000 individuals of principal component analysis-selected European ancestry from UK Biobank and ~20.000 individuals of principal component analysis-selected South Asian ancestry from two replication cohorts. We find positive associations ($p < 0.00039$) between FROH and the total number of International Classification of Diseases and Related Health Problems, revision 10 (ICD-10) subchapters with at least one diagnosis and those within respiratory and endocrine chapters, especially type II diabetes. We show that individuals with an FROH equivalent to that of offspring of first cousins have a 10% higher chance of having a diagnosis in an ICD-10 subchapter (p 5.67 x 10-14, RR 1.10 (1.08-1.13)) in individuals in UK Biobank of European ancestry, which was reproduced in the two replication cohorts. The results were robust to adjustment for measured confounders, but any remaining social confounding cannot be ruled out.

P99 - Cross-sectional and longitudinal characterisation of the developmental phenotype in 22q11.2 duplication syndrome

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Background: 22q11.2 duplication is a recurrent copy number variant (CNV), associated with a wide spectrum of physical and neurodevelopmental features and a high rate of familial transmission. In this study, we aim to contribute to the clinical and neurodevelopmental phenotype of this recurrent CNV.

Methods: We conducted a retrospective chart review and analysed the digital medical records of 28 patients with proximal 22q11.2 duplications, focusing on physical, developmental and behavioural features, including longitudinal data in a subgroup (n=11). Additionally, the phenotypes of patients with de novo (n=8) and inherited (n=13) 22q11.2 duplications were compared.

Results: Patients demonstrate heterogeneous phenotypic representations with variable major congenital anomalies. Dysmorphic features are noted in 64% of patients. Common physical anomalies include nutritional problems (57%), failure to thrive (33%), transient hearing impairment (52%), neurological abnormalities (39%), congenital heart defects (33%) and abnormal head size (32%). Developmental, speech-language and motor delay are common in infancy, while attention (64%), learning (60%) and motor problems (52%) are typically reported at primary school age. Attention-deficit/hyperactivity disorders are diagnosed in 44%. Average full-scale intelligence quotient is in the borderline range (FSIQ 79), with one-third of patients functioning in the borderline range (FSIQ 71-84) and one-fifth of patients having mild intellectual disability (FSIQ 55-70). Longitudinal IQ-data (n=11) indicate that almost two-third of patients have a relative stable cognitive trajectory, whereas one-third show a growing into deficit profile. In patients with de novo duplications, there is a trend of more failure to thrive, while more patients with inherited duplications attend special education.

Discussion: The current cohort of patients with proximal 22q11.2 duplication seems to be representative for what has been described in literature so far. Both inter- and intra-familial heterogeneity is noticed among patients with 22q11.2 duplication. However, the genetic-first approach in this chart review may introduce bias in the described phenotype and therefore not cover the whole spectrum of presentations in patients with 22q11.2 duplication, because mainly patients with discernible phenotypes have been discovered so far. Only index patients with medical or cognitive problems were included, which might result in an ascertainment bias. To delineate the complete phenotype of patients with 22q11.2 duplication by means of unbiased methods, future studies should also include family members with 22q11.2 duplication diagnosed through segregation analysis.

Conclusion: The present study confirms a wide heterogeneous physical, neurodevelopmental and behavioural phenotype in patients with proximal 22q11.2 duplications, and provides for the first time longitudinal IQ-data in a subgroup of patients. When children are diagnosed with 22q11.2 duplications prenatally or early in life, healthcare professionals should be aware of an increased risk of nutritional problems, heart defects and hearing problems, and should initiate neurodevelopmental support early in life, given the high risk of developmental delay, learning and attention problems.