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Abstracts Autumn symposium 2017

Abstracts Talks

T01-T16

T01 - Next-generation sequencing of the mtDNA and exome is the preferred, first strategy to identify known and novel causes of mitochondrial disease

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Mitochondrial disorders are among the most common inherited neuromuscular disorders, caused by both mitochondrial DNA (mtDNA) and nuclear gene defects. Limited genotype-phenotype correlations and extreme clinical and genetic heterogeneity make selection of candidate genes impossible for the majority of patients. Next-generation sequencing (NGS) has therefore revolutionized the identification of known and novel genetic defects in these patients. For patients with either a primary mitochondrial disorder or a mitochondrial cause as part of the differential diagnosis, NGS of the entire mtDNA is performed to detect and quantify point mutations and deletions, followed by whole exome sequencing (WES) to identify mutations in nuclear genes. A disease-causing mtDNA mutation is found in about 10% of patients. For de novo mtDNA mutations recurrence risks are very low, whereas for carriers of heteroplasmic mtDNA mutations, preimplantation genetic diagnosis (PGD) can be offered to prevent disease transmission by transferring embryos with a mutant load below the threshold of expression. We have demonstrated that the method is reliable and that most carriers produce embryos below the threshold. So far 31 cycles have been performed, resulting in 6 pregnancies and healthy children. To identify nuclear gene defects, we apply WES and analysis of a panel of known mitochondrial disease genes, which, if negative, is followed by open exome analysis with functional validation. By WES we detected disease-causing mutations in ~ 50% of patients, including autosomal recessive cases, patients with de novo mutations, as well as patients with multi-genic disease manifestations. The defective genes are mainly associated with mitochondrial protein metabolism (e.g. AARS2, CLPP, MTFMT, MTO1, QRSL1), OXPHOS function (e.g. COQ7, NDUFS7, NDUFA12, NDUFAF5, TMEM126A, TMEM126B) or mtDNA maintenance (e.g. C10orf2, DNA2, FBXL4, RRM1, RRM2B). In addition, we identify causal genes for which no mitochondrial function or localization is reported (e.g. ADD3, IER3IP1, LMOD3, NBAS, RELN, SLC16A2), most often in the patients were mitochondrial disease is part of the differential diagnosis. Identification of the genetic defect is not only important for diagnostics, but can also be crucial for therapeutic intervention. Our patients with COQ7, SLC25A32, SLC19A3 and TMEM126B defects showed clear clinical improvement upon treatment with respectively coenzyme Q, riboflavin, biotin/thiamine and high fat-diet.

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Keywords: mitochondrial disease, NGS, WES, PGD, therapy

T02 - Germline de novo mutation clusters arise during oocyte aging in genomic regions with increased double-strand break incidence

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Clustering of mutations has been found in somatic mutations from cancer genomes and in germline de novo mutations (DNMs). We identified 1,755 clustered DNMs (cDNMs) within whole-genome sequencing data from 1,291 parent-offspring trios and investigated the underlying mutational mechanisms. We found that the number of clusters on the maternal allele was positively correlated with maternal age and that these consist of more individual mutations with larger intra-mutational distances compared to paternal clusters. More than 50% of maternal clusters were located on chromosomes 8, 9 and 16, in regions with an overall increased maternal mutation rate. Maternal clusters in these regions showed a distinct mutation signature characterized by C>G mutations. Finally, we found that maternal clusters associate with processes involving double-stranded-breaks (DSBs) such as meiotic gene conversions and de novo deletions events. These findings suggest accumulation of DSB-induced mutations throughout oocyte aging as an underlying mechanism leading to maternal mutation clusters.

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Keywords: de novo mutations, mutagenesis, clustered mutations, double-strand breaks

T03 - Candidate gene identification based on 3D chromatin interactions: Genetics behind complex diseases just got even more complex

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Introduction: Up to date, genome wide association studies (GWASs) have identified thousands of robust and reproducible genomic loci linked to complex diseases and traits. Their interpretation and the identification of associated candidate genes remains a major challenge. Many of disease susceptibility loci co-localize with DNA regulatory elements, which influence gene expression through chromatin interactions. We have utilised the involvement of regulatory regions in the genetic component of complex disease to identify novel candidate genes in Inflammatory Bowel Disease (IBD), Coronary Artery Disease (CAD) and Kidney Function (KF) related traits. We used a method to study the 3D conformation of the chromatin (circular chromosome conformation capture-sequencing, 4C-seq) to systematically determine the genes that are physically interacting with regulatory elements that overlap the disease associated variants. Results: We assayed regions physically interacting with susceptibility loci for IBD, CAD and KF, in relevant cell types (monocytes, lymphocytes, intestinal epithelial cells, coronary endothelial cells, renal endothelial cells and renal epithelial cells) and altogether generated 780 individual 4C-seq datasets. Using this approach we have linked 1650 novel candidate genes to 193 susceptibility loci. Strikingly, the vast majority of these genes are located outside the boundaries of haplotype blocks. Many of them are critically involved in processes linked to studied diseases. Furthermore, the analysis of expression quantitative trait loci revealed that the expression of many novel candidate genes is genotype dependent. Conclusion: We have systematically applied a novel approach for identification of candidate genes. The utilisation of the variants in regulatory part of the genome dramatically increases the searching space for candidate genes compared to the classical genetic approaches and is in line with the omnigenic model for complex diseases.

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Keywords: Chromatin interactions, GWAS, DNA regulatory elements, candidate genes, 4C-seq

T04 - Refining embryo transfer strategies using embryonic ploidy status in PGD for translocations

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The use of NGS and array technology in couples undergoing PGD for chromosomal disorders allows for simultaneous genome-wide ploidy analysis of all embryos. Whether this data can be used to optimise PGD treatment is unknown. We performed an observational, multicentre study covering a 3-year period (2014-2016) during which 117 couples underwent 171 PGD cycles for structural chromosomal abnormalities. Comprehensive chromosome testing was performed by array CGH. The included study cohort comprised first attempt PGD cycles with day 3 single blastomere biopsy and fresh single embryo transfer (n=73). In all centres only embryos with a balanced karyotype for the chromosomes comprising the PGD indication were eligible for transfer. Two of the three centres received no ploidy information for non-indication chromosomes and ranked eligible embryos for transfer solely based on morphology. The third centre did receive this information and ranked embryos based on both chromosomal constitution and morphology, prioritising the balanced/euploid ones. In all, 73 embryo transfers met the inclusion criteria. There were 38 transfers in the two centres that did not use ploidy information, resulting in 20 ongoing pregnancies. Twenty-seven transfers involved a balanced/euploid embryo, resulting in 19 ongoing pregnancies (70%), and 11 transfers involved a balanced/aneuploid embryo, resulting in one ongoing pregnancy (9%). There were 35 transfers in the center that did use ploidy status to rank embryos, resulting in 8 ongoing pregnanies. Twenty-seven transfers involved a balanced/euploid embryo, resulting in 7 ongoing pregnancies (26%), and 8 transfers involved a balanced/aneuploid embryo, resulting in one ongoing pregnancy (13%). All transfers in all centres taken together, 54 transfers involved a balanced/euploid embryo, resulting in 26 ongoing pregnancies (48%), and 19 transfers involved a balanced/aneuploid embryo, resulting in two ongoing pregnancies (11%) (p=0.004). Aneuploidy of non-indication chromosomes involved a trisomy 19 and a trisomy X. Miscarriage rates, defined as the percentage of cycles where a biochemical pregnancy did not result in an ongoing pregnancy, were 4% for balanced/euploid and 50% for balanced/aneuploid embryos. The biopsied blastomere-inferred embryo genotype of the non-indication chromosomes is thus predictive of the implantation potential. Whether ranking embryos for transfer accordingly improves PGD treatment outcome should be further investigated.

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Keywords: PGD, human embryo, translocation, aneuploidy

T05 - A retrospective analysis of genetic testing in pediatric patients while introducing rapid targeted whole genome sequencing for critically ill newborns.

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Introduction Next generation DNA sequencing (NGS) is increasingly implemented in routine clinical practice. However, time to diagnosis is still long and in many patients a genetic diagnosis is never found. Meanwhile, knowledge of the genetic cause of a disease can be crucial during clinical-decision making, especially in critically ill patients. Rapid whole exome sequencing (WES) and whole genome sequencing (WGS) are feasible, with turn-around-times (TATs) of ~72 hours, but are expensive, and logistics are complex. Since their clinical utility has not yet been demonstrated prospectively, rapid WES or WGS are not embedded in routine clinical practice in most hospitals. We aimed to assess speed and diagnostic yield of standard genetic diagnostics and rapid targeted WGS. Methods Data were retrospectively collected from all children younger than one year that were referred to the clinical geneticist from May 2014 until January 2016 in a tertiary care hospital (University Medical Centre Groningen). During this period, targeted rapid-WGS was implemented for critically ill children in this age group. Patients with a clear clinical diagnosis were excluded for rapid WGS. We measured diagnostic yield and TAT for routine diagnostics and rapid targeted WGS. Data collection will be expanded to four additional hospitals. Results Results are preliminary with data from one hospital. A total of 495 patients was referred to a clinical geneticist, of whom 341 had genetic testing. In 96 cases (28%) routine testing resulted in a diagnosis, of which 42% were chromosomal and 58% monogenetic disorders. Mean time to diagnosis of monogenetic disorders was 91 days (range 5 to 289). Rapid WGS was performed in 23 cases and a diagnosis was found in 7 cases (30%). Mean time to diagnosis of rapid WGS was 12 days (range 5â€“23). In the rapid WGS group, one chromosomal disorder and six monogenetic disorders were found. Conclusion For all children younger than one year the diagnostic yield was 28% for routine genetic diagnostics with a mean time to diagnosis of 91 days. The diagnostic yield of rapid targeted WGS in critically ill children was 30%, with a mean time to diagnosis of 12 days. Considering that patients with evident clinical diagnoses were excluded for rapid WGS, this yield is surprisingly good. The difference in time to diagnosis might affect quality and costs of care, especially in critically ill infants. A prospective study to evaluate these issues is warranted.

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Keywords: NICU, Critically Ill Newborns, Critically ill Children, Rapid Whole Exome Sequencing, Rapid Whole Genome Sequencing, NGS

T06 - Fetal Fraction Evaluation in Non-Invasive Prenatal Testing (NIPT)

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An important factor for quality control of any non-invasive prenatal test (NIPT) is the percentage of fetal DNA in samples. Currently we determine fetal fraction for all male samples based on the number of reads aligned to the entire and subset of the Y-chromosome. Concerned that shipping time may influence the fetal fraction we evaluated fetal fraction as a function of shipping time. Between April 1 and June 29, 2017, 6,836 NIPTs were carried out at the VUmc. The number of days it takes between drawing blood and preparing the sample for testing ranged from the same day to five days (mean/median of 1.89/2 days), with a subset of samples (1.2%) also taking six or seven days. All samples were shallow whole-genome sequenced and analyzed utilizing a mean of 15,649,383 50bp reads. No significant correlation was found between fetal fraction and shipping time. Currently we only determine fetal fractions for male fetuses, but this limits this method of quality control to half of the samples. An alternative to estimating fetal fraction by Y chromosome mapped reads without the need for paired end sequencing, is to estimate the fetal fraction by the distribution of reads mapped around nucleosome positions on the autosomal chromosomes. We have implemented this in the tool SANEFALCON (Straver et al. 2016). Using 3,582 male fetal samples, we found a correlation in fetal fraction determination by Y chromosome mapping and nucleosome position profiling with SANEFALCON. These promising results demonstrate fetal fraction can also be determined in female fetuses from single read shallow whole-genome sequenced NIPT samples. To conclude, we demonstrate that extended shipping time (up to 7 days) does not impact fetal fraction and that fetal fraction of both sexes can be determined in future NIPT runs utilizing nucleosome position profiling.

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T07 - RTTN is located at the centrosomes during mitosis and regulates centriole duplication

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Primary microcephaly (MCPH) is a malformation of cortical development, characterized by an OFC = 2SD compared to the age-and sex-matched norm. MCPH most often manifests itself as consequence of an autosomal recessive mutation. Rotatin (RTTN) is a gene previously associated with MCPH, although not much is known about its´ function. Our group previously localized RTTN to the microtubule-based primary cilium, necessary for proper neuronal proliferation associated with WNT and SHH signaling. As Drosophila analogue Ana3 is localized at the centrosome and basal body, necessary for the formation of the mitotic spindle pole, and RNAi knockdown of Ana3 leads to multipolar spindles, RTTN might have a function in mitosis and neuroproliferation. This project aims to elucidate the function of RTTN in normal and abnormal brain development.To date RTTN mutations have been clinically reported in polymicrogyria and microcephaly patients. Our group has gathered patient material from a cohort of 17 patients with homozygous or compound heterozygous RTTN mutations. Immunocytochemistry of endogenous RTTN in control and patient fibroblasts is being performed. Transfection with pcDNA3.1-Myc/HisA(-) containing wtRTTN has been optimized in HEK293T cells for overexpression studies. Mass spectrometry after c-Myc affinity bead immunoprecipitation of transfected RTTN in HEK293T will be performed to determine interacting partners. Quantification of RTTN mRNA with qPCR show residual transcript in all patients, supporting our hypothesis that complete lack of RTTN mRNA is embryonically lethal in human. Immunocytochemistry of healthy human fibroblasts show localization at the centrosome during the mitosis and cytokinesis. This is in accordance with the location of Drosophila melanogaster analogue Ana3, and gives a first indication that RTTN plays a role in mitosis. Preliminary data of RTTN/?-tubulin staining in patient fibroblasts show abnormal quantity of centrosomes at each pole of the spindle during mitosis and cytokinesis. Conclusion Residual transcription of RTTN seems to be crucial for organism vitality. Recessive mutations in RTTN result in reduced expression and induce cortical malformations ranging from microcephaly to polymicrogyria. Our preliminary data indicate that RTTN is located at the centrosomes during mitosis and that recessive RTTN mutations lead to a default in centriole duplication, resulting in aberrant neuroproliferation and cortical development.

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Keywords: Rotatin, centrosome, mitosis, cortical malformations, gene function

T08 - Defining quality standards for clinical whole exome sequencing: a national collaborative study of the Dutch Society for Clinical Genetic Laboratory Diagnostics (VKGL)

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Clinical whole exome sequencing (WES) has proven to be very effective for diagnosing heterogeneous genetic diseases, and has therefore been adopted in standard Dutch genetic diagnostic services. Quality standards have been described for gene panel based next generation sequencing, but for clinical WES these have not been determined yet. In this study, we present a nationwide quality assessment scheme within all eight university medical centers in the Netherlands in order to improve and harmonize quality and to formulate a quality standard for clinical WES. The exome of Genome in a Bottle (GIAB) sample NA12878 was sequenced and processed by the Dutch genetic diagnostic centers according to their own standard diagnostic protocols. Anonymized VCF and BAM files were collected and used for comparisons. VCF files were used to calculate variant detection sensitivity and precision using the GIAB high confidence call set (v2.19). BAM files were used to calculate informative exome coverage statistics based upon a standardized target defined by all coding exons of UCSC and Ensembl +/- 20bp intron flanks. Results showed that single nucleotide variant (SNV) detection sensitivity varied between 96.3% and 99.1% (average 98.1%) and that INDEL detection sensitivity varied between 86.1% and 96.1% (average 90.4%). Precision of SNV detection was between 98.0% and 99.8% (average 98.9%), and precision for INDEL detection was between 74.8% and 97.0% (average 91.0%). The mean coverage of the exome varied between 55X and 152X. Completeness of the Exome (defined as the percentage of bases with 15x informative coverage) varied between 90.0% and 96.4% (average 93.5%). Downsampling analysis indicated that completeness is a good predictor for quality and can be used to determine the required sequencing output for a desired quality threshold. The nationwide quality assessment scheme shows an acceptable concordance between the Dutch genetic diagnostic centers, although harmonisation of lab protocols and bioinformatics may lead to improved concordance and quality. We propose the following quality standards for a clinical WES based upon the GIAB high confidence call set (v2.19): a minimum sensitivity of 97% for SNVs and 85% for INDELs, and a minimum precision of 98% for SNVs and 85% for INDELs. These quality assurance standards will be deposited as field standards at the Dutch accreditation council for use in ISO15189 laboratory accreditation.

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Keywords: Diagnostics, Clinical testing, Quality, Exome sequencing, Bioinformatics

T09 - Transcriptional regulation in hypertrophic cardiomyopathy due to MYBPC3 founder mutations

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Background: Next-generation sequencing technologies have emerged to profile DNA locations of regulatory regions, often referred to as promoters and enhancers. Regulatory regions recruit transcription factors (TFs) to their DNA sequence through TF binding sites (TFBMs) allowing cells to precisely control the timing, localization and the level of gene transcription. Differential chromatin activity of DNA regulatory regions can be probed by the presence of histone 3 lysine 27 acetylation (H3K27ac) mark through chromatin immunoprecipitation and sequencing (ChIP-seq). Since the aberrant regulation of transcriptional programs in the affected tissue is a common hallmark of human disease, we hypothesized that the changes in H3K27ac occupancy would yield valuable information about the underlying pathways and candidate genes. Motivation: To date, differential activity of chromatin regulatory regions has not been investigated in any human form of cardiomyopathy. As a proof of principle, we have focused on a well-defined cohort of hypertrophic cardiomyopathy due to founder mutations in the cardiac myosin binding protein C - MYBPC3 (n=14) and compare them to controls (n=5). Methods and results: Human septal cardiac tissue has been used to produces H3K27ac ChIPseq libraries and matching RNAseq libraries. Principal component analysis using top variable regions (ChIPseq) and genes (RNAseq) in all samples supports the division between patients and controls. Using ChIPseq we have detected 7,216 regions with more activity (hyperacetylated) and 7,214 regions with less activity (hypoacetylated) in disease and control group (FDR<0.05). In RNAseq data, we were able to detect 445 and 492 genes showing up- and downregulation, respectively (FDR<0.05). Next, we have performed circular chromosomal conformation capture followed by sequencing (4C-seq) on IPS-derived cardiomyocyte culture from a healthy individual on to systematically determine the genes that are physically interacting with selected regulatory regions showing differential histone acetylation between patients and controls. Conclusion: Using an integrative chromatin analysis based on differential H3K27ac occupancy of cardiac regulatory DNA regions and cardiac gene transcription coupled with 3D chromatin interactions, we have annotated the major effector genes and networks involved in hypertrophic cardiomyopathy due to MYBPC3 mutations.

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Keywords: chromatin transcriptional regulation, hypetrophic cardiomyopathy, MYBPC3 founder mutation

T10 - Evolution of dihydropyrimidine dehydrogenase (DPD) diagnostics in a time-period of seven years

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Background: Treatment with fluoropyrimidines, the main chemotherapeutic agents used in many types of cancer, is not well tolerated in a subgroup of patients. The enzyme responsible for degradation of fluoropyrimidines is dihydropyrimidine dehydrogenase (DPD), the first and rate-limiting enzyme of the pyrimidine degradation pathway. DPD dysfunction leads to an increased exposure of active metabolites, which can result in severe or even fatal toxicity. Fluoropyrimidine treatment can be optimized based on DPD activity. Methods: We provide an overview of seven years DPD diagnostics (n=1194). In this time-frame the test has evolved from a single enzyme measurement using Ultra-High Performance Liquid chromatography (UHPLC) in peripheral blood mononuclear cells (PBMCs) to a combined enzymatic and genetic test of four variants in the DPYD gene (DPYD*2A, DPYD*13, c.2846A>T and 1129-5923C>G). Results: Patients in the group tested for four variants (n=814) with either one variant have a lower enzyme activity than the overall patient group. The majority of patients with the DPYD*2A variant (83%) consistently showed a decreased enzyme activity. Only seventeen (22%) of the 77 patients with a low enzyme activity (tested for four variants) carried a variant. Complete DPYD sequencing in a subgroup with low enzyme activity and without DPYD*2A variant (n=47) revealed 10 genetic variants, of which four have not been described previously). Conclusions: We did not observe a strong link between DPYD genotype and enzyme activity. There is no doubt that DPD status should be determined before treatment with fluoropyrimidines to save patients´ lives and prevent unnecessary side effects. Our study in combination with literature shows that there is a discrepancy between the DPD enzyme activity and the presence of clinically relevant SNPs. Therefore at this moment a combination of a genetic and enzymatic test is preferable for diagnostic testing.

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Keywords: fluoropyrimidines, dihydropyrimidine dehydrogenase (DPD), genetic variant, pharmacogenetics

T11 - CLASSIFICATION OF LMNA-VARIANTS BY MEANS OF ZEBRAFISH PHENOTYPING

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Implementation of next-generation sequencing (NGS) techniques in human genetics improved the diagnostic yield, but also resulted in an increase of many genetic variants with unknown clinical significance (VUS). Despite the presence of in silico prediction programs and databases to predict the functional consequences of a variant, the effect of the majority of variants still remains unknown. Functional follow up is therefore crucial to enable further classification of a variant as pathogenic or not. In this proof-of-concept project we are characterizing 20 variants in LMNA by means of phenotypic evaluation in zebrafish embryos. To this end, we have set up a phenotyping pipeline to evaluate cardiomyopathy in zebrafish. The parameters that we evaluate are: heart morphology, pericardial edema, heart rate and fractional shortening. The cardiac read-out parameters were validated using another known genetic cardiomyopathy model (tnnt2a-loss-of-function) in zebrafish. In the next step we characterized the phenotype of a morpholino-mediated lmna model. We observed a concentration-dependent increase of the lmna-knockdown embryos displaying cardiac edema and a significantly reduced heart rate. Additionally, significant cardiac arrhythmia was observed in the knockdown condition. We now are evaluating 20 genetic variants in our model; 5 known (pathogenic and non-pathogenic) controls from the literature and 15 VUS, found in our cardiomyopathy patient cohort. We intend to use this diagnostic phenotyping pipeline as a routine functional evaluation of LMNA-variants, detected in cardiomyopathy patients, leading to improved diagnostics. If desired, this pipeline can be extended to include other genes or phenotypes.

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Keywords: Laminopathy zebrafish

T12 - Towards automated sharing of genetic variants between genome diagnostics laboratories and beyond: an initiative of the Dutch diagnostic data sharing consortium

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The use of genomic information in both research and clinic has expanded enormously the last decade. Exome analyses have become part of routine diagnostics. Yet, the interpretation of the obtained data is a huge challenge for laboratories, because information on individual DNA variants is often absent in public databases. In order to improve patient care the Dutch genome diagnostic laboratories decided to share their data , most importantly the individual variant pathogenicity classifications and observed variant frequencies. When a variant has been previously carefully classified by one or more expert centers, there would be less need to (re-)evaluate this variant when encountered for the first time by another center. Towards this goal we created a central national database with connections to each of the labs for automated bi-directional sharing. The MOLGENIS software platform and VARDA platform are used to share variant classifications and frequencies, respectively. Currently the database contains 46.500 classified DNA variants in 4.500 genes, of which 40.000 variants have been classified by only one laboratory and 6.500 were classified by multiple laboratories. For 650 variants (10%) classifications differed, but only 2 variants had an opposite classification. First data has already been shared with the LOVD3 share database. The goal is to make these highly curated data also publically accessible by submitting data to international databases.

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Keywords: datasharing, variants, VKGL, WES

T13 - Ciliary phenotyping in urine-derived patient cells to determine the pathogenicity of novel variants in ciliopathy genes

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Objective Ciliopathies are rare hereditary disorders caused by dysfunction of the cilium, a small signaling organelle present on nearly every human cell. Ciliopathies display extensive clinical and phenotypic heterogeneity, which complicates accurate diagnosis of patients. Studies of urine-derived renal epithelial cells (URECs) of ciliopathy patients vs controls allow for functional interpretation of variants of unknown significance detected by next-generation sequencing techniques. The aim of this study was to determine the pathogenicity of two novel IFT140 variants in a patient with visual impairment, vertical nystagmus, progressive hearing impairment, short stature, and mild skeletal abnormalities. Methods Mutations in IFT140, encoding an intraflagellar transport complex-A (IFT-A) protein, are associated with skeletal ciliopathies. Cilium length and ciliogenesis frequency was evaluated by immunofluorescence (IF) cytochemistry using markers of the ciliary axoneme. Dysfunction of the retrograde IFT-A complex can be visualized by IF staining showing an accumulation of IFT88 at the ciliary tip. Results The ciliary phenotype in URECs from the patient showed normal ciliogenesis, cilium length, and ciliary localization of IFT140, however, they showed abnormal retrograde IFT. About 40% of the cilia from the patient´s URECs showed an accumulation of IFT88 in the tip, whereas this was not seen in URECs from the healthy mother or controls, and only in 10% of the healthy father´s URECs. Conclusion URECs can be used to study the pathogenicity of variants in ciliopathy genes like IFT140. The novel mutations found in IFT140 cause abnormal intraflagellar transport, which supports the clinical diagnosis of short-rib thoracic dysplasia 9. Functional studies in urine-derived patient cells prove to be an attractive non-invasive procedure to facilitate accurate diagnosis of ciliopathy patients.

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Keywords: diagnosis, ciliopathy, IFT140, urine-derived cells

T14 - De novo missense mutation clustering identifies candidate neurodevelopmental disorder genes

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Haploinsufficiency (HI) is the most common mechanism through which dominant mutations exert their effect and cause disease. Typically, these pathogenic mutations are spread throughout the gene, and result in absence of protein product. In contrast, non-haploinsufficiency (NHI) mechanisms, such as gain-of-function and dominant-negative mechanisms, are often characterized by the spatial clustering of missense mutations within a gene, thereby affecting only particular regions or base pairs of a gene . Here we exploit this property and developed a method to specifically identify genes with significant spatial clustering patterns of de novo mutations in large patient cohorts. We applied our method to a dataset of 4,061 de novo missense mutations from published exome studies of patient-parent trios with (neuro)developmental disorders (NDDs) and identified 15 genes with statistical clustering of mutations. Among the 15 genes that we identified there was a strong enrichment for known NDD genes (12 out of 15, p=1.65e-04) thereby supporting our approach. Strikingly, 11 out of these 12 genes are known to act through a disease mechanisms other than HI and for 8 of these known genes we found extensive functional evidence supporting NHI mechanisms. Interestingly, the identified genes are significantly less tolerant to population variation than known HI genes (p=8.59e-03) and are significantly depleted for truncating mutations (p?1.00e-05) in the patient cohort. The 3 genes that were not previously linked to NDDs (ACTL6B, GABBR2 and PACS2) are involved processes that are known to be disrupted in NDDs. Through a collaboration we found that multiple patients with the same PACS2 mutation have been independently identified that all share clinical phenotypes. Finally, we performed 3D-modeling of protein structures to show that, unlike known HI genes, clustering mutations are unlikely to affect protein folding and more likely to disturb protein interactions/complex formation (p=1.26E-03). In conclusion, we developed a method for the identification of disease genes based on the significance of spatial mutation clustering within a gene. We identified three genes with similar clustering patterns that we propose as candidate NDD genes. Our findings support the notion that these mutations mostly exert their pathogenic effect through disease mechanisms other than HI.

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Keywords: de novo mutations, intellectual disability, bioinformatics

T15 - RNAseq in 296 phased trios provides a high resolution map of genomic imprinting

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Combining allelic analysis of RNAseq data with phased genotypes in family trios provides a powerful method to detect parent-of-origin biases in gene expression genome-wide. We report findings in 296 family trios from two large sequencing studies: 131 blood samples from the Genome of the Netherlands (GoNL), and 165 lymphoblastoid cell lines (LCL) from three ethnicities in the 1000 Genomes Project. Based on parental haplotypes, we identified >2.5 million transcribed heterozygous SNPs phased for parental origin in 31,955 Gencode genes, and employed a robust pipeline for measuring allelic expression incorporating stringent correction for potential mapping bias to the reference genome. We performed simulation experiments, assessing several statistical approaches for the detection imprinting. We identified a total of 59 imprinted genes (10% FDR), with 23 (39%) observed in both populations. Our analysis identified 31 genes that have been previously reported as imprinted, while 28 represent putative novel imprinted genes. We identified multiple gene clusters where novel imprinted transcripts showing weak parental expression bias were located adjacent to known strongly imprinted genes. For example, we identified PXDC1, a gene which lies adjacent to the paternally-expressed gene FAM50B, as showing a 2:1 paternal expression bias. Similarly ADAM23 lies ~130kb distal to ZDBF2, also exhibits ~2-fold over-expression from the paternal allele. Other novel imprinted genes had promoter regions that coincide with sites of parental bias of DNA methylation identified in uniparental disomy samples, thus providing independent validation of our results. Using the stranded nature of the RNAseq data in LCLs we identified multiple loci with overlapping sense/antisense transcripts showing opposing imprinting patterns, eg. RB1/LPAR6 and KCNQ1OT1/KCNQ1. We also identified examples of isoform-specific imprinting, and at many loci also observed clear evidence of read through of imprinted transcription beyond gene annotations. Based on this we applied a sliding window approach to analyze parental expression bias across the entire genome, identifying multiple regions outside of annotated transcripts with evidence of imprinted transcription, suggesting putative novel imprinted lncRNAs. Our data provide the first robust map of imprinted gene expression in the genome, identifying many novel imprinted genes, and providing new insights into the nature of genomic imprinting.

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T16 - SMPD4 mutations link primary microcephaly and severe encepahlopathy to aberrant cytokinesis and membrane ceramide metabolism

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The importance of complex lipid metabolism in neurometabolic disorders has long been known in relation to deficiencies of lysosomal enzymes, i.e. linked to progressive neurodegeneration. Their role in neurodevelopmental processes, e.g. cell division, instead, starts to be explored, thanks to modern technologies such as mass spectrometry. A putative neutral sphingomyelinase, distinct from the acid lysosomal sphingomyelinase deficient in Niemann-Pick type A/B, is coded by SMPD4. SMPD4 knock-down by RNA interference in HeLa cells causes dysregulation of lipid composition and localization, with accumulation of different ceramide-containing lipids, but sphingomyelin, leading to abnormal cytokinesis, the latest phase of mitosis leading to final splitting into daughter cells (Ekin Atilla-Gokcumen et al. 2014). In four children from a large consanguineous pedigree we identified by RNASeq in the linkage area identical non-coding homozygote SMPD4 mutations leading to lack of normal transcript. The mutation was undetected by whole exome sequencing, being outside the captured coding areas, and by Sanger sequencing because of an interfering pseudogene. All children presented at birth with congenital arthrogryposis, microcephaly with consistent simplified gyral pattern and mild cerebellar hypoplasia on brain MRI and died soon after birth of central hypoventilation and untreatable seizures. One girl survived up to three years: her OFC remained around â€“ 5SD and her post-mortem brain MRI showed diffuse white matter volume loss and thin corpus callosum, simplified gyral pattern, without signs of dys/demyelination. Additionally, five similarly affected children from two unrelated families were found to have biallelic SMPD4 mutations, for a total of nine affected individuals. Our observations identify a novel pathway in the pathogenesis of cerebral malformation.

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Abstracts Posters

P01-P31

P01 - Skewed X-inactivation is common in the general female population

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X-inactivation is a well-established dosage compensation mechanism ensuring that genes on the X-chromosome are expressed at comparable levels in males and females. Female carriers of X-linked recessive disorders may develop symptoms in case of preferential inactivation of the chromosome carrying the healthy allele. Skewed X-inactivation is often explained by negative selection of one of the alleles. We demonstrate that unbalanced expression of the paternal and maternal X-chromosome is common in the general population and that the random nature of the X-inactivation mechanism is sufficient to explain the preferential expression of one of the two X-chromosomes. To demonstrate this, we analysed blood-derived RNA-seq data from female children from the Genome-of-the-Netherlands (GoNL) project. In the GoNL project trio whole genome sequencing was performed, making it possible to establish the parental origin of all heterozygous X-alleles. We calculated the median ratio of the paternal counts over the total counts at all heterozygous SNPs on the X-chromosome covered by more than 10 reads. Median ratios could be far away from 0.5, and we observed expression of only one of the X-chromosomes in two individuals. These empirical observations can be explained by a theoretical model in which random X-inactivation occurs in an embryonic stage where 8 cells give rise to the blood cell compartment . Our observations are consistent with more commonly applied assays measuring the methylation status of the AR locus, but now extend these observation to the entire X-chromosome. Strikingly, we find that many of the genes known to escape X-inactivation demonstrate a similar skewing as the non-escapee genes, and are not escaping from X-inactivation in the blood. Collectively, our data suggest that skewed X-inactivation is common in the population and that this may contribute to manifestation of symptoms in carriers of recessive X-linked disorders.
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Keywords: Epigenetics, X-linked disorders, X-inactivation, RNA-seq, genome sequencing

P02 - Full-length mRNA sequencing uncovers a widespread coupling between transcription and mRNA processing

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The full control of gene expression requires tight coordination of regulatory mechanisms at the transcriptional and post-transcriptional level. To gain insight in this process we studied the interdependence of transcription, splicing and polyadenylation events on single mRNA molecules. Using full-length mRNA sequencing in MCF-7 breast cancer cells and three human tissues, we found an unexpectedly high number of genes that demonstrate interdependency between transcription and mRNA processing events, which can span the entire length of the mRNA molecules. Our analysis shows that alternative poly(A) sites coupled with alternative splicing events are depleted for known poly(A) signals and enriched for MBNL binding motifs, supporting a dual role of MBNL proteins in regulating splicing and polyadenylation. We predict thousands of open-reading frames from the sequence of full-length mRNAs, facilitating a more sensitive proteo-genomics analysis of MCF-7 mass-spectrometry data. Our findings demonstrate that our understanding of transcriptome complexity is far from complete and provides a framework to reveal largely unresolved mechanisms that coordinate transcription and mRNA processing.

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Keywords: full-length RNA sequencing; alternative splicing; proteomics; mass spectrometry; alternative polyadenylation; MBNL protein family; single-molecule sequencing; transcriptome; RNA structure; post-transcriptional regulation

P03 - A Zebrafish Model for Pontocerebellar Hypoplasia.

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Pontocerebellar hypoplasia (PCH) represents a heterogeneous group of congenital neurodegenerative diseases. Patients suffer from severe mental and motor impairments, caused by atrophy of the pons and cerebellum. So far, ten subtypes (PCH1-10) are described based on clinical features and/or genetic causes. Most genetic aberrations are located in genes with a function in transfer RNA (tRNA) metabolism pathways, e.g. tRNA Splicing Endonuclease 54 (TSEN54) or Cleavage And Polyadenylation Factor I Subunit 1 (CLP1). Hitherto, it is unknown if and how faulty tRNA processing leads to pontine and cerebellar degeneration. To elucidate the consequences of aberrant tRNA processing, a zebrafish line with a nonsense mutation in tsen54 was created using ENU mutagenesis. Survival of homozygous offspring was assessed and pathologically examined. Zebrafish with aberrant tsen54 did not survive over 26 day post fertilization (dpf); Knockout zebrafish died between 15dpf and 26dpf. The total body volume increase between 7dpf and 19dpf was less than that of controls. Brain volumes did not showed to be more severely effected then other tissue. We show here that the a knockout of tsen54 results in early dead between 15dpf and 26dpf. Additionally general hypoplasia, with no special effect on the brain, was seen. This deviates from findings in humans with PCH2a carrying TSEN54 mutations were only the pons and cerebellum are affected. Further research must show if aberrant levels of tRNA are present in the fish and if these are comparable to human levels.

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Keywords: Pontocerebellar Hypoplasia, TSEN54, Zebrafish

P04 - ASSOCIATION OF RS182429 VARIANT OF THE T-CELL ACTIVATION RHO-GTPASE ACTIVATING PROTEIN (TAGAP) IN PAKISTANI RHEUMATOID ARTHRITIS PATIENTS

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Variations in TAGAP have been associated with various autoimmune diseases including Rheumatoid Arthritis (RA). Its role in the activation of T-cells is what might make this gene important in autoimmune diseases. Genome-wide association studies suggest association between TAGAP and RA in various populations. This is the first study that sought to investigate any association of TAGAP with RA in Pakistani population. For this, two single nucleotide polymorphisms of TAGAP were investigated which have been implicated in RA liability of European patients (rs182429 A/G and rs212389 A/G). We genotyped these variants in 186 Pakistani RA patients and 185 controls using Taqman genotyping assays. Genotypic and allelic frequency distributions were estimated for both variants. Genotypic frequency distribution of rs182429 showed significantly higher frequency of heterozygous AG genotype in cases (48.92%) than in controls (36.2%) while homozygous GG genotype frequency showed contrary results (Cases: 12.37%; Controls: 25.41%). Allele frequency distribution of rs182429 showed significantly higher allele A frequency in patients than in controls. Higher AG genotype frequency in patients and GG in controls suggests former a disease susceptible and later a protective genotype in Pakistani population. Similarly, higher allele A frequency in cases make it a disease susceptibility allele, while G allele has contrary role in RA for Pakistani population. These observations confirmed the association of rs182429 of TAGAP with RA in Pakistan. Conversely, variant rs212389 showed no association with RA in this population. Moreover, exploring functional significance of these SNPs may lead to identification of novel therapeutic targets for RA.

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Keywords: Rheumatoid arthritis; disease susceptibility; single nucleotide polymorphism; genotyping; case/control study

P05 - Direct visualization of repetitive genome structures for the genetic diagnosis of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the more common inherited myopathies affecting 1:8,500 individuals. FSHD is caused by misexpression of DUX4 in skeletal muscle. DUX4 is a transcription factor normally expressed in cleavage stage embryos but silenced in somatic tissue. The DUX4 gene is embedded within each unit of the D4Z4 macrosatellite repeat array on chromosome 4, which is polymorphic in size normally ranging between 8-100 units, and adopts a repressive chromatin structure in somatic tissue. Derepression of DUX4 in FSHD muscle can be established by two mechanisms: commonly by a contraction of the D4Z4 repeat to sizes between 1-10 units (FSHD1) or more rarely by mutations a the chromatin repressor SMCHD1 (FSHD2). The genetic diagnosis of FSHD can be very challenging because of several complicating factors. Amongst these are the size of the repeat ranges from 3.3kb (1 unit) to >330kb and the observation that DUX4 expression in skeletal muscle depends on the chromosomal background of the D4Z4 repeat due to the polymorphic nature of the DUX4 polyadenlyation signal. FSHD diagnosis is not amenable to short read NGS and therefore the genetic diagnosis of FSHD is routinely performed by Southern blotting, preferably on high molecular weight DNA and by pulsed field gel electrophoresis, elucidating FSHD-associated repeat contractions and discriminating between the different D4Z4 variants by different restriction enzymes and fluorescent or radioactive DNA probes. As the Southern blot method is laborious, complex and time consuming we tested two innovative alternatives to diagnose FSHD. Molecular combing is a method in which whole genome 200-2,000 kb DNA fibers are stretched on a glass slide. Hybridization of locus specific fluorescence probes generate a genomic Morse code in which the D4Z4 repeat size on the different chromosomes can be established by detection and analysis software. Genome mapping by Bionano Genomics is established by enzymes to create nicks in genomic DNA after which fluorescent nucleotides are incorporated which generate a unique sequence specific pattern. Linearized genomic DNA strings are generated using NanoChannel arrays after which the unique sequence specific pattern is visualized and analyzed by dedicated software. Our results show that both technologies are uniquely suitable to create high resolution maps of repetitive regions in the genome and can accurately diagnose FSHD.

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Keywords: Genome structure, direct visualisation, FSHD, DNA rearrangements, molecular combing

P06 -Multidisciplinary Dutch Guideline for Genotyping in Case of Fetal Ultrasound Anomalies

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Pregnant women in the Netherlands can choose to have a fetal ultrasound. When anomalies are detected, women can opt for an amniocentesis or chorionic villus biopsy. The standard tests performed are QF-PCR followed by karyotyping or array. When results are normal, additional genotyping is performed following local practice as there are currently no standard testing protocols for all Dutch perinatal centers.
A team of clinical geneticists, clinical laboratory geneticists, gynecologists, a pediatrician and a pediatric neurologist spent two years preparing a multidisciplinary guideline for standardizing additional fetal genotyping in the Netherlands. The team worked with the mandate of their professional association and was assisted by the experienced staff of the Dutch Foundation of Medical Specialists. Guidelines were developed according to the Appraisal of Guidelines for Research & Evaluation II (Brouwers 2010, www.kennisinstituut.nl), with quality of evidence and strength of recommendations assessed following GRADE methodology (Schünemann 2013). Patient perspective was also investigated.
The resulting guidelines provide evidence-based recommendations for relatively frequent fetal conditions: nuchal translucency >3.5 mm, ventriculomegaly, agenesis of the corpus callosum, skeletal dysplasias and club feet.
The guideline recommends:

- For nuchal translucency >3.5 mm with associated anomalies (e.g. congenital heart defect), genotyping for Noonan syndrome is indicated.
- If a skeletal dysplasia is suspected, additional genotyping depends on the fetal anomalies on ultrasound and the time needed for analysis.
- In cases of isolated nuchal translucency >3.5 mm, ventriculomegaly, agenesis of the corpus callosum or club feet, there is no evidence-based indication for performing additional genotyping.
- Multidisciplinary counseling and treatment should be performed in a prenatal diagnostic center by a multidisciplinary team.

The guideline will be implemented following approval by professional associations.
Evidence-based research in this field remains limited by the low numbers of cases included in studies, the diversity of the congenital anomalies and the low incidence of specific syndromes. In future, prenatal whole exome sequencing will be essential and allow detection of a wide variety of genetic syndromes in the fetus.

Ref: Brouwers MC, Kho ME, Browman GP, et al. AGREE Next Steps Consortium. AGREE II: advancing guideline development, reporting and evaluation in health care. CMAJ. 2010;182(18):E839-42.
Schünemann H, Brożek J, Guyatt G, et al. GRADE handbook for grading quality of evidence and strength of recommendations. Updated October 2013. The GRADE Working Group, 2013.P06

P07 - Diagnosing Danon disease: a case-report of a young boy and his deceased mother

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Case description: We describe a previously symptomless 14-year-old boy suffering out-of-hospital cardiac arrest due to an extremely hypertrophic cardiomyopathy. He was resuscitated and an ICD was placed. Physical examination was normal, no dysmorphologic features were seen and no signs of muscular myopathy or other neurological problems were found. His mother died at the age of 31 years because of dilated/hypertrophic cardiomyopathy; diagnosed at that time as having peri-partum dilated cardiomyopathy. She was waiting for a cardiac transplantation, which is the only curative treatment in cardiomyopathy caused by Danon´s disease. Genetic analysis: Whole exome sequencing revealed a hemizygous mutation in the LAMP2 gene in leucocytes of the boy (c.127delT, p.Tyr43fs (exon 2)). Sanger sequencing of DNA isolated from the mother´s preserved cardiac cells showed that she was heterozygous for the same mutation. The mutation had occurred de novo in the mother. Morphological and immunohistochemical (IHC) analysis of cardiac muscle cells (mother): Autophagic vacuoles were observed with glycogen accumulation. There was a clear negative LAMP2-protein expression by IHC (scattered coloration of the protein was visible, probably due to X-inactivation in a female patient). Discussion: Danon's disease is a rare X-linked inborn error of metabolism and is characterized by the combination of cardiomyopathy, muscle weakness and intellectual disability. This case illustrates the difficulty of diagnosing Danon's disease in patients without the traditional symptoms, and an unclear family history. Clinicians should not rule out genetic causes of cardiomyopathy in patients without a positive family history for HCM/DCM, nor based on not observing expected clinical findings in the patient. Exome based panels are helpful in diagnosing these type of rare conditions.

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Keywords: Danon Disease Antopol disease glycogen storage cardiomyopathy glycogen storage disease type IIB GSD IIB lysosomal glycogen storage disease without acid maltase deficiency pseudoglycogenosis II vacuolar cardiomyopathy and myopathy, X-linked Inborn error of metabolism Cardiomyopathy

P08 - A specific de novo missense mutation in SMARCB1 causes severe intellectual disability and hydrocephalus

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Here, we describe four patients with a similar de novo missense mutation (c.110G>A; p.Arg37His) in SMARCB1, with a phenotype that does not resemble Coffin Siris syndrome. The mutations were identified by whole exome sequencing. All four patients have profound intellectual disability (ID) with absent verbalization and severe motor delay. Furthermore, they had an enlarged choroid plexus and overproduction of cerebrospinal fluid. This was severe in three patients, who developed hydrocephalus and required shunting and multiple shunt revisions. In one patient, surgical removal of the choroid plexus was necessary. The remaining patient showed transient signs of increased intracranial pressure. Other shared features were severe neonatal feeding difficulties, congenital heart- and kidney anomalies, congenital eye abnormalities, joint hypermobility, obstructive sleep apnea and a history of anemia. The SMARCB1 gene encodes a subunit of the SWI/SNF-complex involved in chromatin remodeling. Different mutations in this gene can give rise to very different phenotypes. Loss of function mutations cause the rhabdoid tumor predisposition syndrome [MIM: 609322], characterized by an increased risk for rhabdoid tumors in childhood. In addition, several loss of function mutations have been described in families with schwannomatosis [MIM:162091]. Missense mutations in exon 8 and 9, which encode the SNF5 domain of the SMARCB1 protein, are known to cause Coffin Siris syndrome [MIM: 614608], a syndrome characterized by intellectual disability (ID), coarse facial features and fifth digit anomalies. The mutation identified in the patients that we described is located in the N-terminal region of the gene, which contains a winged helix DNA binding domain. As shown by homology modeling, the Arg37 amino acid specifically is important for the function of this domain, presuming that an alteration of this amino acid will affect the DNA binding domain. Based on the observation of a recurrent mutation, we suggest that this mutation exerts a gain of function effect. In conclusion, a p.Arg37His mutation in the DNA binding domain of SMARCB1 causes a syndrome characterized by severe ID and hydrocephalus, therewith broadening the spectrum of syndromes associated with mutations in SMARCB1.

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Keywords: SMARCB1, intellectual disability, hydrocephalus

P09 - A zebrafish model for small-fiber neuropathy

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Small-fiber neuropathy (SFN) patients experience a spectrum of painful sensations; they have aberrant temperature responses and in nerve biopsies the density of the small-fibers is decreased. Gain-of-function mutations in SCN9A and SCN10A, have been identified as an underlying genetic cause. To further unravel the genetic aspects of SFN, members of our Consortium (Propane) apply unbiased sequencing approaches to identify genetic variants in a large patient population with SFN. Our aim is to develop a zebrafish model of SFN which allows us to test the pathogenicity of these identified variants; in order to do this we set up and validated a panel of read-out parameters reflecting SFN in zebrafish. Our read-out panel is based on clinical-diagnostic tests and exists of behavioral tests and morphological characteristics. For the behavioral experiments a customized ZebraBox has been established which allows us to quantify the temperature response. A zebrafish line called; sensory:GFP is being used as morphological read-out. Validation of this panel has been performed using various methods (overexpression, morpholino-mediated knockdown and a knockout line). By applying this panel we have demonstrated that expressing the human pathogenic SCN9A p.(I228M) mutation in zebrafish results in an aberrant temperature sensitivity. Furthermore, these zebrafish have a decreased density of the small nerve fibers, meaning that we have created a zebrafish model of SFN. Our next step is to test the pathogenicity of variants that are identified within our SFN patient population. Furthermore, this panel will be used to test the analgesic properties of novel compounds.

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Keywords: Zebrafish, small-fiber neuropathy, model and SCN9A

P10 - Wole Exome Sequencing as first line diagnostic test for all genetic disorders

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Whole Exome Sequencing (WES) has been adopted by many laboratories to detect single nucleotide variants (SNVs) and copy number variants (CNVs) in a diagnostic setting . It was recently shown that WES is more cost effective and less time consuming if performed for all patients regardless of their genetic disorder. This, however, requires WES to detect the vast majority of clinically relevant SNVs and CNVs that are currently being detected using Sanger sequencing, panel-based WES, and microarray-based CNV analysis. Hitherto, a SNV and indel sensitivity of =97% and =85%, respectively, was proposed by the VKGL. In this retrospective study, we set out to determine the sensitivity of WES to identify SNVs, indels and CNVs previously reported to be of diagnostic relevance. We compiled a list of clinically relevant genes consisting of 3,623 genes that are tested by our in-house diagnostic gene panels. For these genes we extracted all SNVs and indels that had been evaluated for their clinical relevance or are reported in HGMD professional. This resulted in a total of 147,760 class 3 â€“ 5 variants in 51,714 exons. Similarly, we extracted all pathogenic CNVs from our in-house microarray database, resulting in 1,754 CNVs. The sensitivity of WES towards those 149,514 SNVs and CNVs was determined on the basis of the median coverage in 50 exomes from 25 men and 25 women. Exon-wise analysis showed that 97% of all exons of clinically relevant genes and 98% of all exons with known pathogenic variants are sufficiently covered (= 20x). In total, at 96.9% of the genomic positions with pathogenic variants the coverage allows correct detection of SNVs and indels. Our in-house pipeline detects CNVs from WES if they encompass at least three baits. This holds true for 87.4% of the potentially pathogenic CNVs, while the detectability is slightly higher for sequence gains (89.2%) than for losses (85.8%). Taking SNVs, indels and CNVs together, we are able to detect 96.8% of all disease-causing variants. In conclusion, we evaluated the possibility for WES to replace Sanger sequencing and array-based diagnostics. Our results show WES is able to identify 96.8 % of the reported disease-causing variants, which is in close proximity of the guidelines proposed by the VKGL. Whereas some regions are insufficiently covered by WES to identify disease-causing variants, these gaps may be filled by adapting targeting kits or by using other targeted re-sequencing technologies in parallel to WES.

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Keywords: Technologies, WES, Diagnostics, Variant Detection

P11 - Multi-cancer phenotype including colorectal and breast cancer of patients with biallelic NTHL1 mutations

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Biallelic germline mutations affecting NTHL1 predispose to the development of adenomatous polyposis and colorectal cancer (CRC), but the previously described clinical features of families and individuals suggest a broader spectrum of malignancies associated with this germline aberration. In this study, we identified eleven novel unrelated index patients with biallelic NTHL1 germline mutations by genotyping 2,670 individuals with colorectal polyposis or familial CRC using NTHL1 targeted sequencing n=828, p.Gln90* genotyping n=1842, or by alternative discoveries. Combined with the clinical characteristics of six previously published families, we collected clinical data of 27 individuals with biallelic NTHL1 mutations 13 men/14 women from 17 families. Next to adenomatous polyps, 25 individuals 93% developed one n=10 or multiple n=15 malignancies. In total, 13 types of tumours were encountered, affecting the colorectum, breasts, duodenum, endometrium, urothelium, brain, skin, head and neck region, hematologic sytem, cervix, prostate, thyroid, and pancreas. By Kaplan-Meier analyses we calculated the cumulative risk for CRC and extracolonic cancer to be 42% 95%CI 25-66 and 60% 95%CI 41-80 by age 60, respectively. Furthermore, whole-exome sequencing data of four different tumour types derived from individuals with biallelic NTHL1 mutations showed a unique somatic mutational signature, characterized by an enrichment of C>T transitions at non-CpG sites. Individuals with biallelic NTHL1 germline mutations have a high lifetime risk to develop a wide range of benign and malignant tumours. We recommend that NTHL1 germline mutation testing is included in gene panel analyses for individuals with multiple primary malignancies or adenomatous polyposis. At present, surveillance is advised for the colorectum, breasts, and duodenum, and may be considered for the endometrium.

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Keywords: NTHL1, adenomatous polyposis, colorectal cancer, breast cancer, lifetime risk, mutational signature

P12 - Breakpoint characterization of a rare alpha-thalassemia deletion using Next Generation Sequencing

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We describe the characterization of a breakpoint fragment of a rare alpha0-thalassemia deletion found in a family of North European origin. Targeted sequencing of the breakpoint fragment using TLA and capturing probes were used to enrich for the region of interest. The breakpoint was determined by Next Generation Sequencing. Hemoglobinopathies are the most common monogenic disorders in the world population. Alpha-thalassemia is characterized by the reduced synthesis of the alpha-globin chain of the tetrameric haemoglobin protein leading to microcytic hypochromic anemia in carriers and HbH disease or the lethal Hb Bart´s Hydrops Foetalis Syndrome in homozygotes. The most common molecular causes of alpha-thalassemia are deletions, usually one of both duplicated alpha-genes are deleted but less commonly large deletions of unknown length can be detected by MLPA. Targeted Locus Amplification (TLA, Cergentis) was used in combination with capture baits designed to recognize fragments from the alpha- and beta-globin gene clusters to enrich for the breakpoint fragment in isolated DNA of the carrier, followed by NGS. The breakpoint sequence was confirmed by designing gap-PCR primers and subsequent amplification and direct sequencing of the breakpoint fragment.

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Keywords: thalassemia, Next Generation Sequencing, TLA

P13 - ATAD3A deletions: a challenge in prenatal diagnosis

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The ATAD3 gene cluster is part of the ATPase family AAA-domain containing proteins consisting of three paralogs, ATAD3A, ATAD3B and ATAD3C located in tandem on chromosome 1p36.33. The ATAD3 genes encode mitochondrial membrane proteins that contribute to the stabilization of large-mitochondrial protein complexes. Recently, deletions in the ATAD3 gene cluster were described in neonates with fatal congenital pontocerebellar hypoplasia. A pregnant female, G3P2, was referred because the loss of a previously child due to severe pontocerebellar hypoplasia, intracranial calcifications of basal ganglia, corneal clouding, multiple contractures and underdeveloped lung alveoli. Sonography of this pregnancy at 20 weeks gestational age (GA), revealed no abnormalities. However, at 28 weeks GA, multiple congenital aberrations were detected. The second pregnancy resulted in a normal child. A third pregnancy presented at 30 weeks with pontocerebellar hypoplasia and progressive microcephaly, reminiscent of the clinical picture of their earlier child. SNP array analysis revealed no aberrant CNVs and therefore fast whole exome sequencing (WES) was performed for the foetus, the diseased sister and both parents. Analysis of the exome of the foetus revealed a homozygous variant in the ATAD3B gene for which only the mother was a heterozygous carrier, suggesting a deletion on the paternal allele. Further analysis of this gene cluster demonstrated biallelic deletions of the ATAD3B/ATAD3A genes in both children and heterozygous deletions in both parents. Due to the highly homologous genes in this gene cluster it was not possible to map the exact breakpoints using the SNP array and WES results. In our neonatal clinic two additional patients with biallelic ATAD3A deletions and one patient with two ATAD3A mutations, that lead to infantile lethality, were detected indicating that it is essential to diagnose this abnormality prenatally, as it has a major impact on the obstetric and neonatal care. The results described above suggest that neither SNP array nor WES is sufficient for a precise reconstruction of the genetic defect, and that other laboratory screening methods needs to be developed and implemented.

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Keywords: whole exome sequencing, prenatal diagnostics, SNP array

P14 - Functional analysis of variants of uncertain clinical significance in the RAS and mTOR signaling pathways.

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The mechanistic target of rapamycin (mTOR) complex 1 (TORC1) is an essential protein kinase complex that controls cell growth and metabolism. The RAS GTPase is a critical component of signaling pathways controlling cell proliferation and differentiation. Activation of TORC1 signaling triggers activation of a RAS-dependent compensatory pathway. Mutations in different components of the TORC1 and RAS signaling pathway are associated with a broad spectrum of inherited and somatic diseases. We have investigated the effects of variants of uncertain clinical significance (VUS) identified in genes encoding components of the TORC1 and RAS signaling pathways. Our work has provided insight into the likely pathogenicity of the tested VUS, the genetic risks in the families segregating the tested variants, into possible genotype-phenotype correlations, and into structure-function relationships. Investigating the effects of VUS on TORC1 and RAS signaling is a useful adjunct to standard genetic testing and has been implemented in our laboratory to help improve molecular diagnostic testing. We will present an overview of our work.

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Keywords: TORC1 and RAS signaling pathway, mutations, VUS

P15 - Diagnostic clinical resequencing of polyposis-predisposition genes using single molecule Molecular Inversion Probes

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Polyposis, the formation of numerous polyps in the colon and rectum, is strongly associated with a heritable genetic predisposition and colorectal cancer development. Molecular diagnosis of hereditary polyposis is of great importance for the clinical management of patients and their relatives. To date, multiple high-penetrant genes are known that predispose to polyposis. Therefore, a fast, robust and cost-effective assay is warranted that enables resequencing of these genes. By making use of single molecule Molecular Inversion Probes (smMIPs) resequencing of gene panels is now possible with high sensitivity and in a time- and cost-effective manner1. Recently, we have successfully implemented smMIP-based resequencing for the breast cancer-related genes BRCA1, BRCA2 and PALB2 in our routine diagnostic workflow. This has led to minimal amount of rework and a shortened turnaround time. Here, we present our smMIP-based resequencing workflow of polyposis-predisposition genes in a diagnostic clinical setting and its diagnostic yield. 1 O´Roak et al, Science, (2012) 338(6114):1619-22

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P16 - Evaluation of an expanded carrier screening offer in a non-commercial setting

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Since May 2016, expanded carrier screening for 50 severe recessive disorders is available in a non-commercial hospital setting in Amsterdam, in order to facilitate informed reproductive decision-making. The screening is available for couples without a priori increased risk (no family history). Couples can apply for counseling via the website www.dragerschapstest.nl, or by physicians´ referral. Pre- and posttest counseling is provided by genetic professionals at the AMC outpatient clinic. External geneticists can send in blood samples as well. Outcome and impact of testing in the first year was evaluated. Methods A capture-based next generation sequencing strategy is used. Only pathogenic variants are reported (individual reports). CNV analyses is included. Reimbursement is possible for couples with high-risk indication (HRI) based on e.g. ancestry/consanguinity/family history. Pre- and posttest questionnaires were completed including reasons for testing, knowledge, psychological impact, and satisfaction. Partners could opt for parallel (both partners at the same time) or sequential testing. Results In the first year, 67 couples (46 with HRI) and 19 individuals (17 with HRI) visited the outpatient clinic. One couple refrained from testing. Thirty-three couples (50%) choose parallel testing. Most important reason to have testing was to avoid severe illness in the child (41%), and to avoid having a child with a severe illness (27%). Eventually, 122 individual test results were completed within 12 months, including 9 partners sequentially tested after positive tested carriers and 22 additional requests from outside the AMC. Carrier status of one (n=31 persons), two (n=5), three (n=1) or four (n=1) mutation(s) was identified. Fewer carriers were identified in the HRI group (27%) compared to the general risk group (42%). Eighty-four (69%) individuals tested negative. No carrier couples were found. Most individuals (85%) considered pre-test counseling necessary; 79% did not think this should be offered by the general practitioner. Overall, 43% believed that the costs of testing were too high. Conclusions Analyses shows that about one third of tested individuals were carriers. Although the test was primarily designed for couples with no a priori risk, two-thirds reported HRI. Our results show that, although interest in testing by couples from the general public is still relatively low, offering expanded carrier screening within regular healthcare is feasible.

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Keywords: carrier screening; expanded carrier screening; recessive; questionnaires

P17 - The Leiden experience in diagnostics for short stature: gene panel based mutation analysis by next generation sequencing on the Ion Torrent PGM

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Aims Short stature is usually classified based on phenotypic characteristics, such as pre- or postnatal onset, proportionate or disproportionate stature, and biochemical data. Defining a specific genetic diagnosis in a young child has important therapeutic consequences. Furthermore, it provides insight in recurrence risks, plays a role in family planning and contributes to knowledge on prognosis and surveillance. Routine diagnostic procedures usually include targeted gene-by-gene testing using Sanger sequencing, MLPA and SNP-array analysis which is laborious and time consuming and often does not lead to a definite diagnosis. The LUMC in Leiden is one of the Dutch national centres of expertise for growth disorders and skeletal dysplasia´s. Here we present the results of the implementation of gene panel based analysis for growth disorders, with which we aim to enhance the diagnostic efficiency and yield. Methods Gene panel based sequencing was implemented using a custom made Ion AmpliSeqTM kit followed by sequencing on the Ion TorrentTM Personal Genome Machine. Genetic variants are identified using the SeqNext module in Sequence Pilot software from JSI. Using this approach we simultaneously analyze 14 genes (COMP, FGFR3, GH1, GHR , GHSR, IGF1, IGFALS, IGFBP3, IGF1R, NPR2, NPR3, PAPSS2, SHOX, STAT5B). In addition MLPA for GH1, GHR, IGF1, IGFALS, IGF1R, SHOX and STAT5B was performed. Results With this approach we have so far identified (likely) pathogenic mutations in 24 out of 138 tested patients. Of these, 5 carried pathogenic mutations in GH1, GHR or COMP and 15 carried one or two variants of uncertain clinical significance (VUCS). Copy number variations were detected in 4 patients using MLPA (3 in SHOX and 1 in GH1). A total of 17 different VUS in 8 different genes were detected. Four patients had a variant in 2 different genes which might have been missed in a classical gene-by-gene approach. Conclusion Although our approach has a similar diagnostic yield as conventional gene-by-gene analysis, it allows a more efficient and complete analysis of growth related genes.

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P18 - Counselors´ experiences with uncertainties concerning Next Generation Sequencing. A focus group study.

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Background: Next Generation Sequencing (NGS) enables the analysis of multiple genes to improve the identification of a hereditary predisposition. NGS based panel testing for cancer is increasingly used in the diagnostic setting. However, panel tests for cancer may yield a higher level of uncertainty, for example by the increased identification of variants of unknown significance. So far, it remains unknown how uncertainties concerning panel tests and the communication thereof with counselees are experienced by counselors. Consequently, it is unknown whether counselors experience difficulty with these uncertainties. Therefore, we aimed to explore counselors´ perceptions of and experiences with uncertainty and the communication thereof with counselees concerning decisions about NGS based panel testing. Methods: Six focus groups were conducted in six academic medical centers in The Netherlands. In total, 38 counselors participated; group size ranged between 4 and 10. Counselors´ socio-demographic characteristics were assessed in a questionnaire. Topics discussed were the uncertainties experienced by counselors as well as dilemma´s and needs in the communication thereof. Focus groups were audio recorded and transcribed verbatim. The transcripts were analyzed inductively by two independent coders to provide insight in counselors´ experiences. Results: Counselors reported several uncertainties related to panel tests, such as incidental findings and inconclusive test results. These uncertainties were not necessarily experienced as problematic. However, most counselors reported difficulty in deciding to what extent and even whether uncertainties should be communicated to patients before testing. This was particularly the case for less experienced counselors. Most counselors reported to inform patients more extensively after the test, in order to restrict their information about uncertainties to those relevant for that specific patient. Counselors indicated that their needs mainly concern general agreements between counselors and between centers about the extent and manner of communicating uncertainties before testing. Conclusion: Counselors perceive various uncertainties regarding NGS based panel testing. Deciding what uncertainties to communicate is experienced as challenging. Based on these results, further research will inventory how best to communicate uncertainties concerning NGS based panel testing before testing.

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Keywords: NGS based panel testing; uncertainty; decision making; counselors; focus groups.

P19 - Targeted resequencing of coding and cardiac non-coding regulatory regions related to genes implicated in dilated cardiomyopathy

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Background: Dilated cardiomyopathy (DCM) is the most common type of disorder of cardiac muscle with a very high morbidity and mortality, leading to chronic end-stage heart failure or sudden cardiac death. In dilated cardiomyopathy, mutations in over than 40 genes encoding crucial elements of cardiomyocytes have been detected. However, the coding variants in DCM genes explain inheritance in only a third of DCM patients.
Motivation: It has been described that regulatory sequences of genes, promoters and enhancers, regulate the time, location and levels of gene expression programs. Variants in those regulatory elements can alter the binding affinity of transcription factors, thereby changing or even diminishing the expression of a regulated gene even though the gene itself is not mutated. The regulatory processes that mediate biological mechanisms of DCM remain incompletely understood and in genetic diagnostics of cardiomyopathies regulatory sequences have largely been ignored.
Methods and Results: We have performed a custom-designed targeted next generation sequencing of 113 genes previously linked to dilated cardiomyopathy, including their coding sequences, untranslated regions and cardiac-specific cis-regulatory elements (promoters and enhancers) in 38 Dutch DCM patients. Regulatory elements were designed based on a full promoter sequence spanning 1kb from transcription start site of each gene including 5'UTR sequence and known cardiac DNAse hypersensitivity sites within a window of +/-30kb from gene start. Using standard settings we have detected a possible coding pathogenic mutation in 17/36 DCM patients. Next, we have focused on 16,434 variants detected in regulatory sequences in at least one DCM patient. Variants were further prioritized using various filters, mainly based on overlap with known (cardiac) transcription factor-binding sites and regulatory signals from other types of chromatin methods (e.g. H3K27ac ChIPseq). Using various filters, we have narrowed down the list of potential disease causing non-coding variants to 0-3 per patient. In addition, we have used genotype data from 499 GoNL controls to test the mutational load in DCM cohort.
Conclusion: For the first time we have created lists of variants in regulatory elements (promoters, enhancers) of gene involved in dilated cardiomyopathy. We have performed an important exercise before moving to whole genome sequencing analyses of diagnostic panel and exome sequencing negative DCM patients.

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Keywords: targeted resequencing, dilated cardiomyopathy, coding and non-coding mutation, transcription factor binding site

P20 - Genome-wide H3K27ac chromatin profiling in healthy and remodeled human myocardium

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Background: The activity of DNA regulatory regions, such as promoters and enhancers, is a crucial factor in transcriptional regulation and can be probed by chromatin immunoprecipitation sequencing (ChIPseq) of the H3K27ac histone mark. Active promoters and enhancers contain hotspots for transcription factor binding sites (TFBSs) and are considered candidate regions harboring disease-related non-coding mutations. However, there is a lack of information about the locations of those highly tissue specific regulatory regions in cardiac tissue and how their activity differs in health and disease. Materials and results: To address this gap of knowledge we have performed H3K27ac ChIPseq in septal tissue from patients with cardiac remodelling/hypertrophy due to pressure overload caused by aortic stenosis (n=20) and healthy controls (n=5). Using Cisgenome, 40,745 and 16,734 acetylated regions were detected in at least one sample in the disease and control group, respectively. Next, we have detected 5,634 regions with more activity (hyperacetylated) and 5,724 regions with less activity (hypoacetylated) in disease and control group (DeSeq, FDR<0.05). Genes annotated to differentially acetylated regions using a +/- 5kb window were mostly enriched for cardiac function, fibrosis formation and altered cell metabolism (ToppFun, STRING). The identification of TFBSs using AME yielded 18 and 45 transcription factor (TF) binding motifs that were enriched in hyper- and hypoacetylated regions, respectively. Data-linkage analysis using BioGRID revealed 10 TF-based mechanisms that were encoded by genes in the hypoacetylated regions involved in unfolded protein response (UPR) to endoplasmic reticulum (ER) stress. Validation experiments in the murine TAC model showed RNA downregulation of UPR and ER stress targets in response to left ventricular pressure overload. Conclusion: This is the first study providing a unique overview of the H3K27ac activity in both pressure overloaded and healthy myocardium in human. Next to the detection of novel candidate genes involved in the pathogenesis of cardiac remodeling (UPR and ER stress targets), we provide a valuable resource of genomic locations of regulatory elements in healthy and remodelled human cardiac tissue. These regions can be used for interpretation of non-coding mutations found in whole genome sequencing (WGS) or genome wide association studies (GWAS) data sets of many cardiac traits, including genetic cardiomyopathies.

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Keywords: chromatin immunoprecipitation sequencing, histone modification, translational regulation, human myocardium

P21 - Enabling exome sequencing in non-genetic clinical practice: fast-WES as a routine diagnostic test

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Although whole exome sequencing (WES) is increasingly applied in clinical genetics, it is mostly used to replace the gene-by-gene sequencing paradigm that we were used to. However, several studies have shown that the possible applications of WES stretch beyond clinical genetics: in other clinical situations, particularly in the neonatal intensive care unit (NICU), an unbiased tool to make a diagnosis can be extremely useful. Several hurdles, such as proven soundness and cost-efficacy, need to be taken before a test can be implemented in a routine clinical setting. To our opinion the long turnaround time for WES has been the major barrier. The full standard WES procedure sometimes takes over six months in diagnostic labs. We managed to reduce the waiting period from several months to one week through developing a protocol that combines Agilent´s SureSelectXT workflow with rapid Illumina NextSeq sequencing. The sequencing workflow is seamlessly integrated with our bio-informatics pipeline and in-house analysis software, enabling delivery of WES reports based on high quality exome data within two weeks. We will present our workflow, as well as examples underscoring the utility of fast-WES for physicians and parents in the context of NICU. We believe that fast-WES opens many new possibilities for the introduction of WES in routine clinical practice.

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P22 - Towards molecular understanding of ZBTB18 mutations: pointing the finger?

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Background: ZBTB18 (ZNF 238) has previously been identified as contributing factor for the 1q43-44 deletion syndrome phenotype (Balliff et al., 2012). For agenesis of the corpus callosum (ACC), a key feature of this syndrome, ZBTB18 is the postulated candidate gene. Patients with mutations in this gene present with Intellectual Disability (ID) and frequent co-occurrence of ACC, hypotonia, microcephaly, growth problems and variable facial dysmorphologies, which is explained by a key role of ZBTB18 in cortical and cerebellar development. Objective: We present five new cases with a de novo ZBTB18-gene mutation, review the literature in order to substantiate a genotype-phenotype correlation and provide insight in the Zinc fingers structural change by 3D-modeling. Design and subjects: Routine diagnostic exome sequencing was performed. Patients´ phenotypes were compared to literature cases and patients identified via GeneMatcher, in order to include all ZBTB18 mutation cases known so far. Bioinfomatic modelling was done to assess the effect of the mutations in the C2H2 zinc finger domain of the ZBTB18 protein on DNA binding properties. Results: We present five new patients with a ZBTB18 mutation (3 missense and 2 frameshift mutations). Only one out of three patients who underwent MRI had corpus callosum abnormalities. Hypotonia, microcephaly, growth retardation and facial dysmorphologies were inconsistently present. We present the fourth case with the p.Arg464His mutation, which is a recurrent de novo mutation located within the C2H2 zinc finger domain of the ZBTB18 protein. 3D-modeling showed the effect of mutations in ZBTB18´s zinc finger structure differ. Conclusion: Our data contribute to further delineate the heterogeneous phenotype of ZBTB18 mutations. We partially explain the variability and incomplete penetrance of ACC by the extent that the mutations in the zinc finger domains C2H2 structure disrupt its DNA interaction.

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Keywords: ZBTB18, ZNF238, zinc finger, corpus callosum agenesis

P23 – Praktische Haalbaarheid van een Populatie-Brede Dragerschapstest voor Paren met Kinderwens Aangeboden door de Huisarts in Noord-Nederland

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Introductie: In een eerdere studie hebben we aangetoond dat de huisarts volgens professionals en de doelgroep zelf als meest geschikte aanbieder wordt gezien voor een populatie-brede dragerschapstest voor paren met kinderwens. In deze pilot-implementatiestudie onderzochten we de haalbaarheid van het aanbieden van een dergelijke test door de huisarts. De test is ontwikkeld door het UMCG en test op dragerschap van 50 ernstige autosomaal recessieve ziekten. Methoden: Voorafgaand aan de studie volgden alle deelnemende huisartsen een training gericht op kennis en counselingsvaardigheden en werd hun kennis geëvalueerd. Het test-aanbod, met verplichte pre-test counseling door de huisarts, werd gedaan aan alle vrouwen (18-40) met partner en kinderwens van de deelnemende praktijken. Een genetisch consulent superviseerde de eerste twee gesprekken van elke huisarts. In deze mixed-methods studie is de haalbaarheid van het test-aanbod door de huisarts onderzocht. Haalbaarheidscriteria: 1) = 20% van huisartsen heeft extra supervisie nodig 2) = 80% van de consulten vinden plaats binnen 20 minuten (dubbelconsult) 3) huisartsen verwijzen = 20% van 'normaal´-risico paren naar de klinische genetica. Semigestructureerde interviews met 10/13 deelnemende huisartsen over hun ervaringen met en ideeën over het test-aanbod zijn kwalitatief geanalyseerd. Met een kwantitatieve vragenlijst werd de tevredenheid van deelnemers over de counseling door de huisarts gemeten. Van elk consult noteerden huisartsen de consultduur (response n=108(83%)). Resultaten: In totaal hebben 130 consulten plaats gevonden. Alle geïnterviewde huisartsen voelden zich, na de training, in staat om adequaat te counselen. 1) 0/13 deelnemende huisartsen hadden extra supervisie nodig. 2)58% van consulten vond plaats binnen 20 minuten (mediaan 20, IQR 18-28). 3) huisartsen verwezen geen enkel 'normaal´ risico paar naar de klinische genetica. Volgens de huisartsen waren onder andere administratieve factoren zoals ontbrekende gegevens van de partner en voorkennis van de deelnemers van invloed op de duur van het consult. 91% van de deelnemende patiënten was (zeer) tevreden met de counseling door de huisarts. Conclusie: Ons onderzoek laat zien dat het aanbieden van een populatie-brede dragerschapstest aan paren met kinderwens door de huisarts haalbaar lijkt. Echter een voorafgaande training wordt als voorwaarde gezien. Verder onderzoek zal moeten uitwijzen of en hoe de consultduur verminderd kan worden.

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Keywords: preconceptiescreening [expanded preconception carrier screening], dragerschapstest, huisarts, haalbaarheid

P24 - Identification of recurrently mutated genes in ADHD by targeted sequencing

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Introduction and objectives: Attention-Deficit/Hyperactivity Disorder (ADHD) is a multifactorial neuropsychiatric disorder with complex genetic background, hampering the identification of underlying genetic risk factors. We aim to identify recurrently mutated genes in patients with ADHD by a targeted sequencing approach. We will use single molecule molecular inversion probes (smMIPs) to specifically sequence the coding regions of selected ADHD candidate genes. Methods: We selected genes, that have previously shown to harbor (rare) genetic variants associated with ADHD. ADHD candidate genes were selected by reviewing various studies: 1) whole exome sequencing (WES) studies in ADHD families and a case-control cohort, 2) exome-chip study in ADHD cases and controls, 3) copy number variations (CNV) analyses, and 4) the genes, most strongly associated with ADHD, from a recent genome-wide association study (GWAS). We designed smMIPs that cover the entire coding sequence of all selected genes. After optimization of the smMIPs protocol, DNA samples of 142 Dutch adult ADHD patients and 141 healthy controls from the International Multicentre persistent ADHD CollaboraTion (IMpACT) will be analyzed. Finally, sample size will be increased by analyzing additional samples from international IMpACT sites. Results: In total, 57 genes were selected for this study. Visual inspection of 5357 smMIPs probes, covering all coding regions, using the UCSC genome browser is currently ongoing. Discussion: Besides common genetic variants, recently identified by GWAS, we hypothesize that studies focusing on rare and low-frequency genetic variants will reveal an additional source of genetic variation for ADHD. Our study applies a targeted sequencing approach of ADHD candidate genes and although we will focus on low-frequency genetic variation, we hypothesize that the accumulation and combination of several common and rare variants, each with probably small or intermediate effect, underlies ADHD etiology.

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P25 - NPHP1 gene deletions cause ESRD in 0.9% of adult-onset cases

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Background Nephronophthisis (NPH) is the most prevalent (15%) genetic cause for end-stage renal disease (ESRD) in children. ~16% is caused by homozygous full gene deletions of the autosomal recessive NPHP1 gene. However, little is known about the prevalence of these mutations in adult-onset ESRD. With data generated to perform genome-wide association studies in adult-onset ESRD patients, we aimed to determine the prevalence of homozygous NPHP1 full gene deletions. Methods Renal transplant recipients were genotyped using the Affymetrix Axiom Tx GWAS Array, containing ~780,000 markers accross the genome with probes to cover a priori copy number variant (CNV) regions. CNVs (e.g. deletions and duplications) were determined based on median log2 ratios and B-allele frequency patterns. All findings were independently validated. In this abstract we report on 1272 cases, all Caucasian, from the TransplantLines-Genetics cohort. As we are currently analyzing ~4300 additional samples of various ethnicities, from the DeKAF Genomics, GoCAR, Dublin and Vienna cohorts (part of iGeneTRAiN), we will soon be able to report on ~5500 cases. Cases are included in the analysis when they had adult-onset ESRD, defined as start of renal replacement therapy (RRT) at any age ?18 years. Results 1250 cases in the TransplantLines-Genetics cohort met the age criteria, of whom 11 (0.9%) showed a homozygous deletion of the NPHP1 gene. Median age at start of RRT was 35 years (range 18-42), with eight cases aged ?30. Notably only three out of 11 cases (27%) were diagnosed as having NPH. The other cases (8/11, 73%) were noted as chronic kidney disease with unknown etiology (n=5), glomerulonephritis (n=1), sporadic primary reflux nephropathy (n=1) and autosomal dominant polycystic kidney disease (n=1). Conclusion NPH is a classical pediatric kidney disease. However, we show that homozygous NPHP1 full gene deletions alone cause 0.9% of all adult-onset ESRD in our dataset, with the majority of NPHP1 cases ?30 years of age. Considering that other types of mutations in NPHP1 were not analyzed, and the other 19 known NPH genes were not even investigated, NPH is a relatively frequent cause of adult-onset ESRD. As only 27% of NPHP1 cases were registered clinically as NPH, these results warrant wider application of genetic testing in adult-onset ESRD.

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Keywords: Genetics, copy number variant, end-stage renal disease, cystic kidney disease, nephronophthisis

P26 - Comprehensive histological and molecular analysis of PMS2 associated malignancies; a separate entity among MMR deficient tumours?

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Background Lynch syndrome (LS) related cancers have a different genetic background and histology compared to sporadic colorectal cancers (CRC) and show a different treatment response and survival. Up to now most studies focused on MLH1, MSH2 and MSH6 carriers, but data on PMS2 associated tumours is currently lacking. We now aim to unravel the histological and molecular hallmarks of PMS2 associated CRC. Methods We obtained informed consent of PMS2 mutation carriers and were able to collect 20 CRCs for analysis. Histological hallmarks were scored by an experienced pathologist. Moreover, to get an impression of the somatic tumour spectrum, we used the Ampliseq Cancer Hotspot panel (version 2) on isolated tumour DNA. This panel covers mutation hotspots in 50 genes (~2800 COSMIC mutations), including well known somatically mutated genes such as KRAS, APC and TP53. The same panel was used to analyse control cohorts consisting of MLH1 mutation carriers and sporadic CRC. Results PMS2 associated CRCs showed a number of LS associated hallmarks: 81% were right-sided, 43% had Crohn´s like infiltrate (missing: 15%) and 81% (missing: 15%) showed microsatellite instability. However, a majority (65%) hardly had any tumour infiltrating lymphocytes, a hallmark of Lynch associated tumours. The molecular analysis showed a relatively low proportion of TP53 and APC mutations compared with sporadic controls and a high percentage of a specific FBXW7 mutation (c.1393C>T, p.Arg465Cys). Notably, 5/20 CRCs had this transition, where the controls had none. We also found a relatively rare KRAS mutation in exon 4 (c.436G>A, p.Ala146Thr) occurring three times in the PMS2 cohort but not in the control cohort and once in MLH1 associated CRCs. Strikingly we found CTNNB1 mutations in 14/25 (60%) of MLH1 associated CRCs, but none in the PMS2 cohort. Discussion This study illustrates the separate entity of PMS2 associated CRCs, with histological and molecular characteristics that overlap with both MLH1 associated as well as sporadic CRCs. These findings indicate the possibility of a specific route to tumorigenesis in PMS2 carriers, which may suggest a different risk of developing interval cancers compared to MLH1 carriers. As current surveillance guidelines are based on studies comprised of mostly MLH1 and MSH2 carriers, clinical studies are necessary to study the possibility of elongation of surveillance colonoscopy intervals within PMS2 carriers.

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Keywords: Lynch Syndrome, PMS2, oncogenetics, colorectal cancer, cancer surveillance, mismatch repair

P27 - Pontocerebellar hypoplasia with spinal muscular atrophy (PCH1): identification of SLC25A46 mutations in the original Dutch PCH1 family

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Introduction Pontocerebellar Hypoplasia (PCH) is a group of rare neurodegenerative disorders with prenatal onset, mainly but not exclusively affecting pons and cerebellum. PCH is both clinically and genetically heterogeneous, comprising 10 types that are distinguished on differences in genotype and/or phenotype. PCH1 is characterized by the additional presence of anterior horn cell degeneration. At the extreme end of the PCH1 spectrum, patients lack spontaneous respiration and die shortly after birth. Mutations in EXOSC3 are an important cause of milder forms PCH1, but the genetic cause in these severely affected patient remained unknown until recently. In this study we show that mutations in the SLC25A46 gene, involved in mitochondrial fission and fusion, cause PCH1 in the Dutch family that was originally described to delineate the PCH1 phenotype. In line with this, mutations in SLC25A46 gene were previously identified in a range of optic atrophy spectrum disorders, including Charcot Marie Tooth neuropathy and congenital lethal PCH. Methods & Results With whole exome sequencing, a heterozygous mutation leading to a premature stop codon (c.691C>T | NM_138773; p.R231* | NP_620128) in exon 8 of the SLC25A46 gene was identified, but no additional truncating or missense SLC25A46 mutation was detected. Analysis of patient fibroblast mRNA showed monoallelic expression of the SLC25A46 allele with the stop mutation in exon 8, indicating loss of expression of the other allele. To explain this loss of expression, we proceeded with whole genome sequencing and detected a heterozygous deletion of ~2.4 kb encompassing exon 3 of the SLC25A46 gene. Exon 3 contains 58 nucleotides and this deletion therefore results in a frameshift. Immunoblot showed absence of SLC25A46 protein in patient fibroblasts, suggesting instability of the SLC25A46 mRNA or its protein. Conclusion We identified the causal SLC25A46 variants in the original Dutch family that was exemplary for the delineation of PCH1 as a distinct clinical subtype. We suggest classifying SLC25A46-associated PCH1 as PCH1D (mutations in VRK1, EXOSC3 and EXOSC8 are associated with PCH1A, PCH1B and PCH1C, respectively). PCH1D is clinically distinguishable from other PCH1 subtypes by optic nerve involvement, respiratory failure and early death and is at the most severe end of the broad spectrum of SLC25A46-related conditions.

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P28 - Novel ACAN mutations in four children with short stature from two families without consistently advanced bone age

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Objective Heterozygous ACAN mutations have been reported to cause short stature associated with advanced bone age and various other clinical features. We used whole exome sequencing to identify the genetic cause of short stature observed in 4 children belonging to 2 families, and found two novel ACAN mutations. This enabled us to further expand the phenotype of patients with ACAN mutations. Methods The exome was sequenced in 2 siblings and both parents from one family (A), and in two siblings from another family (B). All children were born at term with normal birth weights. The children (2.6-7.6 yrs) presented with short stature (height -3.3 to -2.4 SDS), SH/H ratio ranging from +0.75 to +3.0 SDS, and armspan/H ratios ranging from 0.93 to 1.0. Bone ages varied from delayed (7 and 24 months) in two children to equal to chronological age and advanced (+1.1 yrs) in one child. At follow-up, bone age was still delayed in 1 patient, consistent with chronological age in 2 children, and advanced in 1. In both families 1 parent also had short stature, suggesting autosomal dominant inheritance. The exome sequences were analysed with a stringent post-sequencing annotation pipeline including a gene panel of 109 genes for filtering of the data. Results In all 4 children a heterozygous nonsense mutation in the ACAN gene was identified, inherited from the mother in family A (height -4.1 SDS), segregation analysis in family B is still ongoing. Several of their family members are known with short stature. The mutations were located in the G1 domain (c.706C>T,p.(Arg236*)) in family A, and in the GAG attachment region (c.6673C>T,p.(Gln2225*)) in family B. In three short relatives belonging to family B severe osteochondritis dissecans was observed as an extra clinical manifestation. Conclusion Two novel heterozygous nonsense mutations in ACAN were identified in two families with short stature, with osteochondritis dissecans segregating with short stature in one family. Remarkably, the bone age was only advanced in one of the four children, whereas in the other three children the bone age varied from delayed to consistent with chronological age. This indicates that the absence of advanced bone age should not be considered a contraindication for testing for ACAN mutations in children with short stature.

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Keywords: ACAN, short stature, advanced bone age, osteochondritis dissecans, genetics

P29 - Major contributors identified for increase in diagnostic yield in our 10 years of experience in genetic testing for cardiomyopathies; data sharing, titin (TTN) mutations and stricter clinical inclusion criteria

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Introduction: Inherited cardiomyopathies are defined by structural and functional abnormalities of the myocardium, characterized by extreme genetic and clinical heterogeneity. In the Erasmus Medical Center genetic testing is offered to patients with hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and noncompaction cardiomyopathy (NCCM). Materials and Methods: From 2006 till 2012 we performed Sanger sequencing of eight sarcomere genes. In the past four years, we applied a targeted next generation sequencing (NGS) 52 gene panel approach for ~900 patients. For the targeted approach we included the Titin gene (TTN), encoding the largest known human protein that plays a central role in sarcomere organization. We evaluated the diagnostic yield by comparing NGS gene panels with Sanger sequencing. Results: Sanger sequencing of eight genes resulted in a pathogenic mutation in 40% of the HCM patients. Using NGS, the majority of mutations in HCM were detected in genes, previously tested with Sanger sequencing. The increase in diagnostic yield from 40% to 50% is mainly achieved by data sharing and by re-evaluating patients with stricter clinical inclusion criteria. NGS in DCM and NCCM patients led to a doubling of the diagnostic yield, from 15% to 30%, mainly explained by truncating mutations in TTN in exons 259-359, encoding the A-band of Titin. Conclusions: Targeted NGS has proven most beneficial for NCCM and DCM patients, resulting in doubling the diagnostic yield. The increased yield for HCM patients was mainly achieved by stricter clinical inclusion criteria together with data sharing, resulting in reclassification of many missense variants.

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P30 - MAMLD1 deletions in three patients with proximal hypospadias

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Hypospadias is a congenital malformation that has a prevalence of 4-43:10.000. Distal or (sub)glandular hypospadias is far more common than proximal hypospadias. In most cases of isolated distal hypospadias inheritance is multifactorial, while the more proximal anomalies are part of the spectrum of Disorders of Sex Development (DSD) and can have a variety of genetic defects. MAMLD1 is one of the genes associated with hypospadias, although functional studies in mouse KO models do not support this as the male knockout mice do not show hypospadias and have normal fertility. Patients with a contiguous syndrome involving the MTM1 gene and MAMLD1 have been described as well as patients with deletions encompassing IDS and MAMLD1 not showing hypospadias. We present the findings in two sibs with proximal hypospadias and a very small deletion of MAMLD1 confirmed with MAQ assay and a third patient with Hunter syndrome and hypospadias carrying a deletion encompassing the IDS gene, which extends to the first exon of transcript 1 of MAMLD1.

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P31 - A zebrafish model of classic galactosemia: paving the way for new insights on this metabolic disorder

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Classic galactosemia (CG) is an inherited metabolic disorder caused by a severe deficiency of galactose-1-phosphate uridylyltransferase (GALT), a key enzyme in galactose metabolism. Current standard of therapy â€“ lifelong galactose-restricted diet â€“ is life-saving in the neonatal period but fails to prevent the development of chronic complications in brain and ovaries. We have developed a zebrafish model of CG, aiming to gain new insights on brain and gonadal damage throughout development. Knockout (KO) galt zebrafish were generated using a TALEN-strategy. Biochemical and clinical phenotypes of KO fish were thoroughly investigated. Analysis of galt-enzyme activity revealed a severe impairment in KO embryos, as well as in brain and gonads of adult fish. Following exposure to galactose, KO embryos accumulated high levels of galactose-1-phosphate (substrate of GALT). qPCR was performed to evaluate galt tissue-specific expression (brain and gonads) in adult wildtype (WT) fish. Other key enzymes of galactose metabolism (galk1, gale, aldose reductase and ugp) were also investigated by qPCR and catalytic activity in WT and KO fish. Neurological impairment was evaluated in WT and KO embryos (5 dpf) and juvenile fish (4 weeks old) by quantifying motor activity. KO and WT embryos showed similar activity, whereas KO juvenile fish showed a decreased motor activity. Fertility was evaluated by periodic crossings of WT, heterozygous and KO fish. Breedings involving KO female exhibited an increased number of unsuccessful crossings and a lower egg quantity. Egg quality was unaffected. Further phenotypic characterization in CG zebrafish is underway. We have crossed galt KO fish with reporter lines that carry brain- and gonad-specific promoters driving GFP expression (mbp:GFP and vasa:GFP respectively), which will allow studying brain and gonadal damage from embryonal stage to adulthood. We have successfully developed a zebrafish model of CG that recapitulates the human phenotype. The unique features of zebrafish allow to successfully model this metabolic disorder, providing a valuable platform to study its pathogenic mechanisms and onset, as well as to develop new therapeutic strategies.

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