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Abstract Talks

T01-T16

T01 - NGS expanded carrier screening in the Netherlands: initial implementation results

K. Abbott, T. Dijkhuizen, M. Veldhuis, J. Schuurmans, I. van Langen, R. Sinke

Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands

Introduction: Expanded carrier screening (ECS) has broadened in recent years from high risk population-targeted testing to general public screening. and the main challenge now is choosing the most applicable test design for the intended population. Here we describe the ECS test developed at our department of Genetics and our initial results. Materials and Methods: Based on focus group discussions, we designed and implemented a couple-based ECS multi-gene NGS test using Agilent SureDesign. Our first design included 50 rare, early onset and serious recessive Mendelian conditions. The test was later expanded to 70, including a few more prevalent but treatable conditions like cystic fibrosis. Concentrating on couple-based screening, emphasis was on the combined risk for having affected children. The a priori risk of being a carrier couple is approximately 1 in 150 and increases for those referred for medical reasons (e.g. consanguinity). Only results with high predictive value regarding affected offspring were reported in the combined result. Results: A total of 169 couples were tested, 52 potential high-risk couples and 117 general public couples as part of an population-based implementation study. 5 couples, referred for diagnostic reasons, shared carriership of one of the diseases tested. All remaining couples tested normal. Reporting times averaged at 38 days, and in some cases results were returned within 2 weeks. Conclusions: Our combined approach to ECS testing allows for a fast, simplified procedure to report combined risk to couples, eliminating the burden of individual findings. Based on these results a broader implementation of the test (e.g. general public via their GP) has recently been started. Future international discussions will guide further development of such important screening tests.

E-mail: k.m.abbott@umcg.nl
Keywords:

T02 - Aggregation of population-based genetic variation over protein domain homologues via MetaDome strongly improves diagnostic prediction of missense variants

Laurens van de Wiel, Daan Gilissen, Hanka Venselaar, Joris A. Veltman, Gerrit Vriend and Christian Gilissen

Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, 6525 GA, the Netherlands Centre for Molecular and Biomolecular Informatics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, 6525 GA, the Netherlands Bio-informatica, HAN University of Applied Sciences, Nijmegen, 6525 EN, the Netherlands Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, 6525 GA, the Netherlands Institute of Genetic Medicine, International Centre for Life, Newcastle University, Newcastle upon Tyne, NE1 3BZ, United Kingdom

The growing availability of human genetic variation has given rise to novel methods of measuring genetic tolerance and better interpret variants of unknown significance. We have developed a novel concept based on protein domain homology in the human genome to improve variant interpretation. For this purpose we map population variation from the Exome Aggregation Consortium (ExAC) and pathogenic mutations from the Human Gene Mutation Database (HGMD) onto 5,250 Pfam protein domains that cover 41% of all protein coding sequences. We observed that 71% of pathogenic missense variants from HGMD are found in such a protein domain. We then aggregated population-based and disease-causing genetic variants across 30,853 homologous protein domains into 2,750 meta-domains. We find that genetic tolerance is consistent across 97% of domain homologues in different genes ($p<0.05$, Bonferroni corrected), and that patterns of genetic tolerance faithfully mimic patterns of evolutionary conservation (Pearson 0.97, p-value < 1e-308). Interestingly, we find that 2,201 of the aggregated domain positions (0.47%) are not evolutionary conserved, but still highly intolerant to normal variation while also containing one or more disease causing missense variants. Residues that are not evolutionary conserved, but nevertheless depleted of population-based variation are especially strong predictors of sites of pathogenic missense variants. An informative example concerns domain positions 17 and 21 in the "EGF-like domain" (PF00008) that are depleted of population-based variation in 60 genes and cause disease in at least 3 of these genes (NOTCH3, JAG1, and CRB2). Summing across all genes, we observed that the presence of pathogenic missense variants at aligned homologous domain positions is often paired with the absence of population variation and vice versa. We realized that this type of information could be of great benefit to genetic diagnostics and that therefore it would be helpful to have an easy-to-use web service that could provide access to this wealth of information without the need for a bioinformatics intermediate. Therefore we created the MetaDome web service. MetaDome provides a schematic protein representation with annotation of pathogenic variants found in a gene of interest and those that are homologously related. MetaDome can intuitively be used to visualize genetic tolerance and help interpret variants of unknown significance.

E-mail: laurens.vandeWiel@radboudumc.nl
Keywords: variant interpretation; functional variation; protein domain homology; evolutionary conservation; genetic tolerance

T03 - Development of an innovative method for comprehensive preimplantation genetic testing

Jeroen Meekels, Jos Dreesen, Marion Drüsedau, Chris van Uum, Kasper Derks, Han Brunner, Christine de Die-Smulders, Aimee Paulussen, Masoud Zamani Esteki

Maastricht University Medical Centre+ GROW School for Oncology and Developmental Biology

Preimplantation genetic testing (PGT), formerly called preimplantation genetic diagnosis (PGD), is an optional method for couples who wish to circumvent transmission of genetic disorders to their offspring. PGT is now being performed in three forms dedicated for different genetic conditions, namely PGT for monogenic disorders (PGT-M), PGT for structural chromosomal aberrations (PGT-SR) and PGT for aneuploidies (PGT-A). Current PGT methods, however, are laborious and time-consuming and therefore represent substantial barrier to effective reproductive genetic care. For instance, conventional PGT-M requires family- and locus-specific designs causing a long preparation time, and current PGT-SR may produce carrier offspring and cannot help all the couples afflicted with complex genetic aberrations. Couples with (multiple) small structural aberrations (<10 Mb) or with consanguinity cannot be helped using the current PGT-SR and PGT-M methods, respectively. Consanguineous couples usually have very similar haplotypes or haplotype blocks, making it difficult to distinguish between paternal and maternal haplotypes. Although it is also possible to combine the different PGT methods, it would be more invasive for the preimplantation embryos as it requires different PGT methods on different (single-cell) biopsies derived from the same embryo. Moreover, the current PGT-A methods merely profile copy-number states of the chromosomes and not their segregation origin (i.e. meiotic versus mitotic). Recently we developed a conceptual workflow, termed haplarithmisis (Greek for haplotype numbering), enabling both haplotyping and copy number typing. Haplarithmisis reconstructs genome-wide haplotype architectures and determines the copy number as well as parental and segregational origin of those haplotypes. We recently demonstrated that this method can be routinely applied as a generic method for PGT at the single cell level through tracing the disease alleles genome wide as well as determining numerical and structural chromosomal anomalies. Here we developed a next-generation sequencing haplarithmisis-based method to analyze PGT couples having disorders and/or aberrations, which are (nearly) impossible to diagnose using the current PGT practice. Being able to use haplarithmisis for these cases will drastically: (i) shorten the PGD preparation time for these families, (ii) improve their well-being and (iii) make the PGT procedure less invasive for preimplantation embryos.

E-mail: jeroen.meekels@mumc.nl
Keywords: Preimplantation genetic testing (PGT) Embryo selection Next generation sequencing (NGS) Haplarithmisis

T04 - Functional characterization of variants of uncertain significance in BRCA2: Fifty shades of BRCA2 deficiency

R.L.S. Mesman; F.M.G.R. Calleja; G. Hendriks; P. Devilee; C.J. van Asperen; H. Vrieling; M.P.G. Vreeswijk

Human Genetics, LUMC, Leiden, Zuid-Holland, Netherlands; Clinical Genetics, LUMC, Leiden, Zuid-Holland, Netherlands

During the last 20 years, genetic testing to identify pathogenic variants in BRCA1 and BRCA2 has become routine clinical practice. However, quantitative assessment of the cancer risk associated with the occurrence of a variant turned out to be less straight forward as anticipated. For many intronic and missense variants lack of clinical and family data prevents reliable estimation of their cancer risk (variants of uncertain significance (VUS)). Furthermore, recent findings challenge the existing dogma that truncating variants and variants in the canonical splice sites are always associated with high cancer risk. We have optimized and validated a previously developed (Kuznetsov et al., 2008; Hendriks et al., 2014) mouse embryonic stem cell (mES) based model system that allows functional analysis of all types of BRCA2 variants, including variants that may affect RNA splicing. The procedure involves the generation of a desired mutation in BRCA2 present in a bacterial artificial chromosome (BAC) and its subsequent introduction into conditionally knock out mBRCA2 mES cells. The performance of the assay was validated using a series of clinically proven pathogenic (Class 4/5; n=15) and neutral (Class 1/2; n=20) missense variants. Of 60 clinically relevant missense VUS, eight variants did not complement the lethality conferred by removal of the endogenous mBrca2 gene. The ability to perform homologous recombination, one of the key functions of BRCA2, varied among BRCA2 variants that were capable of complementing mBrca2 deficiency between 30-120% of wild type. Recently, a number of the hypomorphic BRCA2 variants were shown to be associated with moderate risks of breast cancer in a large scale case control study (Shimelis et al., 2017). To assess whether naturally occurring BRCA2 mRNA isoforms might be able to rescue the deleterious effects predicted to occur for exon deletions or nonsense variants, we are currently assessing the functional implications of single exon deletions and nonsense variants. Preliminary data indicate for some exons that nonsense variants are not only not lethal but still display considerable levels of homologous recombination. Ongoing efforts are now focused on establishing the relationship between functional results and the associated cancer risk.

E-mail: r.l.s.mesman@lumc.nl
Keywords: BRCA2, variants of uncertain significance, functional assays, homology directed repair

T05 - Rapid whole exome sequencing as a diagnostic test for fetal multiple congenital anomalies on ultrasound

N. Corsten-Janssen, J.C.D. Diphoorn, J. el Mecky, K. Bouman, J.B.G.M. Verheij, R. Kinds, A.J. Scheper, I.M. van Langen, R.J. Sinke, R.H. Sijmons, H. Westers, B. Sikkema-Raddatz, C.C. van Diemen

University Medical Center Groningen

Introduction Identifying the cause of fetal anomalies seen on ultrasound provides important information to improve perinatal management. The conventional genetic test (microarray) leads to a diagnosis in ~40% of fetuses with multiple congenital anomalies. Whole Exome Sequencing (WES) may improve diagnostic yield, but implementing prenatal WES is challenging due to uncertainties around fetal phenotyping, variant interpretation, ethical/counseling issues of incidental findings and variants of unknown clinical significance, and the requirement of short turnaround times. In this study, we implement WES in prenatal care to increase the number of genetic diagnoses. Methods Phase 1: Retrospective trio WES analysis (blind) of six fetuses, with known postnatal genetic diagnosis, on the fetal phenotype only using filtering with our custom virtual gene panel (~3,800 disease genes, excluding late-onset disease genes) or with additional filtering using human phenotype ontology (HPO) terms. Phase 2: Prospective study of rapid trio WES analysis next to conventional genetic tests for twenty-five fetuses with ultrasound abnormalities. Inclusion criteria: at least two congenital malformations on ultrasound or one congenital malformation in addition to a previous pregnancy with a fetus with a similar phenotype. Questionnaires and interviews with parents about the test will be conducted to study the impact on couples. Results Phase 1: WES analysis on fetal DNA resulted in a coverage of >95% and could confirm five out of six known diagnoses. HPO filtering was not helpful. One causal pathogenic PTPN11 mutation was missed due to low coverage of the variant. Modeling the WES pipeline shows a theoretical turn-around time of 10 working days after the invasive procedure. Phase 2: five fetuses have been included (May 2018), resulting in a genetic diagnosis in three: a de novo mutation in SAMD9 (MIRAGE syndrome), compound heterozygous mutations in PEX1 (Zellweger syndrome) and homozygous mutations in POMGNT1 (Walker Warburg syndrome). The turnaround time was 8-14 working days. Results of the interviews and questionnaires are not available yet. Conclusion Our retrospective analysis and the preliminary results from our prospective study show that implementing WES as a routine test in the prenatal setting is challenging, but technically feasible and has a promising diagnostic yield.

E-mail: c.c.van.diemen@umcg.nl
Keywords: prenatal diagnostics, whole exome sequencing

T06 - Functional Analysis of PALB2 Genetic Variants that Associate with Breast Cancer

Rick Boonen, Chantal Stoepker, Nandi Celosse, Bas Vroling, Fergous Couch, Peter Devilee and Haico van Attikum

LUMC, NKI, bio-prodict, Mayo Clinic

Heterozygous carriers of germ-line mutations in the DNA repair gene PALB2 are at a highly increased lifetime risk for developing breast cancer. Therefore, PALB2 now takes a valid place on breast cancer predisposition gene panel tests and is becoming widely included in breast cancer clinical genetics practice and risk management. While truncating PALB2 mutations that fully impair protein function are known to increase cancer risk, the interpretation of missense variants of unknown significance (VUSs) is still in its infancy. Here we describe the development of a cDNA-based system for the semi high-throughput functional analysis of VUSs in PALB2. Since PALB2 plays a key role in homologous recombination (HR), we evaluated the effect of PALB2 VUSs on protein function using a mouse embryonic stem (mES) cell-based homology-directed repair assay. In addition, the effect of PALB2 VUSs on cellular sensitivity to PARP inhibitor treatment was determined as an alternative measure for HR. By assessing the ability of PALB2 VUSs to rescue the HR defect in Trp53/Palb2 double knockout mES cells, we identified several novel VUSs in PALB2 that completely impair PALB2 function. Further analysis showed that some VUS located in the WD40 domain strongly affect protein function by impairing PALB2 stability, whereas others fully disrupt the interaction with BRCA1. The outcome of our functional assays may contribute to the interpretation of VUSs and their influence on the risk and treatment of breast cancer.

E-mail: r.a.c.m.boonen@lumc.nl
Keywords: PALB2; Homologous Recombination (HR); Breast Cancer; Variant of uncertain significance (VUS); DNA repair

T07 - Genetic causes of obesity: diagnostic yield of 18% in a tertiary pediatric obesity cohort

L Kleinendorst, O Abawi, AE Brandsma, MH Jongejan, EFC van Rossum, B van der Zwaag, ELT van den Akker, MM van Haelst

Klinische Genetica, AMC. Centrum Gezond Gewicht, Kinderendocrinologie, Erasmus MC-Sophia. Klinische Genetica, VUmc. Kindergeneeskunde, Maasstad ziekenhuis. Kindergeneeskunde, Franciscus Gasthuis. Centrum Gezond Gewicht, Endocrinologie, Erasmus MC. Afdeling Genetica, UMC Utrecht.

Introduction: Obesity is predominantly considered a multifactorial disorder. In unselected patient cohorts, an underlying genetic diagnosis can be established in only a minority of cases. These patients typically present with severe obesity in early childhood. Establishing a genetic diagnosis can lead to personalized treatment, reduce stigma and support reproductive decision-making. This study provides an overview of obesity-associated mutations and copy number variations (CNV´s) identified in a selected, tertiary pediatric obesity population. Methods: In 184 obese children, referred to the pediatric obesity center Centrum Gezond Gewicht between 2012 and 2018, diagnostic sequencing of 52 obesity-associated genes and CNV detection by SNP-microarray analysis were performed to identify genetic causes of obesity. On clinical suspicion, specific additional diagnostics (e.g. Prader-Willi diagnostics, whole exome sequencing) were performed. Results: The median age at intake was 10.0 years (range 0.7–18 years); 110 patients were female (60%). Mean BMI-SDS was +3.7. In 33 patients (18%), an underlying genetic cause was identified, leading to a diagnosis of genetic obesity. Thirteen different genetic diagnoses were established, most frequently MC4R-associated obesity (5 heterozygous patients, 3 homozygous or compound heterozygous), leptin receptor deficiency (5 patients) and 16p11.2 deletion syndrome (3 patients). In an additional 16% of the patients, a novel CNV or sequence variant of unknown clinical significance (VUS) was shown in obesity-associated genes, for which the role in the phenotype has yet to be confirmed. Conclusion: A definitive diagnosis of genetic obesity was made in 18% of our patients with childhood obesity. This may increase, if follow-up studies in patients with VUS confirm a causal role for their variants. Our results indicate that 'genetic obesity´ reflects a heterogeneous group of conditions, with 13 different genetic diagnoses made in this pediatric cohort. This diagnostic yield is higher than similar clinical studies and shows that genetic testing is highly relevant in selected obese patients, especially when personalized treatment becomes available in the near future.

E-mail: l.kleinendorst@amc.uva.nl
Keywords: genetic obesity, pediatric obesity, early-onset obesity, high diagnostic yield

T08 - The molecular convergence of Kleefstra Syndrome Spectrum

B. Mossink1,3, M. Frega1,3, J. Keller1,3, J. Qu4, P. Koerner4, S. Jansen1,3, H. van Bokhoven1,2, H. Zhou1,4, D. Schubert2,3, N. Nadif Kasri 1,3

1 Department of Human Genetics, Radboudumc, Nijmegen (the Netherlands) 2 Department of Cognitive Neurosciences, Radboudumc, Nijmegen (the Netherlands) 3 Donders Institute for Brain Cognition and Behaviour, Nijmegen (the Netherlands) 4 Department of Molecular Developmental Biology, Radboud Institute for Molecular Life Sciences, Nijmegen (the Netherlands)

Kleefstra syndrome (KSS) is a neurodevelopmental disorder caused by pathogenic mutations in any of the functionally distinct epigenetic modifiers EHMT1, MBD5, MLL3 or SMARCB1. We exploited this genetic heterogeneity to decipher the molecular pathophysiology of KSS. Previously we examined a combination of cellular and electrophysiological approaches, by directly comparing shRNA-mediated knockdown of each KSS gene in cultures of cortical neurons. Examination of neuronal network development on microelectrode arrays showed that loss of function leads to hyperactive networks with altered organization. Patch-clamp electrophysiology disclosed that KSS gene deficiency causes increased excitability and reduced inhibition, and these findings were recapitulated in Ehmt1+/- mice. Using the same knockdown approach, we here we investigated the molecular changes that could underlie the hyperactivity that we observed in KSS gene-deficient networks by RNA-sequencing. We found that knockdown of different KSS genes resulted in distinguishable gene expression patterns. Interestingly, gene ontology annotation of differentially expressed genes detected from EHMT1-, SMARCB1- and MLL3-deficient cultures shared high similarity in their associated biological functions. In addition, when cellular components were assessed, differentially expressed genes detected from all knock-down conditions were very similar. Taken together, these data show that KSS target genes share similar roles in regulating neuronal structures and activity, with a prominent enrichment for genes that directly affect neuronal excitability and synaptic function. By uncovering the molecular and functional convergence points, we gain mechanistic insight not only into KSS but to other phenotypically congruent neurodevelopmental disorders.

E-mail: britt.mossink@radboudumc.nl
Keywords:

T09 - De novo mutations in CNOT1, a master regulator of gene expression on DNA, RNA, and protein level, cause neurodevelopmental delay

Sinje Geuer1*, Sreehari Kalvakuri2*, Machteld Oud1, Inge van Outersterp1, Michael Kwint1, Anna Ruiz3, Jenny Morton4, Christopher Griffith5, Karin Weiss6, Candace Gamble7, James Bartley8, Mari Mori9, Hilary Vernon10, Kendra Brunet11, Claudia Ruivenkamp12, Paul Kruszka6, Alexandra Afenjar13, Kimberly Nugent14, Tjitske Kleefstra1, Rolf Bodmer2, Maximilian Muenke6, Lisenka ELM Vissers1*, Arjan PM de Brouwer1*

1 Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands. 2 SBP Medical Discovery Institute, 10901 North Torrey Pines Rd, La Jolla, California, USA. 3 Laboratori de Genètica, UDIAT-Centre de Diagnòstic, Corporació Sanitària Parc Taulí, Sabadell, Spain 4 Clinical Genetics Unit, Birmingham Women's Hospital, Birmingham, UK 5 Department of Pediatrics, University of South Florida, Tampa, Florida, USA 6 National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA 7 Cook Children´s, Fort Worth, Texas, USA 8 Pediatric Specialty Clinics, Loma Linda University, Loma Linda, California, USA 9 Division of Human Genetics, Hasbro Children´s Hospital, The Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA 10 McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, Maryland, USA 11 Porcupine Health Unit, Timmins, Ontario, Canada 12 Department of Clinical Genetics, Leiden University Medical Centre, Leiden, The Netherlands 13 Département de Génétique (Pr Soubrier), Hôpital Pitié-Salpêtrière, Paris, France 14 Children's Hospital of San Antonio, San Antonio, Texas, USA

Over the last decade, exome and genome sequencing studies have shown that a vast majority of neurodevelopmental disorders such as developmental delay (DD) can be explained by a de novo mutation in one of thousands of genes involved in neurodevelopment. Yet, many genes underlying DD still await discovery. Here, we report 17 patients with DD and a heterozygous de novo mutations in CNOT1. Deep-phenotyping of these patients revealed a spectrum of features centered around DD including intellectual disability, motor delay, speech delay, seizures, hypotonia, and behavioral problems, such as autism. CNOT1 is a member of the CCR4-NOT complex, which is a master regulator that orchestrates different levels of gene expression. It has been implicated in several aspects of mRNA and protein expression, including transcription initiation, elongation, mRNA degradation, ubiquitination, and protein modification. Population genetic signatures for CNOT1 indicate that it belongs to the 2% human protein-coding genes most intolerant to variation (RVIS -1.9), and that CNOT1 is depleted from loss-of-function mutations in large databases (pLi=1, z-score=7.44), both suggestive for mutations being under strong purifying selection. CNOT1 functions as a scaffolding protein binding other subunits of the CCR4-NOT complex, such as CNOT2, CNOT4, and CNOT7 through CNOT11. To elucidate the pathophysiological effects of the de novo variants observed in our patients on CNOT1 scaffolding capacity, we generated 8 different CNOT1 mutation constructs and transfected these in COS and IMCD3 cells. Whereas analysis of the mutation-specific constructs is still ongoing, we have so far been able to confirm the interaction of wildtype (wt) CNOT1 with its partners CNOT2, CNOT4 and CNOT8. To further examine the effect of CNOT1 de novo mutations on neurodevelopment, we generated mutation-specific Drosophila models, which showed learning and memory defects. Introduction of human wt CNOT1 was able to rescue this phenotype. Moreover, the introduction of the mutation-specific constructs were not, supporting our hypothesis that CNOT1 impairment results in DD. In summary, we show that de novo CNOT1 mutations cause neurodevelopmental delay. In addition, our data highlight the role of CNOT1 as a master regulator in CCR4-NOT complex formation, demonstrating the essential central role of the CCR4-NOT complex in normal human brain development.

E-mail: lisenka.vissers@radboudumc.nl
Keywords: Neurodevelopmental disorders Intellectual disability de novo mutations CNOT1 Drosophila model Functional genomic follow-up

T10 - The Use of Pericytes in a Novel Cell-based Strategy for Correcting the Muscular Phenotype in Myotonic Dystrophy type I

AUSEMS Rosanne1,2,3, RAAIJMAKERS Renée1,2,3, VAN DEN BROEK Walther4, GOURDON Geneviève5,6, WANSINK Derick4, VAN ENGELEN Baziel2,3, VAN BOKHOVEN Hans1,3

1. Department of Human Genetics, Radboud university medical centre, Nijmegen, The Netherlands 2. Department of Neurology, Radboud university medical centre, Nijmegen, The Netherlands 3. Donders lnstitute for Brain Cognition and Behavior, Radboud university medical centre, Nijmegen, The Netherlands 4. Department of Cell Biology, Radboud university medical centre, Nijmegen, The Netherlands 5. Inserm UMR 1163, Paris, France 6. Imagine Institute, Paris, France.

Introduction: The most common inheritable form of muscular dystrophy in adults, myotonic dystrophy type 1 (DM1), is caused by an expanded (CTG•CAG)n repeat in the DMPK/DM1-AS gene pair. Transcription of the repeat produces expanded (CUG)n and (CAG)n repeat-containing transcripts that respectively sequester RNA-binding proteins and produce toxic homopolymeric proteins through a process called RAN translation. Ultimately, expression of these toxic RNAs and proteins results in a severe myopathy, characterized by muscle weakness and wasting. We are exploring the possibility of a cell-based therapy to combat these muscle problems in DM1 patients, based on a distinct class of myogenic progenitor cells called pericytes (PCs), also known as adult mesoangioblasts (aMABs). Using autologous ex vivo gene-edited PCs, we aim to systemically stimulate the limited process of myogenic regeneration in DM1 skeletal muscle. We have successfully isolated PCs from skeletal muscle of genetically confirmed DM1 patients and a mouse model and are currently investigating their myogenic behaviour and growth in vitro. Methods: DMSXL mice express a human DMPK transgene with ~1300 repeats and replicate several muscle symptoms of DM1. Skeletal muscle tissue from the DMSXL mice, wild type litter mates and patient biopsies were cultured as explants under specific conditions to promote progressive increase in the proportion of PCs. ALP+/CD31- PCs were sorted from the heterogeneous population of weakly adherent cells by flow cytometry. These PCs were cultured in vitro and used for characterization of gene expression, cell growth and myogenic fusion characteristics. Since the DM1 repeat is unstable in stem cells, repeat length was carefully monitored throughout the process. Results: We succeeded in isolating ALP+/CD31- PCs from DM1 human and mouse skeletal muscle. PCs were characterized with specific markers via immunocytochemistry and RT-qPCR. Expanded DMPK RNA expression and nuclear (CUG)n RNA aggregates (foci) were determined. Human PCs hold myogenic potential as they efficiently differentiated into multinucleated myotubes. Discussion: We are currently optimizing CRISPR/Cas9-mediated excision of the (CTG)n repeat, like we have performed earlier in human and mouse myoblasts. To test the ability of PCs to reconstitute muscle fibers in vivo, we aim to apply intra-arterial injections with genetically corrected and fluorescently labeled PCs in DM1 mouse models.

E-mail: rosanne.ausems@radboudumc.nl
Keywords: pericytes, adult mesoangioblasts, myotonic dystrophy, cellular therapy, CRISPR/Cas9

T11 - Filamin-C: genotype-phenotype correlation in patients with cardiomyopathy and/or myopathy

J.A.J Verdonschot2, A. de Haan1, J.G.J.Hoeijmakers3, G.R.F. Claes1, A. van den Wijngaard1, I.P.C Krapels1, H. G. Brunner1

1Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, The Netherlands 2Department of Cardiology, Maastricht University Medical Centre, Maastricht, The Netherlands 3Department of Neurology, Maastricht University Medical Centre, Maastricht, The Netherlands

Introduction Mutations in filamin-C (FLNC) are known to cause skeletal myopathies with variable cardiac involvement. The expanding clinical spectrum of FLNC mutations has recently included cardiomyopathies without skeletal muscle involvement. Currently, there are no studies reporting whether the genotype could explain differences in neurological and cardiac involvement. Furthermore, it is currently unknown whether all patients with FLNC mutations are at equal risk of developing myopathy, cardiomyopathy, or both. Methods Clinical data from patients with FLNC mutations described in literature was reviewed. In addition, patients with myopathies and/or cardiomyopathies have been evaluated using whole-exome sequencing. In eleven patients, FLNC mutations were identified and clinical data from neurological and cardiac examination was collected. Results A total of 102 variants were identified in our patients and in literature (55 missense and 47 truncating variants). In patients with dilated cardiomyopathy and arrhythmogenic cardiomyopathy truncating variants were more frequently observed (resp. n=42; n=7) than missense variants (resp. n=17, n=1). In hypertrophic cardiomyopathy missense variants were more frequent (n=25) than truncating variants (n=2). Although data is limited, both missense and truncating variants are associated with proximal and distal myopathies. The majority of these variants are private and are associated with a single phenotype. Thirteen variants were reported in multiple families and in eight of these variants more than one phenotypes was observed. The phenotype could not be correlated to mutations occurring in specific exons or domains; mutations in FLNC associated with (cardio)myopathies were detected throughout the entire gene. Conclusions Truncating mutations in FLNC seem to be associated with dilated cardiomyopathy and arrhythmogenic cardiomyopathy; missense variants with hypertrophic cardiomyopathy. However, based on the type and the location of the mutation we were not able to predict which patients are at risk of developing a pure skeletal, a pure cardiac of a mixed phenotype.

E-mail: e.vanhoutte@mumc.nl
Keywords: Myopathy Cardiomyopathy FLNC Filamin-C

T12 - Functional analysis of BRCAness in female cancers: translation to clinical applications

L.M. van Wijk1, M.P.G. Vreeswijk1 , M. Meijers1, S. Vermeulen1, M. de Jonge2, N.T. ter Haar2, F.G. Talens8, K.A.T. Naipal6, T.G. Meijer6, N.S. Verkaik6 ,R.A.E.M. Tollenaar4, W.E. Mesker4, A. Jager7, C.D. de Kroon5, J.R. Kroep3, K.N. Gaarenstroom5, M.Vugt8, D.C. van Gent6, T. Bosse2, H. Vrieling1.

1) Human Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Pathology, Leiden University Medical Center, Leiden, Netherlands; 3) Medical Oncology, Leiden University Medical Center, Leiden, Netherlands; 4) Surgery, Leiden University Medical Center, Leiden, Netherlands; 5) Gynaecology, Leiden University Medical Center, Leiden, Netherlands; 6) Genetics, Erasmus University Medical Center, Rotterdam, the Netherlands; 7) Medical Oncology, Erasmus University Medical Center, Rotterdam, the Netherlands; 8) Medical Oncology, University Medical Center Groningen.

Background: Breast and ovarian tumors harbouring BRCA1- or BRCA2 pathogenic mutations respond very well to treatment with Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) due to their deficiency in DNA damage repair via homologous recombination (HR). However, tumors may also display HR-deficiency independent of BRCA1 or BRCA2 mutations (i.e. BRCAness). Assessment of the HR efficacy of tumors might therefore allow identification of an additional group of cancer patients that could benefit from PARPi treatment. Experimental procedure: Fresh tumor samples were collected from patients following surgery or ascites/pleural fluid drainage. Samples were ex vivo irradiated with 5 Gy ionizing radiation, fixed after 2 hours incubation at 37°C and embedded into paraffin. The ability of replicating tumor cells to accumulate RAD51 protein at DNA double strand breaks (RAD51 foci) was used as functional read out for HR proficiency. Results: To date, the HR-status of 67 ovarian, 70 breast and 25 endometrial samples has been determined. HR-deficiency was observed in 21% of ovarian (n=14), 16% of breast (n=11) and 24% of endometrium (n=6) samples. In about 35% of the HR-deficient tumors, germline and somatic BRCA mutations were detected, while one breast tumor displayed BRCA1 promotor methylation. Apparently, germline BRCA testing is not sufficient to identify all HR-deficient tumors. Genetic analyses of other HR genes in non-BRCA mutated HR-deficient ovarian, breast and endometrial tumors are ongoing. Conclusion: Functional analysis of BRCAness in tumor tissue is a promising new tool to identify HR-deficient tumors which comprise a significant proportion of ovarian, breast and endometrial tumors and allows identification of additional cancer patients that may benefit from treatments targeting deficiencies in HR.

E-mail: L.M.van_Wijk@lumc.nl
Keywords: BRCA1/BRCA2-BRCAness-homologous recombination deficiency-PARPi

T13 - Hypermorphic and hypomorphic AARS alleles in patients with CMT2N expand clinical and molecular heterogeneities

Marian A.J. Weterman1, Molly Kuo2,3, Susan B. Kenter4, Sara Gordillo4, Dyah Karjosukarso4, Ryuichi Takase5, Marieke Bronk4, Stephanie Oprescu6, Marit de Wissel4, Fred van Ruissen4, Ron J.W. Witteveen7, Henriette M.E. Bienfait8, Martijn Breuning1, Ya-Ming Hou5, Marianne de Visser9, Anthony Antonellis2,6,10, and Frank Baas1

1Department of Clinical Genetics, LUMC, Leiden, the Netherlands; 2Cellular and Molecular Biology Program, University of Michigan Medical School, Ann Arbor, Michigan, USA; 3Medical Scientist Training Program, University of Michigan Medical School, Ann Arbor, Michigan, USA; 4Department of Clinical Genetics and Genome Analysis AMC, Amsterdam, the Netherlands; 5Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia USA; 6Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA 7Department of Neurology, Alrijne Hospital, Leiden, the Netherlands, 8Department of Neurology, Spaarne Gasthuis, Haarlem, the Netherlands; 9Department of Neurology, Academic Medical Center, Amsterdam, the Netherlands; 10Department of Neurology, University of Michigan Medical School, Ann Arbor, Michigan, USA.

Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed enzymes implicated in several dominant and recessive disease phenotypes. The canonical function of ARSs is to couple an amino-acid to a cognate tRNA. We identified three novel disease-associated missense mutations in the alanyl-tRNA synthetase (AARS) gene in three families with dominant axonal Charcot-Marie-Tooth (CMT) disease. Two mutations (p.Arg326Trp and p.Glu337Lys) are located near a recurrent pathologic change in AARS, p.Arg329His. The third (p.Ser627Leu) is in the editing domain of the protein in which hitherto only mutations associated with recessive encephalopathies have been described. Yeast complementation assays demonstrated that two mutations (p.Ser627Leu and p.Arg326Trp) represent loss-of-function alleles, while the third (p.Glu337Lys) represents a hypermorphic allele. Further, aminoacylation assays confirmed that the third mutation (p.Glu337Lys) increases tRNA charging velocity. To test the effect of each mutation in the context of a vertebrate nervous system, we developed a zebrafish assay. Remarkably, all three mutations caused a pathological phenotype of neural abnormalities when expressed in zebrafish, while expression of the human wild-type mRNA did not. Our data indicate that not only functional null or hypomorphic alleles, but also hypermorphic AARS alleles can cause dominantly inherited axonal CMT disease.

E-mail: m.a.j.weterman@lumc.nl
Keywords: Charcot-Marie-Tooth disease; dominant; neurodegenerative; amino-acyl tRNA synthase

T14 - Addition of a 161-SNP Polygenic Risk Score to family history-based risk prediction: impact on clinical management recommendations in non-BRCA1/2 breast cancer families

Inge M.M. Lakeman (1#), Florentine S. Hilbers (1#), Mar Rodriguez-Girondo (2) , Andrew Lee (3), Maaike P.G. Vreeswijk (1), Antoinette Hollestelle (4), Caroline Seynaeve (4), Hanne E.J. Meijers-Heijboer (5), Jan C. Oosterwijk (6), Nicoline Hoogerbrugge (7), Edith Oláh (8), Hans F.A. Vasen (9,10), Christi J. van Asperen (11) and Peter Devilee (1,12) #these authors contributed equally to this manuscript

1. Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands 2. Department of Medical Statistics, Leiden University Medical Center, Leiden, the Netherlands 3. Department of Public Health and Primary Care, Centre for Cancer Genetic Epidemiology, University of Cambridge, Cambridge, United Kingdom 4. Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, the Netherlands 5. Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands 6. Department of Genetics, Universtity Medical Center Groningen, University of Groningen, Groningen, the Netherlands. 7. Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands 8. Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary. 9. The Netherlands foundation for the Detection of Hereditary Tumours, Leiden, the Netherlands 10. Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, The Netherlands 11. Department of Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands 12. Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands

PURPOSE Approximately 18% of the familial breast cancer risk is explained by 182 Single Nucleotide Polymorphisms (SNPs), but these are presently not used to guide clinical management in breast cancer families tested negative for BRCA1/2 pathogenic variants. We explored whether a genetic test that incorporates a SNP-based Polygenic Risk Score (PRS) is clinically meaningful in unexplained (i.e., non-BRCA1/2) high-risk breast cancer families. PATIENTS AND METHODS Two cohorts were used: a family-based cohort including 323 female non-BRCA1/2 breast cancer cases and 262 unaffected female relatives and a hospital-based cohort including 357 female incident breast cancer cases and 327 female population controls. SNP genotyping was performed for all individuals and the 161-SNP PRS (associated with overall breast cancer) was calculated and standardised to population controls (sPRS). The association of the sPRS with breast cancer in high-risk breast cancer families was estimated using a Cox-type random effect regression model adjusted by family history. Updated individualized breast cancer lifetime risk scores were derived by combining the calculated BOADICEA breast cancer lifetime risk with the effect of the sPRS. RESULTS The mean sPRS for incident cases was 0.35 (SD=0.9). For the family-based cohort the mean sPRS in unaffected and affected relatives was 0.53 (SD=0.9) and 0.70 (SD=0.9), respectively. Within the high-risk, family-based cohort, we found a significant association between sPRS and breast cancer, HR=1.16, 95%CI=1.03-1.28, P=0.026. Addition of the PRS to risk prediction based on family history alone changed screening recommendations in 14.7%, 11.5%, and 19.8% of the women according to breast cancer screening guidelines from the USA, United Kingdom and the Netherlands (NCCN, NICE, and IKNL), respectively. CONCLUSION Our results support the application of the PRS in risk prediction and clinical management of women from genetically unexplained breast cancer families.

E-mail: i.m.m.lakeman@lumc.nl
Keywords: Genetics, Breast cancer, Polygenic Risk Score, Risk prediction

T15 - Analysis of sibling pairs´ de novo mutations suggests limited influence of environmental and familial factors to germline mutation rate

Jakob M. Goldman1,2, Tychele Turner3 , Wendy S. W. Wong4, Marianne Jonker5, Joris A. Veltman2,6, John Niederhuber7,8* & Evan E. Eichler3,9,10* & Christian Gilissen2*

1: Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands. 2: Department of Human Genetics, Donders Centre for Neuroscience, Radboud University Medical Center, Nijmegen, the Netherlands 3: Department of Genome Sciences, University of Washington School of Medicine, Seattle, USA 4: Inova Translational Medicine Institute (ITMI), Inova Health Systems, Falls Church, Virginia, USA 5: Radboud Technology Center Biostatistics, Radboud University Medical Center, Nijmegen, the Netherlands. 6: Institute of Genetic Medicine, International Centre for Life, Newcastle University, Newcastle upon Tyne, United Kingdom 7 Inova Translational Medicine Institute (ITMI), Inova Health Systems, Falls Church, Virginia, USA 8 Johns Hopkins University School of Medicine, Baltimore, Maryland, USA 9 Howard Hughes Medical Institute, The Rockefeller University, New York, USA 10 Howard Hughes Medical Institute, University of Washington, Seattle, USA

De novo mutations (DNMs) are genetic mutations that happen in the gametes of parents and are present in the germline of the offspring. Studies of whole-genome sequencing estimate the human DNM rate as on average one mutation per 108 bases per generation. On an individual level, the number of DNMs is positively correlated with the age of the father at conception and to a weaker extent also the age of the mother at conception. In spite of this, about 40% of the differences in the numbers of DNMs between individuals are still unexplained. Here, we investigate whether family-specific influences, such as the genetic make-up of parents, can explain some of the remaining variation. We analyzed three different large-scale whole-genome sequencing cohorts. Our first cohort was sequenced with complete Genomics technology and consisted of 820 healthy individuals and their parents from the Inova Premature Birth Study, including 36 families with multiple offspring. The second cohort was sequenced with Illumina technology and consisted of 1,291 healthy individuals from the Inova Childhood Longitudinal Cohort Study, including 43 families with multiple offspring. Our final cohort consisted of 521 sibling pairs and their parents as part of the Simon Simplex cohort, where one sibling was affected with Autism spectrum disorder. The fraction of variation in individual DNM counts explained by the age of father and mother at conception were 39%, 37% and 46%, respectively. In 83 dizygotic twin pairs, we observe differences in DNM counts that are not statistically different from the differences between parental-age matched unrelated children (Wilcoxon rank sum test $p>0.2$), suggesting that any family-specific effects are of small effect size. We used a linear mixed-effects model to estimate the fraction of variation in individual DNM counts explained by potential family-specific factors. With the given cohorts, there is limits to the statistical power of this analysis. A weighted mean estimate of the variance explained is 4.7%; the upper confidence interval borders are 23%, 34% and 9% for the cohorts 1,2 and 3, respectively. Together, these results suggest that family-specific influences are likely small and that therefore the number of DNMs of an individual has little predictive value for the number of DNMs in siblings.

E-mail: jakob.goldmann@radboudumc.nl
Keywords: de novo mutations, mutation mechanisms, whole genome sequencing, families

T16 - Towards personalized treatment of genetically classified refractory epilepsies using Human Induced Pluripotent Stem Cells (hIPSCs) as an ex-vivo tool

E.J.H. van Hugte, M. Frega, J. Nicolai, H.J.M. Majoie, H.J. Schelhaas, J.S. Verhoeven, J.H.L.M van Bokhoven, N. Nadif Kasri

RadboudUMC, afdeling humane genetica, Nijmegen Academisch Centrum voor Epileptologie Kempenhaeghe, Afdeling Epileptologie, Heeze School of Mental Health and Neuroscience, Universitair Medisch Centrum Maastricht

Dravet syndrome (DS) is a severe epileptic encephalopathy (EE) manifesting in the first year of life. DS is characterized by recurrent and prolonged seizures, behavioral problems and developmental delay. ln addition, EEs are associated with substantial cognitive and neuropsychological decline as a result of seizures during brain development. lt is therefore of crucial importance to timely control the seizure activity. Although numerous antiepileptic drugs are available, it remains a challenge for clinicians to select the appropriate one. Choosing treatment is based on a rational trial and error approach, but an effective predictive tool to investigate treatment options is lacking. ln 70%-80% of the cases, DS results from de novo mutations in the SCN1A gene, which encodes the voltage gated nav1.1 channel. Conversely, not every patient with a mutation in the SCN1A develops the same symptoms. GEFS+ (generalized epilepsy with febrile seizures plus) is a milder clinical phenotype that arises from SCN1A mutation. In addition to SCN1A, other genes have been associated with a DS phenotype. Both this genetic and phenotypic heterogeneity add to the difficulties of finding an appropriate treatment for DS. Previous research has focused on knock-out mouse models to address these issues. However, animal models can neither recapitulate mutation-specific effects nor genetic background factors and are not suitable for devising personalized medicine interventions. Hence, there is a strong need for patient-specific disease models.The development of human induced pluripotent stem cells (hiPSC) that are reprogrammed from a patient's own blood cells can facilitate this. These hiPSCs can be differentiated into induced neurons (iNeurons), which are subsequently cultured into a neuronal network, providing a patient specific predictive tool to model DS and explore treatment options in vitro. Therefore, this research aims to generate a patient neuronal network (PNN) by co-culturing excitatory and inhibitory iNeurons. The PNN is cultured on micro-electrode arrays (MEAs), that are able to record neuronal network activity by embedded micro-electrodes. By assessing developing and mature PNNs, the MEAs allow us to study the patient specific network fingerprint, providing a predictive tool for drug efficacy and side effects.

E-mail: eline.vanhugte@radboudumc.nl
Keywords: Epilepsy, human induced Pluripotent Stem Cells, brain-on-a-chip, neuronal networks, Micro-electrode arrays, anti-epileptic drugs, Dravet Syndrome

Abstracts Posters

P01-P22

P01 - Identification and functional genomic characterisation of genetic loci associated with human healthspan

A. Zenin, Y. Tsepilov, S. Sharapov, E. Getmantsev, L. Menshikov, P. Fedichev, Y. Aulchenko

Gero LLC (Moscow, Russia); Novosibirsk State University (Russia); Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia); National Research Center Kurchatov Institute (Moscow, Russia); Moscow Institute of Physics and Technology (Russia); University of Edinburgh (UK); PolyOmica ('s-Hertogenbosch, Netherlands)

Quality of life in an aging population directs the focus of longevity science to the regulatory pathways controlling healthspan. We used the UK Biobank (UKB) cohort and observed that the risks of major chronic diseases increased exponentially and doubled every eight years, i.e., at a rate compatible with the Gompertz mortality law. Assuming that aging drives the morbidity rates acceleration, we built a risk model to predict the age corresponding to the end of healthspan depending on their age, gender, and the genetic background. Using the UKB sub-population of genetically Caucasian individuals as a discovery, and other subpopulation as replication cohorts, we identified new loci associated with healthspan at the whole-genome level of significance. We observed the strongest genetic correlations between healthspan and all-cause mortality (as derived from parental survival, with correlation equal to -0.76). Other strongly (absolute correlation > 0.3) genetically correlated traits included life-history (metrics of obesity, age at first birth), and lifestyle traits (smoking behaviour). We thereby conclude that the healthspan offers a promising new way to interrogate the genetic architecture of human longevity. Using a large database including several billions of genetic associations with complex traits and "omics" phenotypes, we performed functional genomic investigation into molecular pathways underlying healthspan, and explored which (modifiable) traits are likely to be causatively related to the healthspan.

E-mail: y.s.aulchenko@polyomica.com
Keywords: longevity, ageing, healthspan, genetics, functional genomics, genome-wide association study, biomarkers, targets

P02 - High rate of deleterious de novo Copy Number Variations in patients with syndromal Hirschsprung Disease

Erwin Brosens 1, Katherine C. MacKenzie 1, Jonathan Windster 1,2 , Bianca de Graaf 1, Robert van der Helm 1 , Rutger Brouwer 3, Wilfred van Ijcken 3, Yolande van Bever, 1 Bert Eussen 1, Pim Sloots 2, , Dick Tibboel 2, Annelies de Klein 1 , René Wijnen 2, Maria Alves 1, Hirschsprung Disease Consortium , Alice Brooks 1 & Robert Hofstra 1

1Department of Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands 2 Department of Paediatric Surgery, Erasmus Medical Centre, Rotterdam, Netherlands 3 Centre for Biomics, Erasmus Medical Centre, Rotterdam, Netherlands

Hirschsprung disease (HSCR) is characterized by an absence of enteric ganglia. Approximately 18% of patients have additional dysmorphisms or major congenital anomalies or associated developmental disorders as autism or intellectual disability. Some HSCR patients have a known monogenetic syndrome or chromosomal syndrome in which HSCR can be a variable feature . In others, the genetic etiology is unknown. We hypothesized that rare Copy Number Variations (CNVs) could contribute to the etiology of HSCR. CNV can affect the expression of a dosage-sensitive gene important for the development of all affected organ systems and disturbed biological processes, result in a contiguous gene syndrome in which genes individually contribute to the phenotype or unmask a recessive HSCR predisposition gene in combination with a rare variant on the other allele. Surprisingly, the rate of rare CNVs in syndromic HSCR was very high. Ten out of sixteen patients had a rare CNV, four of these autosomal CNVs were de novo and one was located on the X-chromosome . Seven of these patients were homozygous for at least four out of eight frequently associated predisposing haplotypes. Determining a relationship of these rare CNVs with the development of HSCR proved to be challenging as neither clinical phenotypes nor CNVs had overlap. We hypothesized that genes affected should be dosage sensitive, expressed in the developing intestine (CN loss and gain) and that variant intolerant genes in CN losses also have deleterious variation in other HSCR patients. We could determine the expression of 152 out of 388 candidate genes in the developing intestine. Of these BCAS3, ABHD16A, INTS2 and CRKL were variant intolerant and had deleterious variants in an independent HSCR cohort. KMT2C, GEM and TRPM3 were located within a CN gain. The impact on enteric nervous system development of changes in expression of these main candidate genes are evaluated using zebrafish genetic models. Moreover, we are determining the CNV load in a larger cohort to minimize selection bias as only a limited number of patients were evaluated. However, given the high frequency of rare de novo deleterious CNV affecting developmental important genes screening of syndromic HSCR patients and their parents seems warranted.

E-mail: e.brosens@erasmusmc.nl
Keywords: Copy Number Variation; syndromic Hirschsprung disease; de novo

P03 - NGS Assurance Spike In for sequencing protocols

Kasper WJ Derks, Marion Drusedau, Rob GJ Deckers, Bart de Koning, Aimee DC Paulussen

Maastricht Universitair Medisch Centrum +

Introduction: SASI-Seq was introduced by Quail et al in 2014 as a sample assurance spike-in (SASI) for illumina sequencing. Many sequencing based lab procedures could benefit from the SASI-seq and our lab incorporated the sample tracking concept into the NIPT labprocedure. In there it has proven its added value by preventing sample swaps indicating flowcell swaps and revealing human typing-errors. However, in its current state the SASI-seq is not suitable for all sequencing based protocols. Method: We modified the SASI-seq concept into NASI (NGS Assurance Spike In), which is suitable for more sequencing based applications. For two important novel NGS protocols in our lab, i.e. molecular inversion probes (MIPs) - used for fast and cheap gene panel sequencing - and single cell preimplemantion genetic testing using genome wide haplotyping, the NASI was developed and tested. The NASI was added after DNA isolation for the MIP protocol and after the whole genome amplification of DNA isolated from a single blastomere for PGT. Results: We show our first results with NASI in the MIPs and PGT pipeline and its applicability with regard to preventing sample mix-ups and report cross-contamination between samples.

E-mail: kasper.derks@mumc.nl
Keywords: NGS, SASI, sample tracker, Illumina

P04 - Dissecting human epidermal commitment in healthy and diseased hiPSC models by single-cell RNA-seq

Eduardo Soares1, Quan Xu1, Qingqing Li3, Jieqiong Qu1, Yuxuan Zheng3, Hetty Raeven1, Fuchou Tang3, Huiqing Zhou1,2

1Department of Molecular Developmental Biology, Faculty of Science, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen, Nijmegen, The Netherlands. 2Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. 3- Department of Obstetrics and Gynecology Third Hospital, Biomedical Institute for Pioneering Investigation via Convergence & Center for Reproductive Medicine, College of Life Sciences, Peking University, Beijing 100871, China.

The transcription factor p63 is essential for normal epidermal development. In humans, heterozygous mutations in the DNA binding domain of p63 causes ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome (EEC). In order to understand how p63 mutations can lead to developmental defects we established an efficient feeder-free protocol to derive keratinocytes (iKeratinocytes) from human induced pluripotent stem cells (hiPSCs). Here we use bulk and single-cell RNA-seq to dissect epidermal commitment of normal and p63 mutant hiPSCs at different stages. We show that control hiPSCs can fully commit into the epidermal fate. At the molecular level, the transcriptional state of normal iKeratinocytes closely resembles the transcriptional state of human primary keratinocytes. Functionally, control iKeratinocytes are able to stratify, similarly to primary keratinocytes. In contrast, hiPSCs cell lines carrying two EEC mutations (R204W and R304W) do not fully commit into the epidermal fate. Instead, we observed increased cell death and delayed maturation of the epidermal commitment, in a heterogeneous manner. Bulk and single cell RNA-seq analyzes shows repression of different competing transcriptional programs is impaired on EEC-patient hiPSCs. By repressing these competing transcriptional programs using small molecules inhibitors, we can partially rescue the diseased phenotype. Taken together, our data show that normal human epidermal commitment can be fully recapitulated in vitro and highlight that proper p63 regulation is essential during epidermal commitment.

E-mail: edusoaress@gmail.com
Keywords: stem cells, single-cell transcriptome, cellular identity, EEC-syndrome

P05 - Supporting DNA variant interpretation: the LOVDatabases

Ivo FAC Fokkema, Julia ALH Lopez Hernandez, Mark Kroon, Johan T den Dunnen

Human Genetics and Clinical Genetics, Leiden University Medical Center, Leiden, Nederland

The simplest, and cheapest, answer to the possible consequences of a variant found in a genome is history. Has the variant been seen before and, when yes, where and what were the associated consequences in relation to the health of the individual. The Global Variome shared LOVDatabases, build on a >20 year history of collecting and sharing such data, contain a wealth of information on Individuals (patients), Phenotypes (disease/traits), Screenings and the Variants found, and can be queried in many ways. While the databases do contain information from published literature, most data has not yet been published but was submitted directly to LOVD (functionality includes automated data submission). When performing DNA diagnostics it is essential to check LOVD, preventing easily available information to be missed. Facilities supported by LOVD include variant queries (through the website or the API) across all public database installations, containing information from >3.6 million unique variants linked to >450,000 individuals. For genes with active curators (incl. BRCA, colon cancer, CFTR, etc.) specific "Summary records" resume all supporting information towards the conclusion whether the variant is or is not associated with a disease (pathogenicity). "Classification records" show the evaluation (classification) regarding variant pathogenicity as shared by diagnostic labs. Records labelled "In vitro (cloned)" show the results of assays performed to analyse the functional consequences of variants. Where available, LOVD shows the parental origin of a variant linked to haplotype information. The RNA field, not available in most other database, shows whether the consequences of a variant have been analysed on RNA level and what these consequences were. This adds essential evidence to the predicted consequences at the protein level, critical information for the diagnosis in diseases like Duchenne/Becker muscular dystrophy. Based on some examples we will show how the LOVD functionality can be used best to retrieve variant or phenotype information. The HVP shared LOVD is a community driven initiative operating under the auspices of Global Variome, a UK charity. Per gene, gene variant home pages link to other resources and data views in the major genome browsers. Data are shared with public repositories incl. UCSC, EBI and ClinVar, and copies can be download from the gene home pages. URLs: databases.LOVD.nl/shared, www.LOVD.nl, www.LOVD.nl/3.0/search

E-mail: I.F.A.C.Fokkema@lumc.nl
Keywords: database, variant, classification, phenotype

P06 - De novo mutations in PPP2CA, the Cα subunit of PP2A, cause a neurodevelopmental disorder resembling the phenotype caused by mutations in genes encoding for subunits A and B

Sandra JANSEN1*, Sara REYNHOUT2*, Dorien HAESEN2, Siska VAN BELLE2, Sonja A. de MUNNIK1, Ernie M. H. F. BONGERS3, Jolanda SCHIEVING4, Carlo MARCELIS3, Jeanne AMIEL5,6, Marlène RIO6, Heather McLAUGHLIN7, Roger LADDA8, Susan SELL8, Marjolein KRIEK9, Cacha M. P. C. D. PEETERS-SCHOLTE10, Paulien A. TERHAL11, Koen L. van GASSEN11, Nienke VERBEEK11, Sonja HENRY12, Jessica SCOTT SCHWOERER12, Saleem MALIK13, Nicole REVENCU14, Carlos R. FERREIRA15, Ellen MACNAMARA15,16, Hilde M.H. BRAAKMAN17, Bert. B. A. de VRIES1, Christopher T. GORDON5,6, Lisenka E. L. M. VISSERS1* & Veerle JANSSENS2*

1 Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, P.O. Box 9101, 6500 HB, Nijmegen, the Netherlands. 2 Laboratory of Protein Phosphorylation & Proteomics, Dept. of Cellular & Molecular Medicine, University of Leuven (KU Leuven), Leuven, Belgium & Leuven research Institute for Neuroscience & Disease (LIND), Leuven, Belgium. 3 Department of Human Genetics, Radboud University Medical Center, P.O. Box 9101, 6500 HB, Nijmegen, the Netherlands. 4 Department of Neurology, Radboud University Medical Center, P.O. Box 9101, 6500 HB, Nijmegen, the Netherlands. 5 Laboratory of Embryology and Genetics of Human Malformations, Paris Descartes-Sorbonne Paris Cité University & Institut National de la Santé et de la Recherche Médicale (INSERM) U1163, Institut Imagine, 75015 Paris, France. 6 Service de Génétique, Hôpital Necker-Enfants Malades, Assistance Publique - Hôpitaux de Paris (APHP), 75015 Paris, France. 7 GeneDx, Gaithersburg, MD 20877, USA. 8 Penn State Hershey Children's Hospital, Hershey, Pennsylvania. 9 Department of Genetics, Leiden University Medical Centre, Leiden. 10 Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands. 11 Department of Genetics, University Medical Centre Utrecht, P.O. Box 85500, 3508 GA, Utrecht, The Netherlands. 12 University of Wisconsin, United States. 13 Comprehensive Epilepsy Program, Jane and John Justin Neuroscience Center, Cook Children's Medical Center, Fort Worth. 14 Centre de Génétique Humaine, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Belgium. 15 Office of the Clinical Director, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland. 16 NIH Undiagnosed Diseases Program, Common Fund, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland. 17 Department of Neurology, Academic Center for Epileptology, Kempenhaeghe & Maastricht UMC+, Heeze, the Netherlands

Reversible protein phosphorylation is of utmost regulatory importance in neuronal signaling. Type 2A Protein Phosphatases (PP2A) play an important role in this process and are highly expressed in brain by catalyzing phospho-Ser/Thr dephosphorylations in diverse substrates. PP2A holoenzymes comprise a catalytic C, a scaffolding A, and a regulatory B-type subunit, which determines substrate specificity and physiological function. De novo mutations in several genes encoding A- and B-type subunits have recently been implicated in intellectual disability/developmental delay (ID/DD). Now, we describe 12 individuals with a de novo mutation in PPP2CA, encoding the catalytic Ca subunit. Individuals had mild to severe ID/DD, behavioral problems, diverse brain abnormalities and various types of epilepsy. Interestingly, comparison of these individuals with previously reported individuals with a mutation in subunits A or B showed an overlapping phenotype. PPP2CA de novo mutations covered the entire mutation spectrum including 6 presumed loss-of-function mutations as well as 1 recurrent and 4 non-recurrent missense mutations. Remarkably, functional studies showed complete null alleles in only two cases, hinting towards a haploinsufficiency mechanism, but in eight other cases functional characterization of the mutants showed mutation-specific biochemical distortions, including altered binding to the A subunit, specific B-type subunits and to B"´/STRN subunits, as well as impaired phosphatase activity. Thus, in these latter eight cases, a dominant-negative mechanism could not be excluded. Overall, we conclude that PP2A biogenesis and activity are severely compromised in individuals with ID/DD carrying de novo PPP2CA mutations, underscoring the importance of PP2A dysfunction in genetic brain disorders.

E-mail: sandra.jansen1@radboudumc.nl
Keywords: PPP2CA, PP2A, intellectual disability, syndrome, de novo mutation, epilepsy

P07 - Mosaic mutation detection using single molecule molecular inversion probes (smMIPs) for autoinflammatory disorder diagnostics

B.P. Kant, M.J. Koudijs, E.C. Carbo, S.M.C. Savelberg, F.F.M. Mulder, J. Frenkel, A. Simon, M.A. Swertz, J.K. Ploos van Amstel, M.E. Van Gijn

University Medical Center Utrecht Radboud University Medical Center University Medical Center Groningen

Background: The diagnosis of systemic autoinflammatory diseases (SAID) patients is difficult which can result in a delayed treatment and irreversible organ damage. In several patients with an autosomal dominant form of SAID, low grade mosaicism for mutations has been detected. Even mosaic mutations with an allele frequency <5% can result in severe forms of SAID. Currently many laboratories use Next Generation Sequencing (NGS) to detect mutations in SAID genes. However, NGS deep sequencing is not able to discriminate mosaic mutations with a low allele frequency from PCR or sequencing artefacts. Moreover, even if the genetic region is highly covered one cannot reliably exclude false negatives with allele frequencies <5%. Methods: A sensitive deep sequencing assay using single molecule molecular inversion probes (smMIPs) was designed and validated for mosaic SAID gene mutation detection using Illumina's sequencing platform. Data analysis was performed using SeqNext software (JSI). Results: Our results show the accurate detection of variant allele frequencies as low as 1%. In the first patient series apart from NLRP3 mosaic mutations, a known pathogenic TNFRSF1A mutation with an allele frequency of 1.65% was detected, confirming the relevance of the technology. Conclusions: The smMIPs test for SAID gene mutation detection is able to efficiently discriminate between mosaic mutations and sequencing artefacts and can reliably exclude the presence of mosaic mutations with an allele frequency =3%. It is a flexible, time- and cost effective assay to use in a diagnostic setting to prevent misdiagnosing of SAID patients with mosaic mutations.

E-mail: b.kant@umcutrecht.nl
Keywords: SAID NGS smMIPs mosaicism

P08 - Precise breakpoint detection of balanced and unbalanced structural variation in whole genome sequencing data using haplotype blocks created by linked-reads

J. Knijnenburg(1), M. Kriek(1), H.A. van Duyvenvoorde(1), N. Pappas(2), Y. Ariyurek(3), H.P.J. Buermans(3), K.M.L. Hertoghs(4), M.E.Y. Laurense-Bik(1), K. Kuipers-Heijboer(1), F. Baas(1)

1) Department of Clinical Genetics, LUMC, Leiden, the Netherlands 2) Department of Biomedical Data Sciences, LUMC, Leiden, the Netherlands 3) Department of Human Genetics, LUMC, Leiden, the Netherlands 4) GenomeScan, Leiden, the Netherlands

Introduction: Routine diagnostics of genome wide balanced and unbalanced structural variation is still dependent on classical techniques such as karyotyping and (SNP-)array. Both techniques result in a rough breakpoint detection where especially karyotyping can result in significant misinterpretation of chromosomal breakpoints. New sequencing technologies such as long range haplotyping by mapping linked-reads to generate haplotype blocks (Chromium Genome Solution, 10x Genomics, San Francisco USA) are expected to overcome this problem, although repeat sequence elements at breakpoints in the genome may occasionally interfere with a precise detection. Materials and Methods: To test whether this NGS based approach with linked-reads is suitable for routine diagnostics, a small series of patients, including one with a de novo balanced translocation (8;17) and one with a duplication detected with SNP-array, were analysed. Results: The expected structural variants based on previous knowledge were identified and in both patients precise breakpoints of these variants could be detected. In the translocation patient, the breakpoint differed at least 15 Mb from the estimated breakpoint and proved SOX9 to be causative for the phenotype of the patient. In the second patient the orientation of the duplication fragment could be defined to be in tandem, making a gene disruption to be causal for the phenotype unlikely. Conclusions: Structural variant analysis using WGS with linked-reads promises to be a feasible technique for detection of balanced and unbalanced structural variants in the genome and may be a next step in replacing classical cytogenetic techniques for genome wide screening.

E-mail: j.knijnenburg@lumc.nl
Keywords: linked-read whole genome sequencing, structural variation, haplotype blocks

P09 - ARMC9 Organizes a Joubert Syndrome-associated Protein Module at the Primary Cilium

Latour B(1), Van De Weghe JC(2), Rusterholz TDS(3), Grout ME(2), Aldinger KA(4), Shaheen R(5), Dempsey JC(2), Maddirevula S(5), Cheng YH(2), Phelps IG(2), Gesemann M(3), Goel H(6), Birk OS(7), Alanzi T(5), Rawashdeh R(5), Khan AO(8); University of Washington Center for Mendelian Genomics, Bamshad MJ(9), Nickerson DA(10), Neuhauss SCF(3), Dobyns WB(11), Alkuraya FS(12), Bachmann-Gagescu R(13), Doherty D(14), Roepman R(1)

(1)Department of Human Genetics, Radboud University Medical Center, 6500 HB Nijmegen, the Netherlands; Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, 6525 GA Nijmegen, the Netherlands. (2)Department of Pediatrics, University of Washington, Seattle, WA 98195, USA. (3)Institute of Molecular Life Sciences, University of Zurich, 8057 Zurich, Switzerland. (4)Department of Pediatrics, University of Washington, Seattle, WA 98195, USA; Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA 98101, USA. (5)Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh 12713, Saudi Arabia. (6)Hunter Genetics, Waratah, NSW 2298, Australia; University of Newcastle, Callaghan, NSW 2308, Australia. (7)Genetics Institute, Soroka Medical Center and National Institute for Biotechnology in the Negev (NIBN), Ben Gurion University, Beer Sheva 8499000, Israel. (8)Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh 12713, Saudi Arabia; Eye Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates. (9)Department of Pediatrics, University of Washington, Seattle, WA 98195, USA; Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA. (10)Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA. (11)Department of Pediatrics, University of Washington, Seattle, WA 98195, USA; Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA 98101, USA; Department of Neurology, University of Washington, Seattle, WA 98195, USA. (12)Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh 12713, Saudi Arabia; Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh 11533, Saudi Arabia; Saudi Human Genome Program, King Abdulaziz City for Science and Technology, Riyadh 12371, Saudi Arabia. (13)Institute of Molecular Life Sciences, University of Zurich, 8057 Zurich, Switzerland; Institute of Medical Genetics, University of Zurich, 8952 Schlieren, Switzerland. (14)Department of Pediatrics, University of Washington, Seattle, WA 98195, USA; Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA 98101, USA.

BACKGROUND: Primary cilia are microtubule based, mother centriole derived antenna-like organelles that exist on the surface of most eurkaryotic cell types. Mutations in genes that are essential for ciliary function give rise to severe Mendelian disorders termed ciliopathies. The ciliopathy Joubert syndrome (JS) is a recessive genetic disorder characterized by hypotonia, ataxia, abnormal eye movements, and variable cognitive impairment. The hallmark symptom of JS is a distinctive brain malformation known as the "molar tooth sign." Additional variable symptoms include polydactyly, encephalocele, and progressive retinal dystrophy. So far, more than 35 genes that have been implicated in JS but many patients remain molecularly undiagnosed. We have recently characterized mutations in ARMC9 as being causative of JS. AIM: We have scrutinized the ARMC9-associated protein module to delineate its cellular functions and identify the mechanisms of disease. APPROACH: We made use of protein interactome studies, including yeast two-hybrid screening for binary interactors using retinal, kidney and fetal brain cDNA libraries, tandem affinity proteomics, in silico analysis of in-house and publicly available protein and WES datasets, and disease gene modelling in zebrafish and ciliated cells. RESULTS: ARMC9 patient derived fibroblasts do not display any gross ciliary defects in architecture, ciliogenesis, or trafficking however microtubule stability is compromised due to a decreased levels of post translation modification, a trend previously observed in cell lines with mutations the JS-associated genes KIF7 and CEP41. We identified both novel and previously defined JS-associated proteins in a network centered around ARMC9, localizing to the basal body during G0/G1. CRISPR/Cas9-induced armc9 mutant knockout zebrafish display a ciliopathy phenotype (sinusoidal body shape, decrease numbers of ventricular and nose pit cilia). CONCLUSION: Our studies shed light on the convergent molecular mechanism of disease by elucidating a novel functional Joubert syndrome-associated module, and pinpoint novel functional candidate genes involved in JS and allied ciliopathies.

E-mail: brooke.latour@radboudumc.nl
Keywords: Joubert Syndrome, Ciliopathy, ARMC9; basal body; cilia; primary cilium; zebrafish

P10 - Genetic influences on fertility: What can we learn about female fertility from genetic studies of twinning

The spontaneous rate of twinning is about 1 in 80 live births, which means that about 1 in 40 individuals is a twin. In contrast to Monozygotic (MZ) twinning, Dizygotic (DZ) twinning has a familial component, with mothers of spontaneous DZ twins having a higher number of DZ twin pairs in their pedigrees than the general population. In Caucasians, two thirds of twins are DZ and dichorionic diamniotic (DCDA), commonly referred to as fraternal twins since they develop from two separate oocytes fertilized at the same time. The underlying biological mechanism for DZ twinning is the release and fertilization of multiple oocytes, for which both animal models and human data suggest multifactorial inheritance. We established the Twinning Genetics Consortium (TGC) to characterize the genetic basis of spontaneous DZ twinning and performed the first genome-wide association study (GWAS). We report for the first time compelling evidence that sequence variation at the FSHB and SMAD3 loci increases the odds of DZ twinning, based on 3 discovery cohorts from the Netherlands, Australia and Minnesota (USA). The findings were replicated in a large cohort from Iceland by Decode. We show that variants in these two genes are significantly associated with a broad range of fertility and reproductive traits in women including age at menarche, age at menopause, age at first and last child, and lifetime parity. We estimate the relative risk of twin birth given the novel alleles and explore the genetic relationship of DZ twinning and fertility traits using polygenic risk scores (PRSs), and show that higher DZ twinning PRS is associated with having children, greater lifetime parity, and earlier age at first child. We expect substantial interest in the risk estimates for women, who are carriers of these variants, which we report based on population data from Iceland. We hypothesize that the results will also be of importance to investigations into ovarian response to follicle-stimulating hormone (FSH) stimulation for assisted reproductive technology (ART), making these findings relevant to research into female infertility. Twinning is associated with common perinatal and maternal morbidities and by understanding the genetic basis of human DZ twinning we concomitantly identify loci conferring susceptibility (or conversely resistance) to these prevalent perinatal comorbidities.

E-mail: hmbarek@gmail.com
Keywords:

P11 - Alzheimer´s disease biomarkers in cerebrospinal fluid and on amyloid PET in two patients with GRN mutations and early onset dementia

S.W.R. Nijmeijer(1), P. Scheltens(2), Y.A.L. Pijnenburg(2), R. Vervenne-van Spaendonk(1), C.M. Aalfs(1)

1Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands 2Alzheimer Center & Department of Neurology VU University Medical Center, Amsterdam, The Netherlands

Background: Heterozygous loss-of-function mutations in the progranulin gene (GRN) cause hereditary frontotemporal lobar degeneration (FLTD) in most patients (1-3). Here, we describe two patients with pathogenic GRN mutations and signs compatible with early onset Alzheimer´s disease (AD) pathology. Case descriptions: Clinical characteristics, test results and family histories are shown in Table 1. In both patients, a clinical diagnosis of AD was made. Furthermore, in patient 1, cerebrospinal fluid (CSF) biomarkers were analysed showing decreased Amyloid-beta-42 levels (674 ng/L), increased t-tau (649 ng/L) and increased p-tau (78 ng/L) levels, compatible with AD. In patient 2, the Amyloid-PET revealed amyloid deposition consistent with AD pathology (Figure 1). DNA-analysis revealed pathogenic mutations in the GRN-gene in both patients. Discussion Mutations in the progranulin gene (GRN) are associated with hereditary frontotemporal lobar degeneration (FTLD) (1-3). FTLD usually causes frontotemporal dementia, characterised by personality change and language impairment as presenting symptoms. It has previously been described that some GRN mutation carriers may present with early memory impairment, clinically mimicking AD (4, 5). Furthermore, GRN mutations are also found in cohorts of clinically diagnosed AD patients (6, 7). A possible explanation might be that in these patients, FTLD pathology causes a clinical syndrome resembling AD. However, in the patients presented here, CSF (patient 1) amyloid-PET biomarkers (patient 2) were compatible with underlying AD pathology rather than FTLD as a cause for their early onset dementia. CSF biomarkers compatible with AD (8) and even neuropathological evidence for AD (9) have previously been described in GRN mutation carriers, suggesting that GRN mutations may not only cause FTLD but also other forms of neurodegeneration (9). The cases presented here illustrate the phenotypic variability of GRN mutations and further support the hypothesis that in addition to FTLD, GRN mutations might also predispose to AD pathology.

E-mail: s.w.nijmeijer@amc.nl
Keywords: Alzheimer´s disease Amyloid PET Biomarkers GRN mutation hereditary frontotemporal lobar degeneration (FLTD)

P12 - Identification of a Finnish CRADD founder mutation underlying 'thin´ lissencephaly

Daniel L Polla1, Elisa Rahikkala2, Michaela K. Bode3, Tuomo Määttä4, Teppo Varilo5, Thyrza Loman1, Anju K Philips5, Mitja Kurki6, Aarno Palotie6, Kristiina Avela7, Päivi Vieira8, Jarmo Körkkö9, Olli Pietiläinen6, Valérie Jacquemin10, Isabelle Prison10, Marc Abramowicz11, Kai Pihlajamaa12, Heikki Leutola11, Arjan PM de Brouwer1, Outi Kuismin2, Irma Järvelä5 ,Hans van Bokhoven1

1 Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands. 2 Department of Clinical Genetics, PEDEGO Research Unit and Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland 3 Department of Clinical Radiology, Oulu University Hospital, Medical Research Center Oulu, Oulu, Finland 4 Disability Services, Joint Authority for Kainuu, Kainuu, Finland 5 Department of Medical Genetics, University of Helsinki, Helsinki, Finland 6 Massachusetts General Hospital, Boston, MA, USA; Harvard Medical School, Boston, MA, USA; Broad Institute of MIT and Harvard, Cambridge, MA, USA 7 Helsinki University Hospital, Department of Clinical Genetics 8 Department of Children and Adolescents, Oulu University Hospital, PEDEGO Research Unit and Mecial Center Oulu, University of Oulu , Oulu, Finland 9 Northern Ostgorbothnia Hospital District, Center for Intellectula Disability Care, Oulu, Finland 10 Laboratoire 323, Institut Imagine (Inserm U1163), Paris, France 11 Medical Genetics, IRIBHM, Université Libre de Bruxelles, Brussels, Belgium 12 Symlab, Kajaani, Finland

Lissencephaly (LIS) is a rare genetic condition characterized by agyria or pachygyria that may occur as an isolated brain abnormality or in association with a specific syndrome, such as Miller-Dieker syndrome or Norman-Roberts syndrome. "Thin" lissencephaly (TLIS) is a specific subphenotype of LIS characterized by intellectual disability, megalencephaly, frontal predominant pachygyria and seizures. Previously, five autosomal recessive mutations have been identified in CRADD in individuals of different ethnicities, including one missense mutation of Finnish ancestry, leading to TLIS. Here we report the c.509G>A (p.Arg170His) homozygous TLIS founder mutation in CRADD in the Finnish population. By using exome sequencing and targeted Sanger sequencing, this mutation was found in a total of 22 individuals from 15 families originating from Northern or Northeastern Finland. Patients show mild to moderate intellectual disability, delayed speech development, aggressive behavior in half of the patients, EEG abnormalities in five patients and megalencephaly was identified in only one patient. The hallmark of the disease is predominantly frontal or frontotemporal pachygyria, which can be visualized by brain MRI. This study provides additional information about the phenotypic spectrum of patients with LIS caused by a founder mutation in CRADD. Compared with other types of LIS, the CRADD c.509G>A (p.Arg170His) homozygous founder mutation causes clinically a relatively mild phenotype characterized by non-syndromic ID and frontal or frontotemporal pachygyria. Additional genetic and/or environmental factors may play a role in the phenotypic presentation. Since TLIS is the hallmark of the disease in patients with the Finnish founder mutation, brain imaging studies are essential to confirm the potential pathogenicity of CRADD variants of uncertain significance. This highlights the importance of performing brain MRI supporting the molecular diagnosis for individuals with intellectual disability.

E-mail: daniel.lopopolla@radboudumc.nl
Keywords: Intellectual disability, exome sequencing, founder mutation, lissencephaly, brain MRI

P13 - Pericyte-derived iPSCs as a cell-based treatment for the neuromuscular phenotype in Myotonic Dystrophy type 1

Renée Raaijmakers 1,2,3, Rosanne Ausems 1,2,3, Derick Wansink 4, Baziel van Engelen 2,3, Hans van Bokhoven 1,3

1 Department of Human Genetics, Radboud university medical center, Nijmegen, The Netherlands. 2 Department of Neurology, Radboud university medical center, Nijmegen, The Netherlands. 3 Donders Institute for Brain, Cognition and Behaviour, Radboud university, Nijmegen, The Netherlands. 4 Department of Cell Biology, Radboud university medical centre, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands.

Myotonic dystrophy type 1 (DM1) is a multisystemic neuromuscular disorder leading to significant functional impairment and restricted social participation. An unstable expanded CTG-trinucleotide repeat in the 3´ untranslated region (UTR) of the DM protein kinase (DMPK) gene leads to RNA gain-of-function toxicity by creating abnormal long transcripts. Affected individuals range from the congenital to the late-onset form, with the number of triplets ranging from 37 to >5000. Currently, no curative treatment for myotonic dystrophy exists. However, cell-based strategies show potential for treating DM1. Pericytes (PCs), or adult mesoangioblasts (aMABs), a heterogeneous population of mesenchymal cells associated with postnatal vasculature, show myogenic potency similar to satellite cells. PCs show great promise for cell-based therapies as they are able to pass the blood-brain barrier and can be delivered systemically to colonize muscle tissue and contribute to regeneration of the dystrophic muscle. Despite their stem cell properties, it remains a challenge to reach a sufficient amount of PCs for clinical treatment. The use of induced pluripotent stem cells (iPSCs) to reach unlimited amounts of cells circumvents this bottleneck. Research on Duchenne muscular dystrophy and Limb-girdle muscular dystrophy has shown that pericyte-derived iPSCs (PC-iPSCs) are as pluripotent as fibroblast-derived iPSCs. However, PC-iPSCs possess a durable epigenetic memory, which results in a strong myogenic commitment. As repeat length varies between tissues and expands in iPSCs, we will determine the (CTG)n in each cell type as well as in blood and muscle tissue. To develop healthy autologous cells, PC-iPSCs from DM1 patients we aim to genetically excise the repeat from the 3´ UTR of DMPK by dual CRISPR/Cas9-cleavage. We have demonstrated that this technique is effective in excising the CTG-repeat, thereby normalizing DMPK function without detrimental effects on other genes in the DM1 locus. After gene editing, we will differentiate the corrected PC-iPSCs into iPSC-derived aMAB-like cells (IDEMs). Previous research in other muscular dystrophies has shown that transplantation of IDEMs led to the restoration of the depleted progenitor cells. The therapeutic potential of IDEMs on the muscular phenotype in DM1 will be examined via intramuscular and systemic injection in relevant DM1 mouse models.

E-mail: renee.raaijmakers@radboudumc.nl
Keywords: Myotonic dystrophy type 1, pericyte, adult mesoangioblast, PC-iPSC, CRISPR/Cas9, IDEM, cell therapy

P14 - Genetic mosaicism in basal cell naevus syndrome

M.G.H.C. Reinders (1,2), B. Cosgun (1,2), L.M.C. Gijezen (1), C.N. van Oosterhoud (3), N.W.J. Kelleners-Smeets (1,2), M. Vreeburg (3), P.M. Steijlen (1,2), K. Mosterd (1,2) and M. van Geel (1,2,3)

1. Department of Dermatology, Maastricht University Medical Center, Maastricht, The Netherlands 2. GROW, School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands 3. Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands

Background Basal cell naevus syndrome (BCNS) is an autosomal dominant disorder most commonly caused by a germline mutation in the PTCH1 gene and typically characterized by multiple basal cell carcinomas (BCCs), maxillary keratocysts and cerebral calcifications. About 85% of patients have a positive PTCH1 mutation analysis on blood. Mutations in other participants of the hedgehog pathway, like PTCH2 and SUFU, as well as genetic mosaicism has been described in BCNS. Objective To unravel the underlying genetics in patients with clinical suspicion of BCNS and negative PTCH1 Sanger mutation analysis on blood. Methods We used quantitative techniques like Restriction Fragment Length Polymorphism (RFLP) and Droplet Digital PCR (ddPCR) to detect low-grade PTCH1 mosaicism in blood. Analysis with Molecular Inversion Probes and Next Generation Sequencing was performed on different BCCs of an individual patient to identify a shared PTCH1 mutation. Results Low-grade PTCH1 postzygotic mosaicism was detected with RFLP and ddPCR in a patient with a clinical diagnosis of BCNS. In another patient with multiple BCCs on one side of the body we found a shared PTCH1 mutation in different BCCs. This finding was indicative for type 1 segmental mosaicism. Conclusion BCNS can be caused by genetic mosaicism. Segmental distribution of BCCs, may be present but not always visible. Mosaic BCNS can be diagnosed by using more sensitive techniques for mutational analysis or by comparing the genetic profiles of different BCCs from the same patient. Finding the underlying genetic cause is important to provide personalized medicine and adequate genetic counseling.

E-mail: marieke.reinders@mumc.nl
Keywords: basal cell naevus syndrome, PTCH1, postzygotic mosaicism

P15 - Colorectal polyps and carcinoma in Birt-Hogg-Dubé syndrome

I. van de Beek, I. Glykofridis, R. Wolthuis, J.J.P. Gille, E.J. Meijers-Heijboer, R.J.A. van Moorselaar, A.C. Houweling

VUmc departments of: Clinical Genetics, Oncogenetics and Urology

Introduction Birt-Hogg-Dubé syndrome (BHD) is associated with benign skin lesions called fibrofolliculomas, lung cysts with an increased risk for pneumothorax and an increased risk for renal cell carcinoma. It is caused by mutations in the FLCN gene. In the first description of familial fibrofolliculomas, one of the patients had several adenomatous colon polyps and colorectal carcinoma (CRC). Despite multiple additional publications, it has never become clear whether colorectal polyps and carcinomas are really part of the BHD spectrum. The Dutch guideline on BHD advises colorectal surveillance in families with at least one patient with both BHD and CRC. In the VUmc diagnostic laboratory, 401 FLCN mutation carriers, both index patients and their family members, have been identified until July 2016. Also, 384 of their family were found not to carry the mutation that occurred in their family. We aimed to compare the occurrence of CRC and polyps between these two groups. Methods The patient details were supplied to PALGA; the nationwide network and registry of histo- and cytopathology in the Netherlands. They provided us with anonymized pathology data of colon and rectum from all patients, divided in the BHD and non-BHD group. Results There was no significant difference between the mean age of the BHD and non-BHD group as of July 2016 (54.4 vs 52.2 years, p=0.06). There were slightly more patients with CRC in the BHD group, but the difference was not significant (3.6% vs 2.6%, p=0.47). There was also no significant difference between the age of the first CRC (61.9 vs 67.4, p=0.27). Considering colorectal polyps, more individuals in the mutation group were diagnosed with at least 1 polyp (12.2% vs 6.3%, p=0.004). However, the total number of polyps per person did not differ (3.4 vs 3.3 polyps, p=0.91). Discussion and conclusion We should consider a possible bias that occurred in our data because of colorectal surveillance in some of the BHD patients. This might have caused an underestimation of the risk for CRC, since removal of polyps might prevent developing CRC. On the other hand, the higher number of patients with at least 1 polyp might also be caused by this screening bias. This is supported by the absence of a difference in the total number of polyps per person. Considering previous literature and our own data, there is no proof for a substantial association between BHD and CRC. The discussion about colorectal surveillance in BHD patients is ongoing.

E-mail: i.beek@vumc.nl
Keywords: Birt-Hogg-Dubé syndrome, colorectal carcinoma, colorectal polyps, colorectal surveillance

P16 - Uptake of genetic counselling and predictive DNA testing in hypertrophic cardiomyopathy: a 10-years follow-up study

Lieke M. van den Heuvel (1), Maxiem O. van Teijlingen (1), Ellen M.A. Smets (2), J.Peter van Tintelen (1) & Imke Christiaans (1)

1 Department of Clinical Genetics, Academic Medical Centre, Amsterdam, The Netherlands
2 Department of Medical Psychology, Academic Medical Centre, Amsterdam, The Netherlands

Introduction: Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease, with symptoms ranging from asymptomatic status to heart failure and arrhythmias, which can cause premature sudden cardiac death (SCD). Predictive DNA testing is advised to at-risk relatives after a pathogenic genetic variant is identified in a proband. Cardiac monitoring and treatment can be offered to identified carriers of the familial variant, while non-carriers can be reassured. In a cohort of 97 HCM probands with a pathogenic variant detected between 1996 and 2005, we found an uptake of 39% of genetic counselling and predictive DNA testing in the first year after detection of the pathogenic variant in the proband. Because of this relatively low uptake, this study aimed to assess whether uptake in this HCM cohort increases in time after a follow-up period of 10 years or more. Method: Uptake of genetic counselling and predictive DNA testing was retrospectively determined using patient charts and pedigrees. First-degree relatives, and second-degree relatives in case of a deceased connecting first-degree relative suspected of HCM, of 10 years or older were considered eligible. Results: In total, 635 relatives were eligible for genetic counselling and predictive DNA testing, of which 532 first-degree- and 103 second-degree relatives. Of relatives at risk, 55.3% attended for genetic counselling, of which a majority of 68.4% in the first year after detection of the pathogenic variant in the proband. Of relatives attending genetic counselling, 98.3% attended for predictive DNA-testing. Median time between detection of the pathogenic variant in the proband and counselling of eligible relatives was 6 months (range 0-184 months). Opting for genetic counselling occurred significantly more frequently in first-degree relatives ($p < .001$), in relatives diagnosed with HCM ($p = .007$), and in families with SCD in a first-degree relative ($p = < .001$). Discussion: These results demonstrate that after a median follow-up of 14.8 years (i.e., 178 months) over half of relatives at risk attended genetic counselling and predictive DNA testing in this HCM cohort. Our results suggest that part of these relatives might be not or insufficiently informed about their genetic risks. Further research on the effectiveness of interventions facilitating family communication and increasing awareness among probands, relatives and healthcare professionals is needed to improve uptake of genetic counselling.

E-mail: l.m.vandenheuvel@amc.uva.nl
Keywords: Uptake, relatives at risk, genetic counselling, predictive DNA testing, hypertrophic cardiomyopathy, follow-up

P17 - Improving diagnostic yield for filaggrin; hidden mutations in the Dutch population

Frank S. van Leersum1, Carolien N. van Oosterhoud2, Stijn A.I. Ghesquiere2, Roel R.H.F.J. Brandts2, Peter M. Steijlen1, Michel van Geel1,2

1. Afdeling Dermatologie, Maastricht Universitair Medisch Centrum 2. Afdeling Klinische Genetica, Maastricht Universitair Medisch Centrum

Molecular diagnostics with conventional Sanger sequencing for ichthyosis vulgaris (IV) has been hampered by the notoriously difficult to analyse filaggrin (FLG) gene, caused by its homologous and polymorphic repeated units. By implementation of single molecule molecular inversion probes (smMIPs) and next generation sequencing (NGS), an alternative screening strategy for analysis of the entire coding region of the FLG gene becomes feasible. Genetic analysis of the whole gene instead of screening for only population-specific mutations, would improve diagnostic yield by scrutinizing also for rare family-specific mutations or specific mutations in ethnicities not previously studied. The smMIP-NGS strategy is easy to implement, affordable and since exclusion of NGS-duplicate-reads is possible, mutation-percentages can be related and assigned to polymorphic duplicated filaggrin-repeat-unit 8 and 10. In a cohort of previously screened Dutch patients (N=70) for only the population-specific mutations, retrospectively the whole FLG gene was analysed. Since all known mutations result in premature protein termination, focus of attention was on identifying nonsense and small insertion or deletion mutations. In several (8/70) of the screened patients additional novel truncating mutations were identified, elucidating their previously unexplained (more severe) clinical presentations. This study emphasises the need for screening the entire FLG gene for mutations, to improve the diagnostic yield in IV and identify hidden variants in the homologous repeated units of the gene. Herein, the smMIP-NGS method proves to be a reliable straightforward strategy to boost clinical diagnostics for IV and opens possibilities to facilitate patient stratification in large cohort studies.

E-mail: frank.van.leersum@mumc.nl
Keywords: Filaggrine, ichthyosis vulgaris, small-molecule molecular inversion probes, next generation sequencing

P18 - Prenatal diagnosis of Walker-Warburg Syndrome: A case report

M.M. van Veghel-Plandsoen1, M.I. Srebniak1, L.C.P. Govaerts1, J.M.J. Cornette2, R. M. Verdijk3, , I.A. Groenenberg2, S. Galjaard2, R.J. H. Galjaard1, M. Wilke1

1. Department of Clinical Genetics, 2. Department of Obstetrics and Gynecology, 3. Department of Pathology

Bi-allelic mutations in the isoprenoid synthase domain-containing (ISPD) gene are the second most common cause of Walker-Warburg syndrome (WWS). The autosomal recessive multisystem disorder is characterized by complex eye and brain abnormalities with congenital muscular dystrophy (CMD) and aberrant a-dystroglycan (aDG) glycosylation. Because of the severity of the problems caused by WWS, most affected individuals die in early childhood. Ultrasound markers of WWS only become overt later in pregnancy but when suspected , subtle features can be recognized by dedicated first trimester ultrasound with high frequency probes. A women presented with a history of 2 termination of pregnancies at 17 and 13 weeks of gestation based on ultrasound findings of ventriculomegaly, occipital encephalocoele and on pathology neuronal migration abnormality with normal SNP-array . Whole exome sequencing (WES) for neuronal migration disorders was offered to the family and performed on DNA derived from the first affected fetus. A heterozygous pathogenic mutation c.679C>T, p.Gln227* in the ISPD gene was detected. No second mutation could be found. Sanger-sequencing of the parents confirmed the paternal origin of this truncating mutation. Careful reconsideration of the clinical symptoms lead to the re-evaluation of the ISPD region of the SNP array derived from the mother and the fetuses and revealed a heterozygous loss of an approximately 8kb band in 7p21.2 including exon 10 of the ISPD gene. A specific MAQ analysis was developed to confirm the deletion in DNA of the mother as well as in all fetuses (at that time three affected pregnancies) and narrowed the deletion region to arr[hg19] 7p21.2 (16127283_16133734)x1. Sanger sequencing and the MAQ assay were offered for early prenatal diagnosis using chorion villus sampling in this family and after the detection of a fourth affected fetus, a healthy (carrier) girl was born. Unraveling the underlying etiology for the neurological abnormalities and offering the possibility of early genetic diagnosis could only be achieved through a close corporation between clinicians, counselors and laboratory specialists of the genetic , pathology and obstetric departments .

E-mail: m.plandsoen@erasmusmc.nl
Keywords: Walker-Warburg Syndrome; SNP-array; Whole exome sequencing; MAQ analysis

P19 - Impaired fertility and motor activity in a zebrafish model of classic galactosemia

J.M. Vanoevelen1,3, A.I. Coelho1,2,3, M. Hascovic1,2,3, B. Elshout1,2, B. van Erven1,2,3, J. van Heukelom1,2, J. Bierau1, X. Huang4, G.T. Berry4, Vos R.5, Rubio-Gozalbo M.E.1,2,3

1 Department of Clinical Genetics, Unit Clinical Genomics, Maastricht University Medical Centre, Maastricht, The Netherlands 2 Department of Pediatrics, Maastricht University Medical Centre, Maastricht, The Netherlands 3GROW - School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands 4The Manton Center for Orphan Disease Research, Division of Genetics and Genomics, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA 5Department of Methodology and Statistics, School for Public Health and Primary Care (CAPHRI), Maastricht University, Maastricht, The Netherlands

Classic galactosemia is an inherited metabolic disorder caused by galactose-1-phosphate uridylyltransferase (GALT) deficiency, a key enzyme in galactose metabolism. Current therapy (galactose-restricted diet) 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 classic galactosemia, aiming to gain new insights on brain and gonadal damage throughout development. An inheritable lesion in the zebrafish galt gene, giving rise to a galt loss-of-function, was generated using a TALEN-approach. Biochemical, morphological and behavioral 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). Neurological impairment was evaluated in WT and KO embryos (5 dpf), juvenile fish (4 weeks old) and adult fish (3 and 9 months) by quantifying motor activity (swimming behavior). KO and WT embryos showed similar activity, whereas KO juvenile and adult fish showed a significantly decreased motor activity, reminiscent of the impaired motor skills in galactosemia patients. Fertility was evaluated by performing regular crossings of WT, heterozygous and KO fish. Crosses involving KO females exhibited an increased number of unsuccessful crossings and a lower egg quantity whereas egg quality remained unchanged. Histological analysis of the gonads revealed that the number of follicles per ovary was reduced in KO females. Additionally, the distribution of follicles in different stages of maturity was altered in KO vs. WT. Further phenotypic characterization of galactosemic zebrafish is currently underway. We have crossed galt KO fish with reporter lines that carry brain (mbp:GFP) and gonad-specific (vasa:GFP) fluorescent tags, allowing the study of brain and gonadal damage from embryonal stage to adulthood. In conclusion, we have generated a galt loss-of-function line in zebrafish that recapitulates important hallmarks of classic galactosemia ie. reduced fertility and neurological impairments.

E-mail: j.vanoevelen@maastrichtuniversity.nl
Keywords: classic galactosemia, zebrafish

P20 - Introducing Next Generation Sequencing in Curaçao: results of the first 11 intellectual disability (ID) panels

Eline A. Verberne, Marjan M. Weiss, Mieke M. van Haelst

VU University Medical Center

Introduction: Until recently, clinical genetics was not part of regular medical care in Curaçao. Since 2011 a clinical genetics outpatient clinic has been established at the St. Elisabeth Hospital in Curaçao. Patients are evaluated by a clinical geneticist, who visits the island twice a year. If indicated, blood is send to the Netherlands for genetic testing. 65 out of 205 patients (32%) present with intellectual disability. Here, we show the results of the first 11 Intellectual Disability (ID) gene panels that were performed. Methods: ID panel analysis (virtual panel by WES) was performed in eleven cases with intellectual disability or general developmental delay associated with congenital anomalies and/or dysmorphic features. Samples were captured with the Nimblegen SeqCap EZ MedExome capture kit or Agilent SureSelectXT Human All Exon V5 or V6 capture library and sequenced on an Illumina HiSeq2500 or HiSeq4000. Different versions of the ID panel were performed, containing 657 to 1156 known ID genes. Written informed consent was obtained from the parents of all patients. Results: In 8 cases likely disease-causing mutations were found in DYRK1A, ATRX, ARID1B, SATB2, MTOR, BRAF, SON and MYCN. In three patients the ID panel revealed no variants of unknown clinical significance or (likely) pathogenic variants. Conclusion: Here we present the results of the first 11 ID panels performed in patients in Curacao. In 8 out of 11 patients a likely disease causing mutation was found. We show that recognizing dysmorphic features associated with these syndromic conditions can be challenging in this population because of the different ethnic backgrounds. Due to budgetary restrictions for genetic care in Curaçao, very strict inclusion criteria for ID panel analysis results in a high diagnostic yield of 73%.

E-mail: e.verberne@vumc.nl
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P21 - Titin cardiomyopathy leads to altered mitochondrial energetics, increased fibrosis and long-term life-threatening arrhythmias.

Job A.J. Verdonschot, MD, MSc (1,2) Mark R. Hazebroek, MD (1) Kasper W.J. Derks, PhD (1,2) Arantxa Barandiarán Aizpurua, MD (1) Jort J. Merken, MD (1) Ping Wang, PhD (2) Jörgen Bierau, PhD (2) Arthur van den Wijngaard, PhD (2) Simon M. Schalla, MD, PhD (1,3) Myrurgia A. Abdul Hamid, MD (4) Marc van Bilsen, PhD (1) Vanessa P.M. van Empel, MD, PhD (1) Christian Knackstedt, MD, PhD (1) Hans-Peter Brunner-La Rocca, MD, PhD (1) Han G. Brunner, MD, PhD (2,5) Ingrid P.C. Krapels, MD, PhD (2) Stephane R.B. Heymans, MD, PhD (1,6,7)

1. Department of Cardiology, Maastricht University Medical Centre, Center for Heart Failure Research, Cardiovascular Research Institute Maastricht (CARIM) 2. Department of Clinical Genetics and School for Oncology & Developmental Biology (GROW), Maastricht University Medical Center 3. Department of Radiology and Nuclear Medicine, Maastricht University Medical Center 4. Department of Pathology Maastricht University Medical Centre 5. Department of Human Genetics, Radboud University Medical Center, Donders Institute for Brain, Cognition and Behaviour, Nijmegen 6. Department of Cardiovascular Research, University of Leuven, Belgium 7. Netherlands Heart Institute (ICIN), Utrecht, The Netherlands

Aims: Truncating titin variants (TTNtv) are the most prevalent genetic cause of dilated cardiomyopathy (DCM). We aim to study clinical parameters and long-term outcomes related to the TTNtv genotype, and determine the related molecular changes at tissue level in TTNtv DCM patients. Methods and results: A total of 303 consecutive and extensively phenotyped DCM patients (including cardiac imaging, holter monitoring and endomyocardial biopsy) underwent DNA sequencing of 47 cardiomyopathy-associated genes including TTN, yielding 38 TTNtv positive (13%) patients. At long-term follow-up (median of 45 months, up to 12 years), TTNtv DCM patients had increased ventricular arrhythmias compared to other DCM, but a similar survival. Arrhythmias are especially prominent in TTNtv patients with an additional environmental trigger (i.e. virus infection, cardiac inflammation, systemic disease, toxic exposure). Importantly, cardiac mass is reduced in TTNtv patients, despite similar cardiac function and dimensions at cardiac magnetic resonance. These enhanced life-threatening arrhythmias and decreased cardiac mass in TTNtv DCM patients go along with significant cardiac energetic and matrix alterations. All components of the mitochondrial electron transport chain are significantly upregulated in TTNtv hearts at RNA-sequencing. Also, interstitial fibrosis was augmented in TTNtv patients at histological and transcript level. Conclusion: TTNtv lead to pronounced cardiac alterations in mitochondrial function, with increased interstitial fibrosis and reduced hypertrophy. Those structural and metabolic alterations in TTNtv hearts go along with increased ventricular arrhythmias at long-term follow-up, with a similar survival and overall cardiac function.

E-mail: job.verdonschot@mumc.nl
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P22 - Combination therapy in fragile x syndrome; possibilities and pitfalls illustrated by targeting the mglur5 and gaba pathway simultaneously

Shimriet Zeidler, Helen de Boer, Renate K. Hukema, Rob Willemsen

Department of Clinical Genetics, Erasmus University Medical Center, P.O. Box 2040 3000 CA Rotterdam, The Netherlands

Fragile X syndrome (FXS) is the most common monogenetic cause of intellectual disability and autism. The disorder is characterized by altered synaptic plasticity in the brain. Synaptic plasticity is tightly regulated by a complex balance of different synaptic pathways. In FXS, various synaptic pathways are disrupted, including the excitatory metabotropic glutamate receptor 5 (mGluR5) and the inhibitory ?-aminobutyric acid (GABA) pathways. Targeting each of these pathways individually, has demonstrated beneficial effects in animal models, but not in patients with FXS. This lack of translation might be due to oversimplification of the disease mechanisms when targeting only one affected pathway, in spite of the complexity of the many pathways implicated in FXS. In this report we outline the hypothesis that targeting more than one pathway simultaneously, a combination therapy, might improve treatment effects in FXS. In addition, we present a glance of the first results of chronic combination therapy on social behavior in Fmr1 KO mice. In contrast to what we expected, targeting both the mGluR5 and the GABAergic pathways simultaneously did not result in a synergistic effect, but in a slight worsening of the social behavior phenotype. This does implicate that both pathways are interconnected and important for social behavior. Our results underline the tremendous fine-tuning that is needed to reach the excitatory-inhibitory balance in the synapse in relation to social behavior. We believe that alternative strategies focused on combination therapy should be further explored, including targeting pathways in different cellular compartments or cell-types.

E-mail: s.zeidler@erasmusmc.nl
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