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Humane Genetica

Dutch Society of Human Genetics
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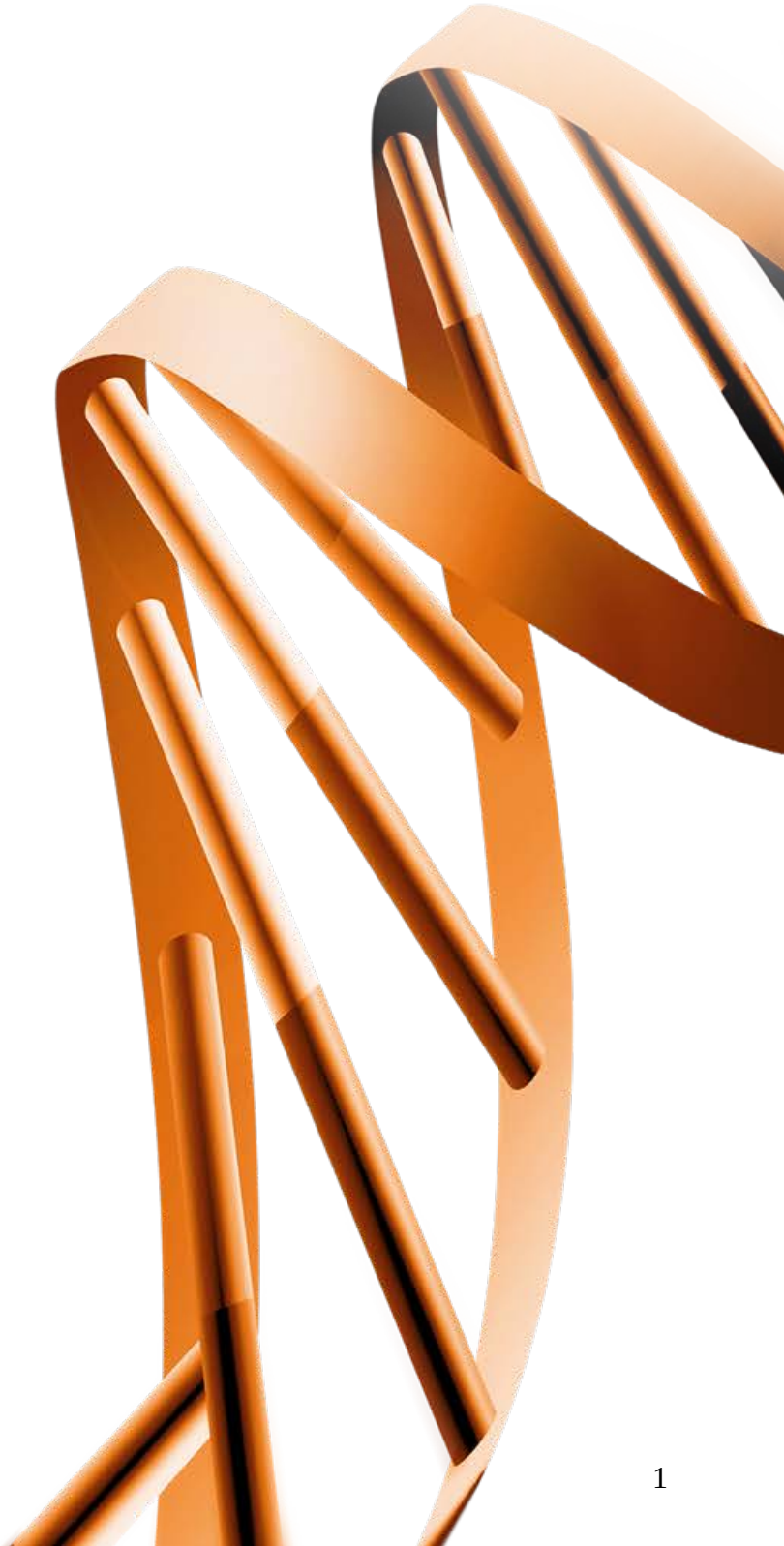

Autumn Symposium

October 6, 2016

VERENIGING KLINISCHE GENETICA NEDERLAND

VKGL

Nederlandse Associatie voor Community Genetics en Public Health Genomics

nederlandse vereniging voor Humane Genetica

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Dear Participants,

It is with great pleasure that we welcome you to the annual autumn symposium in the tradition of the NvHG.

This year is again a good year for genetics.
We have seen progress in the fields of diagnostics, counseling, community genetics and research. The decision by the minister to make the non-invasive pre-natal testing available to all is a good example of the relevance of our field for society.

2016 was also the year of the first First Joint Meeting of the Belgian and Netherlands Human Genetics Societies, held in Leuven. That meeting was a great success and has fostered the interactions with our colleagues in Belgium.

At todays, one-day symposium you will hear scientific presentations and see posters from all disciplines of our society. The Young Investigators are again competing for the best presentation and poster award and we will announce the winner of the famous NvHG Young Investigator Award for the best thesis in genetics.

This meeting would not be possible without the generous support from our sponsors. We thank all of them. I encourage all participants to show their appreciation of this support by talking to the representatives from all the stands.

We thank you for your scientific support.

Frank Baas

General information

Venue
Academisch Medisch Centrum
Collegezaal 4
Meibergdreef 9
1105 AZ Amsterdam
Tel.: 020 566 9111 https://www.amc.nl

Registration
Open on Thursday October 6, 2016: 09:00 – 11:00 hrs

Abstracts
Abstracts are grouped in:

- Abstracts Guest Speakers G 01 to G 02
- Abstracts Talks: T 01 to T 12
- Abstracts Posters : P 01 to P 24

Posters
Poster boards have a size of 200 cm (height) en 120 cm (width)

Language
The official language of the biannual spring meeting will be English

Accreditation
Accreditation forms are available at the registration desk (GAIA ID number: 265879)

Badges
You are requested to hand in your badge at the end of the symposium

Presentations
You are requested to timely hand in an USB stick with your presentation to the chairperson of your symposium session

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BIOKÉ
Attn. Finance
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Professional Diagnostics
Transistorstraat 41
1322 CK Almere The Netherlands

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Novogene Bioinformatics Technology Co. Ltd
Xi Wan, PhD
Regional manager, NGS service

Phone: 0031 – 681318439 (the Netherlands)
Email: wanxi@novogene.com; xwan2010@gmail.com
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nederlandse vereniging voor Humane Genetica

The NVHG Autumn Symposium 2016 - Dutch Society of Human Genetics
Nederlandse Vereniging voor Humane Genetica (www.nvhg-nav.nl)

Program Thursday October 6, 2016

09:00- 10:00	**Registration (open until 11.00 hrs)**
10:00-10:05	Opening Frank Baas, chair NVHG
10:05-10:30	**Robert Hofstra** *(ErasmusMC, Rotterdam)* *G 01* Diagnostic Functional Genetics
10:30- 10:45	**Saskia van der Crabben** (*UMC, Utrecht)* *T 01* Chromosome breakage syndrome with severe lung disease through destabilized SMC5/6 complex; an international collaboration
10:45- 11:00	**Lisenka Vissers** (*Radboudumc, Nijmegen)* *T 02* De novo loss-of-function mutations in SON disrupt RNA-splicing of genes essential for brain development and metabolism, causing an intellectual disability syndrome
11:00- 11:15	**Svitlana Pasteuning** (*LUMC, Leiden)* *T 03* New function of the myostatin/activin type I receptor (ALK4) as a mediator of muscle atrophy and muscle regeneration
11:15- 11:30	**Grazia Mancini** (*ErasmusMC, Rotterdam)* *T 04* Integrator Complex subunit INTS1 and INTS8 gene mutations cause severe syndromic intellectual disability
11:30- 11:45	**Izabela Krzyzewska** *(AMC, Amsterdam)* *T 05* Novel epigenetic loci associated with Beckwith Wiedemann Syndrome
11:45- 12:00	**Saskia Maas** *(AMC, Amsterdam)* *T 06* Phenotype, Cancer Risks and Surveillance in Beckwith-Wiedemann Syndrome Depending on Molecular Genetic Subgroups
12:00- 12:30	NVHG Algemene Ledenvergadering

12:00- 13:30 **Lunch / posterviewing**

13:30- 14:00 **Lude Franke** *(UMCG Groningen)*
Improving diagnostic yield using multi-omics

14:00- 14:15 **Diane van Opstal** *(ErasmusMC, Rotterdam)* *T 07*
Placental SNP array studies for confirmation of abnormal NIPT reveal the remnants of postzygotic chromosome instability seen in cleavage stage embryos

14:15- 14:30 **Ellen van Binsbergen** *(UMC, Utrecht)* *T 08*
Novel pathogenic CNVs identified by a de novo CNV bait strategy

14:30- 14:45 **Roy Straver** *(VUMC, Amsterdam)* *T 09*
Detection of Copy Number Aberrations in Exome Sequencing Data Based on a Within-Sample Comparison Scheme

14:45- 15:00 **Mariëlle van Gijn** *(UMC, Utrecht)* *T 10*
Towards automated sharing of genetic variants between VKGL laboratories; first results from the VKGL datasharing working group

15:00- 15:15 **Coffee – tea break and posterpresentations**

15:15- 15:30 **Suzanne Sallevelt** *(MUMC, Maastricht)* *T 11*
A comprehensive strategy for exome-based preconception carrier screening

15:30- 15:45 **Alexander Hoischen** *(Radboudumc, Nijmegen)* *T 12*
Ultra-sensitive mosaic mutation detection for clinical applications

15:45- 16:00 Annual Award 2016 and the Young Investigator Award 2016

16:00 **Drinks / Closure**

G 01

Diagnostic Functional Genetics

Robert Hofstra

Dept. Clinical Genetics, Erasmus MC, Rotterdam

From gene panel analysis and whole exome sequencing in diagnostic settings a surge of variants of uncertain clinical significance (VOUS) and 'class 4' variants (probable pathogenic, but not sure yet) is already flooding those labs and genetic counsellors. While in the Sanger era already new variant functional testing was a recognized heavy burden, but needed. The large dependence on research project based evaluation of diagnostic question has shown not to be effective. It is of great importance to draw extra attention to ways in which we can investigate the functional effects of variants in a fluent and controlled diagnostic setting.

The current challenge is to develop such functional diagnostic tests to investigate the pathogenicity of new variants. In our department we have started development and diagnostic implementation of such tests in some of our expertise areas. Actual examples of diagnostic functional tests are in the field of Ciliopathies, the mTOR signaling pathway and Neurofibromatosis. These tests make use of patient material or of cell model systems in combination with transfection techniques. Most of these assays make use of biochemical read-outs. Additionally the possible contribution of zebrafish models for diagnostic assays with phenotypic read-outs will be presented.

Email: r.hofstra@erasmusmc.nl

Chromosome breakage syndrome with severe lung disease through destabilized SMC5/6 complex; an international collaboration

T 01

Saskia N. van der Crabben1,11, Marije P. Hennus2,11, Grant McGregor3,11, Deborah I. Ritter4,11, Sandesh C.S. Nagamani5,6, Owen S. Wells3, Magdalena Harakalova1, Ivan K Chinn6-7, Aaron Alt3, Lucie Vondrova8, Ron Hochstenbach1, Joris M. van Montfrans9, Suzanne W. Terheggen-Lagro10, Stef van Lieshout1, Markus J. van Roosmalen1, Ivo Renkens1, Karen Duran1, Isaac J. Nijman1, Wigard P. Kloosterman1, Eric Hennekam1, Jordan S. Orange6-7, Peter M. van Hasselt11, David A. Wheeler4-5, Jan J. Palecek8, Alan R. Lehmann3, Antony W. Oliver3, Laurence H. Pearl3, Sharon E Plon4-7,12 Johanne M. Murray3,12 and Gijs van Haaften1,12.

Departments of 1Medical Genetics (Center for Molecular Medicine), 2Pediatric Intensive Care, Wilhelmina Children´s Hospital, University Medical Center Utrecht (UMCU), Utrecht, The Netherlands. 3Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9RQ, United Kingdom. 4Human Genome Sequencing Center, Baylor College of Medicine, Houston TX, USA. 5Molecular and Human Genetics, 6Texas Children´s Hospital and 7Pediatrics, Baylor College of Medicine, Houston TX, USA. 8Central European Institute of Technology and Faculty of Science, Masaryk University, Brno, Czech Republic. Departments of 9Pediatric Immunology and Infectious Diseases, 10Pediatric Pulmonary Diseases and of 11Metabolic Diseases, Wilhelmina Children´s Hospital, UMCU, Utrecht, The Netherlands. 11 Junior and 12 senior authors contributed equally.

We describe a new disorder: Lung disease, Immunodeficieny and Chromosome breakage Syndrome (LICS). Two sibling-pairs, from two unrelated families (respectively from the Netherlands and USA), were analyzed for their severe pulmonary disease following viral pneumonia with evidence of combined T- and B-cell immunodeficiency, resulting in early infantile death. Independent whole exome sequencing revealed biallelic missense mutations in the NSMCE3 gene. The two groups became aware of each other´s work through www.genematcher.org: patients showed an identical clinical picture and shared missense variants. NSMCE3 encodes a subunit of the SMC5/6 complex, essential for DNA damage response and chromosome segregation. The NSMCE3 missense changes disrupt interactions within the SMC5/6 complex, leading to destabilization of the complex. Evidence of defective DNA damage response was demonstrated including: chromosome rearrangements in patient primary fibroblasts, micronuclei, sensitivity to replication stress and DNA damage and defective homologous recombination. This study is an example of the power of data sharing in uniting researchers studying rare genetic diseases. Only through international collaboration this new chromosome breakage syndrome could be unraveled, thereby expanding the clinical spectrum of breakage syndromes and offering families the possibility of genetic counseling.

E-mail: s.n.vandercrabben@umcutrecht.nl
Keywords: Lung disease, Immunodeficieny and Chromosome breakage Syndrome (LICS), DNA damage, NSMCE3, defective homologous recombination, data sharing

De novo loss-of-function mutations in SON disrupt RNA-splicing of genes essential for brain development and metabolism, causing an intellectual disability syndrome.

T 02

Lisenka E.L.M. Vissers1, Jung-Hyun Kim2, Deepali N. Shinde3, Margot R.F. Reijnders1, Natalie S. Hauser4, Rebecca L. Belmonte5, Gregory R. Wilson5, Daniëlle G.M. Bosch1, Paula A. Bubulya6, Vandana Shashi7, Slavé Petrovski8,9, Joshua K. Stone2, Eun Young Park2, Joris A. Veltman3,10, Margje Sinnema10, Connie T.R.M. Stumpel10, Jos M. Draaisma11, Joost Nicolai12, Helger G. Yntema1, Kristin Lindstrom13, Bert B.A. de Vries1, Tamison Jewett14, Stephanie L. Santoro15,16, Julie Vogt17, Kristine K. Bachman18, Andrea H. Seeley18, Alyson Krokosky19, Clesson Turner19, Luis Rohena20,21, Maja Hempel22, Fanny Kortüm22, Davor Lessel22, Axel Neu23, Tim M. Strom24, 25, Dagmar Wieczorek26,27, Nuria Bramswig27, Franco A. Laccone28, Jana Behunova28, Helga Rehder28, Christopher T. Gordon29, Marlène Rio30, Serge Romana31, Sha Tang3, Dima El-Khechen3, Megan T. Cho32, Kirsty McWalter32, Ganka Douglas32, Berivan Baskin32, Amber Begtrup32, Tara Funari32, Kelly Schoch7, Alexander P.A. Stegmann10, Servi J.C. Stevens10, Dong-Er Zhang33,34,35, David Traver35, Xu Yao33, University of Washington Center for Mendelian Genomics, The Deciphering Developmental Disorders Study, Daniel G. MacArthur36,37,38, Han G Brunner1,10, Grazia M. Mancini39, Richard M. Myers40, Laurie B. Owen2, Ssang-Taek Lim41, David L. Stachura5, Eun-Young Erin Ahn2,41

1) Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, 6500 HB, Nijmegen, The Netherlands 2) Mitchell Cancer Institute, University of South Alabama, Mobile, AL 36604, USA 3) Ambry Genetics, Aliso Viejo, CA 92656, USA 4) Medical Genetics and Metabolism, Valley Children's Hospital, Madera, CA 93636, USA 5) Department of Biological Sciences, California State University Chico, Chico, CA 95929, USA 6) Department of Biological Sciences, Wright State University, Dayton, OH 45435, USA 7) Department of Pediatrics, Division of Medical Genetics, Duke University School of Medicine, Durham, NC 27710, USA 8) Department of Medicine, The University of Melbourne, Austin Hospital and Royal Melbourne Hospital, Victoria, 3010, Australia 9) Institute for Genomic Medicine, Columbia University, New York, NY 10027, USA 10) Department of Clinical Genetics and School for Oncology & Developmental Biology (GROW), Maastricht University Medical Center, 6202 AZ, Maastricht, The Netherlands 11) Department of Pediatrics, Radboudumc Amalia Children's Hospital, 6500 HB, Nijmegen, The Netherlands 12) Department of Neurology, Maastricht University Medical Center, 6299 HX, Maastricht, The Netherlands 13) Division of Genetics and Metabolism, Phoenix Children's Hospital, Phoenix, AZ 85016, USA 14) Department of Pediatrics, Section on Medical Genetics, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA 15) Nationwide Children's Hospital, Columbus, OH 43205, USA 16) Ohio State University College of Medicine, Columbus, OH 43210, USA 17) West Midlands Regional Genetics Service, Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom 18) Geisinger Medical Center, Danville, PA 17822, USA 19) Department of Pediatrics, Division of Genetics, Walter Reed National Military Medical Center, Bethesda, MD 20889, USA 20) Department of Pediatrics, Division of Genetics, San Antonio Military Medical Center, Fort Sam Houston, TX 78234, USA 21) Department of Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA 22) Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, 20246, Hamburg, Germany 23) Department of Pediatrics, University Medical Center Hamburg-Eppendorf, 20246, Hamburg, Germany 24) Institute of Human Genetics, Helmholtz Zentrum München, 85764, Neuherberg, Germany 25) Institute of Human Genetics, Technical University of Munich, 81675, Munich, Germany 26) Institute of Human Genetics, University Clinic Düsseldorf, Heinrich-Heine-University, D-40225, Düsseldorf, Germany 27) Institute of Human Genetics, University Clinic Essen, University Duisburg-Essen, D-45147, Essen, Germany 28) Institute of Medical Genetics, Medical University of Vienna, Waehringer Strasse 10, 1090 Vienna, Austria 29) Laboratory of embryology and genetics of congenital malformations, INSERM UMR 1163, Institut Imagine,75015, Paris, France. Paris Descartes-Sorbonne Paris Cité University, Institut Imagine, 75015, Paris, France. 30) Département de Génétique, Hôpital Necker-Enfants Malades, 75015, Paris, France. 31) Service de Cytogénétique, Hôpital Necker-Enfants Malades, 75015, Paris, France. Paris Descartes-Sorbonne Paris Cité University, Institut Imagine, 75015, Paris, France. 32) GeneDx, Inc, 205 Perry Parkway, Gaithersburg, MD 20877, USA

33) Moores Cancer Center, University of California San Diego, La Jolla, CA 92093, USA 34) Department of Pathology, University of California San Diego, La Jolla, CA 92093, USA 35) Division of Biological Sciences, University of California San Diego, La Jolla, CA 92093, USA 36) Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, MA 02142, USA 37) Analytical and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA 02114, USA 38) Department of Medicine, Harvard Medical School, Boston, MA 02115, USA 39) Department of Clinical Genetics, Erasmus University Medical Center, 3015 CN, Rotterdam, The Netherlands 40) HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806, USA 41) Department of Biochemistry and Molecular Biology, College of Medicine, University of South Alabama, Mobile, AL 36688, USA

The overall understanding of the molecular etiologies of intellectual disability (ID) and developmental delay (DD) is increasing with next-generation sequencing technologies identifying genetic variants in individuals with such disorders. However, detailed analyses conclusively confirming these variants, as well as the underlying molecular mechanisms explaining diseases, are often lacking. Here we report on an ID syndrome caused by de novo heterozygous loss-of-function (LoF) mutations in SON. Through international collaboration we collected 20 patients with LoF mutations in SON. Deep-phenotyping of the patients revealed common features of ID/DD, malformations of the cerebral cortex, epilepsy, vision problems, musculoskeletal abnormalities and congenital malformations. To further examine the effect of SON haploinsufficiency on embryonic development, we injected zebrafish embryos with son morpholinos, which resulted in a host of developmental defects. Embryos surviving 72 hours post injection progressed to more severe phenotypes, with extreme spinal malformations (22%), head and eye malformations with edema of the brain (37%), and profound developmental abnormalities (10%), mimicking features observed in affected individuals. SON is a nuclear speckle protein with dual abilities to bind to DNA and RNA, and its cellular functions includes the regulation of RNA splicing and gene transcription. Hallmark features of SON knockdown in HeLa cells and human embryonic stem cells are intron retention and exon skipping. Importantly, our analyses of RNA from patients with SON LoF mutations revealed that genes critical for neuronal migration/cortex organization (TUBG1, FLNA, PNKP, WDR62, PSMD3, and HDAC6) and metabolism (PCK2, PFKL, IDH2, ACY1 and ADA) are significantly downregulated due to accumulation of mis-spliced transcripts resulting from erroneous SON-mediated RNA splicing. In summary, we identified de novo LoF mutations in SON as a cause of a novel complex neurodevelopmental disorder characterized by ID/DD and severe brain malformations. Moreover, our data highlight SON as a master regulator governing neurodevelopment, and demonstrate the importance of SON-mediated RNA splicing in human development.

E-mail: lisenka.vissers@radboudumc.nl
Keywords: Novel intellectual disability syndrome; SON-mediated RNA splicing; Intron retention and exon skipping; zebrafish model; regulator of neurodevelopment

New function of the myostatin/activin type I receptor (ALK4) as a mediator of muscle atrophy and muscle regeneration

T 03

Svitlana Pasteuning-Vuhman1, Johanna Boertje-van der Meulen1, Maaike van Putten1 Maurice Overzier1, Peter ten Dijke2, Szymon M. Kielbasa3, Wibowo Arindrarto3, Ron Wolterbeek3, Ksenia V. Lezhnina4, Ivan V. Ozerov4, Aleksandr M. Aliper4, Willem M. Hoogaars5, Annemieke Aartsma-Rus1, Cindy J.M. Loomans1

1Departments of Human Genetics Leiden University Medical Center, Leiden, the Netherlands, 2Molecular and Cell Biology and Cancer Genomics Centre Leiden University Medical Center, Leiden, Netherlands, 3Medical Statistics and Bioinformatics Leiden University Medical Center, Leiden, the Netherlands, 4InSilico Medicine, Inc., Johns Hopkins University, Baltimore, USA, 5Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Move Research Institute Amsterdam, the Netherlands

Skeletal muscle fibrosis and impaired muscle regeneration are major contributors to muscle wasting in Duchenne muscular dystrophy (DMD). Muscle growth is negatively regulated by myostatin and activins. Blockage of these pathways might improve muscle quality and function in DMD. Here, antisense oligonucleotides (AONs) were designed to specifically block the function of ALK4, a key receptor for the myostatin/activin pathway in skeletal muscle. AON-induced exon skipping resulted in specific Alk4 downregulation, inhibition of myostatin activity and increased myoblast differentiation in vitro. Unexpectedly, a marked drop in muscle mass (10%) was found after Alk4 AON treatment in mdx mice. In line with in vitro results, muscle regeneration was stimulated and muscle fiber size was markedly decreased. Notably, when Alk4 was downregulated in adult wild type mice, muscle mass decreased even more (15%). RNAseq analysis revealed dysregulated metabolic functions and signs of muscle atrophy. One of the markers of low glucose level, the asparagine synthetase (Asns), and the key regulators of muscle degradation (Murf-1), muscle atrophy (Atf4) were upregulated in response to Alk4 downregulation. In conclusion, ALK4 inhibition increases myogenesis but also regulates the tight balance of protein synthesis and degradation. Therefore, caution is required when developing therapies that interfere with myostatin/activin pathways.

E-mail: s.k.pasteuning@lumc.nl
Keywords: Duchenne muscular dystrophy, Antisense oligonucleotides, Myostatin/activin pathway, muscle metabolism, muscle mass, DMD mouse model (mdx mice), RNAseq and pathway analyses

Integrator Complex subunit INTS1 and INTS8 gene mutations cause severe syndromic intellectual disability

T 04

Renske Oegema1*, David Baillat6*, Rachel Schot1, Daphne Heijsman2, Peter van der Spek2, Andreas Kremer2, Marjon van Slegtenhorst1, Martina Wilke1, Alice Brooks1, Frans Verheijen1, Leontine van Unen1, Ireneus de Coo3, Rob Willemsen1, Adriana I. Iglesias1, Maarten Lequin4, Eric J. Wagner6*, Maarten Fornerod5*, **Grazia M.S. Mancini**1

Dept. of Clinical Genetics1, Bioinformatics2, Neurology3, Radiology4, Biochemistry5, ErasmusMC Rotterdam, The Netherlands and Dept. of Biochemistry & Molecular Biology6, University of Texas Medical Branch, Galveston TX, USA

The integrator (INT) is formed by 14 protein subunits that bind as a complex to RNA polymerase II and regulate gene expression through enzymatic posttranscriptional trimming of small nuclear U1/U2-RNAs, enhancer RNAs, and assisting promoter-proximal pause-release during mRNA transcription. Initially, we identified by WGS compound heterozygote mutations in INTS8 in three Dutch siblings affected by severe ID, epilepsy, microcephaly and cerebral blindness. One mutation leads to deletion of three amino acids (c.2917_2925del, p.Glu972_Leu974del; simplified as INTS8?EVL) and the other is c.893A>G, predicting a p.Asp298Gly substitution. Brain MRI in all three individuals showed periventricular nodular heterotopia mostly in the frontal regions and cerebellar hypoplasia. Dysmorphic features consisted of broad nasal bridge, prominent glabella, hypertelorism and abnormal overlapping digits. In vitro expression of the INTS8 mutations shows that the c.893G>A at position +1 of exon 8 induces an alternative splicing followed by a premature stop and nonsense mediated decay, while the INTS8?EVL allele produces a protein that impacts the ability of INTS8 to associate with other subunits and destabilizes the INT assembly. Ints8 zebrafish knock-down morphants recapitulate a microcephaly phenotype. Patient fibroblasts have insufficient levels of mature U-RNAs and RNASeq show dysregulation of major genes involved in neurogenesis. Subsequently, three unrelated Dutch individuals born to unrelated parents, presented with a similar phenotype. In two of them by WES (trio analysis) we identified the same homozygote p.Ser1784* mutation in INTS1. In the third sequencing is pending. The mutation affects the C-terminus domain essential for INT complex integrity and snRNA processing. The three individuals present with severe developmental delay, no speech, abnormal gait, renal anomaly and distinctive facial features with hypertelorism, prominent forehead and glabella, broad nasal bridge, downward slant of eyelids, wide spaced incisors or cleft lip/palate, juvenile cataract, sternum abnormality, short stature and overlapping toes. The MRI shows in one mild dysgenesis of cerebellum and pons, enlarged frontal ventricles and irregular gyration. INTS1 is one of the core INT catalytic subunits and homozygote Ints1 knock-out mice do not develop beyond the blastocyst. We conclude that INT is essential in human development, pointing to a novel pathogenic mechanism for ID and brain malformation.

E-mail: g.mancini@erasmusmc.nl
Keywords: Integrator, INTS1, INTS8, RNApolymerase-II, PNH, ID

Novel epigenetic loci associated with Beckwith Wiedemann Syndrome

T 05

I.M. Krzyzewska, M.Alders, S. M. Maas, F.I. Rezwan, P. Henneman, K. van der Lip, A.N. Mul, A. Venema, D. Mackay and M.M.A.M. Mannens

Department of Clinical Genetics, DNA-diagnostics laboratory, Academic Medical Center Amsterdam,TheNetherlands; Department of Pediatrics, Academic medical Center, Amsterdam, The Netherlands; Faculty of Medicine, University of Southampton, Southampton, UK;

Beckwith Wiedemann Syndrome (BWS) is an overgrowth disorder with a predisposition to embryonal tumour development. Most BWS cases show various genetic and epigenetic aberrations in the imprinted region on chromosome 11p15.5. However, 20% of BWS cases cannot be explained by indels and/or DNA-methylation aberrations of this locus. The aim of this study was to reveal new epigenetic loci explaining BWS cases without epimutations of the 11p15.5 locus.We collected DNA samples from 37 BWS patients without any known defects in the methylome according to our diagnostics workflow (KCNQ1OT1, H19; High Resolution Melting Analysis). All patients met the BWS criteria of DeBaun. As positive control group we used 5 patients (HIL) with known alterations of the methylation level in BWS loci; as negative control group we used DNA of 26 healthy subjects. DNA was extracted from whole blood. Bisulphite conversion was performed using a conversion kit of ZYMO®. DNA methylation levels of patients and controls were analyzed using the DNA-methylation 450K array of Illumina. General differences including all cases vs. all controls were analyzed using the R package IMA. Single case vs. control analysis was performed using the recently developed R method of Rezwan et al (Epigenetics, 2015).450K array qc analysis, based on control probes and detection P-value showed no bad quality arrays. No significant differentially methylated probes or regions were detected in the general analysis. Single case vs. control statistical analysis using the method of Rezwan et al showed 5 altered methylation regions in one of the 37 BWS diagnosed patients. These differentially methylated regions were also moderately disrupted in the positive control HIL patients. However, none of these five significantly altered loci represented the critical BWS locus (KCNQ1OT1, H19). The genes situated in these 5 disrupted regions perfectly fit an overgrowth phenotype as described in earlier reports. Our study suggests that the 5 regions in which we found DNA-methylation alterations may be considered as new epigenetic loci for BWS susceptibility. Currently we are studying our findings in the above mentioned study in a population of Silver Russell Syndrome patients, a syndrome that demonstrates a reciprocal phenotype and is associated with (epi)genetic changes reciprocal to BWS.

E-mail: I.M.Krzyzewska@amc.uva.nl
Keywords: epigenetic, BWS

Phenotype, Cancer Risks and Surveillance in Beckwith-Wiedemann Syndrome Depending on Molecular Genetic Subgroups

T 06

Saskia M. Maas a,b, Fleur Vansenne c, Daniel J.M. Kadouch d, Abdulla Ibrahim e,f, Jet Bliek g, Saskia Hopman h, Marcel M. Mannens g, Johannes H.M. Merks a, Eamonn R. Maher e, Raoul C. Hennekam a

a Department of Pediatrics, Academic Medical Center, Amsterdam, the Netherlands b Department of Clinical Genetics, Academic Medical Center, Amsterdam, the Netherlands c Department of Clinical Genetics, University Medical Center Groningen, Groningen, the Netherlands d Department of Plastic surgery, Academic Medical Center, Amsterdam, the Netherlands (currently: Department of Dermatology, Academic Medical Center, Amsterdam, the Netherlands) e Department of Medical Genetics, University of Cambridge and NHR Cambridge Biomedical Research Centre, Cambridge CB2 OQQ,UK f Department of Clinical Genetics, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK g Department of Clinical Genetics, DNA-diagnostics Laboratory, Academic Medical Center, Amsterdam, The Netherlands h Department of Genetics, University Medical Center, Utrecht, The Netherlands

Patients with Beckwith-Wiedemann syndrome (BWS) have an increased risk to develop cancer as a child, especially Wilms tumor and hepatoblastoma. The risk varies depending on the cause of BWS. We obtained clinical and molecular data in our cohort of children with BWS, including tumor occurrences, and correlated phenotype and genotype. We obtained similar data from larger cohorts reported in literature. Phenotype, genotype and tumor occurrence were available in 229 own patients. Minor differences in phenotype existed depending on genotype/epigenotype, similar to earlier studies. By adding patients from the literature we obtained data on genotype and tumor occurrence of in total 1971 BWS patients. Tumor risks were the highest in the IC1 (H19/IGF2:IG-DMR) hypermethylation subgroup (28%) and pUPD subgroup (16%) and were lower in the KCNQ1OT1:TSS-DMR (IC2) subgroup (2.6%), CDKN1C (6.9%) subgroup, and the group in whom no molecular defect was detectable (6.7%). Wilms tumors (median age 24 months) were frequent in the IC1 (24%) and pUPD (7.9%) subgroups. Hepatoblastoma occurred mostly in the pUPD (3.5%) and IC2 (0.7%) subgroups, never in the IC1 and CDKN1C subgroups, and always <30 months of age. In the CDKN1C subgroup 2.8% of patients developed neuroblastoma. We conclude tumor risks in BWS differ markedly depending on molecular background. We propose a differentiated surveillance protocol, based on tumor risks in the various molecular subgroups causing BWS.

E-mail: s.m.maas@amc.uva.nl
Keywords: Wiedemann–Beckwith syndrome; Wilms tumor; hepatoblastoma; neuroblastoma; genotype–phenotype correlation

Placental SNP array studies for confirmation of abnormal NIPT reveal the remnants of postzygotic chromosome instability seen in cleavage stage embryos

T 07

Diane Van Opstal (1), Karin E.M. Diderich (1), Marieke Joosten (1), Joke Polak (1), Wai Yee Cheung (1), Attie T.J.I. Go (2), Dimitri N.M. Papatsonis (3), Anneke Dijkman (4), Lutgarde C.P. Govaerts (1), Malgorzata I. Srebniak (1)

(1) Dpt of Clinical Genetics, Erasmus MC, Rotterdam (2) Dpt of obstetrics and prenatal medicine, Erasmus MC, Rotterdam (3) Dpt of Obstetrics and Gynecology, Amphia Hospital, Breda (4) Dpt of Obstetrics and Gynecology, Reinier de Graaf Groep, Delft

Objective: Since non-invasive prenatal testing (NIPT) revolutionized prenatal screening for fetal chromosome aberrations, cytogenetic investigations of term placentas have become more relevant. The reason is that the fetal part of the cell-free DNA fraction in maternal blood, that is tested with NIPT, is derived from the placenta. Therefore, if amniocentesis after an abnormal NIPT result revealed a normal fetal karyotype, we collected the term placenta after birth in order to confirm that confined placental mosaicism was the origin of the abnormal NIPT findings and to exclude another biological source. Method Placentas of 9 cases with abnormal NIPT results (3 cases of trisomy 16, one trisomy 7, one trisomy 12, one trisomy 21, one trisomy 22, one deletion on 10q and one duplication of 4q) and with a normal karyotype in amniotic fluid, were collected after birth for cytogenetic confirmatory studies. The cytotrophoblast as well as the mesenchymal core of at least 4 chorionic villi biopsies from different quadrants and the umbilical cord were cytogenetically investigated with the Infinium_CytoSNP_850 genotyping array (Illumina) and the results were compared with those from amniotic fluid and/or cord blood. Results In 8/9 cases, confined placental mosaicism was proven. In 3 of these eight abnormal placentas, chromosomal mosaicism demonstrated postzygotic chromosome instability that probably arose during early embryonic development: • In the first case of a 4q duplication with NIPT and with a 10 Mb terminal deletion on chromosome 2 in amniotic fluid, the placenta showed to be mosaic with five different cell lines: four with different 2q terminal deletions (3, 4, 10 and 56Mb) and one with a 4q duplication and 2q deletion of 3 Mb. • The second and third are cases of mosaic trisomy/uniparental disomy (UPD)/biparental disomy involving chromosomes 16 and 21. Conclusion SNP array investigations of the placenta show that this organ may function as the litter-basket of abnormal cell lines derived through chromosomal instability that characterizes normal human embryo development. Moreover, although rarely described in the literature, mosaic UPD is probably more common than generally thought. Furthermore, our study shows that trisomic zygote rescue is not necessarily a single event during early embryogenesis.

E-mail: a.vanopstal@erasmusmc.nl
Keywords: NIPT, cell-free DNA, confined placental mosaicism, chromosome instability, cleavage stage embryo, non-invasive prenatal testing, uniparental disomy, chromosomal mosaicism

Novel pathogenic CNVs identified by a de novo CNV bait strategy

T 08

Ellen van Binsbergen 1, Trijnie Dijkhuizen 2, Markus J. van Roosmalen 1, Janneke Weiss 3, Abeltje Polstra 4, Birgit Sikkema-Raddatz 2, Richard J. Sinke 2, Erik A Sistermans 3, Marielle E. Swinkels 1, Eva H. Brilstra1, Lia Knegt 4, Peter M. van Hasselt 5, Edwin Cuppen 1, Conny M.A. van Ravenswaaij-Arts 2, Hans Kristian Ploos-van Amstel 1, Wigard P. Kloosterman 1

1 Department of Genetics, Center for Molecular Medicine, University Medical Center Utrecht, 3584 CG Utrecht, The Netherlands 2 University of Groningen, University Medical Center Groningen, Department of Genetics, 9700 RB Groningen, The Netherlands 3 Department of Clinical Genetics, VU University Medical Center, 1007 MB Amsterdam, The Netherlands 4 Department of Clinical Genetics, AMC University Hospital, 1105 AZ Amsterdam, The Netherlands 5 Department of Metabolic Diseases, University Medical Center Utrecht, 3508 AB Utrecht, The Netherlands

De novo copy number variations (CNVs) are a common cause of intellectual disability and multiple congenital abnormalities (ID/MCA). Despite the discovery of many disease-associated CNVs in recent years, diagnostic screening often reveals de novo CNVs that have to be classified as variant of unknown significance (VUS). A de novo status of a CNV makes it more likely to be disease-associated, but genotype-phenotype correlation in multiple patients is needed to substantiate causality. In order to detect novel disease associated CNV regions, we analyzed a well-defined set of possibly diagnostically relevant CNVs obtained by arrayCGH in 8,162 ID/MCA patients. For 67.4% of the CNVs inheritance was known resulting in a dataset of 599 de novo CNVs, including known recurrent disease-associated CNVs and non-recurrent CNVs. The de novo non-recurrent CNVs were used as a bait to capture overlapping CNVs and define novel disease-associated regions. We discovered 17 novel or previously weakly supported regions that contain overlapping CNVs in independent patients. Among these are a novel deletion locus on 2q24.1 associated with developmental delay and autism and a duplication region on 16q11.2q12.1 for which we could broaden the clinical phenotype with short stature. Taken together, these results underscore that de novo CNVs are a useful starting point for discovery of novel disease-associated CNV regions. Furthermore, analysis and integration of large CNV data (data sharing) sets is pivotal for completing genome-wide maps of pathogenic CNVs.

E-mail: e.vanbinsbergen@umcutrecht.nl
Keywords: de novo CNV's, novel disease associated regions

Detection of Copy Number Aberrations in Exome Sequencing Data Based on a Within-Sample Comparison Scheme

T 09

Roy. Straver E.A. Sistermans M.M. Weiss M.J.T. Reinders

VU University Medical Center Amsterdam Delft University of Technology

Introduction While whole genome sequencing is becoming more and more affordable, whole exome sequencing (WES) is still the primary method of choice for diagnostic purposes. Due to only targeting known genes, this provides an affordable way to obtain high coverage of likely disease causing variants. Although used for SNP detection, WES can be used to discover pathogenic Copy Number Variants (CNVs) as well. Existing methods to detect CNVs on WES data rely on direct comparison of reads per exon to other samples. Aberrated (deleted and/or duplicated) regions are either called through Hidden Markov Models or require a sequence of aberrated exons. Input data is generally the average read depth over an exon after normalization and PCA or SVD to remove fluctuations. Materials & Methods We developed a method, based on a combination of our previous work (WISECONDOR) and a segmentation algorithm, to find aberrated exons in exome data. The method fully depends on an internal comparison of DNA fragments per probe, rather than the average read depth per exon. This works by comparing the amount of reads mapped to an individual probe to a set of probes known to behave alike in terms of read depth, which is learned through a set of known normal samples. This approach provides both the effect size and z-score per probe. The z-score provides a direct indication of how likely a probe is part of an aberration. Additionally, our method does not force a minimum length to make a call, if there is enough certainty for a single probe to be aberrated it will be called. It does so by using different windows of z-scores to scout the sample for aberrations of any size, followed by a segmentation algorithm to determine the exact start and end positions of detected aberrations. In a first validation experiment, at least 10 known aberrations in our data were correctly identified, sizes ranging 5 Kbp and up. One sample with 2 deleted exons was correctly identified, including the partial deletion of an exon in a neighbouring gene. Conclusion Our work provides an alternative and reliable method to find CNVs in exome data, requiring only a set of previously reference exomes. We do not require resequencing of control exomes in the same run.

E-mail: r.straver@vumc.nl
Keywords: Copy Number Variations, Next Generation Sequencing, Whole Exome Sequencing

Towards automated sharing of genetic variants between VKGL laboratories; first results from the VKGL datasharing working group.

T 10

Marielle van Gijn, VKGL datashare working group

Dept of Genetics from:

Academic Medical Center / Erasmus Medical Center /Leiden University Medical Center /Maastricht University Medical Center / Netherlands Cancer Institute /Radboud University Medical Center /University Medical Center Groningen /University Medical Center Utrecht /VU Medical Center

The VKGL datasharing working group was established in 2013, consisting of clinical laboratory geneticists and bioinformaticians from all 9 genome diagnostics laboratories in the Netherlands. The goal of genetic datasharing is to improve patient care by the sharing of genetic data to more efficiently interpret the enormous amount of DNA variants produced by WES and WGS.

Moreover, sharing of genetic data will lead to harmonization of diagnostic reporting among the different Dutch genetic laboratories.

From a clinical laboratory perspective the most important is the sharing of classified DNA variants. If a variant has been properly classified by an expert previously, there is less need to do duplicate this effort and differences in interpretation and classifications will become clear and can be discussed. The pathogenicity interpretation of rare variants for monogenic diseases depends for a large part on determining its frequency within the population. Therefore we also decided to share variant frequencies based on the studies from the individual centers.

From pilot studies it became clear there was a need for a national central database with connections to each of the labs for automated sharing. The data from the central database will be downloaded to each lab to make the information available within the local variant interpretation software. For the sharing of variant classifications a central database has been developed based on the MOLGENIS software platform. The database will have connections to the different labs for automated data upload and download of VCF or text files. For the sharing of frequencies it was decided that either laboratories use VARDA software to upload VCF files to the database or laboratories calculate the frequencies themselves and use VARDA software to upload these to the database. The first version of the database will be demonstrated. Currently only VKGL laboratories have access to the database but the future goal is to make these data publically accessible and submit to international databases.

E-mail: M.E.vanGijn@umcutrecht.nl
Keywords : datashare working group

A comprehensive strategy for exome-based preconception carrier screening

T 11

Suzanne C.E.H. Sallevelt1, Bart de Koning1, Radek Szklarczyk1, Aimee D.C. Paulussen1, Christine E.M. de Die-Smulders1,2, Hubert J.M. Smeets1,2,3.

1. Department of Clinical Genetics, Maastricht University Medical Center+ (MUMC+) and/or Maastricht University, Maastricht, The Netherlands 2. Research School for Developmental Biology (GROW), Maastricht University, Maastricht, The Netherlands 3. Research School for Cardiovascular Diseases in Maastricht, CARIM, Maastricht University, Maastricht, The Netherlands

Whole exome sequencing (WES) provides the possibility of genome-wide preconception carrier screening (PCS). Here, we propose a filter strategy to rapidly identify the majority of relevant pathogenic mutations, which was developed on WES data from 8 consanguineous and 5 fictive non-consanguineous couples, and subsequently applied to 20 other fictive non-consanguineous couples. Presumably pathogenic variants based on frequency and 1) database annotations (HGMD/ClinVar) or 2) generic characteristics and mutation type (nonsense, frameshift and splice-site mutations), were selected in genes shared by the couple and in the female´s X-chromosome. Unclassified variants, predominantly missense mutations and in-frame deletions/insertions not present in HGMD or annotated pathogenic in ClinVar, were not included. This yielded on average 29 [19-51] variants in genes shared by the consanguineous couples (n=8) and 15 [6-30] by the non-consanguineous (n=25). For X-linked variants the numbers per female were 3 [1-5] and 1 [0-3], respectively. Remaining variants were verified manually. The majority could be quickly discarded, effectively leaving true pathogenic variants. Dependent on the clinical consequences, reproductive choices can be offered to the couple. Addition of gene panels for filtering was not favorable, as it resulted in missing pathogenic variants. We conclude that WES is eligible for PCS, both for consanguineous and non-consanguineous couples, with the remaining number of variants being manageable in a clinical setting. It is important to develop and continuously curate databases with pathogenic mutations in order to further increase sensitivity of WES-based PCS.

E-mail: suzanne.sallevelt@mumc.nl
Keywords: Whole exome sequencing (WES); preconception carrier screening (PCS)

Ultra-sensitive mosaic mutation detection for clinical applications

T 12

Alexander Hoischen1; Rocio Acuna Hidalgo1; Hilal Sengül1, Marloes Steehouwer1; Michael Kwint1; Elke Mersy2; Aimee Paulussen2, Maartje van de Vorst1, Lisenka ELM Vissers1, Marcel Nelen1; Christian Gilissen1; Joris A. Veltman1,2; Jay Shendure3; Anne Goriely4

1) Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands; 2) Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, The Netherlands; 3) Department of Genome Sciences, University of Washington, Seattle, Washington, USA; 4) Weatherall Institute of Molecular Medicine, University of Oxford, UK

Despite the great advances in the next generation sequencing field there is still room for improved targeted re-sequencing assays that combine high throughput with ultra-high sensitivity. We have now further optimized a single-molecule molecular inversion probe (smMIP) based targeted re-sequencing approach1. Single-molecule tracing is enabled using up to 410 (1,048,576) molecular tags. Consensus calling of respective PCR-duplicates allows correction for PCR and sequencing errors. The improved assay allows low-frequency or sub-clonal variant detection with variant levels of <0.05%. This assay provides very robust genotyping accuracy, high throughput, fast turnaround and cost-effectiveness. We anticipate that this or similar assays allow novel applications in which mutations are present in very low relative abundance in any given DNA sample with important new applications beyond cancer genetics. Here we present first successful applications that include: 1.) Low level mosaic mutations as drivers of clonal hematopoiesis in blood of healthy individuals; 2.) Accurate determination of the fraction of mutated alleles for post-zygotic de novo mutations2. 2.) Detection of previously unrecognized mosaic disease causing mutations for rare clinical syndromes. 3.) Detection of known 'paternal age effect disorders´ causing mutations as small clonal events in dissected testis material3. 4.) First evidence for presence or absence of parental alleles in cell free DNA from plasma of pregnant women. References: 1) Hiatt JB, et al. Genome Res. 2013 May;23(5):843-54. 2) Acuna-Hidalgo R, et al. Am J Hum Genet. 2015 Jul 2;97(1):67-74. 3) Goriely A, Wilkie AO. Am J Hum Genet. 2012 Feb 10;90(2):175-200.

E-mail: alexander.hoischen@radboudumc.nl
Keywords: ultra-sensitive sequencing, mosaic mutations, post-zygotic mutations, NIPT, low allelic fraction

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CREBBP Mutations in Individuals Without Rubinstein-Taybi Syndrome Phenotype

P 01

Leonie A. Menke1, Martine J. van Belzen2, Marielle Alders3, Francesca Cristofoli4, the DDD study5, Nadja Ehmke6, Patricia Fergelot7, Alison Foster8,9, Erica H. Gerkes10, Mariëtte J.V. Hoffer2, Denise Horn6, Sarina G. Kant2, Didier Lacombe7, Eyby Leon11 Saskia M. Maas1,3, Daniela Melis12, Valentina Muto13, Soo-Mi Park14, Hilde Peeters4, Dorien J.M. Peters15, Rolph Pfundt16, Conny M.A. van Ravenswaaij-Arts10, Marco Tartaglia13, Raoul C. Hennekam1

1 Department of Pediatrics, Academic Medical Center, Amsterdam, The Netherlands 2 Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands 3 Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands 4 Center for Human Genetics, University Hospitals Leuven, KU Leuven, Leuven, Belgium 5 Wellcome Trust Sanger Institute, Wellcome Genome, Campus, Hinxton, Cambridge, United Kingdom 6 Institute of Medical and Human Genetics, Charité-Universitätsmedizin Berlin, Berlin, Germany 7 Department of Genetics, and INSERM U1211, University Hospital of Bordeaux, Bordeaux, France 8 Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom 9 Clinical Genetics Unit, University of Birmingham, Birmingham, United Kingdom 10 University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, The Netherlands 11 Division of Genetics and Metabolism, Children´s National Health System, Washington, USA 12 Department of Translational Medical Science, Federico II University, Naples, Italy 13 Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, Rome, Italy 14 Department of Clinical Genetics, Cambridge University Hospitals, Cambridge, United Kingdom 15 Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands 16 Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

Mutations in CREBBP cause Rubinstein-Taybi syndrome. By using exome sequencing, and by using Sanger in one patient, CREBBP mutations were detected in 11 patients who did not, or only in a very limited manner, resemble Rubinstein-Taybi syndrome. The combined facial signs typical for Rubinstein-Taybi syndrome were absent, none had broad thumbs, and three had only somewhat broad halluces. All had apparent developmental delay (being the reason for molecular analysis); five had short stature and seven had microcephaly. The facial characteristics were variable; main characteristics were short palpebral fissures, telecanthi, depressed nasal ridge, short nose, anteverted nares, short columella and long philtrum. Six patients had autistic behavior, and two had self-injurious behavior. Other symptoms were recurrent upper airway infections (n=5), feeding problems (n=7) and impaired hearing (n=7). Major malformations occurred infrequently. All patients had a de novo missense mutation in the last part of exon 30 or beginning of exon 31 of CREBBP, between base pairs 5128 and 5614 (codons 1710 and 1872). No missense or truncating mutations in this region have been described to be associated with the classical Rubinstein-Taybi syndrome phenotype. No functional studies have (yet) been performed, but we hypothesize that the mutations disturb protein-protein interactions by altering zinc finger function. We conclude that patients with missense mutations in this specific CREBBP region show a phenotype that differs substantially from that in patients with Rubinstein-Taybi syndrome, and may prove to constitute one (or more) separate entities.
E-mail: l.a.menke@amc.nl
Keywords: CREBBP; exon 30; exon 31; exome Sequencing; intellectual disability, Rubinstein-Taybi syndrome; RSTS; syndrome; mutation; clinical features; case series; genotype-phenotype correlation

Recognizing the tenascin-X deficient type of Ehlers-Danlos syndrome: a cross-sectional study in 17 patients

P 02

Serwet Demirdas1,2; Eelco Dulfer3, Leema Robert4, Marlies Kempers2, Daphne van Beek5, Dimitra Micha5, Baziel G. van Engelen6, Ben Hamel2, Joost Schalkwijk6, Bart Loeys 2,8, Alessandra Maugeri5* & Nicol C. Voermans8*

1 Department of Clinical Genetics Erasmus Medical Center, , Rotterdam, The Netherlands. 2 Department of Human Genetics, Radboud university medical center, Nijmegen, The Netherlands 3 Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands 4 Department of Genetics, Guy´s and St Thomas' Hospital, London, United Kingdom 5 Department of Clinical Genetics, Center for Connective Tissue Research, VU University Medical Center, Amsterdam, The Netherlands 6 Department of Dermatology, Radboud university medical center, Nijmegen, The Netherlands 7 Centre for Medical Genetics, University Hospital of Antwerp/University of Antwerp, Antwerp, Belgium 8Department of Neurology, Radboud university medical center, Nijmegen, The Netherlands * these authors equally contributed to this study

Background: The tenascin-X (TNX) deficient type Ehlers-Danlos syndrome (EDS) is similar to the classical type of Ehlers-Danlos syndrome. Due to the limited awareness among geneticists and the challenge of the molecular analysis of TNXB, the TNX-deficient type EDS is likely to be under diagnosed. We performed an observational, cross-sectional study to improve recognition of this EDS type. Methods: History and physical examination were performed. Results of serum TNX measurements were collected and mutation analysis was performed by a combination of NGS, Sanger sequencing and MLPA. Results: Included were 17 patients of 11 families with autosomal recessive inheritance and childhood onset. All patients had hyperextensible skin without atrophic scaring scarring. Hypermobility of the joints was observed in 16/17 patients. Deformities of the hands and feet were observed frequently. TNX serum level was tested in 11 patients of (7 families). Genetic testing was performed in 10 families; 10 different mutations were detected, all suspected to lead to non-sense mRNA mediated decay. Conclusions: Patients with the TNX deficient type EDS typically have generalized joint hypermobility, skin hyperextensibility and easy bruising. In contrast to the classicclassical type, the inheritance pattern is autosomal recessive and atrophic scaring scarring is absent. Molecular analysis of TNXB in a diagnostic setting is challenging.

E-mail: s.demirdas@erasmusmc.nl
Keywords: Ehlers-Danlos syndrome, EDS, Tenascin-X, TNX, TNXB gene

Towards improved molecular diagnostics for tuberous sclerosis complex

P 03

Marianne Hoogeveen-Westerveld1., Rutger Brouwer2., Peter Elfferich1., Roza Amin1., Jacqueline Boonman1., Rosemary Ekong3., Sue Povey3., Bert Eussen1., Tom Brands1., Wilfred van IJcken2., Ans van den Ouweland1., **Mark Nellist**1.

1. Department of Clinical Genetics, Erasmus Medical Centre, Rotterdam, The Netherlands. 2. Center for Biomics, Erasmus Medical Centre, Rotterdam, The Netherlands. 3.Department of Genetics, Evolution and Environment, University College London, London, UK.

In most individuals with tuberous sclerosis complex (TSC) a pathogenic TSC1 or TSC2 mutation can be detected. This can help establish a diagnosis of TSC and provides useful information for therapeutic and family planning purposes. Unfortunately, in a small proportion of cases, no pathogenic mutation is identified. These individuals are referred to as TSC 'no mutation identified' (NMI). In some TSC NMI cases the pathogenic mutation may not be detectable using standard screening methods while, in other cases, variants of uncertain clinical significance (VUS) are identified. These are often nucleotide changes that are unlikely to prevent TSC1 or TSC2 expression, but could affect the function of the TSC protein complex. We have used a combination of targeted Next Generation Sequencing (NGS) and functional assays to improve the identification and classification of TSC1 and TSC2 variants. To detect mutations missed by conventional screening methods we performed targeted HaloPlex HS NGS of the TSC1 and TSC2 loci in TSC NMI individuals. So far, in a series of 40 cases, we have identified and validated the presence of a pathogenic variant in 26 cases (65%) and detected previously unidentified VUS in 7 cases (18%). Our work demonstrates that NGS technology can help increase the diagnostic yield for the molecular screening of individuals with TSC. To help classify TSC1 and TSC2 VUS, we have studied the effects of ~300 TSC1 and TSC2 variants on TSC complex formation and on signal transduction through the mechanistic target of rapamycin (mTOR) complex 1 (TORC1). Our work has provided insight into the genetic risks in the families segregating the tested variants and into the structure and function of the TSC complex. Investigating the effects of VUS on TSC complex activity is a useful adjunct to standard genetic testing and has been implemented as a diagnostic test in our laboratory. The results of our functional assessments have been submitted to the TSC1 and TSC2 Leiden Open Variation Databases (LOVD) (see www.lovd.nl/TSC1 and www.lovd.nl/TSC2).

E-mail: m.nellist@erasmusmc.nl
Keywords: tuberous sclerosis complex, functional genetics, NGS

Checking the quality of genetic variant and phenotype descriptions

P 04

Zgjim Osmani, Peter E.M. Taschner

Free sharing of high quality data helps to map the human genetic variation, to improve DNA diagnostics and to solve rare diseases worldwide. Novel variants discovered in immigrant populations are often characterized as variants of unknown significance (VUS) because information about their effects are lacking. It is important to publish and share all variant and phenotype information with clinicians world-wide, so they can provide correct diagnoses and proper counselling to patients. The goal of our project is to improve the quality of genetic variant and phenotype descriptions in the literature and databases in support of the Human Variome Project (HVP). Our questions were: Do the requirements in author instructions help to improve the quality of variant descriptions? Do the journals enforce the guidelines regarding to variant submission? The HVP Gene and Disease-specific Database Council has created example author instructions containing guidelines regarding HGVS nomenclature and submission of variants to public databases. These were sent to editors of genetics and genomics journals on a list created by the HVP office. From this list, we have selected journals requiring HGVS nomenclature and variant submission and investigated their January 2016 issues. We have checked whether variants were described according to the Human Genome Society (HGVS) nomenclature and submitted to public databases. The HGVS nomenclature guidelines require specification of genomic references to be able to verify (intronic) variants and their potential effects on splicing. Reference sequences were not reported in 38 out of 114 articles. In total, 17,4 % of all variants are still described incorrectly. In 82 papers, authors have indicated submission of all variants to public databases, yet no variants were found. Only 31 % of all variants are actually submitted to public databases. HGVS guidelines also require reporting at the RNA level, but RNA analysis is frequently neglected. Only 0,6 % of variants in manuscripts have RNA descriptions. Without supporting RNA level evidence, protein predictions caused by altered splicing cannot be verified. RNA analysis was reported in the database for 8,4 % of total variants, demonstrating that submission of variants might improve the quality of variant descriptions. To clarify uncertainties and inconsistencies, we have contacted authors for more information. Examples of common mistakes and recommendations for improvement will be presented.

E-mail: s1082488@student.hsleiden.nl
Keywords: HGVS nomenclature, variant, literature, database, journal, article, Human Variome Project (HVP)

The utility of next generation sequencing in neuromuscular disorders

P 05

Westra D (1), Saris CGJ (2), Voermans NC (2), Kamsteeg EJ (1)

Dept. of (1) Human Genetics and (2) Neurology, Radboud university medical center, Nijmegen, The Netherlands

Introduction Neuromuscular disorders (NMDs) have a wide clinical spectrum with a heterogeneous genetic, phenotypic, and allelic background. A large number of genes have been associated (to date more than 360 genes), multiple genes can have a overlapping phenotype, and a single gene can cause different NMDs. Making a genetic diagnosis via regular methods therefore is relative expensive, labor intensive, and time consuming. Next generation sequencing may be a promising alternative strategy for mutation detection in NMD patients. Methods 557 patients with neuromuscular disorders lacking a genetic diagnosis were tested by exome sequencing followed by targeted bioinformatics analysis for 223 nuclear encoded genes associated with myopathy. Results In 13.3% (74/557) of the patients, we identified the definitive genetic cause of the neuromuscular disorder. Additionally, in 17.6% (98/557) a likely pathogenic variant and/or a variant of uncertain significance (VUS) was suspected, but a second hit was missing or segregation analysis and/or functional studies still needs to be performed. In 12 other patients, based on segregation analysis we could exclude an identified VUS in a myopathy associated gene as the possible genetic cause. Collagen VI related myopathy was the most prevalent NMD in our cohort, followed by RYR1-related myopathy. Discussion Targeted NGS is a time- and cost-effective manner to identify the genetic cause in NMDs. This can improve patient care, counseling, and can give insights for potential new therapies. Nevertheless, in almost 70% of the patients, the NMD could not be explained by a genetic aberration in the screened genes. This may be due to the presence of copy number variations or disease causing variants outside the coding sequences of the associated genes or in currently unknown causative genes.

E-mail: dineke.westra@radboudumc.nl
Keywords: Neuromuscular disorders, next generation sequencing, exome sequencing

The expanding clinical phenotype of Bosch-Boonstra-Schaaf optic atrophy syndrome: 20 new cases and possible genotype-phenotype correlations

P 07

Chun-An Chen, 1,2, **Danielle G.M. Bosch**, 3–6, Megan T. Cho, 7, Jill A. Rosenfeld,1, Marwan Shinawi, 8, Richard Alan Lewis, 1,9, John Mann, 10, Parul Jayakar, 11, Katelyn Payne, 12, Laurence Walsh, 12,13, Timothy Moss, 14, Allison Schreiber, 14, Cheri Schoonveld, 15, Kristin G. Monaghan, 7, Frances Elmslie, 16, Ganka Douglas, 7, F. Nienke Boonstra, 4,6, Francisca Millan, 7, Frans P.M. Cremers,3,5, Dianalee McKnight, 7, Gabriele Richard, 7, Jane Juusola, 7, Fran Kendall, 17,18, Keri Ramsey, 19, Kwame Anyane-Yeboa, 20, Elfrida Malkin, 21, Wendy K. Chung, 20,22, Dmitriy Niyazov, 23, Juan M. Pascual, 24, Magdalena Walkiewicz, 1, Vivekanand Veluchamy, 25, Chumei Li, 26, Fuki M. Hisama 27, Bert B.A. de Vries, 3,6 and Christian Schaaf, 1,2,28

The first two authors and the last two authors contributed equally to this work, and the last two authors are co–senior authors. 1Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA; 2Jan and Dan Duncan Neurological Research Institute, Texas Children´s Hospital, Houston, Texas, USA; 3Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands; 4Bartimeus, Institute for the Visually Impaired, Zeist, The Netherlands; 5Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands; 6Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands; 7GeneDx, Gaithersburg, Maryland, USA; 8Division of Genetics and Genomic Medicine, Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri, USA; 9Department of Ophthalmology, Baylor College of Medicine, Houston, Texas, USA; 10Genetics, Kaiser-Permanente Fresno Medical Center, Clovis, California, USA; 11Nicklaus Children´s Hospital, Miami, Florida, USA; 12Riley Hospital for Children, Indianapolis, Indiana, USA; 13Departments of Neurology, Medical and Molecular Genetics, and Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana, USA; 14Genomic Medicine Institute, Cleveland Clinic, Cleveland, Ohio, USA; 15University of Minnesota Health, Minneapolis, Minnesota, USA; 16South West Thames Regional Genetics Service, St. George´s Healthcare NHS Trust, London, UK; 17VMP Genetics, LLC, Atlanta, Georgia, USA; 18University of Georgia, Athens, Georgia, USA; 19Center for Rare Childhood Disorders, Translational Genomics Research Institute, Phoenix, Arizona, USA; 20Department of Pediatrics, Columbia University Medical Center, New York, New York, USA; 21Nyack Hospital, Nyack, New York, USA; 22Department of Medicine, Columbia University Medical Center, New York, New York, USA; 23Division of Medical Genetics, Department of Pediatrics, Ochsner Clinic Foundation, New Orleans, Louisiana; 24Department of Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, Texas, USA; 25Division of Pediatric Neurology, Akron Children´s Hospital, Akron, Ohio, USA; 26McMaster University Medical Center, Hamilton, Ontario, Canada; 27Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, Washington, USA; 28Texas Children´s Hospital, Houston, Texas, USA.

PURPOSE: Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS) is an autosomal-dominant disorder characterized by optic atrophy and intellectual disability caused by loss-of-function mutations in NR2F1. We report 20 new individuals with BBSOAS, exploring the spectrum of clinical phenotypes and assessing potential genotype-phenotype correlations. METHODS: Clinical features of individuals with pathogenic NR2F1 variants were evaluated by review of medical records. The functional relevance of coding nonsynonymous NR2F1 variants was assessed with a luciferase assay measuring the impact on transcriptional activity. The effects of two start codon variants on protein expression were evaluated by western blot analysis. RESULTS: We recruited 20 individuals with novel pathogenic NR2F1 variants (seven missense variants, five translation initiation variants, two frameshifting insertions/deletions, one nonframeshifting insertion/deletion, and five whole-gene deletions). All the missense variants were

found to impair transcriptional activity. In addition to visual and cognitive deficits, individuals with BBSOAS manifested hypotonia (75%), seizures (40%), autism spectrum disorder (35%), oromotor dysfunction (60%), thinning of the corpus callosum (53%), and hearing defects (20%). CONCLUSION: BBSOAS encompasses a broad range of clinical phenotypes. Functional studies help determine the severity of novel NR2F1 variants. Some genotype-phenotype correlations seem to exist, with missense mutations in the DNA-binding domain causing the most severe phenotypes.

E-mail: D.G.M.Bosch-2@umcutrecht.nl
Keywords: BBSOAS; developmental delay; NR2F1; optic atrophy

Frequent loss of maternal chromosome 11 in SDHAF2, SDHD, and VHL-related paragangliomas, but not in SDHB-related paragangliomas

P 08

Attje S Hoekstra, Erik F Hensen, Ekaterina S Jordanova, Esther Korpershoek, Anouk NA van der Horst-Schrivers, Eleonora PM Corssmit, Frederik J Hes, Jeroen C Jansen, Dirk Kunst, Henri JLM Timmers, Dianna Eccles, Judith VMG Bovée, Peter Devilee, Jean-Pierre Bayley

Leiden University Medical Center VU University Medical Center Erasmus Medical Center Rotterdam University Medical Center Groningen Radboud University Nijmegen Medical Centre University of Southampton School of Medicine

Germline mutations in the succinate dehydrogenase (SDHA, SDHB, SDHC, SDHD, SDHAF2) or Von Hippel-Lindau (VHL) genes cause hereditary paraganglioma/pheochromocytoma. While SDHB (1p36) and VHL (3p25) are associated with autosomal dominant disease, SDHD (11q23) and SDHAF2 (11q13) show a remarkable parent-of-origin effect whereby tumor formation is almost exclusively associated with the paternal allele. Loss of the entire maternal copy of chromosome 11 is a frequent event in SDHD-linked tumors, suggesting an important role for chromosomal 11 loss. Using fluorescent in situ hybridization, microsatellite marker and SNP array analysis, we demonstrate that loss of the entire copy of chromosome 11 is a frequent event in SDHAF2-related PGLs, occurring in 89% of tumors. Analysis of two imprinted differentially methylated regions (DMR), H19-DMR and KvDMR (11p15), indicated selective loss of the maternal copy of chromosome 11. Comparative analysis in related tumors showed that 85% of SDHD and 75% of VHL-related PGLs/PCCs also show loss of maternal chromosome 11p15. By contrast, an absence of chromosome 11 loss was found in 62% of SDHB-mutated PGLs/PCCs, while only 31% showed loss of maternal chromosome 11p15. Copy number profiling revealed frequent loss of 1p in SDHB mutant tumors and indicated that SDHB tumors show greater genomic instability compared to SDHD and SDHAF2. These results show that loss of the entire copy of maternal chromosome 11 is a significant event in SDHAF2, SDHD and VHL-related PGLs/PCCs, but is less significant in SDHB-mutated tumors, suggesting both groups of tumors have distinct genetic etiologies.

E-mail: a.s.hoekstra@lumc.nl
Keywords: Paraganglioma, pheochromocytoma, succinate dehydrogenase, von Hippel-Lindau, loss of heterozygosity, methylation, SNP array.

A splice site mutation in RAB39B causing X-linked mental retardation with macrocephaly

P 09

Virginie J.M. Verhoeven, Leontine van Unen, J. Anneke Kievit, Marjon A. van Slegtenhorst, Mark Nellist, Jasper J. Saris, Frans W. Verheijen

Dept. Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands

Mutations in the small GTPase gene RAB39B have been previously associated with X-linked mental retardation, autism, epilepsy and macrocephaly. Here, we report on two brothers with macrocephaly (OFC > +3 SD) and moderate mental retardation (IQ 50). One sibling also fulfills the criteria for autism. Whole exome sequencing revealed a hemizygous RAB39B c.215G>T, p.(Arg72Ile) variant in both brothers. The mother was heterozygous for the variant, and the father and a third, healthy brother did not have the variant. In silico prediction programs indicated that the RAB39B c.215G>T substitution was likely to destroy the canonical splice donor site of exon 1. We isolated RNA from fibroblasts from one of the affected brothers and four healthy male controls. Reverse transcriptase PCR followed by Sanger sequencing revealed a novel RAB39B c.106_215del, p.Val36Ile fs*18 splice isoform in RNA from the affected sibling, demonstrating that the c.215G>T substitution affects RAB39B splicing and supporting the hypothesis that attenuation of RAB39B expression causes mental retardation and macrocephaly.

E-mail: v.verhoeven@erasmusmc.nl
Keywords: mental retardation, macrocephaly, RAB39B, XLMR

Cost-effectiveness of routine screening for lynch syndrome in endometrial cancer patients up to 70 years of age

P 10

Anne Goverde1,2, Manon CW Spaander1, Helena C van Doorn3, Hendrikus J Dubbink4, Ans MW van den Ouweland2, Carli M Tops5, Sjarlot G Kooi6, Judith de Waard7, Robert F Hoedemaeker8, Marco J Bruno1, Robert MW Hofstra2, Esther W de Bekker-Grob9, Winand NM Dinjens4, Ewout W Steyerberg9, Anja Wagner2, on behalf of the LIMO study group

Departments of Gastroenterology and Hepatology1, Clinical Genetics2, Gynaecology,3 Pathology4 and Public Health9, Erasmus MC, University Medical Center, Rotterdam, the Netherlands. Department of Clinical Genetics5, Leiden University Medical Center, Leiden, the Netherlands. Department of Gynaecology, Albert Schweitzer Hospital, Dordrecht, the Netherlands.6 Department of Gynaecology, Sint Franciscus Gasthuis, Rotterdam, the Netherlands.7 Pathology laboratory Pathan, Rotterdam, the Netherlands.8

Aim To assess the cost-effectiveness of routine screening for Lynch Syndrome (LS) in endometrial cancer (EC) patients =70 years of age. Methods Consecutive EC patients =70 years of age were routinely screened for LS by analysis of microsatellite instability, immunohistochemistry and MLH1 hypermethylation, followed by germline mutation analysis if indicated. Health benefit in life years gained (LYG) was based on the number of LS carriers detected among EC patients and their relatives. We calculated the incremental cost-effectiveness ratio (ICER) for LS screening among EC patients =70 years compared with EC patients =50 years and compared with the revised Bethesda guidelines. Results Screening of 179 EC patients identified 7 LS carriers; only 1 was =50 years and 6 were 51-70 years of age. Germline mutation analysis of relatives additionally identified 27 LS carriers (18 and 9 per age category respectively). LS screening resulted in 74,7 LYG, or 45,4 and 29,3 LYG per age category. The ICER for LS screening in EC patients =70 years compared with =50 years was €5,252/LYG. The revised Bethesda guidelines identified 3/7 (43%) LS carriers among EC patients. The ICER for routine LS screening in EC patients =70 years of age compared with the revised Bethesda guidelines was €6,668/LYG. Both ICERs remained <€16,000/LYG in sensitivity analyses. Conclusion Routine screening for LS by analysis of microsatellite instability, immunohistochemistry and MLH1 hypermethylation in EC patients =70 years is a cost-effective strategy, allowing colorectal cancer prevention in EC patients and their relatives.

E-mail: a.goverde.1@erasmusmc.nl
Keywords: Lynch syndrome; screening; cost-effectiveness; colorectal cancer; endometrial cancer

Performance of BRCA1/2 mutation prediction models in male breast cancer patients

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Setareh Moghadasi Vincent Grundeken Linda A.M. Janssen Nizet H. Dijkstra Wendy A.G. van Zelst-Stams Jan C. Oosterwijk Margreet G.E.M. Ausems Rogier A. Oldenburg Muriel A. Adank Eveline W. Blom Mariëlle Ruijs Theo A.M. van Os Carolien H.M. van Deurzen John W.M. Martens Carolien P. Schroder Juul T. Wijnen Maaike P.G. Vreeswijk Christi J. van Asperen

Department of Clinical Genetics, Leiden University Medical Centre, Leiden, the Netherlands Dutch Breast Cancer Research Group, Amsterdam, the Netherlands Department of Human Genetics, Radboud University Medical Centre, Nijmegen, the Netherlands Department of Genetics, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands Department of Genetics, University Medical Centre, Utrecht, the Netherlands Department of Clinical Genetics, Erasmus Medical Centre, Rotterdam, the Netherlands Department of Clinical Genetics, VU University Medical Centre, Amsterdam, the Netherlands Department Clinical Genetics, Maastricht University Medical Centre, Maastricht, the Netherlands Department of Clinical Genetics, the Netherlands Cancer Institute, Amsterdam, the Netherlands Department of Clinical Genetics, Academic Medical Centre, Amsterdam, the Netherlands Department of Pathology, Erasmus Medical Centre, Rotterdam, the Netherlands Department of Medical Oncology, Erasmus Medical Centre, Rotterdam, the Netherlands Department of Medical Oncology, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands Department of Human Genetics and Department of Clinical Genetics, Leiden University Medical Centre, Leiden, the Netherlands Department of Human Genetics, Leiden University Medical Centre, Leiden, the Netherlands

PURPOSE Female breast cancer patients are offered genetic testing based on guidelines determined by mutation probabilities calculated in prediction models. However, it is not yet known whether these models accurately predict mutation probability in male breast cancer (MBC) patients. As a result, all male patients are currently offered BRCA1 and BRCA2 diagnostic DNA screening. To establish whether existing models can identify which male patients should be tested, we compared the performance of three commonly used BRCA1/2 mutation prediction models, BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm), BRCAPRO and the Myriad prevalence table ('Myriad´). METHODS BOADICEA, BRCAPRO and Myriad were evaluated using the family history data of 307 MBC probands tested for BRCA1/2 at Dutch family cancer clinics, 58 (19%) of whom were BRCA1/2 mutation carriers. We compared the numbers of observed versus predicted mutation carriers and assessed the area under the receiver operating characteristic (ROC) curve (AUC) for each model. RESULTS BOADICEA predicted the total number of BRCA1/2 mutation carriers reasonably accurately (observed/predicted ratio: 0.94). However, when a cut-off of 10% and 20% prior mutation probability was used, BRCAPRO showed better performance (observed/predicted ratio BOADICEA: 0.81 and 0.79, vs. BRCAPRO: 0.97 and 0.94, respectively). Myriad underestimated the number of mutation carriers in up to 68% of the cases. BRCAPRO showed a non-significant, higher AUC than BOADICEA (0.798 vs 0.776), whereas Myriad showed a significantly lower AUC (0.671). CONCLUSION Our results support the use of BRCAPRO and BOADICEA in determining the probability that a MBC patient carries a BRCA1/2 mutation.

E-mail: s.moghadasi@lumc.nl
Keywords: BRCA1/2, Mutation Prediction Models, Male Breast Cancer

Identification and functional assessment of deep-intronic and non-canonical splice defects in abca4 associated with stargardt disease

P 12

Riccardo Sangermano1, Silvia Albert1, Miriam Bauwens2, Nathalie M. Bax3, Stephanie Cornelis1, Valerie Richelle1, L. Ingeborgh van den Born4, Alejandro Garanto1, Carel B. Hoyng3, Rob W.J. Collin1, Elfride De Baere2, Frans P.M. Cremers1

1Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, Netherlands 2Center for Medical Genetics, Ghent University and Ghent University Hospital, Ghent, Belgium 3Department of Ophthalmology, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, Netherlands 4Rotterdam Eye Hospital, Rotterdam, Netherlands

Purpose: Non-canonical ABCA4 splice variants are frequent in Stargardt disease (STGD1), but their effect is poorly understood. Deep-intronic variants have also been shown to affect RNA splicing. About 30% of STGD1 patients carries one or no ABCA4 variant, and we hypothesize that the missing mutations reside in the non-coding regions. Transcript analysis is not straightforward as ABCA4 is specifically expressed in the retina. The purpose of this study is to (1) identify intronic variants in unsolved cases by ABCA4 locus sequencing, and (2) to prove their functional effects as well the effect of non-canonical splice variants, by in vitro splice assays and by studying mRNA defects in patient-derived photoreceptor progenitor cells (PPCs) or retinal organoids. Methods: Haloplex-based locus sequencing was performed in 84 mono-allelic maculopathy cases. After applying splice site prediction programs, the effect of intronic variants was tested in vitro by cloning ~1.5-kb fragments into minigene constructs, transfecting them into HEK293T cells and performing RT-PCR. For selected variants we obtained skin biopsies, generated induced pluripotent stem cells and differentiated them into PPCs or retinal organoids. Subsequently, RT-PCR was performed. Results: By PPC mRNA analyses and minigene assays, the non-canonical c.5461-10T>C and c.6729+5_6729+19del variants were shown to result in exon 39/40 or 48 skipping, respectively, effectively resulting in null alleles. Haloplex sequencing revealed > 80 rare deep-intronic variants which potentially activate cryptic exons. At least 12 were analyzed by minigene splice assays. We found strengthening of splice sites and activation of pseudo-exons for at least two variants. For one of the deep-intronic variants, this result was confirmed by RT-PCR on mRNA extracted from patient-specific organoids. Conclusions: Minigene splice assays and mRNA analysis of patient-derived photoreceptor cells are effective to investigate variants predicted to have an effect on splicing. Two non-canonical splice variants, among which the most frequent severe ABCA4 variant, c.5461-10T>C, were shown to completely inactivate ABCA4 function. Haloplex-based locus sequencing identified 2 novel deep-intronic variants, both of which resulted in pseudo-exon activation. Some of these variants might be amenable for antisense oligonucleotide-based therapy.

E-mail: Riccardo.Sangermano@radboudumc.nl
Keywords: Stargardt disease, splice defects, deep-intronic variants

Towards an integrated database for systemic autoinflammatory diseases

P 13

Ellen C. Carbo 1, K. Joeri van der Velde 2, Bart Charbon 2, Fleur Kelpin 2, Joost Frenkel 3, J.K. Hans Kristian Ploos van Amstel 1, Mariëlle E. van Gijn 1, Morris A. Swertz 2, INSAID Consortium

1 Dept. of Genetics, University Medical Center Utrecht, Utrecht; 2 Dept. of Genetics, University Medical Center Groningen, Groningen; 3 Div. of Pediatrics, University Medical Center Utrecht, Utrecht

Systemic autoinflammatory diseases (SAID) are a growing number of monogenic and multifactorial conditions characterized by sterile inflammation. These can be severe conditions that benefit from a speedy molecular diagnosis, to start a timely treatment and prevent irreversible organ damage. The aim of the European INSAID consortium is to improve the performance of genetic diagnosis and to find new molecular diagnosing criteria for SAID. For this purpose we are creating a European database to integrate and share genetic, phenotype and functional data of SAID patients to speed up the identification of causal variants, new genes and biomarkers in genetically orphan SAID patients. The first version of the INSAID database, build on the open-source MOLGENIS platform, can be used for genetic/genomic queries across variants, variant classifications, and patient phenotypes. Submissions from different labs are presented together in a unified data explorer. Users can search and filter across all variants at once, or browse and search within data from specific labs. Reference databases such as 1000 Genomes, ExAC and Genome of the Netherlands are connected to provide allele frequencies for variants that have been seen in the general population before. Moreover the SAID specific INFEVERS database is connected. Additional variant assessment support tools that aid variant interpretation, such as CADD scores or SnpEff annotations, can be automatically attached to the variants. The resulting system allows multiple labs to classify variants and reach a consensus. In the near future we will expand the online database to include more laboratories, patients, phenotypes, classifications, biomarkers, proteomics and immunomics. Users will be able to automatically upload data for fast, community-driven analysis and interpretation. The MOLGENIS data APIs into R and JavaScript will be used for the analysis and visualization scripts, making all data and graphs interactively accessible to all users. The current online demo version is available at http://www.molgenis.org/infevers. It is being tested and further developed within the INSAID consortium to be tailored towards effective usage in daily practice. We expect one central and easy to use European bioinformatics platform will enable effective statistical clustering and augment in finding new genes and conditions for SAID.

E-mail: e.c.carbo-2@umcutrecht.nl
Keywords: Systemic autoinflammatory diseases, database, data sharing

De novo truncating mutations in the last and penultimate exon of PPM1D cause a novel intellectual disability syndrome

P 14

Sandra Jansen1, Sinje Geuer1, Rolph Pfundt1, Rachel Brough2, Johanna C. Herkert3, Elysa J. Marco4, Marjolein H. Willemsen1, Tjitske Kleefstra1, Mark Hannibal5, Joseph Shieh6, Sally Ann Lynch7, Frances Flinter8, David FitzPatrick9, Alice Gardham10, Birgitta Bernhard 11, Nicola Ragge12, Ruth Newbury-Ecob13, Raphael Bernier14, Malin Kvarnung15, Marja W. Wessels16, Kristin Monaghan17, Petra de Vries 1, Joris A. Veltman1,18, The DDD study, Christopher J. Lord2, Lisenka E. L. M. Vissers1 and Bert B. A. de Vries1

1 Department of Human Genetics, Donders Centre for Neuroscience, Radboud University Medical Center, P.O. Box 9101, 6500 HB, Nijmegen, the Netherlands. 2 The CRUK Gene Function Laboratory and Breast Cancer Now Research Centre, The Institute of Cancer Research, London, SW3 6JB United Kingdom. 3 University of Groningen, University Medical Center Groningen, Department of Genetics, P.O. Box 30.001, 9700 RB, Groningen, the Netherlands. 4 Departments of Neurology, Pediatrics and Psychiatry, University of California, 675 Nelson Rising Ln, Ste 405, San Francisco, California, United States. 5 Division of Pediatric Genetics, Metabolism & Genomic Medicine, University of Michigan Medical School, D5257 MPB, 1500 E Medical Center Dr, Ann Arbor, MI 48109-5718, United States 6 Division of Medical Genetics, Department of Pediatrics, UCSF Benioff Children's Hospital, Institute for Human Genetics, University of California San Francisco, San Francisco, CA 94143-0793, United States 7 Clinical Genetics, Children's University Hospital, Temple Street, Dublin 1, Ireland, UCD Academic Centre on Rare Diseases, School of Medicine and Medical Sciences. 8 Department of Clinical Genetics, Guy's and St Thomas' NHS Foundation Trust, London, UK. 9 MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh, United Kingdom. 10 Department of Clinical Genetics, North East Thames Genetics Service, Great Ormond Street Hospital for Children, NHS Foundation Trust, Great Ormond Street, London WC1N 3JH, United Kingdom. 11 North West Thames Regional Genetic Service (Kennedy Galton Centre), North West London Hospitals, Watford Road, HA1 3UJ, London, United Kingdom 12 Department of Medical genetics, Birmingham Women's Hospital, Oxford Brookes, United Kingdom 13 Department Department of Clinical Genetics, University Hospitals Bristol NHS Foundation Trust, St Michael´s Hospital, Southwell St, Bristol BS2 8EG, United Kingdom 14 Center on Human Development and Disability, University of Washington, Box 357920, Seattle, WA 98195-7920, United States 15 Department of Clinical Genetics, Karolinska University Hospital Solna, Karolinska Institutet, Stockholm, Sweden 16 Department of Clinical Genetics, Erasmus MC University Medical Center, Wytemaweg 80, 3015 CN Rotterdam 17 GeneDx, Gaithersburg, MD 20877, USA 18 Department of Clinical Genetics, Maastricht University Medical Centre, Universiteitssingel 50, 9229 ER, Maastricht, the Netherlands.

Intellectual disability (ID) is a highly heterogeneous disorder with more than 600 known genes involved, yet, a genetic diagnosis remains elusive in ~35-40% of patients with moderate-severe ID. Recent meta-analyses statistically analyzing de novo mutations identified in >5,000 ID patients highlighted PPM1D as a candidate ID gene. PPM1D is a type 2C phosphatase that functions as a negative regulator of cell stress response pathways by mediating a feedback loop of p38-p53 signaling, thereby contributing to growth inhibition and suppression of stress induced apoptosis. We identified 14 patients with mild-moderate ID and a de novo truncating PPM1D mutation. Deep-phenotyping of the patients revealed in addition to ID overlap for behavioural problems (ADHD and anxiety disorder), hypotonia, broad based gait, facial dysmorphisms and periods of fever and vomiting. PPM1D is shown to be expressed during fetal (brain) development and in the adult brain. All mutations were located in the last, or penultimate exon, suggestive of escaping nonsense-mediated mRNA decay. Both PPM1D expression analysis and cDNA sequencing in patient EBV-LCLs support the presence of a stable, but truncated transcript, consistent with this hypothesis. Exposure of patient´s cells to ionizing radiation resulted in normal p53 activation suggesting that p53 signaling is not affected by the

truncated protein. However, a cell growth disadvantage was observed. Thus, we show that de novo truncating PPM1D mutations in the last and penultimate exon cause syndromic ID which provides novel insights in the role of cell cycle checkpoint genes in neurodevelopmental disorders.

E-mail: sandra.jansen1@radboudumc.nl
Keywords: Cell cycle checkpoint Intellectual disability PPM1D Stress response pathways Syndrome Truncating mutation

Candidate gene sequencing in infertile men using single molecule molecular inversion probes

P 15

M.S. Oud1, P.F. de Vries1, D.F.C.M. Smeets1, M.K. O´Bryan2, R.I. McLachlan2, L.E.L.M. Vissers1, A. Hoischen1, L. Ramos3, J.A. Veltman1, M.J. Noordam4

1. Department of Human Genetics, Radboudumc, The Netherlands 2. Department of Anatomy and Developmental Biology, Monash University, Australia 3. Department of Gynaecology and Obstetrics, Radboudumc, The Netherlands 4. Department of Genetics and Cell Biology, Maastricht, The Netherlands

Infertility is defined as the failure to conceive within one year of unprotected sexual intercourse and affects millions of couples each year worldwide. In approximately half of the infertility cases there is a male factor involved. Currently 10-15% of azoospermia (i.e. no sperm in the ejaculate) cases are caused by recurrent microdeletions of the Y chromosome (YCMs). In the majority of the remaining cases the etiology of the infertility remains unknown though many genes have been reported to affect male fertility. Here we describe a novel method to rapidly and affordably screen a large cohort of patients for mutations and structural variations in causal (N=5) and candidate (N=103) male infertility genes and evaluate the use of this method for diagnostic applications. For this study, we included 1,112 idiopathic infertile men that were presented at the outpatient clinics of the Radboudumc and of the Monash University. Of these, 532 men were diagnosed with azoospermia and 580 with a severe form of oligozoospermia, i.e. a total motile count of <5,000,000. We designed a set of 4,575 single molecule Molecular Inversion Probes (smMIPs) that target the coding regions of 5 causal and 103 candidate male infertility genes. To validate the effectiveness of our novel screening method compared to commonly used screening methods, we screened 16 patients with known YCMs, sex chromosome aneuploidies and other chromosomal abnormalities in a blinded fashion. Our results matched all previous diagnoses, and in some cases provided a better characterization of the genomic abnormality. Overall, more than 90% of all targeted regions were sufficiently covered for a reliable variant detection. At this moment we are screening 5 patients with known CFTR mutations and 5 with CYP21A2 mutations. In conclusion, we have developed a method to perform targeted sequencing of known and candidate male infertility genes that can be useful for research and diagnostic applications. The potential of the smMIP screening technology is highlighted as it should not only be able to detect point mutations, but also structural variations of the X and Y chromosome and other chromosomal abnormalities, i.e. the current most diagnosed causes of male infertility. We will now use the smMIP technology to screen our cohort for mutations in the 108 causal/candidate genes to elucidate which genes affect male fertility and the prevalence of mutations in these genes.

E-mail: manon.oud@radboudumc.nl
Keywords: Male Infertility, smMIPs, Targeted Sequencing, Diagnostics

BRCA testing in Ovarian tumors initiated by a PAthologist (OPA): a pre-screen for germline testing and therapy choice

P 16

Arjen R. Mensenkamp (1), Edward M. Leter (2), Joanne A. de Hullu (3), Riki W. Willems (4), Gwendolyn H. Woldringh (1), Michiel Simons (4), Marjolijn Jongmans (1,5), Hans Bulten (4), Marjolijn J.L. Ligtenberg (1,4), Nicoline Hoogerbrugge (1)

1. Department of Human Genetics, Radboud university medical center, Nijmegen 2. Department of Clinical Genetics, Maastricht University Medical Center, Maastricht 3. Department of Obstetrics & Gynecology, Radboud university medical center, Nijmegen 4. Department of Pathology, Radboud university medical center, Nijmegen 5. Department of Medical Genetics, University Medical Center Utrecht, Utrecht

Dutch guidelines advise germline DNA BRCA1 and BRCA2 testing to all women with ovarian cancer (OC). About 20% of all OC patients have a tumor DNA BRCA mutation, of which 75% are germline and 25% somatic mutations. Both may benefit from PARP inhibitor therapy. A tumor DNA BRCA test for all patients with newly diagnosed OC initiated by a PAthologist (OPA) may serve as a pre-screen for germline DNA BRCA testing and as a predictive test for response to PARP inhibitor therapy. Pathologists from multiple laboratories were invited to submit formalin fixed, paraffin embedded (FFPE) samples of all newly diagnosed epithelial OCs for tumor DNA BRCA testing. BRCA testing was performed using a combined approach of single molecule molecular inversion probe(smMIP)-based targeted next generation sequencing (NGS) and BRCA1 Multiplex Ligation-dependent Probe Amplification (MLPA) for the detection of gross deletions and duplications in DNA from these samples. Turnaround time for tumor DNA BRCA testing was calculated from dates of request to test result. Test results were shared with patients by the gynecologic oncologist, who referred patients with a positive tumor DNA BRCA test for germline testing. From October 2015 to the first half of June 2016 78 tumor DNA BRCA tests were requested for 75 women. BRCA testing was not feasible in six of the provided tumor samples, because of low tumor cell percentage (N =3) or low DNA quantity or quality (N = 3). For three of these BRCA testing was performed on a different sample. In 72 women with complete tumor DNA BRCA testing, median age at OC diagnosis was 64 (range 29-86). BRCA mutations were detected in 16 tumors (22%; 10 BRCA1 and 6 BRCA2), of which thirteen were high-grade serous cancer, one low grade serous, one endometroid and one undifferentiated. Median age of women with tumor DNA BRCA mutations was 58 (42-85). Median turnaround time was 14 days (7-86). In 60% (3 out of 5) of the women with tumor DNA BRCA mutations, germline mutations were found. OPA was feasible in most tumors and turnaround times were short. Knowledge on tumor BRCA mutation status can be used to select patients for germline testing and to aid therapy choices shortly after OC diagnosis. Uptake of tumor DNA BRCA testing will be evaluated with data from a national pathology registration database. Patients and physicians experiences will be evaluated by questionnaires. Supported by a grant from AstraZeneca.

E-mail: **ingrid.fakkert**@radboudumc.nl
Keywords: ovarian cancer, hereditary breast and ovarian cancer, tumor DNA testing, BRCA1, BRCA2

Deletion of a 545 kb region proximal of the NDP gene causes Norrie disease

P 17

L. Haer-Wigman1,2, N. De Leeuw1,3, H.G. Yntema1,2, R.W. Collin1,3, D. Lugtenberg1,2, D. Olde Weghuis1,2, R. Pfundt1,3, N.E. Schalij-Delfos4, M.H. Breuning5

1Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands 2Radboud Institute of Molecular Life Sciences, Nijmegen, the Netherlands 3 Donders Institute for Brain, Cognition and Behaviour, Nijmegen, the Netherlands 4Department of Ophthalmology, Leiden University Medical Center, Leiden, the Netherlands 5Department of Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands

Background Norrie disease is a rare, inherited eye disorder causing blindness at birth or in early infancy. Later in life, a subset of patients also develops neurological symptoms as sensorineural deafness, intellectual disability, psychosis etc. Norrie disease is caused by mutations in the NDP gene located on the X-chromosome. Case presentation We investigated a three-month-old baby boy who had wandering eye movements and no visual reaction. Clinical examination by an ophthalmologist determined that the visual impairment was due to bilateral vitreoretinal dysplasia and the differential diagnosis was Norrie disease. There was no known history of visual impairment in the family. Results Sequencing of all three exons of the NDP gene rendered no pathogenic variants. Subsequently, exome sequencing was performed to detect the genetic cause. No causative single nucleotide variants were detected, however, copy number variant analysis of the exome data detected a deletion proximal of the NDP gene, including the EFHC2 gene located upstream. Because all three exons of the NDP gene were detected via Sanger sequencing, it is certain that the NDP gene itself was not part of the deletion. Genome wide array analysis of the mother showed the presence of a heterozygous 545 kb loss in Xp11.3 (arr[hg19] Xp11.3(43,839,073-44,377,451)x1) ending approximately 6,000 bases proximal of the NDP gene. Region-specific FISH analysis in samples from the maternal grandparents revealed that de deletion had arisen de novo in the mother. Conclusion en discussion The Norrie disease of this baby boy is most likely caused by the deletion proximal of the NDP gene. Multiple pathogenic deletions have been described in patients with Norrie disease, yet in these cases always one or more exons of the NDP gene were deleted. We hypothesize that the deletion encompasses a regulatory element that is needed for expression of the norrin protein encoded by the NDP gene. Multiple regulatory elements (enhancers and transcription factor binding sites) are predicted to be present in the deleted gene region. Next to NDP, pathogenic variants in four other genes are known to cause familial exudative vitreoretinopathy. One of these genes, ZNF408, is a transcription factor. It would be very interesting to investigate whether ZNF408 regulates the expression of the NDP gene and has a binding site in the genomic region that is deleted in this patient.

E-mail: lonneke.haer-wigman@radboudumc.nl
Keywords: Norrie disease, NDP, copy number analysis, exome sequencing

Detection of low level mosaic mutations in a gene panel for segmental overgrowth.

P 18

M. Alders, M.A. Haagmans, O.R. Mook, M.M.A.M. Mannens

Department of Clinical Genetics, AMC, Amsterdam, the Netherlands

Somatic mutations in genes of the PI3K/AKT/mTOR pathway causing segmental overgrowth may be present in a very low percentage. This can hinder the identification of causal mutations. Sanger sequencing, but also standard NGS diagnostic pipelines are able to identify mosaic mutations with a detection limit of approximately 10%. Targeted analysis of specific sites is usually used for more sensitive detection of mutations. With the growing number of known mutations there is a need for a high sensitive mutation scanning method to analyze the complete coding sequences of the genes involved rather than analysis of hotspots. We have developed a NGS based gene panel test for segmental overgrowth (AKT1, AKT3, MTOR, PIK3CA, PIK3R2, PTEN, TSC1 and TSC2) especially for the detection of low level mosaicism. Samples are sequenced with high coverage (average 1500-2000 reads, minimum 500 reads) and an analysis pipeline was developed that is able to detect mosaic mutations as low as 1%.

E-mail: m.alders@amc.uva.nl

Expanding the Phenotype of ITPR1-Related Spinocerebellar Ataxia´s

P 19

Tessa van Dijk1, Peter Barth2, Liesbeth Reneman3, Bart Appelhof1, Frank Baas1, Bwee Tien Poll-The2

1 Department of Clinical Genetics, Academic Medical Centre, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands 2 Department of Pediatric Neurology , Academic Medical Centre, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands 3 Department of Radiology, Academic Medical Centre, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands

Mutations in the ITPR1 gene are associated with different types of autosomal dominant Spinocerebellar Ataxia (SCA) and with Gillespie syndrome. Age of onset and severity are variable and hypoplasia/atrophy of cerebellar vermis and hemispheres is mild, if present at all. Here we describe a patient with severe hypoplasia of cerebellar hemispheres, vermis and pons, caused by a de novo missense mutation in the ITPR1 gene identified with whole exome sequencing (WES). Brain MRI showed severe pontine and cerebellar hypoplasia without supratentorial abnormalities or signs of cortical atrophy. Because the MRI was compatible with pontocerebellar hypoplasia (PCH), mutation analysis of the TSEN54, TSEN2, TSEN34, RARS2 and VRK1 genes was performed, but no mutations were identified. Trio analysis of the WES data revealed a de novo missense mutation in exon 56 of the Inositol 1, 4,5-Triphosphate Receptor type 1 (ITPR1) gene (c.7649T>A, NM_001099952.2) which results in the amino acid substitution of a highly conserved Isoleucine by Asparagine (p.I2550N). The mutation was predicted pathogenic by various in silico prediction programs (i.e. SIFT, Polyphen) and was not present in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) or the ExAC database (http://exac.broadinstitute.org/). Recently, mutations in ITPR1 were also identified in patients with Gillespie syndrome, which is characterized by non-progressive cerebellar ataxia and iris hypoplasia. The mutation in our patient is predicted at the border of the transmembrane ion channel pore domain, in close proximity to the heterozygous mutations found in Gillespie syndrome. In conclusion, we broadened the spectrum of ITPR1 related ataxias by identifying a de novo missense mutations in a patient with very severe hypoplasia of cerebellum and pons, mimicking PCH.

E-mail: t.vandijk@amc.nl
Keywords: Pontocerebellar Hypoplasia, ITPR1 gene, Spinocerebellar Ataxia

Functional characterization of CMT-associated LRSAM1 mutations

P 20

Johanna E. Hakonen, Marit B. de Wissel, Rossella Avagliano Trezza, Noam Zelcer, Frank Baas, Marian A.J. Weterman

Department of Clinical Genetics, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands
Department of Medical Biochemistry, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands

Charcot-Marie-Tooth (CMT) disease is a genetically heterogeneous group of inherited neuropathies characterized by motor and sensory deficits due to peripheral axon degeneration. We demonstrated that mutations in LRSAM1 gene are associated with the axonal type of the CMT (CMT2). The precise functional consequence of LRSAM1 mutations in the disease are unknown. Both dominant and recessive (null alleles) have been identified in CMT2 patients. In this study we provide a functional basis for association of LRSAM1 with CMT2. Namely, our results demonstrate how mutations in the LRSAM1 affecting the RING domain disrupt the ubiquitination activity and lead to the loss of target ubiquitination. This functional disruption may explain the role of LRSAM1 in CMT2. This assay can be used as a functional screen for novel LRSAM1 mutations. Here we describe a novel heterozygous missense mutation c.2120C>T (p.Pro707Arg) mutation in the LRSAM1 gene found in a CMT2 patient. The mutation is located in the C-terminal RING domain of the LRSAM1 and disrupts LRSAM1 function.

E-mail: j.e.hakonen@amc.uva.nl
Keywords: Charcot-Marie Tooth disease, peripheral axon degeneration, CMT2, LRSAM1, ubiquitination, E3 RING ubiquitin ligase

A case of unexpected moderate haemophilia A in a female neonate within a family with mild haemophilia A in affected males: what happened?

P 21

M.P.R. Lombardi1 ,P. Lakeman1,2 ,I.B. Mathijssen1,2 , M. Peters2,3.

1 Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands 2 Haemophilia Treatment Center, Academic Medical Center, Amsterdam, The Netherlands 3 Department of Pediatric Hematology, Emma Children´s Hospital, Academic Medical Center, Amsterdam, The Netherlands.

A carrier of the F8 mutation c.1834C>T, p.(Arg612Cys) has Factor VIIIc of 118%. She was pregnant with a female fetus and was counseled that her unborn daughter had a 50% chance of carrying the mutation. Arg612Cys is associated with mild haemophilia A in affected males (Waseem et al., Thromb Haemost 1999; 81: 900-5). In an affected male cousin this mutation resulted indeed in a Factor VIIIc of 19%. No additional precautions during pregnancy were advised apart from regular prenatal and obstetrical care and the policy was to test her daughter´s factor VIIIc value postnatally between age 3 and 6 months. After birth, the daughter surprisingly was diagnosed with moderate Haemophilia A (Factor VIIIc : 4%). Additional investigations were performed to unravel the cause of this unexpected finding. Skewed X inactivation (lyonisation) seemed unlikely, because even in a complete skewed situation (when only affected X chromosomes are activated in each cell), the patient´s clinical features would resemble the mild form of haemophilia A of the affected males in the family. And this would also be the case is she had monosomy X (Turner syndrome) or a submicroscopic deletion on the contralateral X chromosome. These analyses were performed and no abnormalities were detected. Additional F8 gene mutation analysis, however, identified the familial c.1834C>T mutation, but also a second mutation c.1171C>T, p.(Arg391Cys), which is associated with mild to severe haemophilia A (Green PM et al., Br J Haematol. 2008 Oct;143(1):115-28). These two mutations explain the moderate haemophilia A phenotype. The second mutation was not identified in her parents peripheral blood lymphocyte (PBL) DNA. A low recurrence risk for future pregnancies was counseled (<1%). Nevertheless, if a (small) percentage of her father´s germ cells does carry the second mutation, due to paternal germline mosaicism, then this couple is confronted with the possibility of having a more severe form of haemophilia A in their female offspring , if the mother passes the familial mutations as well, compared to their affected male offspring, as their sons only have 50% chance of inheriting their mother´s mild haemophilia A mutation.

E-mail: m.p.lombardi@amc.uva.nl
Keywords: Hemophilia-A, F8, mutation

tRNA Processing Mutations in Pontocerebellar Hypoplasia

P 22

Bart Appelhof, Tessa van Dijk, Veerle R.C. Eggens, Anneloor ten Asbroek, Marian A.J. Weterman, Frank Baas

Department of Genome Analysis, Academic Medical Centre, University of Amsterdam

Pontocerebellar hypoplasia (PCH) represents a heterogeneous group of neurodegenerative disorders with a prenatal onset. So far, ten subtypes are described (PCH1-10), based on genetic and clinical features. Coinciding symptoms are hypoplasia and/or atrophy of the pons and cerebellum and patients suffer from severe cognitive and motor defects. Currently, only symptomatic treatment is available and most patients die before adulthood. Multiple aberrations in different genes have been associated with PCH thus far. The majority of these genes participate in RNA processing, e.g. the tRNA splicing endonucleose (TSEN) genes, arginyl tRNA synthetase 2 (RARS2), cleavage and polyadenylation factor 1 subunit 1 (CLP1) and exosome component 3 (EXOSC3). However, there is still a substantial group of patients where the pathogenic mutation is not yet identified. We performed whole exome sequencing on 25 PCH patients and 5 trios with an unknown genetic cause, aiming to identify novel genes involved in PCH which explain the disease mechanism. So far, we identified over ten candidate mutations of which one is located in RNA 3´-terminal phosphate cyclase (RtcA). The mutation in RtcA is further assed in a zebrafish model. Knockdown of RtcA by using morpholinos results in microcephaly and abnormal brain development. Aberrant movement is also seen in the RtcA knockdown animals. This fits a PCH phenotype. RtcA is a cyclase involved in the tRNA processing pathway. After intron removal by TSEN complex, a tRNA exon with a terminal 2,3-cyclic phosphate is generated. RtcB cleaves the cyclic phosphate resulting in either a 2'- or 3'- phosphate. The latter is a substrate for the ligation reaction, whereas the 2'-phosphate is a dead end product. RtcA can rescue this molecule by again forming a 2,3-cyclic phosphate, enabling proper cleavage by RtcB, ligation and mature tRNA syntheses. Cyclase activity was shown to be absent in fibroblast lysate of the patient. So far, we can conclude that the aberration in RtcA could be the cause of PCH in this patient, however more research is necessary to confirm this.

E-mail: b.appelhof@amc.nl
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ROH-analysis uncovers partial UPD17 causing urea cycle disorder

P 24

Myrthe van den Born1, Grazia Mancini1, Jasper Saris1, Dicky Halley1, George Ruijter1, L. van Zutven1, Johannes Häberle3 and Monique Williams2

1 Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands2 Department of Metabolic diseases, Erasmus Medical Center, Rotterdam, the Netherlands

3 Division of Metabolism and Children's Research Center, Universitäts Kinderspital Zürich, Switzerland

N-acetylglutamate (NAG) is the allosteric activator of carbamoylphosphate synthetase 1 (CPS1) and thus an essential co-factor for the conversion of ammonia into urea in the liver. The enzyme N-acetylglutamate synthase (NAGS) catalyzes the formation of NAG. NAGS deficiency is a rare metabolic disease inherited as an autosomal recessive trait, which causes a block in the urea cycle resulting in hyperammonemia.

We observed a girl with a sudden metabolic encephalopathy at the age of 3 due to hyperammonemia. The patient's development was severely delayed and she developed epilepsy. Metabolic screening (amino acids, organic acids) did not show abnormalities suggestive for an urea cycle defect or organic aciduria. Enzymes (NAGS, CPS1 and OTC) measured in liver samples showed normal activities. No mutations or deletions were found in the genes OTC and CPS1. SNP-array revealed a large region of homozygosity (ROH) at the terminal end of chromosome 17 including the NAGS gene. DNA sequencing and MLPA analysis of NAGS did not show any mutations, deletions or duplications. Additional testing identified a homozygous c.-118-2908C>T mutation in the NAGS enhancer region (Heibel et al, Human Mutation, 2011). This mutation affects a highly conserved nucleotide adjacent to another transcriptor binding site. Further, this mutation has been investigated in a research assay and was found to decrease NAGS expression. The mother was heterozygous for this enhancer mutation; the father did not have the mutation. We hypothesize a monosomic rescue resulting in a partial uniparental disomy (UPD) of chromosome 17. This case highlights the importance of combining SNP-array, metabolic screening, and DNA-examination.