RARE-MED SYMPOSIUM 2022

DECEMBER 8, 2022 - 9.00-17.00 - AULA, GHENT, BELGIUM

RARE-MED SYMPOSIUM

ABSTRACT BOOK

Hosted by RARE-MED

Date: December 8, 2022

Venue: Aula, Ghent, Belgium

WELCOME

ELFRIDE DE BAERE

Elfride De Baere is Professor at Ghent University, Head of Clinic at Ghent University Hospital and Senior Clinical Investigator of the Research Foundation Flanders FWO. She is coordinator of the RARE-MED consortium.

Dear participants,

It is with great pleasure that we welcome you to the second RARE-MED symposium, which will be unique for several reasons. First, we will hear three renowned keynote speakers covering non-coding variation (Nicky Whiffin), RNA therapeutics (Willeke Van Roon-Mom) and functional genomics (Musa Mhlanga) in rare diseases.

Apart from this, 26 young investigators will present their exciting research on rare diseases during the short platform presentations and the 1' poster pitches.

This meeting would not have been possible without the generous support from the different sponsors and without the help of many people including the Organizing Committee and many supporters. A big Thank You to all of them.

Finally, we encourage all participants to partake in scientific discussions. We count on you to make this meeting dynamic and to interact with the speakers.

Welcome to our meeting, enjoy the great science!

Elfride De Baere

on behalf of the Organising Committee,

Sarah Vergult, RARE-MED research professor

Kris Vleminckx, RARE-MED research professor

Frauke Coppieters, RARE-MED research professor

Elfride De Baere, RARE-MED coordinator

Karolien Aelbrecht, project manager

PROGRAM

09:00	**Welcome -** Elfride De Baere, RARE-MED coordinator

ADVANCED DIAGNOSIS & THERAPY

09:05	**Keynote speaker I – Nicky Whiffin** **Interpreting variation in the non-coding genome**
09:50	**1' Poster pitches**
	Annelies Dheedene *- Inherited pathogenic variants in neurodevelopmental disorders: a potential pitfall in trio-based analysis of clinical exomes*
	Eline Van Vooren *- An in vitro enzymatic assay to elucidate the VUS problem in RPE65, a target for retinal gene therapy*
	Karo De Rycke *- Zebrafish as a tool to study cardiovascular effects caused by fibrillin impairment*
	Lukas Nollet *- Serum calcification propensity T50 as a novel biomarker for disease severity in patients with pseudoxanthoma elasticum*
	Marjolein Carron *- Modelling of RCBTB1-associated inherited retinal disease in Xenopus tropicalis indicates a role for RCBTB1 in cell polarity and oxidative stress responses*
	Maria del Rocío Pérez Baca *- A novel neurodevelopmental syndrome caused by loss-of-function of the Zinc Finger Homeobox 3 (ZFHX3) gene*
	Andy Willaert *– Zebrafish Facility Ghent – A new UGent CORE facility*
10:00	**Part I: Short platform presentations (10')**
	Eva D'haene **-** *Structural variants disrupt a critical regulatory region downstream of FOXG1*
	Lisa Dangreau **-** *The pseudoxanthoma elasticum zebrafish model contributes to novel pathophysiological insights and therapeutic strategies in ectopic mineralization.*
	Hannes Syryn **-** *Whole exome sequencing advances a genetic diagnosis in patients with differences of sex development*
10:40	**Coffee break**
11:10	**Part II: Short platform presentations (10')**
	Laurenz De Cock *- The added value of RNA-sequencing in exome variant interpretation*
	Michiel Vanhooydonck *- Establishment of the first reported zebrafish model for thoracic aortic dissection & rupture*
	Sofia Papadimitriou *- The importance of good data quality and proper pathogenicity reporting in the medical genetics field: the case of oligogenic diseases*
11:50	**Keynote speaker II – Willeke Van Roon-Mom** **RNA Therapeutics in neurological and rare diseases**

12:35	**1' Poster pitches**
	Marlies Colman *- Defective collagen biosynthesis in kyphoscoliotic Ehlers-Danlos syndrome due to pathogenic variants in PLOD1 and FKBP14: further insights into the common pathophysiological mechanisms and comparison of clinical characteristics*
	Charlotte Matton *– FOXL2 mutation update for BPES, a syndromic form of POI: in silico assessment and ACMG classification of 394 unique sequence variants, and review of structural variants*
	Lynn Backers *– ATRIP-deficient patient expands molecular and clinical spectrum of seckel syndrome*
	Sophie Debaenst *– Crispant analysis in zebrafish as a tool for rapid functional screening of disease-causing genes for Osteogenesis Imperfecta*
	Lieselot Vincke *- Characterization of the genetic architecture of inherited retinal disease in a consanguineous Iranian cohort*
	Lisa Hamerlinck *– An optimized workflow for CRISPR/Cas9-mediated generation of indels and large deletions in induced pluripotent stem cells and neural stem cells*
12:45	**Platinum Sponsor Talk by Novartis**
12:50	**Lunch & Poster Session**
13:55	**Golden Sponsor Talk by Bionano Genomics**

DISEASE MODELS & FUNCTIONAL GENOMICS

14:00	**Part III: Short platform presentations (10')**
	Leslie Naesens *- Human GTF3A deficiency predisposes to neuroinvasive HSV-1 infection by disrupted transcription of host-derived non-coding RNAs*
	Tamara Jarayseh *- A tapt1 knock-out zebrafish line with aberrant lens development and impaired vision models human early-onset cataract*
	Robin Vroman *- The matrisome of the murine and human dorsal root ganglion: a transcriptomal approach*
	Melissa Pille *- The role of the Wiskott-aldrich Syndrome protein in the development of T cells*
	Alfredo Dueñas Rey *– Identification and characterization of a novel retina-specific lncRNA upstream ABCA4 with a potential role in ABCA4-associated inherited retinal disease*
	Violette Deleeuw *- Defects in the first hybrid domain of fibrillin-1 affect vascular wall homeostasis in the thoracic aorta*
	Münevver Burcu Çiçekdal *- CRISPR/Cas9 mediated disruption of an evolutionary conserved putative enhancer in the mab21l2 locus induces developmental eye anomalies in Xenopus tropicalis*
15:30	**Coffee break**
16:00	**Keynote speaker III – Musa Mhlanga** **A chromatin-regulated biphasic circuit coordinates inflammation and trained immunity**
16:45	**Best Presentation Awards – Closure**
17:00	**Reception**

INVITED SPEAKERS

NICKY WHIFFIN

Nicky Whiffin is a Group Leader and Wellcome Sir Henry Dale Fellow at the Big Data Institute and Wellcome Centre for Human Genetics at the University of Oxford. Nicky is also a visiting scientist at theBroad Institute of MIT and Harvard, a Wellcome Beit award winner and a fellow of St Anne's College and the Centre for Personalised Medicine in Oxford. Nicky leads the Computational Rare Disease Genomics group, which uses large genomic datasets and computational/statistical approaches to identify novel categories of variants, outside of protein-coding regions, that cause rare human disease.

Nicky's undergraduate degree was in Natural Sciences at the University of Cambridge before she studied for a PhD in genetic susceptibility to Colorectal Cancer at the Institute of Cancer Research in London. During her postdoctoral work at Imperial College London, she developed tools and methods to improve interpretation of variants identified in patients with Inherited Heart Conditions.

Interpreting variation in the non-coding genome

Current approaches to identifying genetic diagnoses for individuals with rare disease focusses almost exclusively on regions of the genome that directly encode protein. Through this approach, we only find a likely disease-causing variant for ~one third of individuals. Increasingly, non-coding region variants are being shown to play an important role in rare disease, but we are still limited in which of these variants we can accurately identify, annotate and interpret for a role in disease. In this talk, I will outline our recent approaches to identify untranslated region (UTR) variants that disrupt translational regulation and lead to rare disease. I will share the tools and resources we have created to help annotate and interpret these variants for a role in rare disease, and discuss new clinical guidelines to classify these variant types. Finally, I will discuss on-going work in the group to systematically identify non-coding region variants in rare disease patients in the Genomics England 100,000 Genomes Project.

WILLEKE VAN ROON-MOM

Willeke van Roon-Mom is a full professor of Human Genetics, in particular of translational studies of neurodegenerative disorders. She studied Medical Biology at the Rijksuniversiteit in Groningen, and did her PhD in Auckland New Zealand studying Huntingtons disease. After a Post Doc in New Zealand, she returned to the Netherlands to work at the Human Genetics department at the Leiden University Medical Center where she started her own research group. Her work is highly translational in nature, working in close collaboration with clinical departments and industry. Unique patient-driven fund raising initiatives contribute not only financial input, but also patient perspective to research programs in her group. The main topic of her research is autosomal dominant neurodegenerative diseases that have aberrant protein aggregation as a pathological hallmark. She studies molecular disease mechanisms, identifies biomarkers and then uses this knowledge to develop novel therapies with a focus on RNA targeting antisense oligonucleotide therapies. She is the co-founder and co-lead of the Dutch Center for RNA Therapeutics that aims to develop RNA targeting therapies for patients with ultra-rare mutations.

RNA therapeutics in neurological and rare diseases

Research in my group focuses on hereditary brain disorders with aberrant protein aggregation as a main pathological hallmark. One group of disorders that we study are the polyglutamine disorders, where the disease is caused by a polyglutamine expansion in proteins that are important for normal brain function. This expansion of a stretch of glutamine amino acids causes a toxic gain of function of these proteins. In this presentation I will show examples of the preclinical development of splice modulation RNA targeting therapies for some of these polyglutamine diseases and how we are using this knowledge to develop patient-specific RNA targeting treatments for patients with unique mutations that cause a brain disorder.

MUSA MHLANGA

Musa M. Mhlanga holds a PhD in Cell Biology & Molecular Genetics from New York University School of Medicine (2003). He began his PhD at the Rockefeller University in the laboratory of David Ho where he worked on spectral genotyping of human alleles. He then went on to work on the development of *in vitro* and *in vivo* applications of molecular beacons for their use in visualizing RNA in living cells with Fred Russell Kramer and Sanjay Tyagi at New York University School of Medicine. Upon completion of his doctoral work he was awarded a U.S. National Science Foundation post-doctoral fellowship at the Institut Pasteur in Paris, France to work in the laboratory of nuclear cell biology. There he worked on RNA transport and single molecule visualization and tracking of RNA in living cells. In late 2008 he moved his lab to South Africa to join the Council of Scientific and Industrial Research as the Research Leader of the Synthetic Biology Emerging Research Area.

He is now principal investigator at Radboud University working on gene regulation with a focus on single cell and single molecule biology. His lab is especially interested in nuclear architecture and the interplay of gene expression with coding and noncoding RNA.

A chromatin-regulated biphasic circuit coordinates inflammation and trained immunity.

The biphasic nature of a healthy inflammatory response consists of successive waves of coordinated immune gene activation and attenuation (Bhatt *et al.,* 2012). Complex genetic programmes orchestrate the coordinated transcription of subsets of immune genes, allowing for cycles of pro-inflammation and anti-inflammation to ensue (Medzhitov *et al.,* 2008, Rogatsky and Adelman, 2014). However, the molecular mechanisms that underlie these transcriptional programmes remain poorly understood. During my lecture I will explain how this can be addressed using functional genomics, including chromatin interaction mapping (C-technologies).

SHORT PLATFORM PRESENTATIONS

EVA D'HAENE (SPP1)

Postdoctoral researcher

Department of Biomolecular Medicine

Ghent University

Structural variants disrupt a critical regulatory region downstream of *FOXG1*

Authors:

Eva D'haene, Lisa Hamerlinck, María del Rocío Pérez Baca, Lies Vantomme, Björn Menten, Bert Callewaert, Elfride De Baere, Sarah Vergult

Affiliation(s):

Center for Medical Genetics, Department of Biomolecular Medicine, Ghent University, Belgium

The forkhead box G1 (FOXG1) transcription factor is a crucial regulator during embryonic brain development. Pathogenic variants affecting *FOXG1* cause *FOXG1* syndrome, a congenital form of Rett syndrome. Interestingly, 34 reported individuals with *FOXG1* syndrome related features harbor structural variants (SVs) that disrupt the region downstream of *FOXG1*. Yet, the regulatory mechanisms resulting in aberrant *FOXG1* transcription have not been elucidated.

We identified a *de novo* non-coding deletion in a patient with *FOXG1* syndrome, allowing us to narrow down a ~100kb critical regulatory region affected in all patients with SVs 3' of *FOXG1*. By mapping regulatory interactions via UMI-4C in neural stem cells and neurons, we showed that the *FOXG1* promoter interacts with this region during human neuronal development. Using available epigenomics data, Hi-C interaction maps and *in vivo* enhancer assays in zebrafish embryos, we identified multiple regulatory elements in this region, including a cluster of neuronal enhancers and the distal boundary of the *FOXG1*-containing topologically associating domain (TAD). In addition, through Hi-C and UMI-4C on patient cells we found that deletion of the critical regulatory region impacts *FOXG1* interactions and TAD structure. We are currently validating the impact of elements within this region on *FOXG1* transcription through CRISPR-Cas9 engineered deletions in neural stem cells and neurons.

In summary, we narrowed down a critical regulatory region downstream of *FOXG1* that is affected in a cohort of *FOXG1* syndrome patients, containing both enhancer and architectural regulatory elements. Our results greatly improve the functional annotation and validation of regulatory elements at the *FOXG1* locus, crucial for correct SV interpretation in patients.

LISA DANGREAU (SPP2)

PhD student
Department of Biomolecular Medicine
Ghent University

The pseudoxanthoma elasticum zebrafish model contributes to novel pathophysiological insights and therapeutic strategies in ectopic mineralization.

Authors:

L. Dangreau, L. Nollet, M. Van Gils, A. Willaert, P.J. Coucke, O.M. Vanakker

Affiliation(s):

1. Center for Medical Genetics, Ghent University Hospital, Ghent Belgium
2. Department of Biomolecular Medicine, Ghent University, Ghent, Belgium

Pseudoxanthoma elasticum (PXE) is an autosomal recessive hallmark disorder of ectopic calcification (EC), where EC and fragmentation of elastic fibers result in skin, ocular and cardiovascular symptoms. PXE is most commonly caused by bi-alllelic pathogenic variants in the *ABCC6* gene, encoding an ATP-dependent transmembrane transporter of which the substrate is unknown. With an incompletely understood pathophysiology and being an intractable disease, PXE exemplifies how disease-modeling in zebrafish can help to better understand an EC disorder and provide novel strategies for treatment.

The *ABCC6* gene has two orthologs in zebrafish, *abcc6a* and *abcc6b.* We developed a complete *abcc6a* knockout zebrafish model using CRISPR/Cas9, showing that it has an essential role in controlling mineralization. The model developed hypermineralization of notochord and ribs starting embryonically and progressing in adulthood with development of scoliosis. This indicated a direct relation between loss of *abcc6a* expression and dysregulated osteogenesis.

We went on to show that an excessive DNA Damage Response was present in the *abcc6a-/-* fish using expression analysis of DDR/PARP1 targets with QRT-PCR. PARP1 and the ATM–p21–p53 axis were found to be significantly increased. In addition, PARP1 downstream targets IL-6, signal transducer and activator of transcription 1/3, TET1, and RUNX2 were upregulated.

Finally, we validated our PXE zebrafish as a model for compound screening in EC by showing a reduction of the hypermineralization with known effective drugs such as vitamin K1, etidronate and magnesium citrate. Based on this validation study we demonstrated for the first time that sodium thiosulphate and PARP-inhibition using minocycline are able to attenuate the PXE-related mineralization *in vivo.*

Overall, we demonstrate how to use the PXE zebrafish model in translational research from mechanistic insights to *in vivo* compound screening.

The authors are a member of the International Network on Ectopic Calcification (INTEC・ www.itnintec.com).

HANNES SYRYN (SPP3)

PhD student
Department of Biomolecular Medicine
Ghent University

Whole exome sequencing advances a genetic diagnosis in patients with differences of sex development and corroborates the role of *RXFP2* in autosomal recessive bilateral cryptorchidism and infertility

Authors:

Hannes Syryn[1], Hannah Verdin[1], Julie Van de Velde[1], Frank Peelman[2], Marianne Becker[3], Cécile Brachet[4], Marieke den Brinker[5], Sylvia Depoorter[6], Julie Fudvoye[7], Daniel Klink[8], Philippe Lysy[9], Guy Massa[10], Nele Reynaert[11], Anne Rochtus[11], Willem Staels[12], Marlies Van Loocke[11], Andrew Sinclair[13], Katie Ayers[13], Ross Bathgate[14], Martine Cools[15], Elfride De Baere[1]

Affiliation(s):

[1]Center for Medical Genetics Ghent, Ghent University Hospital; Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[2]VIB-UGent Center for Medical Biotechnology, Ghent, Belgium; Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[3]Pediatric Endocrinology and Diabetology (DECCP), Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg
[4]Paediatric Endocrinology Unit, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles (U.L.B.), Brussels, Belgium
[5]Department of Paediatrics, Division of Paediatric Endocrinology, Antwerp University Hospital, Antwerp, Belgium
[6]Department of Child Endocrinology, General Hospital Sint-Jan Bruges-Ostend, Brugge, Belgium
[7]Department of Pédiatric endocrinology, CHU de Liège, Chênée, Belgium
[8]Division of Pediatric Endocrinology and Diabetes, ZNA Queen Paola Children's Hospital, Antwerp, Belgium
[9]Pediatric Endocrinology, Cliniques universitaires Saint Luc, Brussels, Belgium
[10]Department of Pediatric Endocrinology and Diabetology, Jessa Hospital, Hasselt, Belgium
[11]Department of Paediatric Endocrinology, University Hospitals Leuven, Leuven, Belgium
[12]Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel (UZ Brussel), Department of Pediatrics, Division of Pediatric Endocrinology, Brussels, Belgium
[13]Murdoch Children's Research Institute, Royal Children's Hospital & Department of Paediatrics, University of Melbourne, Melbourne, Australia
[14]Department of Biochemistry and Molecular Biology, University of Melbourne, Australia
[15]Department of Internal Medicine and Pediatrics, Ghent University, Department of Pediatric Endocrinology, Ghent University Hospital, Ghent, Belgium

Background/Aims:

Differences of sex development (DSD) are heterogeneous conditions affecting the development of chromosomal, gonadal or anatomical sex. Here, we investigated the benefits of whole exome sequencing (WES) for the genetic diagnosis in patients with DSD.

Methods:

Between 2016 and 2022, 198 unrelated index patients with a clinical diagnosis of DSD or the broader DSD umbrella underwent WES-based panel testing interrogating the coding regions of 130 genes implicated in DSD, primary ovarian insufficiency, and hypogonadotropic hypogonadism. Variants were extracted and classified according to the ACMG guidelines. Copy number variant (CNV) analysis was performed using the ExomeDepth algorithm. Structural modeling and a cyclic AMP (cAMP) reporter gene assay were used to assess the pathogenicity of an *RXFP2* (NM_130806.5) missense variant.

Results:

In 15% of patients, we identified a likely pathogenic or pathogenic rare variant in 15 distinct DSD genes, including *AR* (8), *NR5A1* (3), *CYP21A2* (2), *SRY* (2), *SRD5A2* (2), *DHX37* (2), *WT1* (2), *TXNRD2*, *HSD17B3*, *HSD3B2*, *MCM8*, *TACR3*, *FGFR1*, *ATRX*, and *RXFP2*. The majority are sequence variants, four are CNVs identified using ExomeDepth. Interestingly, in two brothers displaying bilateral cryptorchidism and infertility, an intragenic *RXFP2* deletion was found in *trans* with a heterozygous missense variant c.229G>A, p.(Glu77Lys). The RXFP2 receptor binds INSL3 and is involved in testicular descent. The pathogenicity of the missense variant was substantiated by *in silico* modeling and *in vitro* functional analysis. The missense variant showed normal expression and ability to bind the ligand INSL3, but the absence of a cAMP signal in response to INSL3 supported loss-of-function.

Conclusion:

We demonstrate the benefit of WES-based genetic testing of DSD in a clinical context and illustrate the important additive value of CNV assessment on WES data. This finding corroborates the role of *RXFP2* in autosomal recessive bilateral cryptorchidism and supports that infertility is part of the phenotype.

LAURENZ DE COCK (SPP4)

PhD student

Department of Biomolecular Medicine

Ghent University

The added value of RNA-sequencing in exome variant interpretation

Authors:

Laurenz De Cock*, Erika D'haenens*, Sarah Vergult*, Lies Vantomme, Annelies Dheedene, Tim Van Damme, Jo Sourbron, Candy Kumps, Bert Callewaert, Olivier Vanakker, Björn Menten

* Shared first

Introduction

Several studies have shown that transcriptomics (RNA-seq) nicely complements whole exome and whole genome sequencing (i.e., WES and WGS) in variant interpretation and leads to a 7-36% increase in diagnostic yield.

However, several hurdles remain to be taken before RNA-seq can be implemented in routine genetic diagnostics. First of all, the tissue under investigation is not always representative for the diseased tissue, and hence, spatiotemporal differences in expression and expressed isoforms might complicate the analysis. Furthermore, nonsense mediated decay (NMD) can mask aberrant events.

Here we present a minimally invasive ready-to-use protocol and analysis pipeline to perform RNA-seq analysis on short term cultured peripheral blood mononuclear cells (PBMCs).

Material and Methods

Short cultured PBMCs are divided in two cultures, one of which is treated with cycloheximide to allow detection of NMD sensitive transcripts. Thereafter, RNA is extracted from both cultures followed by polyA-sequencing. After alignment of the RNA-seq data and gene count generation, the data are respectively processed with OUTRider and FRASER to detect aberrant expression or splicing events. Alternatively, a manual targeted analysis was performed using the integrative genomics viewer (IGV).

Results & Discussion

We have currently sequenced RNA from 50 individuals. Targeted analysis in five of them revealed aberrant splicing events that were often more complex than anticipated, and hence, were not picked up by targeted cDNA sequencing. The experiments with cycloheximide clearly shed a more complex picture on NMD, with several aberrant transcripts escaping NMD. The RNA-seq data of all five targeted examples were crucial for correct interpretation and classification of splice site variants detected by WES.

In conclusion, we present an optimized RNA sequencing protocol and analysis workflow and show its added value for interpretation and classification of putative splice site variants.

MICHIEL VANHOOYDONCK (SPP5)

PhD student

Department of Biomolecular Medicine

Ghent University

The establishment of the first reported zebrafish model for thoracic aortic dissection and rupture

Authors:

Michiel Vanhooydonck[1], Lore Pottie[1], Annekatrien Boel[2], Piyanoot Tapaneeyaphan[1], Marta Santana Silva[1], Adelbert De Clercq[1], Lisa Caboor[1], Matthias Van Impe[3], Andy Willaert[1], Patrick Sips[1], Bert Callewaert[1]

Affiliation(s):

[1] Center for Medical Genetics Ghent (CMGG), Department of Biomolecular Medicine, Ghent University, 9000 Ghent, Belgium
[2] Ghent-Fertility and Stem Cell Team (G-FaST), Department for Reproductive Medicine, Ghent University, 9000 Ghent, Belgium
[3] Biofluid, Tissue and Solid Mechanics for Medical Applications (bioMMeda), Institute of Biomedical Engineering and Technology (IBiTech), Ghent University, 9000 Ghent, Belgium

Per year, 3-4 persons per 100,000 suffer from thoracic aortic dissection (TAD), causing significant morbidity and mortality. Dissections occur often at sites where neural crest and mesodermal derived cell populations interact. In TAD, an interplay between hemodynamic stress, tissue remodeling due to impaired extracellular matrix assembly, aberrant growth factor signaling and cell-matrix mechanosensing weakens the vessel wall. Despite the availability of different mouse models for TAD, the cascade of underlying mechanisms remains largely elusive. Consequently, current treatment options are limited to a pharmacological reduction of hemodynamic stress and surgical repair at a critical diameter.

We therefore developed a zebrafish model for aortic dissection/rupture targeting two genes involved in angiogenesis, *SMAD3* and *SMAD6*. In humans, loss of function (LOF) of *SMAD3* results in thoracic aortic aneurysm and dissection (**TAAD)**, arterial tortuosity and early onset osteoarthritis. *SMAD6* LOF mutations increase the risk for a bicuspid aortic valve and TAAD. In zebrafish, both *SMAD3* and *SMAD6* have 2 paralogues. Using CRISPR/Cas9 gene editing technology, we developed a quadruple knockout (KO): *smad3a*$^{-/-}$;*smad3b*$^{-/-}$;*smad6a*$^{-/-}$;*smad6b*$^{-/-}$. At 5 days post fertilization, quadruple KO embryos showed asymmetrical branching of the aortic arches. Survival of adult quadruple KO zebrafish was severely decreased and all but one quadruple mutants died before the age of one year. A stress-inducing protocol caused sudden death in 60% of the mutant zebrafish. Histology of consecutive sections of the ventral aorta in quadruple mutants stained for elastin showed medial elastolysis, intramural hematomas, aortic dissections and ruptures, which was further supported by 3D reconstructions. RNA sequencing revealed upregulation of melanogenesis as well as *mitfa*, an important transcription factor in neural crest, relevant for the pathogenesis. In conclusion, we successfully developed the first zebrafish model for aortic dissection/rupture. This model will be highly relevant to better understand the pathogenesis underlying TAD and to evaluate potential therapeutic compounds.

SOFIA PAPADIMITRIOU (SPP6)

Postdoctoral researcher

Department of Biomolecular Medicine

Ghent University

The importance of good data quality and proper pathogenicity reporting in the medical genetics field: the case of oligogenic diseases

Authors:

Sofia Papadimitriou[1,2,3], Barbara Gravel[1,2,3], Charlotte Nachtegael[1,2], Elfride De Baere[4], Bart Loeys[5], Miikka Vikkula[6], Guillaume Smits[1,7,8], Tom Lenaerts[1,2,3]

Affiliation(s):

1. Interuniversity Institute of Bioinformatics in Brussels, Université Libre de Bruxelles-Vrije Universiteit Brussel, 1050 Brussels, Belgium;
2. Machine Learning Group, Université Libre de Bruxelles, 1050 Brussels, Belgium;
3. Artificial Intelligence Laboratory, Vrije Universiteit Brussel, 1050 Brussels, Belgium;
4. Center for Medical Genetics, Ghent University Hospital; Department of Biomolecular Medicine, Ghent University, 9000 Ghent, Belgium
5. Center for Medical Genetics, Antwerp University Hospital/University of Antwerp, 2650 Antwerp, Belgium
6. Human Molecular Genetics, de Duve Institute, UCLouvain, Brussels, Belgium
7. Center of Human Genetics, Hôpital Erasme, Université Libre de Bruxelles, 1070 Brussels, Belgium
8. Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, 1020 Brussels, Belgium;

Background/Aims:

Reports of oligogenic cases (i.e. individuals whose disease phenotype can only be explained by the co-occurrence of multiple variants in several genes) have been rapidly increasing, in an effort to close the gap of missing genetic diagnoses. Nevertheless, the quality of this data had never been properly assessed, especially as standards and guidelines for such cases are currently missing. This work, aimed to collect all reported oligogenic cases in one database, OLIDA, assess the quality of the reported information and provide, for the first time, recommendations for their proper reporting.

Methods:

318 research articles reporting oligogenic cases were extracted from PubMed. Independent curators collected the relevant oligogenic information (i) from the articles and (ii) from public relevant databases. With this data, a transparent curation protocol was developed assigning a confidence score to each oligogenic case based on the amount of pathogenic evidence at the genetic and functional level. The collection and assessment of this data led to the creation of OLIDA, the Oligogenic Diseases Database.

Results:

OLIDA contains information on oligogenic cases linked to 177 different genetic diseases. Each instance is linked with a confidence score depicting the quality of the associated genetic and functional pathogenic evidence. The data revealed that the majority of papers do not provide proper genetic evidence excluding a monogenic model, while this evidence is rarely coupled with functional experiments for confirmation. Our recommendations stress the necessity of fulfilling both conditions. The use of multiple extended pedigrees showing a clear segregation of the reported variants, control cohorts of a suitable size, as well as functional experiments showing the synergistic effect of the involved variants are essential for this purpose.

Conclusion:

With our work we reveal the recurrent issues on the reporting of oligogenic cases and stress the need for the development of standards in the field. As the number of papers identifying oligogenic causes to disease is increasing rapidly, initiating this discussion is imperative.

LESLIE NAESSENS (SPP7)

PhD student
Department of General Internal Medicine
Ghent University

Human *GTF3A* deficiency predisposes to neuroinvasive HSV-1 infection by disrupted transcription of host-derived non-coding RNAs

Authors:

Leslie Naesens[*,1,2], Santoshi Muppala[*,3], Jung-Hyun Lee[3,4], Josephine Nemegeer[5,6], Delfien Bogaert[1], Dhiraj Acharya[3,4], Katrien Staes[5,6], Veronique Debacker[1,2], Pieter De Bleser[6,7], Marieke De Bruyne[8,9], Elfride De Baere[8,9], Michiel van Gent[3,4], Bart N. Lambrecht[1,6,10], Jens Staal[6,11], Tessa Kerre[12], Rudi Beyaert[6,11], Jonathan Maelfait[#,5,6], Simon J. Tavernier[#,2,9,11], Michaela U Gack[#,3,4], Filomeen Haerynck[#,1,2]

Affiliation(s):

Affiliations:
[1] Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium
[2] Primary Immunodeficiency Research Lab, Center for Primary Immunodeficiency Ghent, Jeffrey Modell Diagnosis and Research Center, Ghent University Hospital, Ghent, Belgium
[3] Florida Research and Innovation Center, Cleveland Clinic, Port St Lucie, FL, USA
[4] Department of Microbiology, University of Chicago, Chicago, IL, USA
[5] VIB-UGent Center for Inflammation Research, Ghent, Belgium
[6] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
[7] Data Mining and Modeling for Biomedicine, VIB Center for Inflammation Research, Ghent, Belgium.
[8] Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[9] Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium
[10] Laboratory of Mucosal Immunology and Immunoregulation, VIB Center for Inflammation Research, Ghent, Belgium; Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium
[11] Laboratory of Molecular Signal Transduction in Inflammation, VIB Center for Inflammation Research, Ghent, Belgium
[12] Department of Hematology, Jeffrey Modell Diagnosis and Research Center, Ghent University Hospital, Ghent, Belgium

*Leslie Naesens and Santoshi Muppala share first authorship
Jonathan Maelfait, Simon J. Tavernier, Michaela U. Gack, and Filomeen Haerynck share last authorship.

Herpes simplex virus 1 (HSV-1) affects several billion people worldwide and can cause life threatening herpes simplex encephalitis (HSE) in some patients. Monogenic defects in type I interferons (IFN-I) signaling components have been identified in HSE patients, emphasizing the role of inborn errors of immunity (IEI) underlying HSE pathogenesis.

In vitro studies demonstrates that the cytoplasmic RNA sensor RIG-I, which is well known to restrict RNA viruses, also critically contributes to the innate immune responses to DNA viruses including HSV-1. RNA polymerase III (Pol III) has been shown to convert host or virus-derived DNA products into 5'-triphosphate-RNA ligands for RIG-I.

In this study, we identified compound heterozygous mutations in the gene *GTF3A* in a family afflicted by HSE in early childhood. *GTF3A* encodes for the transcription factor TFIIIA, which is part of the Pol III complex and is well known for its essential role in ribosomal biogenesis. We studied primary patient cells as well as several CRISPR/Cas9-edited *GTF3A* mutant cells**.** We confirmed that the patient TFIIIA mutants have an impaired promoter-binding ability. We tested HSV-1 replication in the patient fibroblasts and *GTF3A* mutant cells and observed enhanced viral replication. To understand the underlying mechanism, we took an unbiased approach and searched for novel transcriptional targets of TFIIIA by ChIP-seq analysis and identified the pseudogene *RNA5SP141*. We found that *RNA5SP141* is upregulated following HSV-1 infection and that this induction is abrogated in primary patient cells, in *GTF3A* gene-edited mutant cells, and upon targeted knockdown using siRNA. Finally, we explored the downstream effects of impaired *RNA5SP141* expression on anti-herpesviral immunity and found abrogated RIG-I activation and markedly diminished induction of antiviral genes during HSV-1 infection.

Our work unveils a novel role for TFIIIA by regulating RIG-I-mediated innate immune responses to HSV-1 and expands the genetic etiology of HSE predisposition

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TAMARA JARAYSEH (SPP8)

PhD student

Department of Biomolecular Medicine

Ghent University

A tapt1 knock-out zebrafish line with aberrant lens development and impaired vision models human early-onset cataract

Authors:

Tamara Jarayseh[1], Brecht Guillemyn[1], Hanna De Saffel[1], Jan Willem Bek[1], Delfien Syx[1], Sofie Symoens[1], Yannick Gansemans[2], Filip Van Nieuwerburgh[2], Sujatha Jagadeesh[3], Jayarekha Raja[3], Fransiska Malfait[1], Paul J. Coucke[1], Adelbert De Clercq[1,x], Andy Willaert[1,x,*]

Affiliation(s):

[1]Center for Medical Genetics Ghent, Ghent University Hospital, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[2]Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium
[3]Department of Genetics, Mediscan Systems, Chennai, India
[x]Shared last author
[*] Corresponding author

Bi-allelic mutations in the gene coding for human trans-membrane anterior-posterior transformation protein 1 (TAPT1) result in a broad phenotypic spectrum, ranging from syndromic disease with severe skeletal and congenital abnormalities to isolated early-onset cataract. We present here the first patient with a frameshift mutation in the TAPT1 gene, resulting in both bilateral early-onset cataract and skeletal abnormalities, in addition to several dysmorphic features, in this way further expanding the phenotypic spectrum associated with TAPT1 mutations. A tapt1a/tapt1b double knock-out (KO) zebrafish model generated by CRISPR/Cas9 gene editing revealed an early larval phenotype with eye malformations, loss of vision, increased photokinetics and hyperpigmentation, without visible skeletal involvement. Ultrastructural analysis of the eyes showed a smaller condensed lens, loss of integrity of the lens capsule with formation of a secondary lens and hyperplasia of the cells in the ganglion and inner plexiform layers of the retina. Transcriptomic analysis pointed to an impaired lens development with aberrant expression of many of the crystallin and other lens-specific genes. Furthermore, the phototransduction and visual perception pathways were found to be significantly disturbed. Differences in light perception are likely the cause of the increased dark photokinetics and generalized hyperpigmentation observed in this zebrafish model. In conclusion, this study validates TAPT1 as a new gene for early-onset cataract and sheds light on its ultrastructural and molecular characteristics.

ROBIN VROMAN (SPP9)

PhD student
Department of Biomolecular Medicine
Ghent University

The matrisome of the murine and human dorsal root ganglion: a transcriptomal approach

Authors:

Robin Vroman[1], Rahel Hunter[2], Matthew J. Wood[2], Olivia C. Davis[3], Zoë Malfait[1], Dale S. George[4], Dongjun Ren[5], Diana Tavares-Ferreira[3], Theodore J. Price[3], Richard J. Miller[5], Anne-Marie Malfait[2], Fransiska Malfait[1], Rachel E. Miller[2], Delfien Syx[1]

Affiliation(s):

[1]Center for Medical Genetics, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[2]Division of Rheumatology, Rush University Medical Center, Chicago, Illinois, USA
[3]Department of Neuroscience and Center for Advanced Pain Studies, University of Texas, Dallas, USA
[4]Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA.
[5]Department of Pharmacology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA.

The extracellular matrix (ECM) is a dynamic structure of a large number of molecules that can be divided into six different categories and are collectively called the matrisome. The ECM plays pivotal roles in physiological processes in many tissues, including the nervous system. Intriguingly, alterations in ECM molecules/pathways are associated with painful human conditions and murine experimental pain models. Nevertheless, mechanistic insight into the interplay of normal or defective ECM and pain is largely lacking. We used a transcriptomal approach to investigate the expression and cellular origin of matrisome genes in murine and human dorsal root ganglia (DRG), containing the cell bodies of sensory neurons. Bulk RNA sequencing showed that about 65% of all matrisome genes were expressed in both murine and human DRG, with proportionally more core matrisome genes (glycoproteins, collagens, and proteoglycans) expressed compared to matrisome-associated genes (ECM-affiliated genes, ECM regulators and secreted factors). Examination of the cellular origin of matrisome expression by single cell RNA-seq on murine DRG revealed that core matrisome genes, especially collagens, were expressed by vascular leptomeningeal-like (fibroblast) cell types whereas matrisome-associated genes were expressed by neuronal cells. Cell-cell communication network analysis with the CellChat software predicted an important role for the collagen signaling pathway in connecting vascular cell types and nociceptors in murine tissue, which we confirmed by analysis of spatial transcriptomic data from human DRG. RNAscope in situ hybridization and immunohistochemistry confirmed expression of collagens in fibroblasts surrounding nociceptors in human DRG. This study supports the idea that the DRG matrisome may contribute to neuronal signaling in both mouse and human. The identification of the cellular distribution of murine and human matrisome genes provides a framework to study the role of the ECM in peripheral nervous tissue and its effects on pain signaling in for example heritable connective tissue disorders.

MELISSA PILLE (SPP10)

PhD student

Department of Diagnostic Sciences

Ghent University

The role of the Wiskott-aldrich Syndrome protein in the development of T cells

Authors:

Pille Melissa[1], Sanchez Sanchez Guillem[2,3,4], Goetgeluk Glenn[1], De Munter Stijn[1], Jansen Hanne[1], Billiet Lore[1], Weening Karin[1], Avila John[5], Xue Haipeng[5], Ingels Joline[1], De Cock Laurenz[1], Pascal Eva[1], Deseins Lucas[1], Vermijlen David[2,3,4], Kerre Tessa[6], Davis Brian[5], Vandekerckhove Bart[1]

Affiliation(s):

[1]Department of Diagnostic Sciences, Ghent University, Ghent, Belgium
[2]Department of Pharmacotherapy and Pharmaceutics, Université Libre de Bruxelles (ULB), 14 Brussels, Belgium.
[3]Institute for Medical Immunology, Université Libre de Bruxelles (ULB), Brussels, Belgium
[4]ULB Center for Research in Immunology (U-CRI), Université Libre de Bruxelles (ULB), 17 Brussels, Belgium.
[5]Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Texas, U.S.A.
[6]Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium.

The Wiskott-Aldrich syndrome (WAS) is an X-linked primary immune deficiency caused by a mutation in the WAS gene. This leads to altered or absent WAS protein (WASp) expression and function resulting in thrombocytopenia, eczema, recurrent infections, and auto-immunity. WASp is involved in signalling of the T Cell Receptor (TCR) due to its organisation of actin filaments. The function of peripheral T cells in WAS patients has been studied extensively showing a decreased proliferation, IFNg and IL-2 production and upregulation of activation markers upon CD3/CD28 stimulation. WAS patients also show a reduced number of peripheral blood T lymphocytes. However, it is unclear to what extent and at what stage, WASp deficiency adversely affects the development of T cells. Here, we show the influence of WASp at the positive selection stage in vitro using ATO-cultures. WASp crispr/Cas9 knocked-out HPC-C cells fail to differentiate towards and generate less mature SP CD8+ T compared to normal HPC-C cells. The same was observed when using WAS-Exon2-Stop-eGFP tranduced HPC-C cells in vitro. In vivo mice experiments using WAS KO HPC-C cells and WAS-Exon2-Stop-eGFP transduced HPC-C allowed us to verify the in vitro results along with TCRα and TCRβ repertoire analysis. CD8 SP cells show oligoclonality in TCRα and TCRβ repertoire. Next, we wanted to look into the redundant function of N-WASp in human cells, as Zhang et al (2002) showed in mice. However, single-cell RNA sequencing showed a low presence of WASp in human cordblood and thymi. WAVE2, however, was present in the magnitude as WASp. Therefore, we created a WASnull/WAVE-/- KO (DKO) and looked at T cell development and functionality. DKO cells do not have a significant lower number of SP CD8+ cells. However, DKO CD8+ cells produce less IFNg and IL-2 compared to WAS KO cells. Thus, suggesting a role in the T cell functionality.

ALFREDO DUENAS REY (SPP11)

PhD student

Department of Biomolecular Medicine

Ghent University

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

Authors:

Alfredo Dueñas Rey[1,2], Victor Lopez Soriano[1,2], Zelia Corradi[3], Claire-Marie Dhaenens[4], *ABCA4* smMIPS study group[5], Manon Bouckaert[1,2], Jasper Verwilt[2,6], Avril Watson[7], Majlinda Lako[7], Eva D'haene[1,2], Karla Ruiz[8], Sandro Banfi[8], Miriam Bauwens[1,2], Frans Cremers[3], Steve Lefever[1,2], Elfride De Baere[1,2], Frauke Coppieters[1,2,9]

Affiliation(s):

1. Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.
2. Department of Biomolecular Medicine, Ghent University, Ghent, Belgium.
3. Radboudumc Department of Human Genetics, Nijmegen, Gelderland, Netherlands.
4. Univ. Lille, Inserm, CHU Lille, U1172 - LilNCog - Lille Neuroscience & Cognition, Lille, France.
5. *ABCA4* smMIPS study group: Mubeen Khan, Fanny Depasse, Isabelle Meunier, Bernard Puech, Sabine Delfoort, Heidi Stöhr, Smaragda Kamakari, Marta del Pozo Valero, Carmen Ayuso.
6. OncoRNALab, Cancer Research Institute Ghent, Ghent, Belgium.
7. Newcastle University Faculty of Medical Sciences, Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom.
8. Telethon Institute of Genetics and Medicine, Napoli, Campania, Italy.
9. Department of Pharmaceutics, Ghent University, Ghent, Belgium.

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

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

Results| Short-read RNAseq analysis of ~7,400 transcriptomes of 54 tissues (GTEx) and 152 transcriptomes of adult human retinas revealed a potential novel lncRNA upstream of ABCA4, with expression restricted to the human retina. Long-read sequencing of donor retina identified at least two novel multi-exonic isoforms, for which expression was demonstrated in the outer nuclear layer of adult human retina. The lncRNA is transcribed from an active retinal enhancer interacting with the ABCA4 promoter, suggesting a cis-acting effect on ABCA4. We identified 2 heterozygous novel copy number variants overlapping the lncRNA, representing likely pathogenic

or modifying alleles. Perturbation knockdown studies in human retinal explants are ongoing to further elucidate its function.

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

VIOLETTE DELEEUW (SPP12)

PhD student
Department of Biomolecular Medicine
Ghent University

Defects in the first hybrid domain of fibrillin-1 affect vascular wall homeostasis in the thoracic aorta

Authors:

Deleeuw Violette, Msc[1], Van Impe Matthias, Msc[2], Logghe Gerlinde, Msc[2], Vanhomwegen Marine, Bsc[1], Olbinado Margie, PhD[3], Stampanoni Marco, PhD[3], Segers Patrick, PhD[2], Laura Muino Mosquera, MD, PhD[1,4], Sakai Lynn, PhD[5], Sips Patrick, PhD[1], and De Backer Julie, MD, PhD[1,6]

Affiliation(s):

1: Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
2: Department of Electronics and Information Systems, Ghent University, Ghent, Belgium
3: Paul Scherrer Institute, Villigen, Switzerland
4: Department of Pediatric Cardiology, Ghent University Hospital, Ghent, Belgium
5: Department of Molecular & Medical Genetics, Oregon Health & Science University, Portland, OR
6: Department of Cardiology, Ghent University Hospital, Ghent, Belgium

Background: Aortic dissection and rupture is the main cause of early cardiovascular mortality in patients with Marfan syndrome (MFS). MFS is caused by a fibrillin-1 deficiency, which binds transforming growth factor beta (TGF-beta) via interaction with latent TGF-beta binding proteins (LTBPs). The role of TGF-beta in MFS has been controversial, with earlier studies suggesting that excess release of TGF-beta due to decreased interaction with dysfunctional fibrillin-1 leads to aortic dilation and vascular damage, while other studies have shown an important protective effect.

Aims: We aim to use dedicated mouse models for MFS, with defects interfering with TGF-beta binding, to gain insights into the role of TGF-beta signaling in aneurysm formation and dissection.

Methods: Mice lacking the fibrillin-1 binding site for LTBPs (Fbn1H1Δ/+ and Fbn1H1Δ/H1Δ), mice with a truncated fibrillin-1 (Fbn1GT-8/+), and mice with a combination of both alleles (Fbn1GT-8/H1Δ) were subjected to cardiac ultrasound and ex vivo synchrotron X-ray imaging.

Results: Only Fbn1GT-8/H1Δ mice showed increased mortality due to aortic rupture starting at 4-5 months of age, whereas all other mice had a normal lifespan. Aortic root dilatation occurred in both Fbn1GT-8/+ and Fbn1GT-8/H1Δ mice at 6 months of age, but not in Fbn1H1Δ/+ and Fbn1H1Δ/H1Δ mice. Significant elastic lamellae fragmentation was observed in the thoracic aortic wall of Fbn1GT-8/+ mice, and to a larger extent in Fbn1GT-8/H1Δ mice. Surprisingly, localized elastin fragmentation was also found in the ascending thoracic aorta of Fbn1H1Δ/+ and Fbn1H1Δ/H1Δ mice despite a lack of aneurysm development.

Conclusions: Our data indicate that loss of LTBP binding to fibrillin-1 leads to the development of localized microdissections in the absence of aortic aneurysm, and exacerbates the aortic wall morphology in mice with truncated fibrillin-1. We therefore hypothesize that local TGF-beta sequestration is required to maintain aortic homeostasis.

MÜNEVVER BURCU ÇIÇEKDAL (SPP13)

PhD student

Department of Biomedical molecular biology

Ghent University

CRISPR/Cas9 mediated disruption of an evolutionary conserved putative enhancer in the mab21l2 locus induces developmental eye anomalies in Xenopus tropicalis

Authors:

Munevver Burcu Cicekdal [1,2] , Fabiola Ceroni [3,4] , Richard Holt [3], Thomas Naert[5], Soeren S. Lienkamp[5], Elfride De Baere[2], Nicola K Ragge[3,6], Kris Vleminckx [1]

Affiliation(s):

[1] Department of Biomedical Molecular Biology, Ghent University, Belgium
[2] Center for Medical Genetics and Department of Biomolecular Medicine, Ghent University and, Ghent University Hospital, Ghent, Belgium
[3] Faculty of Health and Life Science, Oxford Brookes University, UK
[4] Department of Pharmacy and Biotechnology, Università di Bologna, Bologna, Italy
[5] Institute of Anatomy, University of Zurich, Zurich, 8057, Switzerland; Swiss National Centre of Competence in Research (NCCR) Kidney Control of Homeostasis (Kidney.CH), Zurich, 8057, Switzerland
[6] West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's and Children's Foundation Trust, UK

Genetic variants that disrupts the function of cis-regulatory elements (CREs), including enhancers and promoters, have been shown to conduce to a variety of human diseases. Here, we aimed to characterize a putative enhancer of MAB21L2, localized in a 113.5kb non-coding homozygous deletion identified in a proband with the phenotype of anophthalmia, micrognathia and microcephaly. The region was evaluated for the presence of putative enhancers using a genome-wide multi-omics database. One putative enhancer (CRE14) within the corresponding 39kb region in Xenopus(X.) tropicalis contained a conserved binding site for OTX2, a TF critically involved in eye development. Furthermore, binding of Otx2 to the CRE14 enhancer was confirmed by ChIP-seq in mouse embryonic stem cells. Our further analysis using ChromHMM software indicated that CRE14 shows the epigenetic marks of a poised enhancer at NF st10.5, and an active enhancer at st12.5, in line with the initiation of mab21l2 expression and start of the eye specification in Xenopus development. CRISPR mediated disruption of the Otx2 TFBS (CRE14 crispants), as well as reproduction of the proband deletion (del crispants) resulted in eye defects, including misshapen eyes, lens defects and ocular coloboma. In addition, diminished levels of mab21l2 transcripts in CRE14 and del crispants were also observed at st20 and 28. Lastly, we explored the reconstruction of eye structures in mab21l2 crispants using in toto light-sheet microscopy. 2D and 3D phenotyping of affected eyes indicated a significant decrease in both volume and sphericity of the retina in CRE14 crispants compared to the control group. In conclusion, we showed for the first time a X. tropicalis disease model targeting a TFBS using CRISPR/Cas9 through a sensitive and accurate evaluation using deep neural network phenotyping, promoting the use of Xenopus as a promising animal model for in-depth investigation of non-coding gene regulatory networks and associated disease related phenotypes.

POSTER PITCHES

ANNELIES DHEEDENE (P1)

Inherited pathogenic variants in neurodevelopmental disorders: a potential pitfall in trio-based analysis of clinical exomes

Authors:

Annelies Dheedene, Erika D'haenens, Evelien Pouillie, Sarah Delbaere, Olivier Vanakker, Bert Callewaert, Björn Menten

Affiliation(s):

Center for Medical Genetics, Department of Biomolecular Medicine, Ghent University & Ghent University Hospital

Background: The use of clinical exome sequencing (ES) has led to a steep increase in the diagnostic yield in patients with neurodevelopmental disorders (NDDs).

Methods: ES data of > 2000 patients and both parents (when available) were analyzed for a panel of 1441 NDD related genes. Variant classification was based on ACMG/AMP and ACGS guidelines.

Results: We identified a class 4 or 5 variant explaining the phenotype in 21% of cases. In 60% of these probands, the variant occurred *de novo,* while in 40% the causal variant(s) was/were inherited. Of the probands with inherited variants, 12.5% had an X-linked disorder, 46% an autosomal recessive disorder, and 41.5% an autosomal dominant disorder. Furthermore, we reported a variant of unknown significance (class 3) in an additional 25% of the probands.

Conclusion: With a diagnostic yield between 21% and 46%, we confirm that exome sequencing is a game-changer in the diagnostic workup of patients with NDDs. Although the majority of causal variants occurred *de novo*, several (likely) pathogenic variants were observed in seemingly healthy parents. Analysis pipelines focusing solely on *de novo* or bi-allelic defects, may therefore miss molecular diagnoses. Our data add evidence that reduced penetrance is currently underreported in several NDDs and we should be cautious to rely on the "*de novo* paradigm for neurodevelopmental disorders" both in laboratory analysis and genetic counseling.

ELINE VAN VOOREN (P2)

An in vitro enzymatic assay to elucidate the VUS problem in RPE65, a target for retinal gene therapy

Authors:

Eline Van Vooren[1,2], Susanne Kohl[3], Bart P. Leroy[2,4,5], Miriam Bauwens[1,2*], Elfride De Baere[1,2*] *Equal contribution

Affiliation(s):

1 Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
2 Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium
3 Department of Ophthalmology, Institute of Ophthalmology, University of Tübingen and Tübingen University Hospital, Tübingen, Germany
4 Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium & Department of Head & Skin, Ghent University, Ghent, Belgium
Division of Ophthalmology & Center for Cellular & Molecular Therapeutics, Children's Hospital of Philadelphia, Philadelphia, PA, USA

Background

Inherited retinal diseases (IRDs) are a clinically and genetically heterogenous group of disorders leading to early-onset vision loss. RPE65 is a known IRD disease gene, encoding a crucial enzyme of the visual cycle that is important for preserving vision. In 2017-2018, Luxturna received FDA and EMA approval, respectively, as first gene therapy to treat IRD patients with biallelic mutations in RPE65. Therapeutic eligibility however requires a clear-cut molecular diagnosis, which is often hampered by the identification of Variants of Uncertain Significance (VUS), providing insufficient evidence concerning their role in the disease and their effect on gene/protein function. This study aimed to develop an in vitro biochemical assay to assess the pathogenicity of coding RPE65 VUS.

Methods and results

The assay is based on simulating the visual cycle via overexpression of visual cycle enzymes including RPE65 in HEK293-F cells. As RPE65 is responsible for the isomerization of all-trans retinyl esters to 11-cis retinol, the read-out consists of HPLC-based analysis of residual retinols, as well as immunoblotting of RPE65 protein. First, missense variants that had not been assessed functionally were compiled from various sources including our in-house database, LOVD, and members of the European Retinal Disease Consortium (ERDC) and the European Reference Network for Rare Eye Diseases (ERN-EYE). Variant classification according to ACMG/AMP guidelines revealed 53 RPE65 VUS to be assayed. Constructs have been generated by cloning the open reading frames of RPE65, CRALBP, LRAT and RDH5 in the multiple cloning sites (MCSs) of a pVitro2 backbone. Expression of bicistronic constructs has been compared to monocistronic constructs. Mutagenesis for the selected VUS has been performed and a first test assaying nine VUS and control variants has been completed.

Conclusion

Functional assessment of 53 RPE65 VUS via the enzymatic assay designed here will improve IRD patient eligibility for Luxturna gene therapy.

KARO DE RYCKE (P3)

Zebrafish as a tool to study cardiovascular effects caused by fibrillin impairment

Authors:

De Rycke Karo[1], Caboor Lisa[1], Horvat Marina[1], Vermassen Petra[1], De Backer Julie[2], Sips Patrick[1]

Affiliation(s):

[1]Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[2]Department of Cardiology and Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium

Background: Marfan syndrome (MFS) is the most common type of fibrillinopathy with a high predisposition to develop aneurysms and dissections of the thoracic aorta. While the development of several mouse models of MFS has contributed greatly to our current knowledge, a thorough understanding of the underlying mechanisms is still lacking. There is a particular need for more flexible *in vivo* models to address this knowledge gap.

Aims: We aimed to generate a relevant zebrafish model to gain insight into the molecular mechanisms relating fibrillin defects to the cardiovascular system.

Methods: The CRISPR/Cas9 system was used to systematically target the three different fibrillin genes (*fbn1*, *fbn2a* and *fbn2b*) in Tg(kdrl:GFP) reporter zebrafish. Time-lapse fluorescent microscopy was used to evaluate the cardiovascular phenotype.

Results: We found that zebrafish lacking *fbn1* and/or *fbn2a* do not show any cardiovascular phenotype during early-stage development. On the other hand, approximately 50% of homozygous *fbn2b* mutant (*fbn2b*$^{-/-}$) zebrafish embryo's show a severe phenotype characterized by endocardial detachment, leading to vascular embolism and premature mortality at 7-9 dpf. Interestingly, the remaining *fbn2b*$^{-/-}$ zebrafish survive until adulthood, but during larval stages already develop a dilation of the bulbus arteriosus, a structure anatomically related to the aortic root in humans. In addition, the caudal vein of all *fbn2b*$^{-/-}$ embryos develops abnormally as a cavernous structure lacking vessel integrity. This phenotype is resolved in embryos retaining normal blood flow and aggravated upon pharmacological inhibition of blood flow during development.

Conclusion: These data indicate that our new *fbn2b*$^{-/-}$ zebrafish model recapitulates cardiovascular complications observed with fibrillin deficiency, and can thus be considered as a relevant model to study the mechanisms underlying MFS pathogenesis. Our preliminary data suggest that there is an interplay between fibrillin deficiency and biomechanical signaling in the regulation of cardiovascular development.

LUKAS NOLLET (P4)

Serum calcification propensity T50 as a novel biomarker for disease severity in patients with pseudoxanthoma elasticum

Authors:

Lukas Nollet [1-3], Matthias Van Gils [1-3], Suzanne Fischer [4], Laurence Campens [5], Swapna Karthik [6], Andreas Pasch [6-9], Julie De Zaeytijd [10], Bart P. Leroy [1,10,11], Daniel Devos [12], Tine De Backer [5], Paul J. Coucke [1,2], Olivier M. Vanakker [1-3]

Affiliation(s):

[1] Center for Medical Genetics, Ghent University Hospital, Belgium
[2] Department of Biomolecular Medicine, Ghent University, Belgium
[3] Ectopic Mineralization Research Group Ghent, Belgium
[4] Laboratory of Experimental Cancer Research, Ghent University, Belgium
[5] Department of Cardiology, Ghent University Hospital, Belgium
[6] Calciscon AG, Nidau, Switzerland
[7] Institute of Physiology and Pathophysiology, Johannes Kepler University Linz, Austria
[8] Department of Nephrology, Lindenhofspital, Bern, Switzerland
[9] Practice for Internal Medicine and Nephrology at Hirschengraben, Bern, Switzerland
[10] Department of Ophthalmology, Ghent University Hospital, Belgium
[11] Division of Ophthalmology, The Children's Hospital of Philadelphia, PA, USA
[12] Department of Radiology, Ghent University Hospital, Belgium

Background: Pseudoxanthoma elasticum (PXE) is an intractable Mendelian disorder characterized by progressive ectopic calcification in the skin, eyes and arteries. Clinical trials assessing potential treatments in PXE are currently severely hampered by the lack of a reliable biomarker for PXE disease severity. Serum calcification propensity T50 is a blood test measuring the transition time from primary amorphous to secondary crystalline calcium-protein particles, hence representing the anti-calcifying buffer capacity of serum. Here, we evaluated serum T50 in PXE patients aiming to investigate its determinants and suitability as a potential biomarker for disease severity.

Methods: Fifty-seven PXE patients were included in this cross-sectional study and demographic, clinical, imaging and biochemical data were collected. PXE disease severity was assessed using the Phenodex scoring system. Serum T50 was measured using a validated, nephelometry-based assay. Multivariate models were then created to investigate T50 determinants and associations with disease severity.

Results: Mean age of patients was 45.2 years, 68.4% was female and mean serum T50 was 347 ± 68 minutes. Multivariate regression analysis identified serum fetuin-A ($p < 0.001$), phosphorus ($p = 0.007$) and magnesium levels ($p = 0.034$) as significant determinants of T50, while no correlations were found with plasma pyrophosphate levels or ABCC6 genotype. After correction for covariates, T50 was found to be an independent determinant of ocular ($p = 0.013$), vascular ($p = 0.013$) and overall disease severity ($p = 0.016$) in PXE. Additionally, T50 significantly associated with markers of arterial stiffness (cfPWV; $p = 0.042$) and subclinical atherosclerosis (cIMT; $p = 0.016$).

Conclusion: Lower serum T50 values — indicative of a higher calcification propensity — were associated with a more severe phenotype in PXE patients. This study indicates for the first time that serum T50 may be a clinically relevant biomarker in PXE and may thus be of importance to future therapeutic trials.

MARJOLEIN CARRON (P5)

Modelling of RCBTB1-associated inherited retinal disease in Xenopus tropicalis indicates a role for RCBTB1 in cell polarity and oxidative stress responses

Authors:

M. Carron[1,2,3], T. Naert[3], G. Ascari[1,2], S. Demuynck[3], T. Van Nieuwenhuyzen[3], T. Van Laethem[1,2], T. Rosseel[1,2], A. Kremer[4], H. Van Landeghem[3], L. Wanneyn[3], S. Hoogstoel[1,2], F. Coppieters[1,2], C. Guérin[4], E., De Baere[1,2], K. Vleminckx[2,3]

Affiliation(s):

1. Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium
2. Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
3. Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium.
4. VIB Bioimaging Core, Ghent, Belgium.

RCBTB1 was found to be implicated in syndromic and non-syndromic inherited retinal diseases (IRD). Patients with biallelic missense variants in RCBTB1 display diverse IRD phenotypes such as retinitis pigmentosa, reticular dystrophy and chorioretinal atrophy. So far the function of this ubiquitously expressed gene remains largely unknown. From our Xenopus tropicalis rcbtb1-/-model and cellular transcriptome analysis, we hypothesize that RCBTB1 is involved in NRF2-regulated protection against oxidative stress in the eye, more specifically in the retinal pigment epithelium (RPE).

A Xenopus tropicalis knockout (KO) animal model was generated using CRISPR/Cas9 gene editing. Histological examination and three-dimensional electron microscopy was performed on retinas of rcbtb1-/- frogs. The rcbtb1-/- animals showed changes in the RPE, similar to observations in human cases, including loss of apical-basal cell polarity, loss of cuboidal cell morphology, spreading of the pigment granules and vacuolisation.

RNA-seq analysis performed on RCBTB1-mutated patients' lymphocytes, treated with H2O2, as well as on embryos from the rcbtb1-/- animal model treated with CdCl2, showed that in both cases the cellular response to oxidative stress was affected. More specifically, NRF2 downstream targets and several metallothionein genes were found to be differentially expressed, both in the animal and cellular models.

In sum, we showed that the Xenopus tropicalis rcbtb1-/- animal model recapitulates the human IRD phenotype. Both in vivo and in vitro functional data indicate possible involvement of RCBTB1 in the cellular response to oxidative stress. This provides insight into the mechanism underlying RCBTB1-associated IRD and uncovers potential future therapeutic opportunities.

MARIA DEL ROCÍO PÉREZ BACA (P6)

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

Authors:

Maria del Rocio Pérez Baca[1,2°] & Eva Z. Jacobs[1,2°], Lies Vantomme[1,2], Pontus Leblanc[1,2], Elke Bogaert[1,2], Annelies Dheedene[1,2], Laurenz De Cock[1,2], Bart Dermaut[1,2], Aidin Foroutan[3,4], Jennifer Kerkhof[4], Haley McConkey[4], ZFHX3 consortium, Björn Menten[1,2], Bo Yuan[5], Bekim Sadikovic[3,4], Sarah Vergult[1,2*] & Bert Callewaert[1,2*]
°shared first authors, *shared last author

Affiliation(s):

[1]Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium
[2]Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[3]Department of Pathology and Laboratory Medicine, Western University, London, ON N6A 3K7, Canada
[4]Verspeeten Clinical Genome Centre, London Health Sciences Centre, London, ON N6A 5W9, Canada
[5]Seattle Children's Hospital, Seattle and Department of Laboratory Medicine and Pathology, University of Washington, WA, USA

Background

Neurodevelopmental disorders (NDDs) result from impaired development and functioning of the brain. Here, we identify loss-of-function variation in *ZFHX3* as a novel cause for syndromic intellectual disability (ID). ZFHX3, previously known as ATBF1, is a zinc-finger homeodomain transcription factor involved in multiple biological processes including cell differentiation and tumorigenesis.

Methods

Through international collaboration, we collected clinical data of 40 individuals with protein truncating variants (PTVs) or (partial) deletions of *ZFHX3*. Via data-mining and multiple *in vitro* models we identified the subcellular localization and binding partners of ZFHX3. To assess the presence of an episignature associated with ZFHX3 haploinsufficiency, we performed DNA methylation analysis on whole blood extracted DNA of five individuals with a *ZFHX3* PTV and three with a (partial) deletion of *ZFHX3*. In addition, we used a reverse genetic approach to characterize the ZFHX3 orthologue in *Drosophila melanogaster*.

Results

Loss-of-function variation of *ZFHX3* consistently associates with (mild) ID, postnatal growth retardation, feeding difficulties, and recognizable facial characteristics as supported by artificial intelligence (Face2Gene). Publicly available and in-house generated expression data show increased expression of ZFHX3 during human brain development and neuronal differentiation. Immunoprecipitation followed by mass spectrometry in neural stem cells and SH-SY5Y shows that ZFHX3 interacts with the chromatin remodelling BRG1/Brm-associated factor complex and the cleavage and polyadenylation complex.

Furthermore, we identified a specific DNA methylation signature associated with ZFHX3 haploinsufficiency in leukocyte-derived DNA. In *Drosophila melanogaster*, ZFH2 is considered the ZFHX3 orthologue. We show that ZFH2 is expressed in the third instar larval brain and that knockdown of ZFH2 results in an adult lethal phenotype suggestive for a key role in development.

Conclusion

Loss-of-function variants in *ZFHX3* are a novel cause for syndromic ID and are associated with a specific DNA methylation episignature. Our results indicate a role for ZFHX3 in chromatin remodelling and mRNA processing.

ANDY WILLAERT (P7)

Zebrafish Facility Ghent – A new Ugent CORE Facility

Authors:

Andy Willaert, Paul Coucke

Affiliation(s):

Center for Medical Genetics Ghent, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium

The Zebrafish Facility Ghent (ZFG) has been established more than 10 years ago at the Center for Medical Genetics (Ghent University Hospital) and is now integrated as an official Ugent CORE Facility. The zebrafish (Danio rerio) is an increasingly popular vertebrate model organism which offers several advantages over other model organisms for basic research, disease modeling and toxicology testing; The low husbandry cost, short reproductive cycle, external fertilization and development, production of large numbers of synchronous and rapidly developing embryos per mating and the optical transparency of zebrafish embryos make it an excellent tool for high-throughput screenings. The availability of a wide range of molecular techniques such as large-scale genome mutagenesis, transgenesis and overexpression/knockdown approaches, have also increased the power of zebrafish as a model organism. Furthermore, due the high genomic and molecular similarities between zebrafish and other vertebrates, many of the important discoveries in zebrafish development are applicable to humans. Finally, the zebrafish model can both refine and reduce animal experiments carried out in traditional rodent systems (3R principle), as zebrafish are not regarded as a laboratory animal until the age of 5 days according to EU directive 2010/63/EU. The ZFG provides services to both UGent and external users. These services mainly include zebrafish caretaking, breeding and genetic management, zebrafish disease modeling and toxicology/behavior testing, besides custom services for more extended projects.

MARLIES COLMAN (P8)

Defective collagen biosynthesis in kyphoscoliotic Ehlers-Danlos syndrome due to pathogenic variants in PLOD1 and FKBP14: further insights into the common pathophysiological mechanisms and comparison of clinical characteristics.

Authors:

Marlies Colman[1], Robin Vroman[1], Tibbe Dhooge[1], Zoë Malfait[1], Sofie Symoens[1], Biruté Burnyté[2], Sheela Nampoothiri[3], Ariana Kariminejad[4], Fransiska Malfait[1], Delfien Syx[1]

Affiliation(s):

1. Ghent University, Center for Medical Genetics, Department of Biomolecular Medicine
Ghent, Oost-Vlaanderen, Belgium
2. Center for Medical Genetics, Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania
3. Department of Pediatric Genetics, Amrita Institute of Medical Sciences & Research Centre, Kerala, India
4. Kariminejad – Najmabadi Pathology & Genetics Center, Tehran, Iran

The autosomal recessive kyphoscoliotic Ehlers-Danlos syndrome (kEDS) is caused by the deficiency of either lysyl hydroxylase 1 (LH1) or the peptidyl-prolyl cis-trans isomerase FKBP22, two proteins participating in collagen biosynthesis in the rough endoplasmic reticulum. Deficiency of LH1 (encoded by PLOD1), which is crucial for collagen crosslinking, was the first biochemically elucidated congenital error of the human collagen metabolism. Defects in FKBP22 (encoded by FKBP14), which acts as a molecular chaperone of types III, IV, VI and X collagen, were found 40 years later in a subset of individuals presenting a kEDS phenotype with a normal LH1 function.

Clinical characteristics of kEDS include congenital muscle hypotonia, early onset kyphoscoliosis, generalized joint hypermobility and vascular fragility, and defects in both PLOD1 and FKBP14 also come with some gene-specific clinical characteristics. Despite the important phenotypic overlap between kEDS-PLOD1 and kEDS-FKBP14, the common pathophysiological pathway remains poorly understood and functional studies on patient-derived material are scarce.

We report the clinical and molecular characteristics of 14 individuals with kEDS-PLOD1 and 3 individuals with kEDS-FKBP14, and compared our findings with previously reported individuals with kEDS-FKBP14 and kEDS-PLOD1. Using patient-derived skin fibroblast cultures, we found evidence of ascorbic acid-dependent upregulation of FKBP22 in kEDS-PLOD1 skin fibroblasts and thus provide the first pathophysiological link between kEDS-FKPB14 and kEDS-PLOD1. In addition, we provided the first evidence for intracellular retention of types III and VI collagen in kEDS-FKBP14, which could explain part of the phenotype. The intracellular accumulation of these collagens was not accompanied by an upregulated unfolded protein response or autophagy using western blot or RT-qPCR analysis.

In conclusion, this study compares the phenotypic features of kEDS-FKBP14 and kEDS-PLOD1 and highlights new insights on the underlying pathophysiological mechanisms and on how these defects in collagen biosynthesis influence the matrix organization and lead to the observed phenotypes.

CHARLOTTE MATTON (P9)

FOXL2 mutation update for BPES, a syndromic form of POI: in silico assessment and ACMG classification of 394 unique sequence variants, and review of structural variants

Authors:

Charlotte Matton[1,2], Eva D'haene[1,2], Julie Van De Velde[2], Hannah Verdin[2], Alfredo Duenas Rey[1,2], Elfride De Baere[1,2]

Affiliation(s):

1 Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
2 Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium

Background/Objectives:

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

Methods:

Variant collection was performed through a literature search on FOXL2 and BPES between 2001-2022. This was completed with in-house variants, identified via clinical genetic testing and downstream research testing in the Center for Medical Genetics Ghent (CMGG). All sequence variants were subsequently classified according to the ACMG standards.

Results:

In total, 836 genetic defects of the FOXL2 region were found. Of these, 88% are intragenic FOXL2 variants, 394 are unique and 64 are novel. The polyalanine tract is a known mutational hotspot of FOXL2, illustrated by the high percentage of pathogenic polyalanine expansions (27%). Reclassification of disease-causing sequence variants revealed 34% class 5, 63% class 4 and 2% class 3 variants. Furthermore, the mutation spectrum is characterized by 8% coding CNVs and 2% non-coding CNVs, all but one located upstream of FOXL2. The remaining 2% are translocations along with chromosomal rearrangements of 3q23.

Conclusion:

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

LYNN BACKERS (P10)

Atrip-deficient patient expands molecular and clinical spectrum of seckel syndrome

Authors:

Lynn Backers[1,2,3], Simon J Tavernier[1,2,3,5], Evi Duthoo[6], Elien Beyls[6], Likun Du[7], Bram Parton[1,2], Veronique De Backer[3,8], Ria Roelandt[9], Hui Wan[7], Mattias Van Heetvelde[1,2], Kim De Leeneer[1,2], Marieke De Bruyne[1], Levi Hoste[3,8], Ans Baeyens[6], Anne Vral[6], Tessa Kerre[8,10], Qiang Pan-Hammarström[7], Filomeen Haerynck[3,8], Kathleen BM Claes[1,2]

Affiliation(s):

[1]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium
[2]Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[3]Primary Immunodeficiency Research Lab, Center for Primary Immunodeficiency, Jeffrey Modell Diagnosis and Research Center, Ghent University Hospital, Ghent, Belgium
[4]Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
[5]Unit of Molecular Signal Transduction in Inflammation, VIB-UGent Center for Inflammation Research, Ghent, Belgium
[6]Department of Human Structure and Repair, Ghent University, Ghent, Belgium
[7]Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden
[8]Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium
[9]VIB Single Cell Core, VIB-UGent Center for Inflammation Research, Ghent, Belgium
[10]Department of Hematology, Jeffrey Modell Diagnosis and Research Center, Ghent University Hospital, Ghent, Belgium

We report the second ATRIP-deficient patient clinically diagnosed with Seckel Syndrome (SS). Besides the typical clinical SS characteristics (primary dwarfism, facial dysmorphia, skeletal abnormalities, microcephaly and mental retardation), the patient suffers from a combined immunodeficiency disorder (CID). Whole exome sequencing (WES) integrated with transcriptomics

revealed a homozygous splice variant (c.829+5G>T) in ATR-interaction protein (ATRIP) leading to out-of-frame skipping of exon 5. RT-PCR showed no expression of the wild type allele and western blot confirms this by showing absence of the ATRIP protein. The ATRIP protein is implicated in the DNA damage response pathway as the interaction partner of the key player ATR. Activation of the main downstream substrate of the ATR-ATRIP complex, CHK1, is decreased and analysis of micronuclei, representative of un- or misrepaired DNA damage, in response to mitomycin C and ionizing radiation revealed defective DNA repair.

Of interest, a role for the ATR-ATRIP complex during the development or functioning of the immune system has not been thoroughly investigated. Other pathogenic variants were ruled out by WES in 460 genes linked to inborn errors of the immune system, while peripheral blood analysis and immunophenotyping revealed low absolute B cell numbers, IgG subclass deficiencies, aberrant T cell subsets, decreased B and T cell maturation and T cell oligoclonality. Analysis of class switch recombination (CSR) junctions in B lymphocytes by high throughput linear amplification showed alterations in the repair mechanisms during CSR, as was published for ATR-deficient Seckel Syndrome patients (doi:10.1084/jem.20050595). Additionally, first results of single cell RNA-Seq likewise point towards a recombination deficiency during B and T cell development.

In summary, we expanded the molecular and clinical spectrum of Seckel Syndrome and further validations will provide insights into the link with the immune phenotype and will contribute to the disease mechanism.

SOPHIE DEBAENST (P11)

Crispant analysis in zebrafish as a tool for rapid functional screening of disease-causing genes for Osteogenesis Imperfecta.

Authors:

Sophie Debaenst; Hanna de Saffel; Jan Willem Bek; Adelbert De Clercq, Tamara Jarayseh; Lauren Sahd; Paul Coucke; Andy Willaert

Affiliation(s):

Center for Medical Genetics Ghent, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium

Osteogenesis imperfecta (OI), characterized by increased bone fragility and skeletal deformities, is a genetically heterogeneous disorder mainly caused by autosomal dominant mutations in collagen type 1. Mutations in non-collagen genes are mostly associated with recessive forms of OI and encode proteins involved in osteoblast differentiation, bone mineralization and collagen processing. However, the genetic spectrum of OI is still expanding and the underlying pathogenic mechanisms are often not yet known. To get a quick insight into these mechanisms, we use the crispant screening approach, a rapid and cost-effective approach for in vivo functional validation, in order to phenotype directly in FO mosaic founder zebrafish. Crispants reduce model generation time, enabling the validation of a large set of genes in a short period of time. We selected 6 OI genes (creb3l1, ifitm5, mbtps2, sec24d, serpinf1 and sparc) and micro-injected CRISPR/Cas9 components targeting the gene of interest in zebrafish embryos with an osteoblast-specific Tg(osx:Kaede) transgenic background. NGS analysis revealed out-of-frame efficiencies higher than 70%, indicating high fraction of knock-out alleles. Phenotypic analysis was performed at 7, 14 and 90 days after fertilization, though fluorescence microscopy (osteoblasts), alizarin red bone staining and micro-CT analysis for quantitative analysis of the skeleton. Crispants for all genes show skeletal abnormalities in adult stages. Taken together, we showed that crispant screening in zebrafish is a promising approach for rapid functional screening of OI candidate genes. Moreover, the crispants have the potential to provide new insights into the role of these genes in skeletal biology and can be used as a tool for osteogenic compound screening.

LIESELOT VINCKE (P12)

Characterization of the genetic architecture of inherited retinal disease in a consanguineous Iranian cohort

Authors:

Lieselot Vincke[1], Kristof Van Schil[2], Marieke De Bruyne[1], Quinten Mahieu[1], Mattias Van Heetvelde[1], Toon Rosseel[1], Ebrahim Al Hajj[3], Sarah Van Malderen[1], Reza Maroofian[4], Fatemeh Suri[5], Miriam Bauwens[1*], Elfride De Baere[1*] *Equal contribution

Affiliation(s):

[1]Center for Medical Genetics Ghent, Ghent University and Ghent University Hospital, Ghent, Belgium, [2]Center for Medical Genetics Antwerp, Antwerp University Hospital, Antwerp, Belgium, [3]Free University of Brussels, Brussels, Belgium, [4]Institute of Neurology, Department of Neuromuscular Diseases, University College London, London, United Kingdom, [5]Ophthalmic Epidemiology Research Center, Research Institute for Ophthalmology and Vision Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Objectives: To uncover the underlying genetic basis of inherited retinal disease (IRD) in 156 unrelated families of Iranian descent, an integrated approach consisting of whole exome sequencing (WES) and autozygosity mapping was used.

Methods: WES was performed in 156 Iranian IRD families, predominantly originating from a consanguineous background (76%). Data-analysis was performed using the in-house Seqplorer tool. Copy number variants (CNVs) were assessed via ExomeDepth. AutoMap was used to determine runs of homozygosity (ROHs). Exome-wide variant assessment was performed with QCI Interpret Translational, Seqplorer and ExomeDepth to identify variants in novel candidate IRD genes. Variants were validated, classified (ACMG/ACGS guidelines) and segregation analysis was performed if possible.

Results: By interrogating 290 known IRD genes using a WES-based analysis, we obtained a molecular diagnosis for 79.6% of the IRD cohort. In total, 161 (likely) disease-associated variants (74 novel) were identified in 79 genes, of which ABCA4, EYS, and CRB1 were the three most implicated ones. In addition, the importance of structural variation (SV) in IRD was demonstrated, with CNVs identified in 5% of the cohort, including novel CNVs in CDH3, CDHR1, CHM and RD3. Moreover, variants were identified in novel retina-expressed candidate IRD genes, including COBL, FRMPD2, SLC26A7, TRAPPC14 and TRPM2.

Conclusion: This integrated study using WES and in-depth variant assessment provides insight into the genetic architecture of IRD in Iran, an underrepresented population. We provided 79.6% of patients with a genetic diagnosis and expanded the molecular spectrum of IRD in Iran by the identification of known and novel variants in the majority of patients, offering perspectives for counseling in their families. Finally, autozygome-guided exome sequencing revealed several novel candidate genes for IRD in unsolved cases.

LISA HAMERLINCK (P13)

An optimized workflow for CRISPR/Cas9-mediated generation of indels and large deletions in induced pluripotent stem cells and neural stem cells

Authors:

Lisa Hamerlinck*, María del Rocío Pérez Baca*, Lies Vantomme, Eva D'haene and Sarah Vergult

Affiliation(s):

Ghent University, Center for Medical Genetics Ghent

The innovative CRISPR/Cas9 technology has transformed our ability to manipulate the genome. As it is not always evident to receive relevant patient material, CRISPR/Cas9 technology provides a cheap and simple alternative to study how dysfunction can cause disease. Here, we present our workflow in human induced pluripotent stem cells (hiPSCs) and neural stem cells (hNSCs) to generate in vitro knock-out models based on indel generation. Moreover, we successfully optimized this workflow to generate kb-sized deletions, thereby making it possible to also study the impact of (non-coding) structural variation in relevant in vitro models.

We select the most promising single guide RNAs (sgRNAs) via in silico analysis. Subsequently, one RNP complex (indel generation) or two RNP complexes in equimolar ratio (larger deletions) are transfected into hiPSCs or hNSCs via nucleofection. After that, successful editing is assessed in the bulk of the cells via DNA isolation and amplification of the target region, followed by targeted next-generation-sequencing (NGS). For indel generation, primers are designed to generate an amplicon containing the theoretical cut site. For larger deletions, both primers outside and inside the deletion are designed. To obtain clonal cell lines, the transfected hiPSCs or hNSCs are single cell isolated through serial limiting dilutions with conditioned medium. Finally, monoclonality is confirmed by targeted NGS (indels) and CNV-seq (kb-sized deletions).

For indel generation, we obtained editing efficiencies ranging between 49-79% and observed that InDelphi correctly predicted the most frequent indels, 1 bp insertion and 1 bp deletion. Via nucleofection of two RNP complexes, we were able to generate clones harboring heterozygous or homozygous deletions ranging in size from 10 to 90 kb. During this process, we observed that it is crucial that both RNP complexes have similar editing efficiency. Via this workflow, it is possible to obtain monoclonal genome edited cells within a time frame of 6-8 weeks. After obtaining an engineerd cell line, these cells can be differentiated to the desired cell lineage and further functional testing can be performed.

GRIET DE CLERCQ (P14)

Long-read sequencing resolves cryptic structural variation in patients with syndromic intellectual disability

Authors:

Griet De Clercq[1], Lies Vantomme[1], Sarah Vergult[1], Bert Callewaert[1], Annelies Dheedene[1], Björn Menten[1]

Affiliation(s):

[1] Center for Medical Genetics Ghent, Department of Biomolecular Medicine, Ghent University, Ghent University Hospital, Ghent, Belgium

Intellectual disability (ID) affects 1-3% of the global population. A genetic diagnosis is crucial for further management and family planning, yet current molecular techniques fail to identify the genetic defect in 40% of patients. This diagnostic gap can be partly explained by missed structural variants (SVs). These are large genomic rearrangements that due to their size are hard to elucidate through conventional methods and can have a devasting impact on the patient's phenotype.

We applied nanopore long-read sequencing (LRS) onto a cohort of 13 proband-parent trios with unexplained ID and 3 single proband cases to identify, or further specify, cryptic (de novo) SVs. On average, we find 21700 SVs per individual and zero to one de novo variant per proband in a trio setting. Five de novo events and single case variants are further unravelled, revealing more complex rearrangements in 3/5 cases. In one case, a genotype-phenotype correlation could be established. Investigation in other patients is currently ongoing.

Moreover, long-read sequencing enables the instant detection of triplet-repeat expansions in our patients.

This study highlights the potential of LRS as a standard molecular method for SV and triplet-repeat identification, uncovering significantly more variants compared to short-read sequencing. Moreover, exact breakpoints can be pinpointed, revealing more complex rearrangements and allowing several unexplained ID cases to receive a concrete genetic diagnosis. We additionally shed light on the de novo SV formation rate in the human genome. Future work consists of finetuning the bioinformatics workflow for SV analysis and investigation of other inheritance modes as to further narrow down the diagnostic gap.