1 Vascular NOTCH3 aggregation load in CADASIL patients is associated with *NOTCH3* variant position

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CADASIL is the most prevalent hereditary cerebral small vessel disease, caused by cysteine altering variants in *NOTCH3* (*NOTCH3^{cys}*). CADASIL vessel pathology is characterized by NOTCH3 protein aggregation and ultrastructural GOM deposition in the media of small (cerebral) arteries. We recently discovered the first known genotype-phenotype correlation in CADASIL, as we found that *NOTCH3^{cys}* variants located in the six proximal epidermal growth factor-repeat domains (EGFr 1-6) of the NOTCH3 protein are associated with a more severe phenotype than *NOTCH3^{cys}* variants in the distal EGFr domains (EGFr 7-34). The molecular mechanisms underlying this genotype-phenotype correlation are unknown.

We hypothesized that EGFr 7-34 *NOTCH3^{cys}* variants have less propensity to aggregate compared to EGFr 1-6 variants. To test this hypothesis, we quantified vascular NOTCH3 protein aggregation in skin biopsies of CADASIL patients with EGFr 1-6 versus EGFr 7-34 *NOTCH3^{cys}* variants (n=12 and n=13 respectively, aged 50-60 years), using NOTCH3 immunohistochemistry and electron microscopy. We found that patients with *NOTCH3^{cys}* variants located in EGFr 1-6 had more vascular NOTCH3 aggregation than patients with EGFr 7-34 *NOTCH3^{cys}* variants (median 48.6% [IQR 48.2] versus 5.2% [11.1], $P=1.3\cdot10^{-5}$). Also, on electron microscopy, patients with EGFr 1-6 variants had more GOM deposits than patients with EGFr 7-34 variants (median 9.8 GOM/1000µm [IQR 15.2] versus 0.0 [1.6], $P=8.2\cdot10^{-5}$). There was no significant association between NOTCH3 aggregation and disease severity. In conclusion, *NOTCH3^{cys}* variant position is associated with vascular NOTCH3 aggregation propensity, providing the first molecular insight in the NOTCH3 genotype-phenotype correlation.

2 KidneyNetwork: Using kidney-derived gene expression data to predict and prioritize novel genes involved in kidney disease

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Introduction

Genetic testing in patients with suspected hereditary kidney disease may not reveal the genetic cause for the disorder as potentially pathogenic variants can reside in genes that are not known to be involved in kidney disease. To help identify these genes, we have developed KidneyNetwork, in which tissue-specific expression is utilized to predict kidney-specific gene functions.

Material and Methods

KidneyNetwork is a co-expression network built upon a combination of 878 kidney RNA-sequencing samples and a multi-tissue dataset of 31,499 samples. It uses expression patterns to predict which genes have a kidney-related function and which phenotypes might result from mutations in these genes. As proof of principle, we applied KidneyNetwork to prioritize rare variants in exome-sequencing data from 13 kidney disease patients.

Results

We assessed prediction performance of KidneyNetwork by comparing it to GeneNetwork and found improved prediction accuracy of kidney-related HPO-terms, as well as an increase in the number of significantly predicted HPO-terms (figure 1). Applying KidneyNetwork to exome-sequencing data of kidney disease patients allowed us to identify *ALG6* as promising candidate gene for kidney and liver cysts.

Conclusion

We present KidneyNetwork, a kidney-specific co-expression network that accurately predicts which genes have kidney-specific functions and can result in kidney disease. We show the added value of KidneyNetwork by applying it to kidney disease patients without a molecular diagnosis. KidneyNetwork can be applied to clinically unsolved kidney disease cases, but it can also be used by researchers to gain insight into individual genes to better understand kidney physiology and pathophysiology.

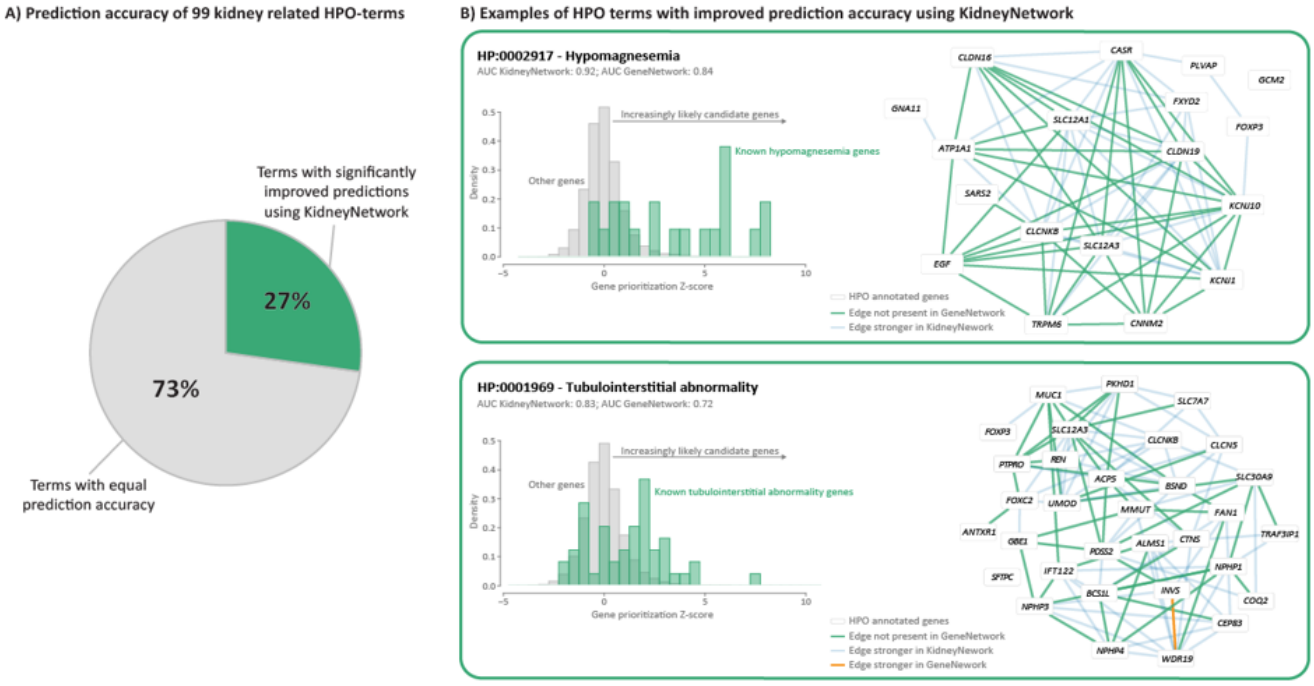

Figure 1. KidneyNetwork performs better for kidney-related HPO terms than GeneNetwork
A) 27% of kidney-related phenotypes are predicted significantly better using KidneyNetwork, as compared to GeneNetwork (a multi-tissue co-expression network we previously developed). B) Density plots of gene prediction scores within two of the most improved phenotypes, hypomagnesemia and tubulointerstitial fibrosis, show higher prediction values for genes annotated for the phenotype, while also predicting potential unknown candidate genes. The networks predicted using KidneyNetwork shows more and stronger correlations between the annotated genes than the networks predicted using GeneNetwork.

3 Genetic diagnoses using chromosomal analyses and exome sequencing in a prospective cohort of fetuses with structural anomalies

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Introduction

Exome sequencing (ES) is increasingly being used in addition to chromosomal analyses to diagnose fetuses with structural anomalies. We aimed to determine the diagnostic yield of genetic testing in these fetuses, report on the phenotype of monogenic disorders and describe our experiences.

Methods

We included fetuses with structural anomalies referred for genetic testing from 2018-2020. When chromosomal analyses (QF-PCR and SNP-array) were normal, fetuses that met the inclusion criteria underwent trio-ES using a phenotype-dependent filtering strategy. We documented the phenotypic characteristics of fetuses and compared them with literature(OMIM/PubMed).

Results

A chromosomal abnormality was found in 23.7% of fetuses (108/456;QF-PCR:18.0%;SNP-array:5.7%). A monogenic disorder was found in 29.8% of fetuses that underwent ES (45/151), and 18 (40.0%) had an inherited variant. The phenotypic characteristics of six fetuses had not previously been described in the monogenic disorders (*CDC42,MYCN,PEX1,POMK,RMRP,THOC6*). Fetuses with a diagnostic genetic variant had a higher proportion of skeletal anomalies compared to fetuses without a diagnostic genetic variant (37.8% vs. 14.2%,p=0.001). Diagnostic genetic variants also occurred more often when there was a maternal history of perinatal loss or a child with congenital

anomalies/intellectual disability (22.2% vs. 7.5%,p=0.007). In five cases, ES results supported pregnancy-related decision-making. Main challenges included interpreting prenatal genotype–phenotype relations, variant filtering strategies and rapid turnaround time.

Conclusions

ES increases diagnostic yield in fetuses with structural anomalies, especially for skeletal anomalies. A prenatal monogenic diagnosis can improve genetic counseling and perinatal care. Future directions should include optimizing inclusion criteria for ES, archiving prenatal genotype–phenotype information in online databases and improving variant filtering strategies.

4 FPLD3-associated PPARγ mutants define subclasses of target genes

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The nuclear receptor PPARγ, encoded by the *PPARG* gene, is the master regulator of adipocyte differentiation and function. How the different domains of PPARγ exactly communicate on an intra- and intermolecular level – including the DNA – on a gene-to-gene level to generate the appropriate transcriptional output in the context of chromatin remains to be defined. Natural *PPARG* mutations, as found in patients with familial partial lipodystrophy subtype 3 (FPLD3), a condition characterized by adipose tissue redistribution causing insulin resistance, type 2 diabetes, and dyslipidemia, can provide novel insights. In two non-consanguineous patients with FPLD3 we identified PPARγ E379K and R212Q. Both mutations – affecting different PPARγ domains – do not interfere in interactions with RXRα, ligand, and cofactors. However, both mutations dramatically impair its adipogenic capacity by affecting the ability of PPARγ to induce an overlapping subset of target genes, including the classical PPARγ target *Lpl*, while retaining the ability to activate other genes including *Acox1*. Genome-wide DNA binding profiles indicate that regulatory regions that require PPARγ for chromatin remodelling are particularly sensitive to these mutations. In addition, the exact nucleotide sequence of the PPARγ binding sites, including the 5' upstream region, can potentially contribute to target gene sensitivity, as for example activation from the *Lpl* PPRE was poor. We propose a model in which recruitment of PPARγ to chromatin is determined by multiple protein-protein and protein-DNA interfaces. Taken together, our findings indicate that relatively subtle molecular defects in PPARγ are sufficient to cause lipodystrophy by dysregulating a subset of PPARγ target genes.

5 A RIPOR2 in-frame deletion is a frequent and highly penetrant cause of adult-onset hearing loss

Background

Hearing loss is one of the most prevalent disabilities worldwide, and has a significant impact on quality of life. The adult-onset type of the condition is highly heritable but the genetic causes are largely unknown, which is in contrast to childhood-onset hearing loss.

Methods

Family and cohort studies included exome sequencing and characterization of the hearing phenotype. Ex vivo protein expression addressed the functional effect of a DNA-variant.

Results

An in-frame deletion of 12 nucleotides in RIPOR2 was identified as a highly penetrant cause of adult-onset progressive hearing loss that segregated as an autosomal dominant trait in 12 families from the Netherlands. Hearing loss associated with the deletion in 63 subjects displayed variable audiometric characteristics and an average age of onset of 30.6 years (SD 14.9 years, range 0-70 years). A functional effect of the RIPOR2 variant was demonstrated by aberrant localization of the mutant RIPOR2 in the stereocilia of cochlear hair cells and failure to rescue morphological defects in RIPOR2-deficient hair cells, in contrast to the wildtype protein. Strikingly, the RIPOR2 variant is present in 18 of 22,952 individuals not selected for hearing loss in the Southeast Netherlands.

Conclusion

Collectively, the presented data demonstrate that an inherited form of adult-onset hearing loss is relatively common, with potentially thousands of individuals at risk in the Netherlands and beyond, which makes it an attractive target for developing a (genetic) therapy.

6 Cancer spectrum and penetrance in a national cohort of patients with a loss-of-function germline *SMARCA4* alteration

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Introduction

Loss-of-function germline *SMARCA4* variants predispose to rhabdoid tumors in children and small cell carcinoma of the ovary hypercalcemic type (SCCOHT) in girls and women. Cancer penetrance is unknown, which complicates the development of surveillance and prevention guidelines. We therefore describe a national cohort of individuals carrying germline *SMARCA4* alterations and their cancer phenotype.

Methods

We have collected clinical and genetic data from individuals with a germline loss-of-function *SMARCA4* alteration through all Dutch DNA diagnostic laboratories.

Results

We have identified 16 individuals from 12 families. In 5 probands the *SMARCA4* variant was detected after cancer diagnosis (4 SCCOHTs, 1 rhabdoid tumor). One patient has inherited the variant from her mother and grandmother, neither of whom developed *SMARCA4*-related cancers. Five probands (mean age 28 years [range 6-51 years] 4 females), all without cancer, carry a *de novo* 19p13.2 deletion including *SMARCA4* and are affected by developmental delay. Finally, in two probands and two of their relatives (mean age 33 years [range 15-54 years] 3 females), also all without cancer, the *SMARCA4* variant was an incidental finding.

Conclusion

Our data reveal an incomplete penetrance for cancer in individuals with germline loss-of-function *SMARCA4* alterations and suggest a possibly lower penetrance in patients with multigene deletions spanning *SMARCA4*. We hypothesize that these larger deletions include additional genes essential for cell survival. Affected cells might not survive in a homozygous deleted state, therefore prohibiting tumor development due to a large deletion affecting the wild type allele, a common somatic alteration in *SMARCA4*-related tumors.

7 APC mosaicism testing in milder polyposis phenotypes reveals *pks+ E.coli* bacteria as possible additional explanation for the development of colorectal adenomas

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Introduction

Mosaic mutations in the *APC* gene have been identified as a common cause (25%) for unexplained polyposis in patients with >20 adenomas. The frequency of *APC* mosaicism remains unknown in milder phenotypes.

Materials and Methods

To test for *APC* mosaicism, we analyzed the *APC* gene in multiple lesions of patients with unexplained colonic polyposis using Next Generation Sequencing. Additionally, patients with milder phenotypes, e.g. >20 adenomas at age >70, were included.

Results

The mosaicism detection rate was 12% (27/232) in the entire cohort, 5.7% in patients with <10 adenomas (2/35) and 7.7% in those with 10-20 adenomas (8/104). Stratified for age, 2.8% (1/36) of patients aged >70 showed with a mosaicism. Besides these "true" mosaicism cases, 21% (50/232) of patients showed a so-called hybrid mosaicism, where multiple, but not all lesions share an identical variant.

Interestingly, 46% (23/50) of hybrids have a specific *APC* splice variant c.835-8A>G in multiple lesions. Together with 7 other recurring *APC* variants, this variant was compatible with the recently described mutational signature caused by colibactin, a compound produced by *pks+ E.coli.* The possible influence of colibactin needs further exploration. Therefore, we are now performing additional analyses like Whole Genome Sequencing.

Conclusions

Our results indicate that *APC* mosaicism also plays a role in milder polyposis phenotypes. Furthermore, a substantial proportion of our cohort had a hybrid mosaicism of which the clinical consequences are not yet clear. In some patients, the presence of *pks+ E.coli* might be the explanation for the development of polyps.

8 **The Molecular Profile of *MSH6*-Associated Colorectal Carcinomas From Patients With Lynch Syndrome**

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BACKGROUND & AIMS
Lynch syndrome (LS) patients carry a germline variant in one of the mismatch repair (MMR) genes (*MLH1, MSH2, MSH6* and *PMS2*) and have a high risk to develop colorectal cancer (CRC). In contrast to other LS CRCs, the molecular profile of *MSH6*-associated CRCs has not been studied on a larger scale. Therefore, this study aimed to examine the molecular profile of CRCs from *MSH6* variant carriers in order to gain more insights in the cancer development in this group of patients.
METHODS
The DNA of 14 *MSH6*-associated CRCs was analyzed using whole-exome-sequencing and was subsequently compared to that of the other LS subgroups. The sequence of mutational events was studied by classifying variants either as being related to MMR-deficiency or not, using COSMIC signature 6.
(PRELIMINARY-)RESULTS
Mutations in *CTNNB1* were more frequently found in *MSH6*-associated CRCs (21%) than in *MSH2*- (6%; P=.295) and *PMS2*-associated CRCs (0%; P=.061). *APC* mutations were found in 36% of the *MSH6*-associated CRCs and frequently co-occurred with mutations in *TCF7L2* and *RNF43*. *KRAS* and *TP53* mutations were prevalent in all four LS subgroups, though the contribution of signature 6 to these mutations was lower in the *PMS2*-associated CRCs (30% & 17%, respectively) than in the *MSH6*- (57% & 50%), *MLH1*- (50% & 63%), and *MSH2*-associated CRCs (88% & 63%).
CONCLUSIONS
Our findings suggest that *MSH6*-associated CRCs can develop via three distinct molecular pathways yet mainly originate from MMR-deficient crypts with an intervening adenoma stage, and could influence future guidelines for screening and treatment.

9 High rate of (epi)genetic predisposing factors and an important role for *DIS3L2* in a nationwide Wilms tumor cohort

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Background

Wilms tumor (WT) is the most common childhood renal tumor, associated with (epi)genetic predisposing factors including Beckwith-Wiedemann Spectrum (BWSp) and *WT1*-related syndromes. In this study, we aimed to determine the prevalence of predisposing factors in relation to phenotypic findings, and to identify novel WT predisposition genes.

Methods

Phenotypic data and diagnostic test results were collected for all children diagnosed with WT in the Netherlands (2015-2020). Comprehensive BWSp testing was performed, followed by germline (trio-) whole exome sequencing (WES).

Results

126 patients were identified, including one familial WT. (Epi)genetic predisposing factors were present in 42/126 patients (33.3%). Heterozygous *DIS3L2* variants were identified as a novel predisposing factor in five patients (4.0%), with a second somatic hit in 4/4 (100%) tumors tested. Twenty patients (15.9%) were diagnosed with BWSp, including patients with a molecular diagnosis in blood-derived DNA (N=8), normal kidney tissue-derived DNA with at least one additional feature of BWSp (N=8), or solely a clinical diagnosis of classical BeckwithWiedemann syndrome (N=4). Four patients without additional BWSp features harbored 11p15 methylation defects in normal kidney tissue. Remaining findings included *WT1*-related syndromes (N=10, 7.9%), Fanconi anemia (N=1), *REST* (N=1) and *NF1* (N=1) mutations. Candidate WT predisposition genes were identified which require validation in larger cohorts.

Conclusions

(Epi)genetic WT predisposing factors, including mosaic 11p15 aberrations, were present in at least 33.3% of patients with WT in this national cohort, with an important role for constitutional heterozygous *DIS3L2* variants. Based on these results, we encourage standard genetic testing after counseling by a clinical geneticist.

10 A clustering of missense variants in the crucial chromatin modifier WDR5 defines a new neurodevelopmental disorder

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WDR5 is a broadly studied, highly conserved protein involved in a wide array of biological functions. Among these functions, WDR5 is a part of several protein complexes that affect gene regulation via post-translational modification of histones. We collected data from ten unrelated individuals with six different rare *de novo* missense variants in *WDR5*; one identical variant was identified in four individuals, and another variant in two individuals. All ten individuals had neurodevelopmental disorders including speech/language delays (N=10), intellectual disability (N=8), epilepsy (N=6) and autism spectrum disorder (N=4). Additional phenotypic features included abnormal growth parameters (N=6), heart anomalies (N=2) and hearing loss (N=2). All six missense variants occurred in regions of the *WDR5* locus known to be extremely intolerant to variation. Three-dimensional structure analyses indicate that all the residues affected by these variants are located at the surface of one side of the WDR5 protein. Five out of the six amino acid substitutions are predicted to disrupt interactions of WDR5 with RbBP5 and/or KMT2A/C, as part of the COMPASS family complexes. Thus, we define a new neurodevelopmental disorder associated with missense variants in *WDR5* characterized by a broad range of associated features including intellectual disability, speech/language impairments, epilepsy and autism spectrum disorders. This finding highlights the important role of COMPASS family proteins in neurodevelopmental disorders.

11 *De novo* recurrent variants in *U2AF2* RNA-binding domains in an intellectual disability syndrome

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The essential pre-mRNA splicing factor U2AF2 recognizes a polypyrimidine-tract splice-site signal at the 3' splicesite and initiates spliceosome assembly. These early steps ensure the high fidelity of splicing, which is vitally important because mistakes in splicing could result in unintended effects leading to dysregulation of abundance of different gene isoforms expression and/or frameshift mutations.

By exome sequencing and international matchmaking we identified 19 unrelated individuals with nine different *de novo* novel missense variants in the RNA-binding domains of *U2AF2*. Four out of the nine variants were recurrent in RNA binding motif 1 (RRM1), where most variants reside, and were found in 14 individuals [c.445C>T (p.Arg149Trp), c.448C>T (p.Arg150Cys), c.449G>A (p.Arg150His), c.556G>A (p.Val186Met)], representing mutation hotspots of *U2AF2*. All of the identified variants reside in the highly intolerant regions. Detailed clinical assessment of the affected individuals show

that they present with developmental delay (DD) or intellectual disability (ID) (20/20), seizures (10/19), often fever related, and brain anomalies (8/14) of whom 5 had corpus callosum hypoplasia or agenesis. In addition, similar facial dysmorphisms were observed.

Functional studies of the recurrent mutations (c.445C>T (p.Arg149Trp), c.448C>T (p.Arg150Cys), and c.449G>A (p.Arg150His)), showed a decreased splice site RNA binding affinity and/or specificity . Additionally, preliminary transcriptome analyses from patients' cells suggest differentially spliced exons in some target genes. In summary, through multi-center collaborations, we demonstrate that *de novo U2AF2* mutations are associated with a novel neurodevelopmental disorder.

12 Impairment of the MSH4-MSH5 heterodimer results in infertility due to male meiotic arrest and premature ovarian failure

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Background

Infertility affects around 10-15% of all couples. Although frequently suspected, a genetic cause for infertility is rarely detected. Therefore, we aimed to decipher the underlying cause for infertility due to impaired meiosis.

Methods

In this study we describe a male index patient with unexplained meiotic arrest (MeiA), and his sister with premature ovarian insufficiency (POI), who underwent exome sequencing. This pair is from the genetically isolated Dutch *DARWIN* population. Furthermore, exome sequencing data of 63 male patients with MeiA from Germany were investigated. To assess the functional consequences of identified variants, we studied immunohistochemical γH2AX staining of testis sections and heterologous expression of identified variants in HEK293T cells.

Results

In the infertile sibling pair we identified a homozygous loss-of-function variant in *MSH4*. Furthermore, we report another four variants in *MSH4*, as well as two variants in *MSH5* in six men with MeiA from the German cohort. γH2AX staining of testis sections of patients with *MSH4*- and *MSH5* variants revealed arrest in early prophase of meiosis I. Heterologous expression of variants in *MSH5* in HEK293T cells showed truncation or complete loss of this protein.

Conclusion

Biallelic pathogenic variants in *MSH4* and *MSH5* lead to infertility in both sexes due to MeiA during meiosis I, resulting in azoospermia in men and POI in women. MSH4 and MSH5 form a heterodimer specifically required for meiotic recombination. Our findings have diagnostic value and potential future therapeutic consequences on eligibility of assisted reproductive technologies for men with azoospermia and women with POI/fertilization failure.

13 **Neurofibromatosis type 1 and the next generation: is preimplantation genetic testing the solution?**

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Introduction

Future parents affected with Neurofibromatosis type 1 (NF1) can opt for preimplantation genetic testing (PGT) to avoid NF1 in their offspring. We aim to identify challenges and pitfalls in PGT for NF1. Methods: We collected data on PGT cycles from the medical files of couples requesting PGT for NF1 between January 1997 and January 2020.

Results

PGT was the reproductive option of choice for 96 couples. PGT was not possible for 14 couples, mostly because the causative variant was not identified or of unknown significance. The origin of the *NF1* mutation was (presumed) sporadic in 63% of the 82 couples proceeding with PGT. PCR with multiple polymorphic markers was most frequently applied, combined with direct mutation analysis if needed. PGT/PCR work-up showed several exceptional situations: in one family two different *NF1* mutations turned out to be causal and in one family with a sporadic male index the mutation was not detected in the sperm cells during PGT work-up. The 'OnePGT' genome wide haplotyping method, based on next generation sequencing, was used for 2 recent families with a familial mutation. A successful PGT test could be developed for 78 couples with 71 different variants in the *NF1* gene. Together, 65 couples underwent 141 PGT cycles and the transfer of 162 unaffected embryos resulted in 39 ongoing pregnancies (pregnancy rate 24,1%/embryotransfer).

Conclusions

PGT is a successful reproductive option for couples with NF1. Test development was possible in almost all cases reviewed despite the fact that most variants were unique and sporadic.

14 Vascular Ehlers-Danlos syndrome – A comprehensive natural history study in the Dutch patient cohort, preliminary results

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Introduction

Vascular Ehlers Danlos Syndrome (vEDS; OMIM 130050; ORPHA 286) is a rare connective tissue disorder, caused by heterozygous pathogenic variants in the *COL3A1* gene. The phenotype is highly variable. vEDS patients are at risk for arterial, bowel and uterine rupture.

Purpose

To perform a national multi-center cohort study in all known Dutch vEDS patients, to provide further insights into the natural history of the disease. This knowledge will allow us to optimize patient care.

Methods

After METC approval, all known Dutch patients carrying a (likely) pathogenic variant in the *COL3A1* gene were invited to participate in the study (n=~130). The phenotype was systematically charted by retrospective and cross-sectional assessment of molecular and clinical data.

Preliminary results

Eighty-seven patients have been included thus far (44 males, mean age 48 years (4-94 years)). Thirty-one of 85 (36%) were index patients (2 missing data). Of the 87 patients, 52 (60%) had a symptomatic history. The main reasons for referral were: family member with (likely) pathogenic variant (60%), arterial dissection (13%) and aneurysm (9%). In total, 30 patients (34%) had aneurysm(s), 29 (33%) dissection(s), 28 (32%) varicose veins and 8 (9%) cardiac valve insufficiency. Six (7%) suffered from (iatrogenic) perforation of the colon. Twentytwo of 62 (34%) did not meet the 2017 criteria suggestive for vEDS.

Conclusion

This national multi-center natural history study of Dutch vEDS patients provides a basis for improving guidelines for diagnosing, follow-up and treatment of vEDS patients worldwide.

15 Whole-exome sequencing 677 aneurysm patients identifies multiple rare variants in the proprotein convertase FURIN causing impaired TGFβ family signaling.

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Aneurysms occur frequently in the abdominal aorta, and occurrence increases with age, resulting in 5-8% of men and 1-2% of women above 65 years being affected. Age-related extracellular matrix remodeling and weakening of the aortic wall is caused by genetic predispositions and major risks factors like hypertension and smoking. Although familial segregation studies have identified over 40 aneurysm genes, altogether (likely)pathogenic variants in these only explain ~4% of cases. In this study we have performed whole-exome sequencing of 677 abdominal aorta aneurysm patients and identified 24 (3.5%) unrelated patients with 13 different rare heterozygous variants in *FURIN*, encoding the proprotein convertase FURIN. Of these 24 patients, 14 had multiple aneurysms and 7 a rupture or dissection. Thoracic aneurysm was observed in 6. More than half of the patients showed a range of extravascular connective tissue features, like atrophic scarring, joint hypermobility, scoliosis or a pectus excavatum. The steady-state protein levels, protease activity and shedding of recombinant FURIN variants were affected. The consequences for maturation of pro TGFβ1 by FURIN-mediated cleavage for downstream SMAD (SMAD2) and non-SMAD (MAPK) signaling, and expression of TGFβ1-responsive *ACTA2* and *COL4A1*, were variably impaired in patient fibroblasts, indicating that TGFβ/BMP family actions are dysregulated in these aneurysms. The range of effects of the recurrent missense pR745Q FURIN in fibroblasts of different patients reflects the influence of individual genetic backgrounds in aorta aneurysms.

Conclusion
Using WES of a large cohort of unrelated patients we identified rare variants of *FURIN* with high prevalence in aortic aneurysm.

16 Clustering of the cardiac transcriptome of dilated cardiomyopathy patients reveals opposite molecular signatures among patients with truncating and missense *TTN* variants

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Introduction

Truncating variants in titin *(TTNtv)* are the most prevalent genetic cause of dilated cardiomyopathy (DCM). Here, we performed unsupervised clustering on the cardiac transcriptome of DCM patients to test whether the transcriptomic profile can detect *TTNtv* and whether missense variants in *TTN* (*TTNmiss*) show a comparable transcriptomic profile.

Methods

RNA was isolated from cardiac biopsies of 92 DCM patients (30 *TTNtv*, 12 *TTNmiss*, 7 pathogenic *LMNA*, 9 other pathogenic variants, and 34 non-genetic). The mRNA-sequencing library was generated and sequenced on the NextSeq-500. The data of 58k transcripts was subjected to dimension reduction and graph-based clustering. Transcriptomic clusters were afterwards associated with GO-biological process and phenotype-genotype data.

Results

Six distinct transcriptomic clusters were identified, among which 3 clusters (C2, C3 and C5) formed a super-cluster which was significantly enriched by *TTN* variants. Cluster 3 (C3) was dominated by *TTNtv* and 3 *TTNmiss*, reflecting a strong upregulation of mitochondrial energy metabolism pathways. Cluster 5 constituted of 5 *TTNtv* and *3 TTNmiss* samples, which showed the opposite: downregulation of metabolic and mitochondrial energy pathways. Interestingly, *TTNtv* patients in C3 had a low left ventricular mass (LVmass) in contrast to patients in C5 which had small hearts with a high LVmass and hypertension.

Conclusions

Transcriptomic clustering revealed two distinct *TTNtv* patient-clusters which show two extremities of cardiac metabolism. Hypertension could be an important mediator in determining the pathophysiology and phenotype of *TTNtv* carriers. DCM patients with *TTNmiss* show similarities to *TTNtv* in the transcriptomic signature, potentially indicating both diagnostic and therapeutic value.