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Results within the research project "Genetics of rare disorders"

Last name, first name, *31.12.1999 ♂ (Index)	Lab nr: 1111111-2222222-3333333
EDTA-blood, received:	Date of request:

Results: A heterozygote variant (NM_001128929.3:c.1706G>A, p.(Ser569Asn)) of uncertain significance in *ROBO2*.

Interpretation:	The patient has possibly a vesicoureteral reflux due to a variant of unknown significance in the *ROBO2*-gene.
Recommendation:	Segregation analysis of the parents of patient.
Further aspects:	We could not detect a clinical relevant variant that explains the cardiomyopathy.

According to § 10 of the German Genetic Diagnostic Law (GenDG), a genetic counseling should be offered to communicate the genetic findings.

In case there are any further questions, please do not hesitate to contact us
Best regards

Human Geneticist | Human Geneticist | Staff Scientist

Symptoms: ESRD with 38 years, hydronephrosis, cardiomyopathy, congenital anomalies of the kidney and urinary tract (CAKUT)

Family history: unclear

Variant summary

Gene, OMIM, Inheritance	Variant(s)	Zygosity	Classification according to ACMG[1]
ROBO2 Vesicoureteral reflux 2 (#610878) autosomal dominant	chr3:77612456; NM_001128929.3: c.1706G>A, p.(Ser569Asn)	heterozygote	unknown significance (PM2_SUP, PP3)

Relevance of the identified variant(s)

c.1706G>A, p.(Ser569Asn)

- MAF: 0% (not reported in the general population (gnomAD), PM2-SUP)
- *in silico*: consistent pathogenic (PP3), highly conserved amino acid
- HGMD / ClinVar: no entries

MAF: Frequency of the allele in the general population, based on GnomAD; GnomAD: Several populations focused on European population; MaxPop: maximal allele frequency within a population or subpopulation; HGMD: a commercial database for variants reported in the literature; ClinVar: a public database for variants reported in the literature and by different laboratories; ACMG: American College for Medical Genetics, an internationally acknowledged authority for clinical genetic diagnostics; [1]Richards et al., Genet Med 2015, PMID:12345678

Variant summary

- A *ROBO2* associated vesicoureteral reflux is related to congenital anomalies of the kidney and urinary tract and characterized by the retrograde flow of urine from the bladder into the ureter that is associated with reflux nephropathy. Latter is a major cause end-stage renal disease in children and young adults (Lu *et al.,* 2007, PMID: 17357069; Elahi *et al.*, 2016, PMID: 26408188)

- Cardiomyopathy has not been associated with pathogen variants in *ROBO2*, yet.

- We recommend a segregation analysis of the parents of the patient regarding the identified variant (DNA or EDTA-blood and consent of the parents is required). A co-segregation of the variant in the family (affected family members also carry the variant and unaffected family members do not carry the variant) will encourage a coherence of the variant in *ROBO2* and the renal symptoms of the patient.

Methods

General aspects of exome sequencing

Whole-exome sequencing (WES) is a method based on the technology of massive parallel sequencing, also called next generation sequencing (NGS). We analyze the majority of coding sequences (exons). The proportions of the covered exons depend on the used *kit* and on the quality of the analysis.
The results can be only interpreted in the context of the patient's medical evaluation, family history, and scientific literature. Please note that the variant's classification may change over time, as more information becomes available.

1. Information regarding Quality

Coverage of more than 20x has been achieved in more than 95 % of target sequences in all family members.

2. Applied methods

a) Strictly following manufacturer's instructions and SOPs, library preparation is done using the Nextera DNA Flex Pre-Enrichment LibraryPrep with Illumina Nextera DNA UD Indexes by Illumina. Target enrichment is achieved by using the Human Core Exome hybridization probes from Twist Bioscience. Paired-end Next-Generation-Sequencing is then performed on a NovaSeq 6000 Instrument using an S1 Reagent *Kit* (300 cycles) by Illumina. The NovaSeq 6000 Instrument is physically located at the facilities of GeneWIZ Europe-an Headquarter & Laboratory, Leipzig.
b) Analysis of the raw data *was* performed using the software Varfeed (Limbus, Rostock) and the variants were annotated and prioritized using the software Varvis (Limbus, Rostock). We have prioritized all potential protein-influencing variants with regard to their pathogenicity and clinical relevance according to all possible inheritance modes.
c) We perform, on an exploratory base, CNV analysis of down to single exon deletion. If we obtain technically valid results (usually through validation with a second method) in a clinically relevant gene, we would inform you. Not reporting single exon deletions or duplications does not mean that these are fully excluded.
d) We report only variants that are located in valid disease associated genes based on OMIM. Exceptions are variants in genes of which we have other sources of information that strongly support disease-gene association. You will not be informed about variants without a clear clinical relevance.

3. Limitations of exome-sequencing

a) We cannot guarantee a complete coverage of all possible variants by this method due to: a) the *kit* and the technique do not cover all coding sequences, b) the technique is not perfect and the coverage and the information regarding deletions and duplications are prone to gaps.
b) We evaluate only variants with a minimum coverage of 10x.
c) The reported variants are NOT validated with Sanger. A Sanger validation is necessary before the results influence a clinical decision. Thus we recommend validation of the variant and segregation analysis using Sanger sequencing. Our institute may run these analyses on clinically routine basis if this is wished by you.
d) We evaluate and report the data based on the available clinical information. However, a final conclusion on the relevance of the variants can only be made by the referring physician after a detailed assessment of clinical symptoms and genetic results.
e) We assess and report the data based on the current literature. We may carry out re-evaluations of data in the future. If we end up with a significantly deviating result, we will inform you. There is no claim to re-evaluate the data.
f) As mentioned in 2c and 3a, deletions and insertions are currently not reliably detected by exome sequencing.

4. Information about incidental findings

a) Exome-sequencing may lead to the identification of variants of medical significance that are not associated with the individual's indication. These findings are called incidental findings and are not the subject of the investigation.
b) If desired and consented by the patient or her/his guardian, we report only pathogenic and likely pathogenic variants in only so-called actionable genes according to the recommendation of the ACMG. Actionable genes are those which, if mutated, could lead to treatable or preventable diseases or recommendations for specific screening programs. These are currently the following 73 genes (ACMG_v3): *ACTA2*, *ACTC1*, ACVRL1, *APC*, *APOB*, ATP7B, *BMPR1A*, *BRCA1*, *BRCA2*, BTD, *CACNA1S*, CASQ2, *COL3A1*, *DSC2*, *DSG2*, *DSP*, ENG, *FBN1*, FLNC, GAA, *GLA*, HFE (p.Cys282Tyr homozygotes only), HNF1A, *KCNH2*, *KCNQ1*, *LDLR*, *LMNA*, *MAX*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *MYBPC3*, *MYH11*, *MYH7*, *MYL2*, *MYL3*, *NF2*, OTC, *PALB2*, *PCSK9*, *PKP2*, *PMS2*, *PRKAG2*, *PTEN*, *RB1*, *RET*, RPE65, *RYR1*, *RYR2*, *SCN5A*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *SMAD3*, *SMAD4*, *STK11*, *TGFBR1*, *TGFBR2*, *TMEM127*, *TMEM43*, *TNNI3*, *TNNT2*, *TP53*, *TPM1*, TRDN, *TSC1*, *TSC2*, TTN, *VHL*, *WT1*.
c) If no variants are reported, please notice, this does not mean that pathogenic variants in the above mentioned genes are generally excluded.

5. Information on candidate genes

This analysis has been performed in a research setting to identify novel genes for rare disorders. As we have identified a variant in a known gene, we did not follow intensively the research aspect.