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Dr. Jane Doe
Paul-Ehrlich-Str. 23
72076 TÜBINGEN
GERMANY

Patient	XXX, XX (*DD.MM.YYYY)
Sex	Female
Patient-ID	#
Sample receipt	xxx
Material	DNA
Report date	xxx

Genetic analysis report – XXX, XX (*DD.MM.YYYY)

Indication	Suspected Joubert syndrome; hypoplasia of vermis, sign of molar tooth in the central part of the brain, hypoplasia of optic nerves, unstable walk, vision problems, enophthalmos
Order	Panel Diagnostics: Joubert Syndrome (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of two pathogenic variants in gene *CEP290*, which are causative for Joubert syndrome in your patient, if present in a compound-heterozygous state.**
- Based upon current scientific knowledge, we did not identify any reportable copy number variants which are likely to be causative for your patient's disease.

Gene	Variant	Zygosity	Heredity	MAF (%)	*in silico* Prediction	Classification
CEP290	**c.5493delA; p.Ala1832Pro*fs**19**	**het.**	**AR**	**< 0.01**	**-**	**pathogenic**
CEP290	**c.1666delA; p.Ile556Phe*fs**17**	**het.**	**AR**	**0.12**	**-**	**pathogenic**

Information for table interpretation:
AD: Describes a trait or disorder requiring only one copy of a gene variant at a particular locus in order to express an observable phenotype. Single heterozygous gene variants can only be causative for the phenotype if the disorder follows the **autosomal dominant** mode of inheritance.
AR: Describes a trait or disorder requiring the presence of two copies of a gene variant at a particular locus in order to express an observable phenotype. In **autosomal recessive** disorders, a single heterozygous variant in one gene cannot by itself be causative for the observable phenotype.
XL: X-linked mode of inheritance
mitochondrial: gene encoded in the mitochondrial DNA. Information on degree of heteroplasmy (heteropl. or homopl.), frequency and read count (variant/total reads).
MAF: The **minor allele frequency** describes the least frequent allele at a specific locus in a given population. The less frequently a variant occurs, the more likely it is pathogenic; however, the prevalence and mode of inheritance of any particular disease must be considered. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

***in silico* Prediction:** The ACMG (American College of Medical Genetics) guidelines recommend using prediction programs to assess the possible pathogenicity of a variant. Each program calculates its predictions based upon different criteria, and the correspondence between a prediction and the actual functional effect of a variant is variable. **These predictions may therefore not serve as the sole basis for the evaluation of pathogenicity.**
Classification: Refers to the possible pathogenicity of a variant, but does not necessarily provide clear evidence of clinical significance. Variants are evaluated based upon current data and specific criteria, and are then classified as follows: pathogenic, likely pathogenic, and uncertain significance. **All variants for which clinical relevance cannot be conclusively confirmed or excluded are referred as variants of unknown clinical significance.**

INTERPRETATION

***CEP290*, c.5493delA; p.Ala1832Pro*fs**19 (het.), NM_025114.4, rs386834158**

***CEP290*, c.1666delA; p.Ile556Phe*fs**17 (het.), NM_025114.4, rs727503855**

OMIM / Reference	Phenotype	Heredity
611755	Leber congenital amaurosis 10 (LCA10)	AR
610188	**Joubert syndrome 5 (JBTS5)**	**AR**
611134	Meckel syndrome 4 (MKS4)	AR
610189	Senior-Loken syndrome 6 (SLSN6)	AR
615991	?Bardet-Biedl syndrome 14 (BBS14)	AR
PMID: 23559409	Nephronophthisis-related ciliopathy	AR
PMID: 26992781	Cone-rod dystrophy	AR
PMID: 23591405; PMID: 25377065; PMID: 26667666	Retinitis pigmentosa	AR

The gene ***CEP290*** encodes the centrosomal protein 290, which is primarily expressed within the eyes, kidneys, and the brain. The protein plays an important role in centrosome and cilia development (Coppieters et al., 2010, PMID: 20690115). Pathogenic variants in *CEP290* are associated with varying autosomal recessive syndromes within the Joubert-syndrome-spectrum, including retinal dystrophy (LCA10), and/or kidney disorders (MKS4 and SLSN6). Clinical features usually appear during the neonatal period to childhood.

We identified two heterozygous variants in gene *CEP290* in your patient.

The detected variant **c.5493delA; p.Ala1832Pro*fs**19** creates a shift in the reading frame, which will most likely result in nonsense-mediated decay of the mRNA transcript. This alteration has already been described as causative for several symptoms within the spectrum of *CEP290*-associated disorders ranging from retinal dystrophy to lethal Meckel Syndrome (also known as 5489delA; i.a. Brancati et al., 2007, PMID: 17564967; Frank et al., 2008, PMID: 17705300; Feldhaus et al., 2020, PMID: 31734136; ClinVar Variation ID: 56739).

The second alteration **c.1666delA; p.Ile556Phe*fs**17** also creates a shift in the reading frame, resulting most likely in nonsense-mediated decay of the mRNA transcript. This variant is one of the most frequently described pathogenic alterations in patients with symptoms within the Joubert syndrome spectrum, mainly retinal dystrophies (i.a. Brancati et al., 2007, PMID: 17564967; Bachmann-Gagescu et al., 2015, PMID: 26092869, suppl. Table S5; Avela et al., 2019, PMID: 31087526; ClinVar Variation ID: 217622).

Based on current scientific knowledge and the according clinical features of your patient, we conclude that both detected variants in the *CEP290* gene are causative for Joubert syndrome in your patient, presuming that they are present in a compound-heterozygous state.

Additional variants may be present within regions which were not analyzed (e.g. introns, promoter and enhancer regions and long repeats). A lower specificity enrichment and/or inaccurate variant calling cannot be ruled out for homologous regions with multiple genomic copies. The occurrence of low frequency somatic mosaicism cannot be reliably assessed using a pipeline optimized for germline variant detection, and may therefore remain undetected. Moreover, detection of large deletions and duplications is not guaranteed by next-generation high-throughput sequencing. Although unlikely, it is also possible that the classification of variants may change in the future due to improvements in scientific understanding.

GENETIC RELEVANCE

Your patient is presumed compound-heterozygous for pathogenic variants in gene *CEP290*. This may be of relevance for future family planning and at-risk family members.

Due to the presumed compound-heterozygosity, one altered *CEP290* allele will be passed on to each of your patient's children, who will be heterozygous carriers.

RECOMMENDATION

Carrier testing of both parents, regarding the identified variants in gene *CEP290* in your patient, may be performed in order to determine their carrier status, as well as to confirm the compound-heterozygous state of these variants.

Testing of adult asymptomatic family members regarding the variants c.5493delA; p.Ala1832Pro*fs**19 and c.1666delA; p.Ile556Phe*fs**17 identified in gene *CEP290* may only be performed following genetic counseling.

We recommend further clinical management/ according to the current guidelines for Joubert syndrome (Parisi and Glass, updated 2017, PMID: 20301500, GeneReviews).

According to § 10 of the German Genetic Diagnostics Legislation, appropriate genetic counseling should be offered with all diagnostic genetic testing. Following the identification of the molecular cause of a genetic disease, genetic counseling has to be offered.

Medical report written by: Scientist 1

Proofread by: Scientist 2

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup
Dr. med. Friedmar Kreuz, M.A.

Consultant for Human Genetics

Dr. rer. nat. Heinz-Dieter Gabriel
PD Dr. biol. hum. Christiane Maier
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Diagnostics

ADDITIONAL INFORMATION

Requested Regions

AHI1, ***ARL13B***, ***ARL3***, ***ARMC9***, ***B9D1***, ***B9D2***, ***C2CD3***, ***CC2D2A***, ***CELSR2***, ***CEP104***, ***CEP120***, ***CEP164***, ***CEP290***, ***CEP41***, ***CPLANE1***, ***CSPP1***, ***EXOC8***, ***FAM149B1***, ***HYLS1***, ***IFT172***, ***INPP5E***, ***KATNIP***, ***KIAA0586***, ***KIAA0753***, ***KIF7***, ***MKS1***, ***NPHP1***, ***OFD1***, ***PDE6D***, ***PIBF1***, ***POC1B***, ***RPGRIP1L***, ***SUFU***, ***TCTN1***, ***TCTN2***, ***TCTN3***, ***TMEM107***, ***TMEM138***, ***TMEM216***, ***TMEM231***, ***TMEM237***, ***TMEM67***, ***TTC21B***, ***ZNF423*** (Joubert Syndrome)

Methods

Sequencing: Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq6000 system. In order to provide a second, independent validation, one or more rare variant(s) identified within the NGS data were additionally sequenced using conventional Sanger sequencing.

NGS based CNV-Calling: (only applicable for nuclear encoded genes) Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth. Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage.

Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g. MLPA. Copy-neutral structural aberrations can not be detected using this method (e.g. balanced translocations and balanced inversions). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

Computational Analysis: Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). The variants were annotated based on several internal as well as external databases.

Diagnostic data analysis: Variants were classified and reported based on ACMG/ACGS-2020v4.01 guidelines (Richards et al., 2015, PMID: 25741868, https://www.acgs.uk.com/quality/best-practice-guidelines/).

Only variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Minor allele frequencies are taken from public databases (e.g. gnomAD) and an in-house database. If an acceptable sequencing-depth per base is not achieved by high-throughput sequencing, our quality guidelines demand local re-sequencing using classical Sanger-technology. Candidate CNV calls are evaluated manually. Potentially pathogenic findings are validated with a second method, like MLPA, on a case by case basis.

In this case, 99.79% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base. **The evaluation of variants is dependent on available clinical information at the time of analysis.** The medical report contains all variants not classified as benign or likely benign according to current literature. Synonymous variants in mitochondrially encoded genes are classified as benign. *In silico* prediction of variants listed in the chart above is calculated on the basis of the output of the programs Mutation Taster, fathmm, Mutation Assessor, SIFT, fathmm-MKL coding, LRT, and PROVEAN according to the following criteria: 100% consensus = pathogenic/benign, ≥ 75% consensus = mostly pathogenic/benign, consensus < 75% or no prediction possible = inconsistent. SpliceAI was utilized to evaluate the consequence of variants on splicing (thresholds: 0.8-1 "splice effect", 0.6-0.8 "possible splice effect", <0.6 "no splice effect"; Jaganathan et al., 2019, PMID: 30661751). The prediction of a splice effect for missense variants is only indicated if a threshold of 0.8 is reached. This prediction can be complemented with additional *in silico* predictions in individual cases.

Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT).