CeGaT

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

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

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

Indication Hearing loss, intellectual disability; positive family history

Order Panel Diagnostics: Non syndromic hearing loss (NSHL) (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

Results

- **Detection of two pathogenic variants in gene *GJB2* in a compound-heterozygous state, which are causative for an autosomal recessive deafness 1A (DFNB1A) in your patient.**
- 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 (%)	Classification
GJB2	**c.269T>C; p.Leu90Pro**	**het.**	**AD, AR, digenic**	**0.12**	**pathogenic**
GJB2	**c.71G>A; p.Trp24***	**het.**	**AD, AR, digenic**	**0.43**	**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).
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 the clinical relevance cannot be conclusively confirmed or excluded are referred to as variants of unknown clinical significance.**

Interpretation

***GJB2*, c.269T>C; p.Leu90Pro (het.), NM_004004.6, rs80338945**

***GJB2*, c.71G>A; p.Trp24* (het.), NM_004004.6, rs104894396**

OMIM / Reference	Phenotype	Heredity
601544	Deafness, autosomal dominant 3A (DFNA3A)	AD
220290	**Deafness, autosomal recessive 1A (DFNB1A)**	**AR, digenic**
149200	Bart-Pumphrey syndrome (Knuckle pads-leukonychia-sensorineural deafness-palmoplantar hyperkeratosis syndrome)	AD
602540	Hystrix-like ichthyosis with deafness	AD
148210	Keratitis-ichthyosis-deafness syndrome	AD
148350	Palmoplantar Keratoderma with deafness	AD
124500	Vohwinkel syndrome (VOWNKL)	AD

The ***GJB2*** gene encodes Connexin 26, a gap junction protein that facilitates the transport of small molecules and ions, such as potassium ions, through cell-to-cell channels. Connexin 26 is mainly expressed in the cochlea and in the skin. In addition to some syndromic diseases, pathogenic variants in *GJB2* also cause non-syndromic autosomal dominant (DFNA3A) and autosomal recessive (DFNB1A) deafness. “Digenic” inheritance in combination with concurrent pathogenic larger deletions affecting the 5' end of *GJB6* and a region upstream of both *GJB6* and *GJB2* has also been frequently reported (ClinGen; del Castillo et al., 2002, PMID: 11807148; Cheng et al., 2005, PMID: 16222667). Digenic inheritance with variants in gene *GJB3* has been suggested in one study, pending further confirmation (Liu et al., 2009, PMID: 19050930).

The variants **c.269T>C; p.Leu90Pro** and **c.71G>A; p.Trp24*** in gene *GJB2* have been identified in your patient in a compound-heterozygous state. Both variants are well known pathogenic variants and have been described to be causative for autosomal recessive deafness 1A (DFNB1A) in multiple cases (Smith and Jones, updated 2016, GeneReviews, PMID: 20301449; HGMD Professional).

The two compound-heterozygous pathogenic variants in gene *GJB2* are causative for an autosomal recessive deafness 1A (DFNB1A) in your patient.

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. Further the degree of heteroplasmy of mitochondrial variants can vary remarkably between different tissues (Wallace & Chalkia 2013; PMID: 24186072). Therefore, it is possible that disease causing variants, deletions and duplications are not detectable in the mtDNA from leucocytes but present in other tissues. The classification of variants may change in the future due to new evidence or improvements in scientific understanding.

Genetic relevance

Your patient is compound heterozygous for two pathogenic variants in gene *GJB2*. This may be of relevance for family planning and at-risk family members.

Individual variants have a 50% probability of being passed on to each respective offspring. One altered *GJB2* allele will be passed on to each of your patient's children, who will be heterozygous carriers.

Recommendation

We recommend further clinical management according to the current guidelines for DFNB1 (Smith and Jones, updated 2016, GeneReviews, PMID: 20301449).

It is possible to investigate further affected family members regarding the variants identified in gene *GJB2*.

Carrier testing of both parents regarding the identified variants in gene *GJB2* in your patient may be performed. Testing of adult asymptomatic family members regarding the variants c.71G>A; p.Trp24* and c.269T>C; p.Leu90Pro identified in gene *GJB2* may only be performed following genetic counseling.

As autosomal recessive deafness 1A (DFNB1A) is to the best of our knowledge not associated with intellectual disability, we recommend further analysis of genes which may be associated with intellectual disability of your patient.

If you wish to perform further genetic analyses, please do not hesitate to contact us.

Genetic counseling should be offered with all diagnostic genetic testing, especially following the identification of the molecular cause of a genetic disease.

Medical report written by: Scientist 1

Proofread by: Scientist 2

With kind regards,

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Diagnostics

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DIN EN ISO 15189:2014

Additional Information

Requested Regions

The whole exome of the individual above was targeted and sequenced. Analysis was restricted to the following gene regions:

ABCC1, ***ACTG1***, ***ATP2B2***, ***BDP1***, ***CABP2***, ***CCDC50***, ***CD164***, ***CDC14A***, ***CDH23***, ***CEACAM16***, ***CIB2***, ***CLDN14***, ***CLDN9***, ***CLIC5***, ***COCH***, ***COL11A2***, ***COL4A6***, ***CRYM***, ***DCDC2***, ***DIABLO***, ***DIAPH1***, ***DIAPH3***, ***DMXL2***, ***ELMOD3***, ***EPS8***, ***EPS8L2***, ***ESPN***, ***ESRP1***, ***ESRRB***, ***EYA4***, ***FAM136A***, ***GAB1***, ***GIPC3***, ***GJB2***, ***GJB3***, ***GJB6***, ***GPRASP2***, ***GRHL2***, ***GRXCR1***, ***GRXCR2***, ***GSDME***, ***HGF***, ***HOMER2***, ***ILDR1***, ***KARS1***, ***KCNQ4***, ***KITLG***, ***LHFPL5***, ***LMX1A***, ***LOXHD1***, ***LRTOMT***, ***MARVELD2***, ***MCM2***, ***MET***, ***MIR96***, ***MPZL2***, ***MSRB3***, ***MT-RNR1***, ***MT-TL1***, ***MT-TS1***, ***MYH14***, ***MYO15A***, ***MYO3A***, ***MYO6***, ***MYO7A***, ***NARS2***, ***NLRP3***, ***OSBPL2***, ***OTOA***, ***OTOF***, ***OTOG***, ***OTOGL***, ***P2RX2***, ***PCDH15***, ***PDE1C***, ***PDZD7***, ***PJVK***, ***PLS1***, ***PNPT1***, ***POLD1***, ***POU3F4***, ***POU4F3***, ***PPIP5K2***, ***PRKCB***, ***PRPS1***, ***PTPRQ***, ***RDX***, ***REST***, ***RIPOR2***, ***ROR1***, ***S1PR2***, ***SCD5***, ***SERPINB6***, ***SLC12A2***, ***SLC17A8***, ***SLC26A4***, ***SLC26A5***, ***SLC44A4***, ***SMPX***, ***SPNS2***, ***STRC***, ***SYNE4***, ***TBC1D24***, ***TECTA***, ***TJP2***, ***TMC1***, ***TMEM132E***, ***TMIE***, ***TMPRSS3***, ***TNC***, ***TPRN***, ***TRIOBP***, ***TRRAP***, ***TSPEAR***, ***USH1C***, ***WBP2***, ***WFS1***, ***WHRN*** (Non syndromic hearing loss (NSHL))

Methods

Sequencing: Protein-coding regions, flanking intronic regions and additional disease-relevant non-coding regions of the nuclear encoded genes, as well as the mitochondrial DNA were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq 6000/NovaSeq X Plus system.

NGS based CNV-Calling: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth (only applicable for nuclear encoded genes). 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. Copy number variants are named according to current ISCN guidelines. NGS based CNV-Calling will not detect balanced rearrangements, uniparental disomy, or low-level mosaicism. Aberrations on the Y chromosome and in the pseudoautosomal region (PAR) cannot be detected with high accuracy. The integration site of duplications cannot be determined by NGS based CNV-Calling.

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 cannot 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) of the nuclear encoded genes and in the mitochondrial DNA with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD and MITOMAP) 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, MITOMAP) 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, 97.79% 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 predictions were performed using the programs MetaLR (Dong et al., 2015, PMID: 25552646), PrimateAI (Sundaram et al., 2018, PMID: 30038395), and SpliceAI (Jaganathan et al., 2019, PMID: 30661751). 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).

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.

Appendix: Additional variants in analyzed genes

Every individual carries many rare variants which are not necessarily causative for genetic disease. The requested analysis yielded variants of uncertain significance that, according to current scientific knowledge, are not likely to be causative for the phenotype of your patient. They have therefore not been included in the medical report. However, they are provided in the following table for transparency.

Based on the currently available scientific data, we do not encourage further investigation of these variants, should the clinical features of your patient remain unchanged.

Please note, that meaningful reevaluation of a rare variant requires additional phenotypic information or new scientific data.

Gene	Position Ref/Alt	Variant	Transcript	Zygosity	MAF (%)	rs-Number
OTOGL	chr12:80764444 C/T	c.6710C>T; p.Pro2237Leu	NM_173591.5	het.	< 0.01	rs770975158
TSPEAR	chr21:45919806 C/A	c.1870G>T; p.Glu624*	NM_144991.3	het.	< 0.01	rs587717339

Please note that manual curation of the variants listed above has been performed without finding evidence for reliable disease relevance.

MAF: The minor allele frequency describes the least frequent allele at a specific locus in a given population (gnomAD). Het = Heterozygous, Homo = Homozygous, Hemi = Hemizygous, Heteropl = Heteroplasmic, Homopl = Homoplasmic. Positions refer to the hg19 genome build. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

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