CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE GmbH Am Strande 7 • 18055 Rostock • Germany

Order no.: xxx **documented by:** xxx
Order received: xx.xx.xxxx
Sample type: blood, CentoCard®
Sample collection date: not available
Report date: xx.xx.xxxx
Report type: Final Report

Patient no.: **xxx**, First Name: **xxx**, Last Name: **xxx**
DOB: **xx.xx.xxxx**, Sex: **x** , Your ref.: **xxx**

Additional report recipient(s):

Test(s) requested: CentoGenome® Solo

CLINICAL INFORMATION

Astigmatism; Attached earlobe; Bilateral sensorineural hearing impairment; Mild myopia; Nail dysplasia; Retinal degeneration; Rod-cone dystrophy
(Clinical information indicated above follows HPO nomenclature.)

Previous deletion/duplication analysis of the *USH2A* (please see order xxx) resulted negative. In a previous external clinical exome testing a heterozygous variant in the *USH2A* gene was identified: c.7524del p.(Arg2509Glyfs*19) (external report not provided).

Clinician suspects: Usher syndrome. Targeted gene request(s): *USH2A* gene.

POSITIVE RESULT
Pathogenic variants identified

INTERPRETATION

Two heterozygous pathogenic variants were identified in the *USH2A* gene. Given NGS methodology does not allow determination of the phase of the two variants (*in cis* or *in trans*), but a compound heterozygous state is likely. Parental targeted testing is highly recommended.

The genetic diagnosis of autosomal recessive Usher syndrome type 2A is thus confirmed.

RECOMMENDATIONS

- Genetic counselling in the familial context is recommended.
- We highly recommend paternal targeted testing to confirm the trans phase of the identified USH2A variants.

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RESULT SUMMARY

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
USH2A	NM_206933.2:c.11864G>A	p.(Trp3955*)	rs111033364	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: Disease causing Conservation_nt: high Conservation_aa: N/A	gnomAD: 0.00012 ESP: - 1000 G: 0.000094 CentoMD: 0.00011	Nonsense Pathogenic (class 1)
USH2A	NM_206933.2:c.7524del	p.(Arg2509Glyfs*19)	rs751176116	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: 0.0000071 ESP: - 1000 G: 0.0000041 CentoMD: -	Frameshift Pathogenic (class 1)

Variant annotation based on OTFA (using VEP v94). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function; splicing predictions: Ada and RF scores. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations.

VARIANT INTERPRETATION

USH2A, c.11864G>A p.(Trp3955*)

The *USH2A* variant c.11864G>A p.(Trp3955*) creates a premature stop codon. According to HGMD Professional 2019.4, this variant has previously been described as disease causing for Usher syndrome 2 by van Wijk et al., 2004 (PMID: 15015129), Le Quesne Stabej et al., 2012 (PMID: 22135276), Shearer et al., 2014 (PMID: 25262649). ClinVar lists this variant as pathogenic (clinical testing/research, Variation ID: 2357). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

USH2A, c.7524del p.(Arg2509Glyfs*19)

The *USH2A* variant c.7524del p.(Arg2509Glyfs*19) creates a shift in the reading frame starting at codon 2509. The new reading frame ends in a stop codon 18 positions downstream. According to HGMD Professional 2019.4, this variant has previously been described as disease causing for Usher syndrome 2 by Bonnet et al., 2011 (PMID: 21569298). ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 517494). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *USH2A* gene have been associated with Usher syndrome type 2A, an autosomal recessive disorder. Usher syndrome type 2 is characterized by congenital, bilateral sensorineural hearing loss that is mild to moderate in the low frequencies and severe to profound in the higher frequencies; intact vestibular responses; and retinitis pigmentosa (RP). RP is progressive, bilateral, symmetric retinal degeneration that begins with night blindness and constricted visual fields (tunnel vision) and eventually includes decreased central visual acuity; the rate and degree of vision loss vary within and among families (OMIM®: 276901; GeneReviews - PMID: 20301515).

INCIDENTAL FINDINGS

We did not detect any class 1 or 2 variants in the genes for which incidental findings are reported based on the ACMG guidelines.

TABULAR LIST OF ADDITIONAL PATHOGENIC AND LIKELY PATHOGENIC VARIANTS

To provide the most comprehensive and relevant genetic information, we list selected variants found in genes associated with **severe and early-onset disease.** The gene selection is based on OMIM® phenotypes and CENTOGENE internal data. We classified these variants at the time of reporting as "pathogenic" and "likely

pathogenic" (see our mutation database CentoMD® for further information). Variants not included and classified in the current release of CentoMD®, and low-quality variants that usually represent technical artifacts, are not included. The complete gene list can be found at https://www.centogene.com/diagnostics/medical-reporting/p-lp-gene-reporting.html (please contact CENTOGENE customer support if the gene list has been updated after this report was issued).

The listed variants may not directly answer the diagnostic request, at least not with the clinical information provided to CENTOGENE or current scientific understanding of relevant genetic disease mechanisms. However, these variants may help to close a potential diagnostic gap regarding the current clinical picture and are therefore provided here for a full diagnostic overview. In case this additional information is used in the further differential diagnosis process, orthogonal validation of relevant variants might be necessary.
Beyond the requested test, variants in genes related to late-onset diseases with unclear (considerably reduced) penetrance and/or cancer-related genes with onset in adulthood are not included in this list. This table does therefore not provide a complete list of potentially relevant genetic variants in the patient. Furthermore, the classification of these variants may change over time. CENTOGENE is not liable for any missing variant in this list and/or any provided classification of the variants at a certain point of time.
Insofar, as the identified variants may indicate (additional) genetic risks or diagnoses in the patient and/or his family and/or inform about reproductive risks, we strongly recommend following applicable local guidelines with regard to informing the patient about such findings. Particularly, if the patient decided not to be informed about "incidental findings" (to avoid any misunderstanding the list given here is not covering "incidental findings" according to ACMG), it should be clarified with the patient whether he/she wants to be informed about these additional variants.

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
SLC26A2	NM_000112.3:c.835C>T	p.(Arg279Trp)	rs104893915	heterozygous	PolyPhen: Probably damaging Align-GVGD: C15 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: moderate Conservation_aa: moderate	gnomAD: 0.00096 ESP: 0.0012 1000 G: 0.0014 CentoMD: 0.00055	Missense Pathogenic (class 1)

Variant annotation based on OTFA (using VEP v94). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function; splicing predictions: Ada and RF scores. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations.

CENTOGENE VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIONS)

Class 1 – Pathogenic
Class 2 – Likely pathogenic
Class 3 – Variant of uncertain significance (VUS)
Class 4 – Likely benign
Class 5 – Benign

Additionally, other types of clinical relevant variants can be identified (e.g. risk factors, modifiers).

METHODS

Genomic DNA is enzymatically fragmented, and libraries are generated by PCR-mediated addition of Illumina compatible adapters. The libraries are paired end sequenced on an Illumina platform to yield an average coverage depth of ~30x. An in-house bioinformatics pipeline including read alignment to GRCh37/hg19 genome assembly, variant calling and annotation is used. Structural variant (SV) calling is based on the HAS pipeline from Illumina. All variants with minor allele frequency (MAF) of less than 1% in gnomAD database, and disease-causing variants reported in HGMD®, in ClinVar or in CentoMD® are considered. While the evaluation is focused on coding exons and flanking +/-20 intronic bases, the complete gene region is interrogated for candidate variants with plausible association to the phenotype. All potential modes of inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and causality. Variants are categorized into five classes (pathogenic; likely pathogenic; VUS; likely benign; benign). All variants related to the phenotype of the patient are reported. SVs of unknown significance are not reported. Variants with low quality and/or unclear zygosity are confirmed by orthogonal methods. Consequently, a specificity of >99.9% for all reported variants is warranted.

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ANALYSIS STATISTICS

CentoGenome® Solo

Targeted nucleotides covered	≥ 10x	98.94%

LIMITATIONS

The genetic results are interpreted in the context of the provided clinical findings, family history, and other laboratory data. Only variants in genes potentially related to the proband's medical condition are reported. Misinterpretation of results may occur, if the provided information is inaccurate and/or incomplete. If the obtained genetic results do not concur with the clinical findings, additional testing should be considered.
Due to technical limitations, repeat expansions cannot be assessed with the applied method. Extremely low-coverage calls (homo/hemizygous or heterozygous calls with less than three or four reads, respectively) are expected to be artifacts based on our extensive validations and are consequently not considered during the analysis.

Potential aberrant splicing is assessed with splice prediction tools. Synonymous variants and intronic variants that are beyond 10 nucleotides from exon-intron boundaries are not considered for aberrant splicing analysis. However, pathogenic splicing variants evidenced by external sources will be reported.

ADDITIONAL INFORMATION

This test was developed and its performance validated by CENTOGENE GmbH. The US Food and Drug Administration (FDA) has determined that clearance or approval of this method is not necessary and thus neither have been obtained. This test has been developed for clinical purposes. All test results are reviewed, interpreted and reported by our scientific and medical experts.

If consent is provided, in line with ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (Genetics in Medicine, 2017; PMID: 27854360), we report incidental findings, i.e. pathogenic variants (class 1) and likely pathogenic variants (class 2) only in the recommended genes for the recommended phenotypes.

To also exclude mistaken identity in your clinic, several guidelines recommend testing a second sample that is independently obtained from the proband. Please note that any further analysis will result in additional costs.

The classification of variants can change over the time. Please feel free to contact CENTOGENE (customer.support@centogene.com) in the future to determine if there have been any changes in classification of any reported variants.

DISCLAIMER

Any preparation and processing of a sample from patient material provided to CENTOGENE by a physician, clinical institute or a laboratory (by a "Partner") and the requested genetic and/or biochemical testing itself is based on the highest and most current scientific and analytical standards. However, in very few cases genetic or biochemical tests may not show the correct result, e.g. because of the quality of the material provided by a Partner to CENTOGENE or in cases where any test provided by CENTOGENE fails for unforeseeable or unknown reasons that cannot be influenced by CENTOGENE in advance. In such cases, CENTOGENE shall not be responsible and/or liable for the incomplete, potentially misleading or even wrong result of any testing if such issue could not be recognized by CENTOGENE in advance.

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