CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE Am Strande 7 • 18055 Rostock • Germany

XXX

Order no.: xxx
Order received: xxx
Sample type: blood, EDTA
Sample collection date: xxx
Report date: xxx
Report type: Final Report

Patient no.: **xxx**, First Name: **xxx**, Last Name: **xxx**
DOB: **xxx**, Sex: **female**, Your ref.: **-**

Test(s) requested: CentoScreen® Paired PACK (sequencing including NGS-based CNV analysis)

CLINICAL INFORMATION

The proband and her partner are asymptomatic and consanguineous. According to the provided pedigree, they have lost a child, they have also a healthy child. Suspected Maple syrup urine disease. The analysis has been requested as carrier screening.

Please see our concurrent report for the husband of this proband ref. xxx.

CARRIER STATUS CONFIRMED

Pathogenic variant identified

INTERPRETATION

A heterozygous pathogenic variant was identified in the *BCKDHA* gene. **The carrier status of the proband for the *BCKDHA* variant is confirmed.**
Considering that we detected the *BCKDHA* variant also in heterozygous state in the partner of this proband, the couple has the 25% of risk of having an affected offspring.

In the remainder CentoScreen panel, we did not detect any pathogenic or likely pathogenic variant by sequencing. We did not detect any clinically relevant variant in the *CYP21A2* gene by Sanger sequencing. We did not detect any copy number variation (CNV) in the panel genes (see limitations) by analyzing the NGS data. No deletion was identified for the exon 7 of the *SMN1* gene by MLPA. We did not detect an expanded allele in the *FMR1* gene by repeat expansion analysis either.

RECOMMENDATIONS

- Proceeding to prenatal testing for the *BCKDHA* variant is now possible.
- Genetic counselling is recommended.

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RESULT SUMMARY

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BCKDHA	NM_000709.3:c.288+1G>A		rs398123496	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: Disease causing Conservation_nt: high Conservation_aa: 2/2 likely splice effect	gnomAD: 0.000016 ESP: - 1000 G: 0.000016 CentoMD®: -	Splicing Pathogenic (class 1)

Variant annotation based on OTFA (using VEP v93). * 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

BCKDHA, c.288+1G>A

The *BCKDHA* variant c.288+1G>A is predicted to disrupt the highly conserved donor splice site of exon 2. According to HGMD Professional 2019.1, this variant has previously been described as disease causing for Maple syrup urine disease by Abiri et al., 2016 (PMID: 26901124). ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 93351) and likely pathogenic (clinical testing, Variation ID: 93351). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *BCKDHA* gene are associated with maple syrup urine disease type Ia, an autosomal recessive disorder. The major clinical features of maple syrup urine disease are mental and physical retardation, feeding problems, and a maple syrup odor to the urine. The keto acids of the branched-chain amino acids are present in the urine, resulting from a block in oxidative decarboxylation (OMIM®: 248600).

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 clinically relevant variants can be identified (e.g., risk factors, modifiers).

METHODS

Genomic DNA is enzymatically fragmented, and regions of interest are enriched using DNA capture probes. The generated library is sequenced on an Illumina platform to obtain at least 20x coverage depth for > 99% of the targeted bases. For the CentoScreen®, the coding regions of the 330 panel genes, 10 bp of flanking intronic sequences, and known pathogenic/likely pathogenic variants within these genes (coding and non-coding) are targeted for analysis. An in-house bioinformatics pipeline, including read alignment to GRCh37/hg19 genome assembly, variant calling, annotation, and comprehensive variant filtering is applied. 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 evaluated. All identified variants are evaluated with respect to their pathogenicity and causality and are categorized into five classes (pathogenic; likely pathogenic; VUS; likely benign; benign). Only pathogenic and likely pathogenic variants (class 1 and class 2) along with few selected risk factor variants are reported. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. 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. The copy number variation (CNV) detection software has a sensitivity of above 95% for all homozygous/hemizygous deletions, as well as heterozygous deletions/duplications and homozygous/hemizygous duplications spanning at least three consecutive exons.

Four locus-specific oligonucleotides were used (according to Keen-Kim et al., 2005) that hybridize upstream and downstream of either the *CYP21A2* gene or its pseudogene *CYP21A1P*. The four primers were combined with each other to obtain 4 different amplicons corresponding to: the normal copy of the gene, pseudogene, gene/pseudogene rearrangement product and the deletion/conversion product. The product of the normal copy of the gene was analyzed by PCR and sequencing of both DNA strands of the entire coding region and the highly conserved exon-intron splice junctions. The reference sequence of the CYP21A2 gene is: NM_000500.7.

The *FMR1* gene was analyzed using the AmplideXTM FMR1 PCR Kit to screen the trinucleotide repeat region in the promoter. The reference

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sequence of the *FMR1* gene is: NM_002024.5.

GENE	PHENOTYPE (OMIM®)	INHERITANCE	RESULTS OF REPEAT EXPANSION ANALYSIS	NORMAL	MUTABLE NORMAL (INTERMEDIATE)	PREMUTATION	PATHOGENIC WITH FULL PENETRANCE	REFERENCE
FMR1	Fragile X syndrome (OMIM: 300624)	X-linked	**Allele 1: 30 ± 1** **Allele 2: 36 ± 1**	<45 CGG repeats	45-54 CGG repeats	55-200 CGG repeats	>200 CGG repeats	Saul *et al*., 2012 (PMID: 20301558)

MLPA (multiplex ligation-dependent probe amplification) analyses were performed using SALSA MLPA probemix P021-B1 provided by MRC-Holland to test for deletions or duplications within or including the SMN1 gene.

GENE (METHOD)	OUTCOME
SMN1 (MLPA)	**2** copies detected
SMN2 (MLPA)	**1** copy detected

Of note: the number of copies of *SMN2* may range from zero to four in healthy individuals (Sangaré *et al*., 2015 - PMID: 24515897; Ogino *et al*., 2003 - PMID: 12673282).

ANALYSIS STATISTICS

CentoScreen® Paired (sequencing including NGS-based CNV analysis)

Targeted nucleotides covered	≥ 20x	99.57%

LIMITATIONS

CentoScreen® is a screening test designed to assess the risk for the proband's offspring to be affected with an autosomal recessive or X-linked recessive disorder. It is not intended to establish a genetic diagnosis for the proband – unaffected or affected. However, the test result may include information about a medical condition of the proband that requires medical follow-up. Please note that a negative result for this panel does not rule out the possibility of a genetic condition in the proband, the proband's partner and/or their offspring. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical or family history, additional testing should be considered.
CentoScreen® panel focuses on 330 genes (list available at www.centogene.com) related to frequently occurring disorders within the population. Pathogenic or likely pathogenic variants outside the panel genes will not be detected. Variants of uncertain significance within the targeted region are not reported. Please note that they may become better understood and reclassified over time. Copy number variations (CNVs) assessment with NGS is limited to 34 genes (*ABCC6, ALDH3A2, COL4A5, CTNS, DBT, DMD, EDA, F8, FANCA, FKTN, GAA, GALC, GBE1, GJB6, GLDC, HBA1, HBA2, HBB, HEXB, HPRT1, HPS3, HSD17B4, IDS, MCOLN1, NEB, OTC, PAH, PCCA, PCDH15, PDHA1, RAPSN, SGCB, STS and XPC*) within the Panel. Any CNVs lying outside the coding regions of these genes will not be reported. Complex genetic events such as inversions, translocations, and repeat expansions, are not analyzed in this test. In addition, due to technology limitations, certain regions may be poorly covered, or not covered at all. In these regions and others encompassing repetitive, high-homology (such as pseudogene homology), and GC-rich sequences, variants can be missed. Recombination of GBA with its pseudogene (*GBAP1*), and inversion of intron 1 and intron 22 within *F8* gene are difficult to sequence and not specifically assessed; therefore, pathogenic variants in these regions may be missed. Evaluation of variants located within repetitive regions of NEB and TTN may have limitations when analyzed using NGS only. For the repeat expansion analysis in the *FMR1* gene, exact number of repeats beyond 200 may not be detectable. Furthermore, mosaic expansions may be missed. 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. Heterozygous CNVs spanning less than three exons cannot reliably be detected, are therefore excluded from routine analysis, and will only be inspected and reported upon medical or technical indication. The CNV detection sensitivity is decreased for repetitive and homologous regions, such as pseudogenes. It is expected that lower quality samples (blood from patients with hematologic disorders, and highly degraded DNA) may generate lower quality NGS data; in these cases, CNV analysis may not be possible to perform. 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 are considered in the analysis.

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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.

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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