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: not available
Report date: xxx
Report type: Final Report

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

Test(s) requested: CentoScreen® Paired PACK

CLINICAL INFORMATION

The proband and his partner are asymptomatic and consanguineous. According to the provided pedigree, they have lost a child and 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 wife 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.

Of note, we tested this proband only for clinically relevant variants in genes for which, we detected a clinically relevant variant in the proband's partner.

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).

ANALYSIS STATISTICS

An overall coverage of 98.18% was achieved (coding region including +/- 10bp).

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

Custom RNA capture baits against 331 panel genes (covering >99% of regions in CCDS and sequence variants including known deep intronic pathogenic variants, splicing, regulatory, any known mutation in CentoMD® 4.0 or HGMD® Professional 2017.3) are used to enrich regions of interest from fragmented genomic DNA. The generated library is sequenced on an Illumina HiSeq 4000 platform. Typically, over 99% of the targeted bases are covered >20x. An end to end in-house bioinformatics pipeline including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering out of low-quality reads and probable artefacts, and subsequent annotation of variants, is applied. All disease-causing variants reported in HGMD®, in ClinVar or in CentoMD® as well as all variants with minor allele frequency (MAF) of less than 1% in gnomAD database are considered. Evaluation is focused on coding exons along with flanking +/-20 intronic bases and all identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized into classes 1 - 5 (please see https://www.centogene.com/genetic-testing/reporting-at-centogene.html). Only Class 1 and Class 2 variants along with few selected risk factor variants are reported. Variants of relevance identified by NGS are continuously and individually in-house validated for quality aspects; those variants which meet our internal QC criteria (based on extensive validation processes) are not validated by Sanger.
Two independent CNV callers were used to determine copy number variations (CNVs) within the panel genes from the NGS data. While one caller uses the Bayesian approach to distinguish biological from technical difference for each target region, the second uses a mixture of

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Poisson models and compares the coverage of small fragments within the target regions against a set of reference samples. Implementation of two callers in tandem offers two alternative approaches to detect CNVs, thereby complementing each other for a robust and sensitive CNV calling pipeline. All clinically relevant CNVs were confirmed with an orthogonal method (MLPA or qPCR) before reporting.

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 331 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. Of note, CNV calls over regions with high homology, repetitive elements or high GC content have reduced sensitivity and may be missed. Please also note that ready to use DNA samples may not fulfill the QC metrics for CNV calling due to shearing or sub optimal processing. Such samples will not be analyzed for CNV calling with NGS data.
Specific genetic events like translocations and repeat expansions may not be reliably detected with Next Generation Sequencing. Recombination of GBA with its pseudogene and inversion of Intron 1 and Intron 22 within F8 gene is not directly assessed and hence may be missed. Analysis of variants lying within repetitive regions of NEB and TTN may have limitations when only sequenced with NGS. In addition, due to limitations in technology, certain regions may either not be covered or may be poorly covered, where variants cannot be confidently detected. 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 consequently are not considered during the analysis.
Repeat Expansion testing may not be able to detect exact number of repeats beyond 200. Mosaic expansions may be missed.
We assess possible effects on splicing based on predictive tools for variants within the splice regions; we do not evaluate putative splicing effects for synonymous variants and intronic variants located outside of the splice regions. However, pathogenic splicing effects suggested by a reputable external source 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.
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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