



XXX

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



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

Test(s) requested: CentoScreen™ Paired PACK panel

CLINICAL INFORMATION

The proband is unaffected. Family history: consanguinity.

Please also see our concurrent report for the proband's partner (xxx).



CARRIER STATUS
Pathogenic variant identified

INTERPRETATION

We detected a heterozygous pathogenic variant in the GCDH gene by sequencing. Pathogenic variants in the GCDH gene are associated with autosomal recessive glutaric aciduria type 1.

There is a 50% chance for the proband's offspring to inherit this variant. Subsequent testing of the proband's partner by sequencing revealed no pathogenic variant in the GCDH gene. Further testing is necessary to determine the risk for their offspring of being affected with the associated disorder.

We did not detect any pathogenic or likely pathogenic variant by sequencing of the remainder of the CentoScreen™ panel genes, including CYP21A2.

We did not detect any copy number variation (CNV) in the panel genes (see limitations) by analyzing the NGS data.

We did not detect any deletion or duplication of the SMN1 exon 7 by dPCR.

We did not detect an expanded allele in the FMR1 gene by repeat expansion analysis. We detected a normal heterozygous allele – please, see the table below. CGG repeats in this patient were found to be interrupted by AGG repeats. In stable normal alleles these AGG triplets are thought to anchor the region during replication and prevent strand slippage (Monaghan et al., 2013 - PMID: 23765048). FMR1-related disorders are inherited in an X-linked dominant manner. Alleles in the normal range have no meiotic or mitotic instability and are transmitted without any increase or decrease in repeat number (Saul and Tarleton, 2012 - PMID: 20301558).

RECOMMENDATIONS

- We recommend proceeding to deletion/duplication analysis of the GCDH gene for the proband's partner to exclude a second possibly pathogenic variant in this family.
- Genetic counselling is recommended.

> Contact Details

Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
dmqc@centogene.com
www.centogene.com

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RESULT SUMMARY

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
GCDH	Chr19(GRCh37):g.13008638C>T NM_000159.2:c.1204C>T p.(Arg402Trp) Exon 11	Het	PolyPhen: Probably damaging Align-GVGD: C65 SIFT: Deleterious MutationTaster: Disease causing Conservation: nt moderate/aa high	gnomAD: 0.00032 ESP: 0.00046 1000 G: - CentoMD: 0.00056	Missense Pathogenic (class 1)

Variant description based on Alamut Batch (latest database available). * AlignGVGD: C0: least likely to interfere with function, C65: most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations

VARIANT INTERPRETATION

GCDH, c.1204C>T p.(Arg402Trp)

The GCDH variant c.1204C>T p.(Arg402Trp) causes an amino acid change from Arg to Trp at position 402. According to HGMD Professional 2017.3, this variant has previously been described as disease-causing for glutaricacidemia type I by Biery et al., 1996 (PMID: 8900227), and others. ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 2085) and likely pathogenic (clinical testing, Variation ID: 2085). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic variants in the GCDH gene are known to cause glutaric aciduria type 1, which is an autosomal recessive metabolic disorder. It is characterized by gliosis and neuronal loss in the basal ganglia and a progressive movement disorder that usually begins during the first year of life (OMIM®: 231670).

ANALYSIS STATISTICS

An overall coverage of 99.97% 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

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.

An in-house developed pipeline was used to determine copy number variations (CNV) within the panel genes (see limitations). The approach utilizes CNV calling based on coverage information of a particular target region when compared to a normalized coverage of control reference data set. Each target region was analyzed as a 100-300bp non-overlapping genomic window. To eliminate variations or noise due to genomic complexity, regions of confidence are selectively probed to eliminate false positives. Fold change values were calculated for each target window against normalized mean of all reference samples. Only regions showing ratio off-set of >2 SD from all genes in the sample were considered as a candidate for CNV. All CNVs were confirmed by an orthogonal method (MLPA or qPCR) before reporting. This approach offers

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a sensitivity of ~90% for detecting CNVs.

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 copy number of SMN1 exon 7 was quantified using digital droplet PCR. Quantification is based on the assumption that molecules are distributed uniformly in partitions and have a Poisson distribution. The reference sequence is NM_000344.3.

GENE	PHENOTYPE (OMIM®)	INHRT.	RESULTS OF REPEAT EXPANSION ANALYSIS	NORMAL	MUTABLE NORMAL (INTERMEDIATE)	PREMUTATION	PATHOGENIC WITH FULL PENETRANCE	REFERENCE
FMR1	FMR1-related (309550)	X-linked	Allele 1: 29±1 repeats Allele 2: 30±1 repeats	<45 CGG repeats	45-54 CGG repeats	55-200 CGG repeats	>200 CGG repeats	Saul et al., 2012 (PMID: 20301558)

The FMR1 gene was analysed using the AmplideXTM FMR1 PCR Kit to screen the trinucleotide repeat region in the promoter. The reference sequence of the FMR1 gene is: NM_002024.5.

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.

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.

Repeat Expansion testing may not be able to detect exact number of repeats beyond 200. Mosaic expansions may be missed. Deletions other than exon 7 in SMN1 are not covered within the assay.

ADDITIONAL INFORMATION

This test was developed and its performance validated by CENTOGENE AG. 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 (dmqc@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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Chief Medical Director

Clinical Scientist

> Contact Details

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dmqc@centogene.com
www.centogene.com

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