Order no.:
Order received: DD/MM/YYYY
Sample type / Sample collection date:
blood, CentoCard / DD/MM/YYYY
Report date: DD/MM/YYYY
Report type: Final Report

Patient no.: , First Name: , Last Name:
DOB: **DD/MM/YYYY**, Sex: **male**, Your ref.:

Test(s) requested: CentoGenome MOx Trio

CLINICAL INFORMATION

Unaffected
(Clinical information indicated above follows HPO nomenclature.)

Consanguineous parents: No.

The proband is the parent of the index patient.

We performed whole exome sequencing for the child of the proband. Please refer to our report [ID Order, Name]. This report reflects exclusively the segregation information for the proband in the context of the family analysis.

CARRIER STATUS CONFIRMED

Pathogenic variant identified

INTERPRETATION

A heterozygous pathogenic variant was identified in the *HEXA* gene. **The carrier status of the *HEXA* variant is confirmed.**

Considering the result of the partner, with each pregnancy of this couple there is a 25% risk for the offspring of being affected.

As a secondary finding, a heterozygous pathogenic variant was identified in the *LDLR* gene. **The finding is consistent with the increased genetic susceptibility to autosomal dominant familial hypercholesterolemia type 1.**

RECOMMENDATIONS

- Retrospective clinical analysis and follow-up for *LDLR*-associated manifestations is recommended.
- Genetic counselling, including reproductive counselling (discussing prenatal and preimplantation diagnoses, if relevant) is recommended.

MAIN FINDINGS

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
HEXA	NM_001318825.1:c.1363G>A	p.(Gly455Ser)	N/A	heterozygous	PolyPhen: Probably damaging Align-GVGD: C55 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high 2/2 likely splice effect	gnomAD: - ESP: - 1000 G: - CentoMD: -	Missense Likely Pathogenic (class 2)

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

HEXA, c.1363G>A p.(Gly455Ser)

The *HEXA* variant c.1363G>A p.(Gly455Ser) causes an amino acid change from Gly to Ser at position 455.The substitution is in close proximity to the highly conserved donor splice site. This variant was previously identified at CENTOGENE in 5 other homozygous patients with overlapping phenotype and decreased enzyme activity. It is classified as likely pathogenic (class 2) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *HEXA* gene are associated with autosomal recessive Tay-Sachs disease (OMIM®: 272800), also known as GM2-gangliosidosis. GM2 gangliosidosis, variant B is marked by accumulation of G2 gangliosides due to hexosaminidase A deficiency. The infantile form (type 1) begins between 3 and 6 months of age.

SECONDARY (INCIDENTAL) FINDINGS

If consent is provided, in line with ACMG recommendations for reporting of secondary (incidental) findings in clinical exome and genome sequencing (Genetics in Medicine, 2021; PMID: 34012068), we report secondary (incidental) findings, i.e., pathogenic variants (class 1) and likely pathogenic variants (class 2) in the recommended genes for the indicated phenotypes.

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
LDLR	NM_000527.2:c.1135T>C	p.(Cys379Arg)	rs879254803	heterozygous	PolyPhen: - Align-GVGD: C0 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.000032 ESP: - 1000 G: 0.000032 CentoMD: -	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.

VARIANT INTERPRETATION

LDLR, c.1135T>C p.(Cys379Arg)

The *LDLR* variant c.1135T>C p.(Cys379Arg) causes an amino acid change from Cys to Arg at position 379. According to HGMD Professional 2021.3, this variant has previously been described as disease causing for hypercholesterolemia by Hobbs et al., 1992 (PMID: 1301956), Romano et al., 2011 (PMID: 21865347), Bertolini et al., 2013 (PMID: 23375686). ClinVar lists this variant (Interpretation: Pathogenic/Likely pathogenic; Variation ID: 251685). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Familial hypercholesterolemia is an autosomal dominant disorder characterized by elevation of serum cholesterol bound to low density lipoprotein (LDL), which promotes deposition of cholesterol in the skin (xanthelasma), tendons (xanthomas), and coronary arteries (atherosclerosis). The disorder occurs in 2 clinical forms: homozygous and heterozygous (Hobbs et al., 1992; PMID:1301956). Mode of Inheritance: Autosomal dominant (OMIM®: 143890)

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 and revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920), variant calling, annotation, and comprehensive variant filtering is applied. Copy number variation (CNV) calling is based on the DRAGEN 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 evaluated. Although the evaluation is focused on coding exons and flanking intronic regions, the complete gene region is interrogated for candidate variants with plausible association to the phenotype. All potential patterns for mode of inheritance are considered. In addition, provided family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and disease causality. Variants are categorized into five classes (pathogenic, likely pathogenic, VUS, likely benign, and benign) along ACMG guidelines for classification of variants. All relevant variants related to the phenotype of the patient are reported. For CentoGenome MOx, if applicable, biochemical analysis is performed upon detection of relevant variants by sequencing. This enhances the diagnosis of metabolic disorders, optimizes variant classification, and helps to ascertain the eventual contribution to the phenotype; the list of enzyme-activity assays and biomarkers can be obtained at www.centogene.com/mox. CNVs of unknown significance are not reported. Mitochondrial variants are reported for heteroplasmy levels of 15% or higher. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Variants with low sequencing quality and/or unclear zygosity are confirmed by orthogonal methods. Consequently, a specificity of > 99.9% for all reported variants is warranted.

ANALYSIS STATISTICS

CentoGenome MOx Trio

Targeted nucleotides covered	≥ 10x	99.55%

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 genetic data or patient information is inaccurate and/or incomplete. If the obtained genetic results are not compatible with the clinical findings, additional testing should be considered.

The genes with mapping issues in GRCh37/hg19 genome assembly, the non-protein-coding disease-associated genes, and genomic regions that are hard to sequence by current technology and are without evidenced relevance for monogenic disorders, are excluded from this analysis. More complex genetic events such as uniparental disomy (UPD), 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, relevant variants can be missed. Extremely low-coverage calls are expected to be artifacts based on our extensive validations and are consequently not considered during the analysis.

The CNV detection sensitivity is decreased for repetitive and homologous regions, such as pseudogenes. Mitochondrial variants with heteroplasmy levels below 15% may not be detected. It is expected that lower quality samples (prenatal, product of conception, blood from patients with hematologic disorders, and highly degraded DNA) may generate lower quality NGS data; in these cases, CNV analysis and/or mitochondrial genome analysis may not be possible to perform. Potential aberrant splicing is assessed with splice prediction tools. Deep intronic variants without strong prediction of aberrant splicing may not be reported, with the exception of known pathogenic splicing variants evidenced by external sources.

ADDITIONAL INFORMATION

This test was developed, and its performance was validated, by CENTOGENE. 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 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.

COPYRIGHT NOTICE

This document contains information from the Online Mendelian Inheritance in Man® (OMIM®) database, which has been obtained under a license from the Johns Hopkins University. This document does not represent the entire, unmodified OMIM® database, which is available in its entirety at http://omim.org/downloads. Regarding OMIM® information: Copyright © 1996 – 2017, John Hopkins University, all rights reserved.

Chief Genomic Officer

Senior Medical Director

Clinical Scientist