



**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: CentoXome MOx Trio**

### CLINICAL INFORMATION

Abnormal CNS myelination; Abnormal heart valve physiology; Coma; Depressed nasal bridge; Developmental regression; Elevated circulating glutaryl carnitine concentration; Encephalopathy; Failure to thrive; Global developmental delay; Hypertelorism; Hypotonia; Infantile onset; Leukodystrophy; Mitral valve prolapse; Motor delay; Relative macrocephaly; Seizure  
(Clinical information indicated above follows HPO nomenclature.)

Age of onset: 4 month(s).

Family history: Unknown.

Consanguineous parents: No.

Clinician suspects: Glutaric aciduria type 1.

Please see the concurrent reports for the parents: XXX and XXX



**POSITIVE RESULT**  
**Pathogenic variants identified**

### INTERPRETATION

Two heterozygous pathogenic variants were identified in the *GLB1* gene in *trans* state. The activity of the enzyme beta-galactosidase was pathologically reduced. **The genetic diagnosis of the autosomal recessive GM1-gangliosidosis is confirmed.**

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

### RECOMMENDATIONS

- Targeted testing for the *GLB1* variant for affected family members, if any, and familial cascade carrier testing is recommended.
- 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.

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**MAIN FINDINGS**

BIOCHEMICAL TESTING				
NAME OF GENE/ENZYME/BIOMARKER	RESULT	REFERENCE	INTERPRETATION	METHOD
<b>beta-galactosidase</b>	<b>&lt;1 (LOD) µmol/L/h µmol/L/h</b> LOD= limit of detection	≥ 28,5 µmol/L/h	pathologic	liquid chromatography mass spectrometry

Tandem mass spectrometry is a screening technology with a sensitivity of nearly 100% and specificity of about 96%. In other words, it is not as specific as enzyme testing in leukocyte preparations. Therefore, there is always an independent confirmation test e.g., genetic testing or specific biomarker analysis mandatory.

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
<i>GLB1</i>	NM_001317040.1:c.639_641del	p.(Leu214del)	rs754077128	heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: 0.000038 ESP: - 1000 G: 0.000034 CentoMD: -	In-frame Pathogenic (class 1)
<i>GLB1</i>	NM_001317040.1:c.1912C>T	p.(Arg638Cys)	rs794727165	heterozygous	PolyPhen: - Align-GVGD: C0 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: weak	gnomAD: 0.000014 ESP: - 1000 G: 0.000012 CentoMD: 0.00019	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**

***GLB1*, c.1912C>T p.(Arg638Cys)**

The *GLB1* variant c.1912C>T p.(Arg638Cys) causes an amino acid change from Arg to Cys at position 638. According to HGMD Professional 2021.3, this variant has previously been described as disease causing for Gangliosidosis GM1 by Santamaria et al., 2006 (PMID: 16941474), Santamaria et al., 2007 (PMID: 17664528). ClinVar lists this variant (Interpretation: Pathogenic; Variation ID: 194596). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

***GLB1*, c.639\_641del p.(Leu214del)**

The *GLB1* variant c.639\_641del p.(Leu214del) is an in-frame deletion of 3 bps in exon 3, which causes the loss of residue Leu at position 214. According to HGMD Professional 2021.3, this variant has previously been described as disease causing for Gangliosidosis GM1 by Yang et al., 2010 (PMID: 20920281), Feng et al., 2018 (PMID: 30267299). ClinVar lists this variant (Interpretation: Conflicting interpretations of pathogenicity; Pathogenic (1), Uncertain significance (1); Variation ID: 684406). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

GM1-gangliosidosis is an autosomal recessive lysosomal storage disease characterized by accumulation of ganglioside substrates in lysosomes. Clinically, patients show variable degrees of neurodegeneration and skeletal abnormalities. There are 3 main clinical variants categorized by severity and variable residual beta-galactosidase activity. Type I, or infantile form, shows rapid psychomotor deterioration beginning within 6 months of birth, generalized central nervous system involvement, hepatosplenomegaly, facial dysmorphism, macular cherry-red spots, skeletal dysplasia, and early death. Type II, or late-infantile/juvenile form (GM1G2; 230600), has onset between 7 months and 3 years, shows generalized central nervous system involvement with psychomotor deterioration, seizures, localized skeletal involvement, and survival into childhood. Mode of Inheritance: Autosomal recessive (OMIM®: 230500)

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## RESEARCH FINDINGS

Research variants (with potential relevance to the described phenotype) are variants in genes with no or only partial experimental evidence for their involvement in human disease.

The data was analyzed focusing on variants affecting protein function (nonsense, frameshift, conserved splice site and missense with high pathogenicity predictions) in genes with supporting evidence on zygosity, segregation or functional importance of the gene. Available literature or experimental data on expression and/or animal models were considered.

However, no such variants could be identified for the patient.

## 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***
<i>LDLR</i>	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). \* AlignGVGD: 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).

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## CARRIERSHIP FINDINGS

In this table we list sequence variants previously ascertained or evaluated and classified in CENTOGENE as "pathogenic" and "likely pathogenic", in selected genes associated with recessive severe and early-onset Mendelian diseases. As only in-house classified variants are presented, it should not be considered a comprehensive list of variants in these genes and does not provide a complete list of potentially relevant genetic variants in the patient. The complete gene list can be found at [www.centogene.com/carriership-findings](http://www.centogene.com/carriership-findings) (please contact CENTOGENE customer support if the gene list has been updated after this report was issued). Orthogonal validation was not performed for these variants. Therefore, if any variant is used for clinical management of the patient, confirmation by another method needs to be considered. Furthermore, the classification of these variants may change over time, however reclassification reports for these variants will not be issued. 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. As the identified variants may indicate (additional) genetic risks or diagnoses in the patient and/or family and/or inform about reproductive risks, we recommend discussing these findings in the context of genetic counselling.

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
<i>TSHR</i>	NM_000369.2:c.1349G>A	p.(Arg450His)	rs189261858	heterozygous	PolyPhen: Probably damaging Align-GVGD: C0 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.00021 ESP: - 1000 G: 0.00020 CentoMD: 0.00013	Missense Pathogenic (class 1)

Variant annotation based on OTFA (using VEP v94). \* AlignGVGD: 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)

- |  |                                |
|--|--------------------------------|
| <b>Class 1</b> – Pathogenic                              | <b>Class 4</b> – Likely benign |
| <b>Class 2</b> – Likely pathogenic                       | <b>Class 5</b> – Benign        |
| <b>Class 3</b> – Variant of uncertain significance (VUS) |                                |

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

## METHODS

Genomic DNA is enzymatically fragmented, and target regions are enriched using DNA capture probes. These regions include approximately 41 Mb of the human coding exome (targeting > 98% of the coding RefSeq from the human genome build GRCh37/hg19), as well as the mitochondrial genome. The generated library is sequenced on an Illumina platform to obtain at least 20x coverage depth for > 98% of the targeted bases. 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. 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. The investigation for relevant variants is focused on coding exons and flanking +/-10 intronic nucleotides of genes with a clear gene-phenotype evidence (based on OMIM® information). 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 CentoXome 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](http://www.centogene.com/mox). 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. Mitochondrial variants are reported for heteroplasmy levels of 15% or higher. The copy number variation (CNV) detection software has a sensitivity of more than 95% for all homozygous/hemizygous and mitochondrial deletions, as well as heterozygous deletions/duplications and homozygous/hemizygous duplications spanning at least three consecutive exons. For the uniparental disomy (UPD) screening, a specific algorithm is used to assess the well-known clinically relevant chromosomal regions (6q24, 7, 11p15.5, 14q32, 15q11q13, 20q13 and 20).

## ANALYSIS STATISTICS

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**CentoXome MOx Trio**

Targeted nucleotides covered	≥ 20x	99.68%
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**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 approximately 0.2 Mb of genomic regions that are hard to sequence by current enrichment technology and are without evidenced relevance for monogenic disorders, are excluded from this analysis. More complex genetic events such as inversions, translocations, and repeat expansions, are not analyzed in this test. The UPD detection is a screening method, and therefore false-positive and false-negative results may occur. 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 (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. 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. Intronic variants that are beyond 10 nucleotides from exon-intron boundaries are not considered for aberrant splicing analysis, 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](mailto: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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