



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

Abnormality of the cerebral white matter; Abnormality of the periventricular white matter; Absent speech; Brain imaging abnormality; Cerebral dysmyelination; Cow milk allergy; Delayed social development; Delayed speech and language development; Dysphagia; Myoclonic seizure; Myoclonus; Seizure
(Clinical information indicated above follows HPO nomenclature.)

Family history: Unknown.
Consanguineous parents: Yes.

Clinician suspects: Tay-Sachs disease.

Please see the concurrent reports for the parents: and



POSITIVE RESULT
Pathogenic variants identified

INTERPRETATION

A homozygous likely pathogenic variant was identified in the *HEXA* gene. In addition, the enzymatic activity of beta-hexosaminidase A is pathologically decreased and the enzymatic activity of total beta-hexosaminidase is within the normal range. **The genetic diagnosis of autosomal recessive Tay-Sachs disease is confirmed.**

Homozygosity of the variant was confirmed by parental analysis.

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 *HEXA* 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
beta-hexosaminidase A	< 0,09 (LOD) µmol/L/h	≥ 2,0 µmol/L/h	pathologic	fluorimetry
total beta-hexosaminidase	9,5 µmol/L/h	≥ 4,5 µmol/L/h	normal	fluorimetry

Fluorimetric assay is an analytical method with a sensitivity of nearly 100% and specificity of 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***
HEXA	NM_001318825.1:c.1363G>A	p.(Gly455Ser)	N/A	homozygous	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). * 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

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. The earliest sign is an incessant startle response to noise. Psychomotor retardation appears after the age of 8 months with hypotonia, amaurosis, and megalencephaly. A cherry-red macular spot may be found but is not specific. Muscular weakness progresses and leads to paralysis. The disorder degenerates into a state of decerebration and is fatal during childhood. Enzymatic activity of the hexosaminidase A is either extremely low or totally absent in leucocytes and cultured in fibroblasts obtained by skin biopsy. In the juvenile form (type 2), onset is between ages 2 and 6 with locomotor ataxia, behavioural disorders, and progressive loss of intellectual capacities, leading to a state of decerebration and death at around the age of 15. The decrease in hexosaminidase A activity is less pronounced than in the infantile form. The adult or chronic form (type 3) may begin around the age of 10, but often the disorder is not diagnosed until adulthood. Two different clinical forms exist. The first is similar to atypical Friedreich disease, with spinocerebellar ataxia but no cardiac or osseous signs, such as scoliosis or flat feet. The second is that of juvenile spinal amyotrophy resembling Kugelberg-Welander's syndrome. Mental capacities and behaviour may or may not be affected. Hexosaminidase A deficiency is found (orphanet.net ORPHA:845).

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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***
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)

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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 (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). * 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.

CENTOGENE VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIONS)

- | | |
|--|--------------------------------|
| Class 1 – Pathogenic | Class 4 – Likely benign |
| Class 2 – Likely pathogenic | Class 5 – Benign |
| Class 3 – 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 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.

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ANALYSIS STATISTICS

CentoGenome MOx Trio

Targeted nucleotides covered	≥ 10x	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 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.

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