CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE • Am Strande 7 • 18055 Rostock • Germany

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

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

Test(s) requested: CentoMetabolic MOx (sequencing including NGS-based CNV analysis)

CLINICAL INFORMATION

Abnormal cerebral morphology; Atrophy/Degeneration affecting the central nervous system; Brain imaging abnormality; Cerebral cortical atrophy; Developmental regression; Hepatosplenomegaly
(Clinical information indicated above follows HPO nomenclature.)

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homozygous pathogenic variant was identified in the *SMPD1* gene. Additionally, the concentration of the biomarker lyso-SM-509 was pathologically increased. **The genetic diagnosis of autosomal recessive Niemann-Pick disease type A/B is confirmed.**

No further clinically relevant variants were detected.

RECOMMENDATIONS

- We recommend parental testing to confirm homozygosity of the *SMPD1* variant in place of compound heterozygosity for a large deletion.
- Genetic counselling, including reproductive counselling (discussing prenatal and preimplantation diagnoses, if relevant) is recommended.

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RESULT SUMMARY

BIOCHEMICAL TESTING				
NAME OF GENE/ENZYME/BIOMARKER	RESULT	REFERENCE	INTERPRETATION	METHOD
lyso-SM-509	**6,3 ng/ml**	≤ 0,9 ng/ml	pathologic	liquid chromatography mass spectrometry

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
SMPD1	NM_000543.4:c.740del	p.(Gly247Alafs*10)	N/A	homozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: - ESP: - 1000 G: 0.000050 CentoMD: 0.00013	Frameshift 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

SMPD1, c.740del p.(Gly247Alafs*10)

The *SMPD1* variant c.740del p.(Gly247Alafs*10) creates a shift in the reading frame starting at codon 247. The new reading frame ends in a stop codon 9 positions downstream. According to HGMD Professional 2021.3, this variant has previously been described as disease causing for Niemann-Pick disease by Galehdari et al., 2013 (PMID: 23724191). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *SMPD1* gene are associated with autosomal recessive inherited Niemann Pick disease type A and B (NP-A/B; OMIM®: 257200, 607616) resulting from a deficiency or reduced activity of the lysosomal enzyme acid sphingomyelinase (ASM) with consecutive accumulation of ceramide-phosphocholin (sphingomyelin) in various tissues (liver, spleen, lymph nodes, adrenal cortex, lung airways, brain, and bone marrow). Most type A babies are diagnosed in the first 6 months of life during a diagnostic evaluation of hepatosplenomegaly and failure to thrive. Additional prominent clinical features are widespread lung damage (interstitial lung disease) with subsequent recurrent lung infections and respiratory failure. A cherry-red spot of the macula is present in 50-100% (dep. on source). They show a rapidly progressive neurodegenerative course, with profound hypotonia (“floppy baby”) and failure to attain milestones. Most type A infants do not survive beyond the third year of life. Type B usually presents in mid-childhood, similar to type A, but not as severe. Clinical symptoms comprise hepatosplenomegaly, recurrent lung infections and thrombocytopenia, short stature and slowed mineralization of bone, cherry-red spot eye abnormality or neurological impairment in about 1/3 of patients. Patients usually survive into adulthood with respiratory and liver failure being the most common causes of mortality (Schuchman, 2017; PMID: 28164782). So far, there is no causative treatment available.

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 clinically relevant variants can be identified (e.g., risk factors, modifiers).

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METHODS

Genomic DNA is enzymatically fragmented, and regions of interest are enriched using DNA capture probes. The final indexed libraries are sequenced on an Illumina platform.

For the CentoMetabolic MOx panel, the coding regions, 10 bp of flanking intronic sequences, and known pathogenic/likely pathogenic variants (coding and non-coding) of the *ABCA1, ABCC2, ABCD1, ABCD4, ADA, AGA, AGL, AGPS, ALAD, ALAS2, ALDOA, ALDOB, ALPL, APOA2, APOA5, APOB, APOC2, APOE, ARG1, ARSA, ARSB, ASAH1, ASL, ASS1, ATP7A, ATP7B, BCKDHA, BCKDHB, CBS, CETP, CLN3, CLN5, CLN6, CLN8, CPOX, CPS1, CTNS, CTSA, CTSD, CTSK, CYP11B1, CYP17A1, CYP19A1, CYP21A2, DBT, DHCR7, ENO3, ENPP1, EPHX2, FECH, FGF23, FUCA1, G6PC, G6PD, GAA, GALC, GALE, GALK1, GALNS, GALT, GBA, GBE1, GHR, GK, GLA, GLB1, GM2A, GNPAT, GNPTAB, GNPTG, GNS, GUSB, GYG1, GYS2, HCFC1, HEXA, HEXB, HFE, HJV, HGD, HGSNAT, HMBS, HPRT1, HSD3B2, HYAL1, IDS, IDUA, ITIH4, KHK, LAMP2, LCAT, LDHA, LDLR, LIPA, LIPI, LMBRD1, LPA, LPL, MAN2B1, MANBA, MCOLN1, MFSD8, MMACHC, MMADHC, NAGA, NAGLU, NAGS, NEU1, NPC1, NPC2, OTC, PAH, PEX1, PEX10, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PEX7, PFKM, PGAM2, PGK1, PGM1, PHKA1, PHKA2, PHKB, PHKG2, PKLR, POR, PPOX, PPP1R17, PPT1, PRKAG2, PYGL, PYGM, RBCK1, SGSH, SLC17A5, SLC25A13, SLC25A15, SLC25A36, SLC2A1, SLC2A2, SLC2A3, SLC3A1, SLC3A2, SLC40A1, SLC6A19, SLC7A7, SLC7A9, SLCO1B1, SLCO1B3, SMPD1, SUMF1, TFR2, TPP1, UGT1A1, UMPS, UROD, UROS, IVD, DLX4, ANTXR2, ABCB4, ABCG5, ABCG8, ACAT1, AGXT, ALDH4A1, ALG3, LDLRAP1, BTD, CD320, CPT1A, DDC, DIABLO, DNAJC5, DPYD, ETHE1, FAH, FBP1, GAMT, GATM, GYS1, HLCS, HPD, LIPC, MMAA, MMAB, MMUT, PCSK9, PDHB, PNPO, PSAP, SI, SLC22A5, SLC25A20, SLC37A4, SLC6A8, TAT* genes are targeted for analysis.

Data analysis, including alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37), variant calling, and annotation is performed using validated in-house software. All identified variants are evaluated with respect to their pathogenicity and causality and are categorized into five classes (pathogenic; likely pathogenic; VUS; likely benign; benign). All pathogenic and likely pathogenic variants are reported. VUS are only considered whenever no relevant pathogenic or likely pathogenic variants have been identified. For CentoMetabolic 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.
CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Variants with low quality and/or unclear zygosity are confirmed by orthogonal methods. Consequently, a specificity of >99.9% for all reported variants is warranted.
The copy number variation (CNV) detection software has a sensitivity of above 95% for all homozygous deletions and heterozygous deletions/duplications spanning at least three consecutive exons.

ANALYSIS STATISTICS

CentoMetabolic MOx (sequencing including NGS-based CNV analysis)

Targeted nucleotides covered	≥ 20x	99.68%

LIMITATIONS

The genetic results are interpreted in the context of the provided clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the provided information is inaccurate and/or incomplete. If the obtained genetic results do not concur with the clinical findings, additional testing should be considered.
The used method is not designed to, and therefore cannot, detect complex genetic events such as inversions, translocations and repeat expansions. In addition, due to technology limitations, certain regions may be either not or poorly covered. In these regions and others encompassing repetitive, high homology, and high CG-rich sequences, 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.
Potential aberrant splicing is assessed with splice prediction tools. Synonymous variants and intronic variants that are beyond 10 nucleotides from exons-intron boundaries are not considered for aberrant splicing analysis. However, pathogenic splicing variants evidenced by external sources will be reported.
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 sensitivity is decreased for repetitive and homologous regions, such as pseudogenes.

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.

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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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