CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE Am Strande 7 • 18055 Rostock • Germany

XXX

Order no.: xxx **documented by:** xxx
Order received: xxx
Sample type: blood, CentoCard®
Sample collection date: xxx
Report date: xxx
Report type: Final Report

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

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

CLINICAL INFORMATION

Abdominal distention; Childhood onset; Coarse facial features; Dysostosis multiplex; Hepatomegaly; Recurrent lower respiratory tract infections
(Clinical information indicated above follows HPO nomenclature.)

Diagnosed condition(s): Lysosomal storage diseases (LSD), skeletal survey shows Mucopolysaccharidosis (MPS).

Family history: Unknown.
Siblings unaffected.
Consanguineous parents: Yes.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homozygous pathogenic deletion encompassing the entire *NAGLU* gene was identified by CNV analysis. The activity of alpha-N-acetylglucosaminidase is pathologically decreased. **The genetic diagnosis of autosomal recessive mucopolysaccharidosis type IIIB is confirmed.**

RECOMMENDATIONS

- Genetic counselling is recommended.
- We recommend parental carrier testing.

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RESULT SUMMARY

BIOCHEMICAL TESTING				
NAME OF GENE/ENZYME/BIOMARKER	RESULT	REFERENCE	INTERPRETATION	METHOD
alpha-N-acetylglucosaminidase	**0,4 µmol/L/h**	≥ 1,5 µmol/L/h	pathologic	fluorimetry

Fluorometric 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.

COPY NUMBER VARIANTS					
GENOMIC COORDINATES	GENE	TRANSCRIPT	AFFECTED EXONS	ZYGOSITY	TYPE AND CLASSIFICATION*
chr17:40688282-40696261	NAGLU	NM_000263	1-6	homozygous	Loss Pathogenic (class 1)

*according to ACMG 2011, modified

VARIANT INTERPRETATION

NAGLU, loss of exons 1-6

The detected loss encompasses exons 1-6 of the gene *NAGLU*, corresponding to the entire gene. The pathogenicity of the variant was assessed based on the mode of action and the data available in HGMD Professional 2019.4, dbVar and CENTOGENE's internal database. Together with the biochemical results, it is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *NAGLU* gene are associated with autosomal recessive mucopolysaccharidosis type IIIB (Sanfilippo B; OMIM®: 252920). Mucopolysaccharidosis type III (MPS III) is a lysosomal storage disease belonging to the group of mucopolysaccharidoses and characterized by severe and rapid intellectual deterioration. The first symptoms appear between the ages of 2 and 6 years, with behavioral disorders (hyperkinesia, aggressiveness) and intellectual deterioration, sleep disorders and very mild dysmorphism. The neurological involvement becomes more prominent around the age of 10 years with loss of motor milestones and communication problems. Seizures often occur after the age of 10. A few cases of attenuated forms have also been reported (orpha.net ORPHA:581).

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® 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.

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 80% for all homozygous deletions and heterozygous deletions/duplications spanning at least three consecutive exons.

ANALYSIS STATISTICS

CentoMetabolic® (sequencing including NGS-based CNV analysis)

Targeted nucleotides covered	≥ 20x	99.29%

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 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.

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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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TECHNICAL SUPERVISOR

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QUALITY CONTROL

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Chief Medical Director

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