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: **male**, Your ref.: **xxx**

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

CLINICAL INFORMATION

Childhood onset; Failure to thrive; Genu valgum; Increased body weight; Joint laxity; Macrocephaly; Short stature (Clinical information indicated above follows HPO nomenclature.)

Normal developmental landmarks achieved.

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

Clinician suspects: mucopolysaccharidosis type 4.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homozygous pathogenic variant was identified in the *GALNS* gene. Additionally, the activity of the enzyme N-acetylgalactosamine-6-sulfate-sulfatase was pathologically decreased. **The genetic diagnosis of autosomal recessive mucopolysaccharidosis type IVA is confirmed.**

RECOMMENDATIONS

- We recommend parental targeted testing to confirm homozygosity of the *GALNS* variant in place of compound heterozygosity for a large deletion.
- Genetic counselling is recommended.

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RESULT SUMMARY

BIOCHEMICAL TESTING				
NAME OF GENE/ENZYME/BIOMARKER	RESULT	REFERENCE	INTERPRETATION	METHOD
galactosamine-6-sulfate sulfatase	**<0,1(LOD) µmol/L/h** LOD = limit of detection	≥ 5,3 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***
GALNS	NM_001323544.1:c.1277C>G	p.(Pro426Arg)	N/A	homozygous	PolyPhen: - Align-GVGD: C15 SIFT: Tolerated MutationTaster: Disease causing Conservation_nt: high Conservation_aa: weak	gnomAD: - ESP: - 1000 G: 0.00035 CentoMD: 0.00032	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

GALNS, c.1277C>G p.(Pro426Arg)

The *GALNS* variant c.1277C>G p.(Pro426Arg) causes an amino acid change from Pro to Arg at position 426. According to HGMD Professional 2019.4, this variant has previously been described as disease causing for Mucopolysaccharidosis IVa by Morrone et al., 2014 (PMID: 24726177). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in *GALNS* gene are associated with autosomal recessive mucopolysaccharidosis type IVA (Morquio A; OMIM®: 253000). Mucopolysaccharidosis type IV (MPS IV) is a lysosomal storage disease belonging to the group of mucopolysaccharidoses and characterized by spondylo-epiphyso-metaphyseal dysplasia. It exists in two forms, A and B. MPS IVA is a spondylo-epiphyso-metaphyseal dysplasia generally diagnosed during the second year of life, after walking acquisition. Skeletal deformities (platyspondyly, kyphosis, scoliosis, pectus carinatum, genu valgum, long bone deformities) become more pronounced as the child grows. Joint hyperlaxity is accompanied by frequent luxations (hips, knees) (orpha.net ORPHA:582).

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	99.78%

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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Chief Medical Director

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