



XXX

Order no.: xxx
Order received: xxx
Sample type: blood, filter card
Sample collection date: xxx
Report type: Final Report
Report date: xxx

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

Test(s) requested: Whole Genome Sequencing (CentoGenome®)

CLINICAL INFORMATION*

Clinical information*: Abnormal myelination, Agenesis of corpus callosum, CNS demyelination, Developmental regression, Difficulty standing, Leukodystrophy

*: Clinical information indicated above follows HPO nomenclature.

Parents are consanguineous.



POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homozygous pathogenic variant was identified in the ARSA gene. **The genetic diagnosis of autosomal recessive metachromatic leukodystrophy is confirmed.**

RECOMMENDATIONS

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

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RESULT SUMMARY

| GENE | VARIANT COORDINATES | ZYGOSITY | IN SILICO PARAMETERS* | ALLELE FREQUENCIES** | TYPE AND CLASSIFICATION*** |
|------|--|----------|--|--|---------------------------------------|
| ARSA | Chr22(GRCh37):g.51065363del NM_000487.5:c.583del p.(Trp195Glyfs*5) Exon 3 | Hom | PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A | gnomAD: - ESP: - 1000 G: - CentoMD: 0.00040 | Frameshift Pathogenic (class 1) |

Variant description based on Alamut Batch (latest database available). * AlignGVGD: C0: least likely to interfere with function, C65: most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations

VARIANT INTERPRETATION

ARSA, c.583del p.(Trp195Glyfs*5)

The ARSA variant c.583del p.(Trp195Glyfs*5) creates a shift in the reading frame starting at codon 195. The new reading frame ends in a stop codon 4 positions downstream. This variant has been confirmed by Sanger sequencing. According to HGMD Professional 2017.3, this variant has previously been described as disease causing for Metachromatic leukodystrophy by Cesani et al., 2016 (PMID: 26462614). ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 93124). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic variants in ARSA gene are associated with metachromatic leukodystrophy, an autosomal recessive disorder. Metachromatic leukodystrophy (MLD) is part of a larger group of lysosomal storage diseases, some of which are progressive, inherited, and neurodegenerative disorders (metachromatic leukodystrophy included). Four types of metachromatic leukodystrophy occur with varying ages of onset and courses (ie, late infantile, early juvenile, late juvenile, adult) (Gieselmann et al., 2010). All forms of the disease involve a progressive deterioration of motor and neurocognitive function.

INCIDENTAL FINDINGS

We did not detect any class 1 or 2 variants in the genes for which incidental findings are reported based on the ACMG guidelines.

ANALYSIS STATISTICS WGS

| AVERAGE COVERAGE (X) | % TARGET BP COVERED | | | | | |
|----------------------|---------------------|-------|-------|-------|-------|-------|
| | 0X | ≥ 1X | ≥ 5X | ≥ 10X | ≥ 20X | ≥ 50X |
| 33.15 | 0.31 | 99.69 | 99.42 | 98.78 | 92.33 | 2.43 |

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

METHODS

Genomic DNA was fragmented by sonication and Illumina adapters were ligated to generated fragments for subsequent sequencing on the

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HiSeqX platform (Illumina) to yield an average coverage depth of ~30X. An end to end in-house bioinformatics pipeline including base calling, primary filtering of low quality reads and probable artefacts, and annotation of variants was applied. CNV calling is based on HAS pipeline. All disease causing variants reported in HGMD®, in ClinVar or in CentoMD® in addition to all variants with minor allele frequency (MAF) of less than 1% in gnomAD database are considered. Evaluation is focused on coding exons along with flanking +/-20 intronic bases, however extended to the complete gene region for candidate genes or in search for a second previously described variant in AR inheritance pattern. All pertinent inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate eventually identified variants. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized in classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported. CNVs of unknown significance are not reported. Reported CNVs are confirmed with another method such as MLPA and qPCR.

Variants of relevance identified by NGS are continuously and individually in-house validated for quality aspects; those variants which meet our internal QC criteria (based on extensive validation processes) are not validated by Sanger.

The ARSA gene was analysed by PCR and sequencing of both DNA strands of the entire coding region and the highly conserved exon-intron splice junctions. The reference sequence is / sequences are: ARSA: NM_000487.5.

LIMITATIONS

Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical findings, additional testing should be considered.

Due to limited read length and other contributing technical limitations, repeat expansions (i.e. in the Huntington gene, the SCA-genes, the myotonic dystrophy repeat region, and other similar regions) cannot be assessed with the applied method. Of note, CNV calls from Whole Genome Sequencing have a limited accuracy and sensitivity, and structural changes below 2 kb at a genome-wide level are not called by our pipeline.

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.

In line with ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (Genetics in Medicine, 2016), we report incidental findings, i.e. pathogenic variants (class 1) and likely pathogenic variants (class 2) only in the recommended genes for the recommended phenotypes.

To also 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 (dmqc@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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Chief Medical Director

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