



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**

**Additional report recipient(s):** xxx

**Test(s) requested: CentoMito® Comprehensive (Large extended screening)**

### CLINICAL INFORMATION

Patient with convulsions and demyelination of the white matter.



**POSITIVE RESULT**  
**Pathogenic variant identified**

### INTERPRETATION

A homozygous pathogenic variant was identified in the BTBD9 gene.  
**This result is consistent with a genetic diagnosis of autosomal recessive biotinidase deficiency.**

### RECOMMENDATIONS

- Biotin supplementation
- Parental carrier testing to confirm homozygosity in place of a heterozygous large deletion
- Genetic counselling

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**RESULT SUMMARY**

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BTD	Chr3(GRCh37):g.15686975C>T NM_001281723.2:c.1618C>T <b>p.(Arg540Cys)</b> <b>Exon 4</b>	Hom	PolyPhen: Probably damaging Align-GVGD: C25 SIFT: Deleterious MutationTaster: Disease causing Conservation: nt weak/aa high	gnomAD: 0.000075 ESP: - 1000 G: - CentomD: -	Missense Pathogenic (class 1)

Variant description based on Alamut Batch (latest database available). \* AlignGVD: 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**

**BTD, c.1618C>T p.(Arg540Cys)**

The BTD variant c.1618C>T p.(Arg540Cys) causes an amino acid change from Arg to Cys at position 540. According to HGMD Professional 2017.3, this variant has previously been described as disease causing for Biotinidase deficiency by Pomponio et al., 1997 (PMID: 9099842), followed by several other publications. ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 1898). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic variants in BTD gene are associated with biotinidase deficiency, an autosomal recessive disorder. Biotinidase deficiency (BTD) is an inherited metabolic disorder in which the body is unable to recycle the vitamin biotin. If this condition is not recognized and treated, its signs and symptoms typically appear within the first few months of life, although it can also become apparent later in childhood. Profound biotinidase deficiency, the more severe form of the condition, can cause seizures, weak muscle tone (hypotonia), breathing problems, hearing and vision loss, problems with movement and balance (ataxia), skin rashes, hair loss (alopecia), and candidiasis. Affected children also have delayed development. Lifelong treatment can prevent these complications from occurring or improve them if they have already developed.

**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**

Targeted amplification of the entire mitochondrial genome is done using two overlapping long range PCRs. The amplicons are run on the Bioanalyzer to assess for any putative deletion within the mitochondrial genome. The amplified products are subsequently tagged and Illumina compatible adapters ligated to generate libraries that are sequenced on Illumina platforms to an average sequencing depth of 1000x or more.

Raw sequence data analysis, including base calling, demultiplexing, alignment to the Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC\_012920) and variant calling are performed using validated in-house software. Following the base calling and primary filtering of low quality reads, standard Bioinformatics pipeline was implemented to annotate detected variants and to filter out probable artefacts. The pipeline confidently detects heteroplasmy levels down to 15%. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized into classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported.

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 sample has been processed by enriching of targeted sequences and sequencing was done by using Next Generation Sequencing Technologies. The entire coding region of nuclear genes including 10 bp of intronic flanking sequences were amplified and sequenced. Raw sequence data analysis, including base calling, demultiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37) and variant calling were performed using validated in-house software. Due to limitations of the method, the target

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sequences of the requested panel might not be covered 100%.

For mitochondrial genome analysis the genes were amplified and sequenced; and raw sequence data analysis, including base calling, demultiplexing, alignment to the Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC\_012920) and variant calling were also performed using validated in-house software. For the CentoMito Comprehensive (Large extended screening panel), the list of analysed genes (nuclear and mitochondrial) is available in our website: <https://www.centogene.com/>. All identified variants were evaluated regarding their pathogenicity and causality, and these were classified in classes 1 - 5 (see above). Analysis does not include copy number variations (CNV) or large deletion/duplications.

**Statistics (CentoMito Comprehensive)**

Overall, 97.13% of the target base pairs of the nuclear genes were covered at least 20x. The average cover was: 135.16x.

Overall, 100% of the target base pairs of the mitochondrial genome were covered at least 1000x. The average cover was: 12820x.

**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.

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](mailto: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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