



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: Hemophagocytic Lymphohistiocytosis panel (sequencing)

CLINICAL INFORMATION*

Abdominal distention, Abnormal bleeding, Abnormality of the coagulation cascade, Acidosis, Elevated C-reactive protein level, Fever, Hepatic failure, Hypoglycemia, Immunodeficiency, Increased serum lactate, Jaundice, Leukocytosis, Metabolic acidosis, Respiratory distress, Sepsis, Thrombocytopenia, Triggered by febrile illness

*: Clinical information indicated above follows HPO nomenclature.



POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homozygous pathogenic variant was identified in the STXBP2 gene. **The genetic diagnosis of autosomal recessive familial hemophagocytic lymphohistiocytosis type 5 is confirmed.**

In the remainder of the panel genes (see methods), no other clinically relevant variant has been identified.

RECOMMENDATIONS

- We recommend parental carrier testing to confirm homozygosity of the STXBP2 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***
STXBP2	Chr19(GRCh37):g.7711208C>T NM_001272034.1:c.1463C>T p.(Pro488Leu) Exon 16	Hom	PolyPhen: Probably damaging Align-GVGD: C0 SIFT: Tolerated MutationTaster: Disease causing Conservation: nt moderate/aa high	gnomAD: 0.0000080 ESP: - 1000 G: - CentoMD: 0.00041	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

STXBP2, c.1463C>T p.(Pro488Leu)

The STXBP2 variant c.1463C>T p.(Pro488Leu) causes an amino acid change from Pro to Leu at position 488. According to HGMD Professional 2017.3, this variant has previously been described as disease causing for Haemophagocytic lymphohistiocytosis type 5 by zur Stadt et al., 2009 (PMID: 19804848), Cote et al., 2009 (PMID: 19884660), Hackmann et al., 2013 (PMID: 24194549). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic variants in the STXBP2 gene are causative for familial hemophagocytic lymphohistiocytosis type 5, an autosomal recessive disorder. Familial hemophagocytic lymphohistiocytosis (FHL) is characterized by proliferation and infiltration of hyperactivated macrophages and T-lymphocytes manifesting as acute illness with prolonged fever, cytopenias, and hepatosplenomegaly. Onset is typically within the first months or years of life and, on occasion, in utero, although later childhood or adult onset is more common than previously suspected. Neurologic abnormalities may be present initially or may develop later; they may include increased intracranial pressure, irritability, neck stiffness, hypotonia, hypertonia, convulsions, cranial nerve palsies, ataxia, hemiplegia, quadriplegia, blindness, and coma. Rash and lymphadenopathy are less common. Other findings include liver dysfunction and bone marrow hemophagocytosis. The median survival of children with typical FHL, without treatment, is less than two months; progression of hemophagocytic lymphohistiocytosis and infection account for the majority of deaths in untreated individuals (OMIM®: 613101; GeneReviews - PMID: 20301617).

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 HiSeqX platform (Illumina) to yield an average coverage depth 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 is applied. CNV calling is based on HAS pipeline. Analysis was restricted to variants obtained within the Panel genes.

The genes PRF1, UNC13D, STX11, STXBP2 included in our Hemophagocytic Lymphohistiocytosis panel are covered 100% with at least 10x read depth. 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

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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 into 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.

Reflex testing to WGS upon negative panel findings, when requested, extends the analysis of variants to clinically relevant genes outside the panel genes based on phenotypic overlap and to research genes (when opted) that may have disease implication based on animal models.

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.

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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