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

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

CLINICAL INFORMATION

Failure to thrive; Iron deficiency anemia; Mitral regurgitation; Perianal abscess; Recurrent otitis media; Scaling skin.

(Clinical information indicated above follows HPO nomenclature.)

Diagnosed condition(s): septic shock with multiple organ failure (MOF) and toxic shock syndrome (TSS).
Hospital admission: PICU due to hypotensive septic shock.

Family history: Yes.
Brother: Death in infancy, Immunodeficiency
Consanguineous parents: Yes.

Clinician suspects: Primary immunodeficiency disease.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homozygous pathogenic large deletion was identified the *HAX1* gene. **The genetic diagnosis of autosomal recessive severe congenital neutropenia type 3 is confirmed.**

No additional clinically relevant variant was identified in the panel genes by sequencing analysis.

RECOMMENDATIONS

- Genetic counselling is recommended.

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RESULT SUMMARY

GENE (TRANSCRIPT, METHOD)	OUTCOME
HAX1 (NM_006118.3; qPCR)	Homozygous pathologic deletion exons 4-7

VARIANT INTERPRETATION

HAX1, homozygous deletion exon 4-7

By NGS-based and qPCR analyses, we detected a homozygous deletion that comprises exons 4 to 7 of the *HAX1* gene. The identified deletion has been reported by Boztug et al., (2010 - PMID: 21108402) in 2 homozygous siblings presenting with severe congenital neutropenia and neurological disease, and by Kurnikova et al., (2011 - PMID: 21344642) in an additional homozygous patient presenting with severe congenital neutropenia. The identified deletion is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in *HAX1* gene are associated with autosomal recessive severe congenital Neutropenia type 3 (OMIM® 610738). Severe congenital neutropenia-3 is an autosomal recessive bone marrow failure disorder characterized by low numbers of neutrophils, increased susceptibility to bacterial and fungal infections, and increased risk of developing myelodysplastic syndrome or acute myeloid leukemia. In addition, patients with *HAX1* mutations affecting both isoform A and B of the gene develop neurologic abnormalities (summary by Boztug et al., 2010).

TABULAR LIST OF ADDITIONAL PATHOGENIC AND LIKELY PATHOGENIC VARIANTS

To provide the most comprehensive and relevant genetic information, we list selected variants found in genes associated with **severe and early-onset disease.** The gene selection is based on OMIM® phenotypes and CENTOGENE internal data. We classified these variants at the time of reporting as "pathogenic" and "likely pathogenic" (see our mutation database CentoMD® for further information). Variants not included and classified in the current release of CentoMD®, and low-quality variants that usually represent technical artifacts, are not included. The complete gene list can be found at https://www.centogene.com/diagnostics/medical-reporting/p-lp-gene-reporting.html (please contact CENTOGENE customer support if the gene list has been updated after this report was issued).

The listed variants may not directly answer the diagnostic request, at least not with the clinical information provided to CENTOGENE or current scientific understanding of relevant genetic disease mechanisms. However, these variants may help to close a potential diagnostic gap regarding the current clinical picture and are therefore provided here for a full diagnostic overview. In case this additional information is used in the further differential diagnosis process, orthogonal validation of relevant variants might be necessary.

Beyond the requested test, variants in genes related to late-onset diseases with unclear (considerably reduced) penetrance and/or cancer-related genes with onset in adulthood are not included in this list. This table does therefore not provide a complete list of potentially relevant genetic variants in the patient. Furthermore, the classification of these variants may change over time. CENTOGENE is not liable for any missing variant in this list and/or any provided classification of the variants at a certain point of time.

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Insofar, as the identified variants may indicate (additional) genetic risks or diagnoses in the patient and/or his family and/or inform about reproductive risks, we strongly recommend following applicable local guidelines with regard to informing the patient about such findings. Particularly, if the patient decided not to be informed about "incidental findings" (to avoid any misunderstanding the list given here is not covering "incidental findings" according to ACMG), it should be clarified with the patient whether he/she wants to be informed about these additional variants.

In accordance with this disclaimer no relevant pathogenic or likely pathogenic variant was identified.

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 is enzymatically fragmented, and regions of interest are enriched using DNA capture probes. The final indexed libraries are sequenced on an Illumina platform. As the enriched target regions cover all panel genes and usually include more genes due to enlarged wet lab backbones, the downstream bioinformatic analysis and the report may include clinically relevant findings exceeding the selected gene panel.

For the CentoImmuno (sequencing including NGS-based CNV analysis) panel(s), the coding regions of the panel genes, 10 bp of flanking intronic sequences, and known pathogenic/likely pathogenic variants within these genes (coding and non-coding) are targeted for analysis. The panel gene list can be obtained here: www.centogene.com/ngspanels-medical-reporting (please contact CENTOGENE customer support if the gene list has been updated after this report was issued). 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 potentially clinically relevant variants are reported. VUSs are not reported in the following cases: the described phenotype(s) is already explained by a detected pathogenic or likely pathogenic variant(s); the detected VUSs are not related to the described phenotype(s); lack of clinical information; for oncogenetic panels. 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.
We performed quantitative PCR assay (qPCR) by using four gene-specific amplicons encompassing the coding exons 4, 5, 6, 7 (or part of it) of the *HAX1*: NM_006118.3 gene(s).

ANALYSIS STATISTICS

CentoImmuno (sequencing including NGS-based CNV analysis)

Targeted nucleotides covered	≥ 20x	99.12%

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.

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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.

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 (customer.support@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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Signature **Signature**

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