CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE GmbH • Am Strande 7 • 18055 Rostock • Germany

Order no.:
Order received: DD/MM/YYYY
Sample type / Sample collection date:
blood, CentoCard® / DD/MM/YYYY
Report date: DD/MM/YYYY
Report type: Final Report

Patient no.: ,First Name: ,Last Name:
DOB: **DD/MM/YYYY**, Sex: fe**male**, Your ref.: **-**

Test(s) requested: CentoXome® Trio

CLINICAL INFORMATION

Unaffected.
Family history: Yes.
Consanguineous parents: No.

We analyzed whole exome sequencing data for the child of the consultand. Please refer to our report [ID Order, Name].
This report reflects exclusively the segregation information for the pro consultand band in the context of the family analysis.

CARRIER STATUS CONFIRMED

Likely pathogenic variant identified

INTERPRETATION

A heterozygous likely pathogenic variant was identified in the *TTN* gene. **The carrier status of the *TTN* variant is confirmed.**

The familial segregation analysis confirms the *trans* phase of the variants in the index patient. Considering the result of the partner, with each pregnancy of this couple there is a 25% risk for the offspring of being affected.

No further clinically relevant variants related to the described family phenotype were detected.

RECOMMENDATIONS

- Targeted testing for affected family members, if any, and familial cascade carrier testing are recommended.
- Genetic counselling, including reproductive counselling (discussing prenatal and preimplantation diagnoses, if relevant) is recommended.

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com

MAIN FINDINGS

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
TTN	NM_001267550.1:c.14212C>T	p.(Arg4738*)	N/A	heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: Disease causing Conservation_nt: weak Conservation_aa: N/A	gnomAD: - ESP: - 1000 G: 0.000051 CentoMD: 0.000043	Nonsense Likely Pathogenic (class 2)

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

TTN, c.14212C>T p.(Arg4738*)

The *TTN* variant c.14212C>T p.(Arg4738*), located in the I-band, creates a premature stop codon. Furthermore, the resulting product is predicted to undergo nonsense-mediated mRNA decay and remove more than 10% of the resulting protein. It is classified as likely pathogenic (class 2) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *TTN* gene have been associated with autosomal recessive *TTN*-related myopathies, also known as autosomal recessive titinopathy. This includes several recessive forms of the disease: limb-girdle muscular dystrophy (OMIM®: 608807), centronuclear myopathy (not an OMIM entity), Salih myopathy (OMIM®: 611705), Emery-Dreifuss-like muscular dystrophy (not an OMIM entity), titinopathy with congenital contractures (not an OMIM entity), minicore myopathy (not an OMIM entity), and distal titinopathy (not an OMIM entity) (Gene-Disease validity for TTN gene by ClinGen Congenital Myopathies Gene Curation Expert Panel and ClinGen Limb Girdle Muscular Dystrophy Gene Curation Expert Panel).

SECONDARY FINDINGS

If consent is provided, in line with ACMG recommendations (ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing; Genetics in Medicine, 2023; PMID: 37347242) we report secondary findings, i.e. relevant pathogenic and likely pathogenic variants in the recommended genes for the indicated phenotypes in this publication.
We did not detect any relevant variants in the genes for which secondary findings are reported.

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).

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com

METHODS

Genomic DNA is enzymatically fragmented, and target regions are enriched using DNA capture probes. These regions include approximately 41 Mb of the human coding exome (targeting > 98% of the coding RefSeq from the human genome build GRCh37/hg19), as well as the mitochondrial genome. The generated library is sequenced on an Illumina platform to obtain at least 20x coverage depth for > 98% of the targeted bases. An in-house bioinformatics pipeline, including read alignment to GRCh37/hg19 genome assembly and revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920), variant calling, annotation, and comprehensive variant filtering is applied. All variants with minor allele frequency (MAF) of less than 1% in gnomAD database, and disease-causing variants reported in HGMD®, in ClinVar or in CentoMD® are evaluated. The investigation for relevant variants is focused on coding exons and flanking +/-10 intronic nucleotides of genes with a clear gene-phenotype evidence (based on OMIM® information). All potential patterns for mode of inheritance are considered. In addition, provided family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and disease causality. Variants are categorized into five classes (pathogenic, likely pathogenic, VUS, likely benign, and benign) along ACMG guidelines for classification of variants. All relevant variants related to the phenotype of the patient are reported. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Variants with low sequencing quality and/or unclear zygosity are confirmed by orthogonal methods. Consequently, a specificity of > 99.9% for all reported variants is warranted. Mitochondrial variants are reported for heteroplasmy levels of 15% or higher. The copy number variation (CNV) detection software has a sensitivity of more than 95% for all homozygous/hemizygous and mitochondrial deletions, as well as heterozygous deletions/duplications and homozygous/hemizygous duplications spanning at least three consecutive exons. For the uniparental disomy (UPD) screening, a specific algorithm is used to assess the well-known clinically relevant chromosomal regions (6q24, 7, 11p15.5, 14q32, 15q11q13, 20q13 and 20).

ANALYSIS STATISTICS

CentoXome® Trio

Targeted nucleotides covered	≥ 20x	99.45%

LIMITATIONS

The genetic results are interpreted in the context of the provided clinical findings, family history, and other laboratory data. Only variants in genes potentially related to the proband's medical condition are reported. Misinterpretation of results may occur if the provided genetic data or patient information is inaccurate and/or incomplete. If the obtained genetic results are not compatible with the clinical findings, additional testing should be considered.
The genes with mapping issues in GRCh37/hg19 genome assembly, the non-protein-coding disease-associated genes, and approximately 0.2 Mb of genomic regions that are hard to sequence by current enrichment technology and are without evidenced relevance for monogenic disorders, are excluded from this analysis. More complex genetic events such as inversions, translocations, and repeat expansions, are not analyzed in this test. The UPD detection is a screening method, and therefore false-positive and false-negative results may occur. In addition, due to technology limitations, certain regions may be poorly covered, or not covered at all. In these regions and others encompassing repetitive, high-homology (such as pseudogene homology), and GC-rich sequences, relevant 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. 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 CNV detection sensitivity is decreased for repetitive and homologous regions, such as pseudogenes. Mitochondrial variants with heteroplasmy levels below 15% may not be detected. It is expected that lower quality samples (prenatal, product of conception, blood from patients with hematologic disorders, and highly degraded DNA) may generate lower quality NGS data; in these cases, CNV analysis and/or mitochondrial genome analysis may not be possible to perform. Potential aberrant splicing is assessed with splice prediction tools. Intronic variants that are beyond 10 nucleotides from exon-intron boundaries are not considered for aberrant splicing analysis, with the exception of known pathogenic splicing variants evidenced by external sources.

ADDITIONAL INFORMATION

This test was developed, and its performance was validated, by CENTOGENE. 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 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.

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com

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.

COPYRIGHT NOTICE

Chief Medical and Genomic Officer Human Geneticist	Human Geneticist	Clinical Scientist

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com