Dr. (Physician)
Institution
Address
Country

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: **male**, Your ref.:

Test(s) requested: CentoXome® Trio

CLINICAL INFORMATION

Difficulty climbing stairs; Difficulty running; Elevated circulating creatine kinase concentration; EMG abnormality; Gowers sign; Lower limb muscle weakness; Myopathy; Proximal muscle weakness in upper limbs.
(Clinical information indicated above follows HPO nomenclature.)

MRI normal.

Family history: Unknown.
Consanguineous parents: No.

Please see the concurrent reports for the parents: [ID Order, Name] and [ID Order, Name].
.

POSITIVE RESULT
Likely pathogenic variant identified
Secondary finding identified

INTERPRETATION

A heterozygous pathogenic and a heterozygous likely pathogenic variants were identified in the *TTN* gene. **This finding is consistent with the genetic diagnosis of autosomal recessive *TTN*-related myopathy.** The familial segregation analysis confirms the *trans* phase of the variants.

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

As a secondary finding, a heterozygous pathogenic variant was identified in the *LDLR* gene. The result is consistent with the increased genetic susceptibility to autosomal dominant familial hypercholesterolemia type 1.

RECOMMENDATIONS

- Targeted testing for affected family members, if any, and familial cascade carrier testing are recommended.
- For the secondary finding, clinical evaluation and follow-up are recommended. Targeted testing for affected and at-risk family members is 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.19426+2T>A	p.?	rs727505178	heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: Disease causing Conservation_nt: high Conservation_aa: N/A 2/2 likely splice effect	gnomAD: - ESP: - 1000 G: 0.00097 CENTOGENE's in-house Biodatabank: 0.00093	Splicing Pathogenic (class 1)
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 CENTOGENE's in-house Biodatabank: 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 CENTOGENE's in-house Biodatabank (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 according to the recommendations of CENTOGENE, ACMG/AMP and ClinGen SVI general recommendations (please, see additional information below).

TTN, c.19426+2T>A p.?

The *TTN* variant c.19426+2T>A is predicted to disrupt the highly conserved donor splice site and results in an in-frame deletion of exon 66 in the I-band. According to HGMD Professional 2022.1, this variant has previously been described as disease causing for Muscular dystrophy, limb-girdle by Topf et al., 2020 (PMID: 32528171), Natera-de Benito et al., 2021 (PMID: 33333461). ClinVar lists this variant (Interpretation: Conflicting interpretations of pathogenicity; Likely pathogenic (1)| Uncertain significance(2); Variation ID: 179861). It is classified as pathogenic according to the recommendations of CENTOGENE, ACMG/AMP and ClinGen SVI general recommendations (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

> Contact Details
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phenotypes in this publication.

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
LDLR	NM_000527.2:c.1135T>C	p.(Cys379Arg)	rs879254803	heterozygous	PolyPhen: - Align-GVGD: C0 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.000032 ESP: - 1000 G: 0.000032 CENTOGENE's in-house Biodatabank: -	Missense Pathogenic (class 1)

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 CENTOGENE's in-house Biodataban (latest database available). *** based on ACMG recommendations.

VARIANT INTERPRETATION

LDLR, c.1135T>C p.(Cys379Arg)

The *LDLR* variant c.1135T>C p.(Cys379Arg) causes an amino acid change from Cys to Arg at position 379. According to HGMD Professional 2021.3, this variant has previously been described as disease causing for hypercholesterolemia by Hobbs et al., 1992 (PMID: 1301956), Romano et al., 2011 (PMID: 21865347), Bertolini et al., 2013 (PMID: 23375686). ClinVar lists this variant (Interpretation: Pathogenic/Likely pathogenic; Variation ID: 251685). It is classified as pathogenic according to the recommendations of CENTOGENE, ACMG/AMP and ClinGen SVI general recommendations (please, see additional information below).

Familial hypercholesterolemia is an autosomal dominant disorder characterized by elevation of serum cholesterol bound to low density lipoprotein (LDL), which promotes deposition of cholesterol in the skin (xanthelasma), tendons (xanthomas), and coronary arteries (atherosclerosis). The disorder occurs in 2 clinical forms: homozygous and heterozygous (Hobbs et al., 1992; PMID:1301956). Mode of Inheritance: Autosomal dominant (OMIM®: 143890).

CARRIERSHIP FINDINGS

In this table we list sequence variants previously ascertained or evaluated and classified in CENTOGENE as "pathogenic" and "likely pathogenic", in selected genes associated with recessive severe and early-onset Mendelian diseases. As only in-house classified variants are presented, it should not be considered a comprehensive list of variants in these genes and does not provide a complete list of potentially relevant genetic variants in the patient. The complete gene list can be found at www.centogene.com/carriership-findings (please contact CENTOGENE customer support if the gene list has been updated after this report was issued). Orthogonal validation was not performed for these variants. Therefore, if any variant is used for clinical management of the patient, confirmation by another method needs to be considered. Furthermore, the classification of these variants may change over time, however reclassification reports for these variants will not be issued. 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. As the identified variants may indicate (additional) genetic risks or diagnoses in the patient and/or family and/or inform about reproductive risks, we recommend discussing these findings in the context of genetic counselling.

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Fax: +49 (0)381 80113 401
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www.centogene.com

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
GAA	NM_000152.3:c.266G>A	p.(Arg89His)	rs200586324	heterozygous	PolyPhen: Probably damaging Align-GVGD: C0 SIFT: - MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.00014 ESP: 0.00023 1000 G: 0 CENTOGENE's in-house Biodatabank: 0.00053	Missense Pathogenic (class 1)

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 CENTOGENE's in-house Biodatabank (latest database available). *** based on ACMG recommendations.

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

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 CENTOGENE's in-house Biodatabank are evaluated. The investigation for relevant variants is focused on coding exons and flanking +/-10 intronic nucleotides of genes with 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, variant of uncertain significance [VUS], likely benign, and benign) according to ACMG/AMP guidelines for classification of variants in addition to ClinGen recommendations. 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 the uniparental disomy (UPD) screening, an in-house 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	99.57%

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

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www.centogene.com

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. The CNV detection sensitivity is decreased for repetitive and homologous regions such as pseudogenes, as well as for events spanning two or less exons. 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.

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 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