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Order no.: 63160141
Order received: 08 Nov. 2023
Sample type / Sample collection date:
blood, CentoCard® / 17 Oct. 2023
Report date: 22 Nov. 2023
Report type: Final Report

Patient no.: **1856309**, First Name: **Matviy**, Last Name: **Semenov**
DOB: **19 Aug. 2015**, Sex: **male**, Your ref.: **CG014726483**

Test(s) requested: *DMD* gene (sequencing including NGS-based CNV analysis)

CLINICAL INFORMATION

Calf muscle pseudohypertrophy; Elevated circulating creatine kinase concentration; Gowers sign
(Clinical information indicated above follows HPO nomenclature.)

Family history: Unknown.
Consanguineous parents: No.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A deletion (one-copy loss, classification: pathogenic) was identified in the *DMD* gene. **The genetic diagnosis of X-linked dystrophinopathy is confirmed.**

RECOMMENDATIONS

- Assessing the eligibility for novel genetic therapies is recommended.
- Maternal targeted testing is recommended to establish whether the detected variant is inherited or *de novo*. Targeted testing for all affected males, if any, and carrier testing for at-risk females in the matrilinear family are recommended.
- Genetic counselling, including reproductive counselling (discussing prenatal and preimplantation diagnoses, if relevant) is recommended.

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MAIN FINDINGS

COPY NUMBER VARIATIONS					
GENOMIC COORDINATES	GENE	TRANSCRIPT	AFFECTED EXONS	ZYGOSITY	TYPE AND CLASSIFICATION*
chrX:31770995-31794906	*DMD*	NM_004006.3	Exon 51	hemizygous	Loss Pathogenic (class 1)

* based on ACMG recommendations

VARIANT INTERPRETATION

DMD, loss

The detected loss encompasses exon 51 (of 79) of the *DMD* gene and is predicted to lead to an out-of-frame deletion (LOVD reading frame checker). The pathogenicity of the variant was assessed based on the mode of action and the data available in HGMD Professional 2022.4, dbVar and CENTOGENE's internal database. According to HGMD professional 2022.4, the loss has been previously described as disease causing for muscular dystrophy, Duchenne/Becker by Sinha et al., 1996 (PMID: 9007319), Ling et al., 2020 (PMID: 31705731) and more. It is classified as pathogenic according to the recommendations of CENTOGENE and ACMG/AMP ClinGen SVI general recommendations (please, see additional information below).

Pathogenic variants (primarily single/multiple exon deletions but also duplications and point variants) in the DMD gene are associated with X-linked inherited muscular dystrophies, ranging from the severe Duchenne muscular dystrophy (DMD; OMIM®: 310200) to the milder Becker muscular dystrophy (BMD; OMIM®: 300376). These variants lead to a prematurely truncated, unstable dystrophin protein which is one component of a large glycoprotein complex (dystrophin-associated glycoprotein complex) in the sarcolemma (plasma membrane) of skeletal muscles, acting as a link between the cytoskeleton and the extracellular matrix. Most boys with DMD present between 3 and 5 years of age. Clinical symptoms include gross motor delay, gait abnormalities (waddling gait), difficulty rising from the ground (Gower's sign) and frequent falls due to progressive proximal limb-girdle type muscular paresis. Highly elevated serum creatine kinase (CK) level is an important diagnostic finding. Dilated cardiomyopathy, arrhythmias, restrictive lung disease with continuous decline in vital capacity and a slight intellectual impairment, are additional features. Untreated patients become wheelchair bound by the age of 12 years and die of cardiorespiratory complications in their late teens to early 20s. In BMD the pathogenic variants maintain the open reading frame, resulting in a shorter lower molecular weight but partly functional dystrophin. Patients develop similar symptoms as DMD but later and with slower development. Cardiac involvement can be present. First symptoms appear by the age of 5-20 years, and life expectancy is reduced to approximately 40 years (Yiu, 2015; PMID: 25752877).
Of note, heterozygous females may present with a classic dystrophinopathy or may be asymptomatic carriers. Penetrance in heterozygous females varies and may depend in part on patterns of X-chromosome inactivation (XCI). The diagnosis of a dystrophinopathy is usually established in a female proband with characteristic clinical findings and elevated CK concentration and/or by identification of a heterozygous pathogenic variant in DMD upon/by molecular genetic testing. Females heterozygous for a DMD pathogenic variant are at increased risk for dilated cardiomyopathy (GeneReviews, PMID: 20301298). (OMIM®: 310200)

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).

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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.
For the DMD gene (sequencing including NGS-based CNV analysis), the coding regions of the gene(s), 10 bp of flanking intronic sequences, and known pathogenic/likely pathogenic variants within the gene(s) (coding and non-coding) are targeted for analysis. 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 oncogenetic analyses. 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 95% for all homozygous/hemizygous deletions, as well as heterozygous deletions/duplications and homozygous/hemizygous duplications spanning at least three consecutive exons.

ANALYSIS STATISTICS

DMD gene (sequencing including NGS-based CNV analysis)

Targeted nucleotides covered	≥ 20x	98.04%

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 (such as pseudogene homology), and GC-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.
Potential aberrant splicing is assessed with splice prediction tools. Synonymous variants and intronic variants that are beyond 10 nucleotides from exon-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 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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