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: **female**, Your ref.:

Test(s) requested: CentoXome® Solo

CLINICAL INFORMATION

Brain imaging abnormality; Elevated circulating creatine kinase concentration; Generalized hypotonia; Hyporeflexia; Leukodystrophy; Proximal muscle weakness; Skeletal muscle atrophy
(Clinical information indicated above follows HPO nomenclature.)

No seizures.

Family history: Yes.
Siblings affected.
Consanguineous parents: Yes.
Clinician suspects: congenital muscular dystrophy.

POSITIVE RESULT
Pathogenic variant identified
Secondary finding identified

INTERPRETATION

A homozygous pathogenic variant was identified in the *LAMA2* gene. **The genetic diagnosis of autosomal recessive *LAMA2*-related muscular dystrophy is confirmed.**

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

As a secondary finding, a heterozygous pathogenic variant was identified in the *BRCA2* gene. This result indicates an increased genetic risk for autosomal dominant hereditary breast and ovarian cancer syndrome.

RECOMMENDATIONS

- If possible, parental targeted testing is recommended to confirm the homozygosity of the identified *LAMA2* variant in place of a compound heterozygosity with a large deletion. Additionally, targeted testing for affected family members, if any, and familial cascade carrier testing are recommended.
- For the *BRCA2* gene variant, targeted testing for any affected and adult at-risk family members is recommended.
- Genetic counselling, including reproductive counselling (discussing prenatal and preimplantation diagnoses, if relevant), is recommended.

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MAIN FINDINGS

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
LAMA2	NM_000426.3:c.5476C>T	p.(Arg1826*)	rs747349942	homozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: Disease causing Conservation_nt: weak Conservation_aa: N/A	gnomAD: 0.000028 ESP: - 1000 G: 0.000028 CentoMD: 0.000043	Nonsense 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 CentoMD® (latest database available). *** based on ACMG recommendations.

VARIANT INTERPRETATION

LAMA2, c.5476C>T p.(Arg1826*)

The *LAMA2* variant c.5476C>T p.(Arg1826*) creates a premature stop codon. According to HGMD Professional 2022.1, this variant has previously been described in patients with muscular dystrophy (PMID: 9829280, 25214167, 32936536). ClinVar lists this variant as pathogenic/likely pathogenic (Variation ID: 265426). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

The clinical manifestations of *LAMA2* muscular dystrophy (LAMA2-MD) comprise a continuous spectrum ranging from severe congenital muscular dystrophy type 1A (MDC1A) to milder late-onset LAMA2-MD.
MDC1A is typically characterized by neonatal profound hypotonia, poor spontaneous movements, and respiratory failure. Failure to thrive, gastroesophageal reflux, aspiration, and recurrent chest infections necessitating frequent hospitalizations are common. As disease progresses, facial muscle weakness, temporomandibular joint contractures, and macroglossia may further impair feeding and can affect speech. In late-onset LAMA2-MD, onset of manifestations ranges from early childhood to adulthood. Affected individuals may show muscle hypertrophy and develop a rigid spine syndrome with joint contractures, usually most prominent in the elbows. Progressive respiratory insufficiency, scoliosis, and cardiomyopathy can occur (GeneReviews - PMID: 22675738).

SECONDARY FINDINGS

If consent is provided, in line with ACMG recommendations (ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing; Genetics in Medicine, 2022; PMID: 35802134) we report secondary findings, i.e., relevant pathogenic and likely pathogenic variants in the recommended genes for the indicated phenotypes.

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BRCA2	NM_000059.3:c.6450del	p.(Val2151Phefs*17)	N/A	heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: - ESP: - 1000 G: - CentoMD: -	Frameshift 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 CentoMD (latest database available). *** based on ACMG recommendations.

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VARIANT INTERPRETATION

BRCA2, c.6450del p.(Val2151Phefs*17)

The *BRCA2* variant c.6450del p.(Val2151Phefs*17) creates a shift in the reading frame starting at codon 2151. The new reading frame ends in a stop codon 16 positions downstream. According to HGMD Professional 2022.1, this variant has previously been described as disease-causing for breast cancer by Lin et al., 2016 (PMID: 26824983), Bhaskaran et al., 2019 (PMID: 30702160), Gao et al., 2020 (PMID: 31825140). ClinVar lists this variant (Interpretation: Pathogenic; Variation ID: 254583). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please see additional information below).

Hereditary breast and ovarian cancer (HBOC) syndrome is an autosomal dominant cancer predisposition syndrome caused primarily by germline *BRCA1* or *BRCA2* gene mutations (OMIM®: 604370, 612555). HBOC syndrome is characterized by a substantially increased risk for female and male breast cancer, ovarian cancer (including fallopian tube and primary peritoneal cancers), and to a lesser extent other cancers such as prostate cancer, pancreatic cancer, and, primarily in individuals with a *BRCA2* pathogenic variant, melanoma. Approximately 5%-10% of breast and ovarian cancer cases are hereditary and due to an identifiable pathogenic variant in a disease-causing gene. *BRCA1*- and *BRCA2*-associated HBOC accounts for the majority of hereditary breast and ovarian cancer cases in individuals with a strong family history or an early-onset diagnosis (PMID: 20301425, MedGen UID: 151793). A genetic diagnosis is extremely helpful so that additional screening, surveillance, and interventions can be started. These efforts can also result in risk-reduction and early diagnosis in family members, which increases the chances of successful treatment and survival (PMID: 35150867, PMID: 32862296). (OMIM®: 612555)

CARRIERSHIP FINDINGS

In this table we list sequence variants previously ascertained as, 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.

SEQUENCE VARIANTS

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
KIAA0556	NM_015202.3:c.49C>T	p.(Arg17*)	rs142375551	heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: Disease causing Conservation_nt: moderate Conservation_aa: N/A	gnomAD: 0.000087 ESP: 0.00015 1000 G: 0 CentoMD: 0.00040	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.

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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 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® Solo

Targeted nucleotides covered	≥ 20x	99.20%

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.

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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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