CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE • Am Strande 7 • 18055 Rostock • Germany

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: CentoGenome® Trio

CLINICAL INFORMATION

Childhood onset; Cognitive impairment; Short attention span.
(Clinical information indicated above follows HPO nomenclature).

Family history: Unknown.
Consanguineous parents: No.

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

POSITIVE RESULT
Pathogenic variants identified; secondary finding identified

INTERPRETATION

A hemizygous expansion in the full mutation range was detected in the *FMR1* gene by WGS-based analysis. This result was confirmed by repeat expansion analysis. **The genetic diagnosis of X-linked fragile X syndrome 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 *LDLR* gene. The result is consistent with the increased genetic risk of developing symptoms of autosomal dominant familial hypercholesterolemia 1.

RECOMMENDATIONS

- Targeted testing for all affected males and carrier testing for at-risk females in the family, if any, is recommended.
- Retrospective clinical analysis and follow-up for *LDLR*-associated symptoms is recommended. Targeted testing for affected, if any, 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

GENE (METHOD)					OUTCOME			
FMR1 (WGS + repeat expansion analysis)					**Hemizygous expanded allele in the full-penetrance range (>200 CGG repeats: ~270 repeats)**			
GENE	PHENOTYPE (OMIM®)	INHERITANCE	RESULTS OF REPEAT EXPANSION ANALYSIS	NORMAL	MUTABLE NORMAL (INTERMEDIATE)	PREMUTATION	PATHOGENIC WITH FULL PENETRANCE	REFERENCE
FMR1	Fragile X syndrome (OMIM: 300624)	X-linked	**>200 (~270 CGG repeats)**	<45 CGG repeats	45-54 CGG repeats	55-200 CGG repeats	>200 CGG repeats	Hunter et al., 2019 (PMID: 20301558)

VARIANT INTERPRETATION

By WGS analysis and confirmation by repeat expansion analysis, we detected an allele expansion in the full-penetrance range in the *FMR1* gene by repeat expansion analysis (with ~270 CGG repeats) (please, see the table above). More than 99% of individuals with fragile X syndrome have a loss-of-function variant of FMR1 caused by an increased number of CGG trinucleotide repeats (typically >200) (Hunter et al., 2019 - PMID: 20301558; Schmucker et al., 1999 - PMID: 25227148). Alleles with more than 200 CGG repeats typically show an aberrant methylation pattern of the promoter. This expansion is classified as pathogenic according to the recommendations of CENTOGENE, ACMG/AMP and ClinGen SVI general recommendations (please, see additional information below).

FMR1-related disorders are inherited in an X-linked dominant manner. Fragile X syndrome occurs in individuals with an *FMR1* full mutation or other loss-of-function variant and is nearly always characterized in affected males by developmental delay and intellectual disability along with a variety of behavioral issues. Autism spectrum disorder is present in 50%-70% of individuals with FXS. Affected males may have characteristic craniofacial features (which become more obvious with age) and medical problems including hypotonia, gastroesophageal reflux, strabismus, seizures, sleep disorders, joint laxity, pes planus, scoliosis, and recurrent otitis media. Adults may have mitral valve prolapse or aortic root dilatation. Approximately 50% of females who have a full *FMR1* pathogenic expansion are intellectually disabled; however, they are usually less severely affected than males with a full mutation. The physical and behavioral features seen in males with FXS have been reported in females heterozygous for the *FMR1* full mutation, but with lower frequency and milder involvement. The disease may demonstrate anticipation in some families. It typically occurs when less severely affected premutation or mosaic mutation carriers transmit unstable *FMR1* alleles to their offspring. Mothers and their female relatives who are premutation carriers are at increased risk for fragile X-associated tremor/ataxia syndrome and *FMR1*-related primary ovarian insufficiency (Hunter et al., 2019 - PMID: 20301558).

RESEARCH FINDINGS

Research variants (with potential relevance to the described phenotype) are variants in genes with no or only partial experimental evidence for their involvement in human disease.

The data was analyzed focusing on variants affecting protein function (nonsense, frameshift, conserved splice site and missense with high pathogenicity predictions) in genes with supporting evidence on zygosity, segregation or functional importance of the gene. Available literature or experimental data on expression and/or animal models were considered. However, no such variants could be identified for the patient.

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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.

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 Biodatabank (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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SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
TSHR	NM_000369.2:c.1349G>A	p.(Arg450His)	rs189261858	heterozygous	PolyPhen: Probably damaging Align-GVGD: C0 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.00021 ESP: - 1000 G: 0.00020 CENTOGENE's in-house Biodatabank: 0.00013	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 tagged with Illumina compatible adapter sequences. The libraries are paired-end sequenced on an Illumina platform to yield an average coverage depth of ~ 30x. A bioinformatics pipeline based on the DRAGEN pipeline from Illumina, as well as CENTOGENE's in-house pipeline is applied. The sequencing reads are aligned to the Genome Reference Consortium Human Build 37 (GRCh37/hg19), as well as the revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920). Sequence variants (SNVs/indels) and copy number variations (CNVs) are called using DRAGEN, Manta and in-house algorithms. Variants with a minor allele frequency (MAF) of less than 1% in gnomAD database, or disease-causing variants reported in HGMD®, in ClinVar or in CENTOGENE's in-house Biodatabank are evaluated. Although the evaluation is focused on coding exons and flanking intronic regions, the complete gene is interrogated for candidate variants with plausible association to the phenotype. All potential modes of inheritance are considered. In addition, the provided clinical information and family history 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. Likely benign and benign variants are not reported. CNVs of unknown significance with no apparent relation to the patient's phenotype are not reported. Mitochondrial variants with a heteroplasmy level of 15% or higher are reported. For detection of SNVs and indels in the regions targeted for downstream analysis a sensitivity of 99.9%, a specificity of 99.9%, and an accuracy of 99.9% is achieved. CNV detection software has a sensitivity of more than 95%. 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. Screening of repeat expansions is performed by the ExpansionHunter algorithm for the following genes: *AR*, *ATN1*, *ATXN1*, *ATXN2*, *ATXN3*, *ATXN7*, *ATXN8OS*, *ATXN10*, *CACNA1A*, *CNBP*, *CSTB*, *C9ORF72*, *DMPK*, *FMR1*, *FXN*, *HTT*, *JPH3*, *NOP56*, *PABPN1*, *PHOX2B*, *PPP2R2B*, *PRNP* and *TBP*. The technical results of repeat expansion screenings will be correlated with the clinical information provided. Any repeat expansion called and considered relevant to the phenotype will be confirmed by an orthogonal method. *GBA1* screening is performed using Gauchian algorithm to detect recombination events affecting the region encompassing exons 9-11 (NM_000157.3), a region which has the highest homology to *GBAP1*. Any detected recombination event is reported only when considered relevant to the phenotype. Spinal muscular atrophy (SMA) screening is performed using SMN Caller algorithm to detect the copy number of the *SMN1* gene. Any detected CNV is only confirmed by an orthogonal method and reported when considered relevant to the phenotype. Screening of uniparental disomy (UPD) is performed using an in-house algorithm for Mendelian inheritance errors (MIE) to detect runs of homozygosity (ROH) for the well-known clinically relevant chromosomal regions (6q24, 7, 11p15.5, 14q32, 15q11q13, 20q13 and 20).

The *FMR1* gene was analyzed using the AmplideXTM FMR1 PCR Kit to screen the trinucleotide repeat region in the promoter. The reference sequence of the *FMR1* gene is: NM_002024.5.

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ANALYSIS STATISTICS

CentoGenome® Trio

Targeted nucleotides covered	≥ 10x	99.68%

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.
Genes with mapping issues in the genome assembly used, and genomic regions that are hard to sequence by current technology and are without evidenced relevance for monogenic disorders, are excluded from this analysis. More complex genetic events not mentioned in the methods section, such as inversions and translocations, are not analyzed in this test. 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 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. Deep intronic variants without strong prediction of aberrant splicing may not be reported, with the exception of known pathogenic splicing variants evidenced by external sources. The CNV detection sensitivity is decreased for repetitive regions, homologous regions such as pseudogenes, and for events spanning 2 or less exons. Mitochondrial variants with heteroplasmy levels below 15% may not be detected. It is expected that lower quality samples (e.g., 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, mitochondrial genome analysis, and/or additional integrated screening analyses in this test may not be possible to perform. The repeat expansion algorithm used is not designed to handle complex loci that harbor multiple repeats. Repeats are only genotyped if the coverage at the locus is at least 10x. The Gauchian algorithm can only detect non-recombinant-like variants from a set of 111 known *GBA1* variants and can detect recombination events affecting exons 9-11 only. Therefore, recombinations affecting other regions are not in the scope of this screening. Silent carriers may be missed with the SMN Caller algorithm. The UPD detection is a screening method, and therefore false-positive and false-negative results may occur.

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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