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: **female**, Your ref.:

Test(s) requested: CentoGenome® Trio

CLINICAL INFORMATION

Unaffected
(Clinical information indicated above follows HPO nomenclature)

Consanguineous parents: No.

We analyzed whole genome 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 consultand in the context of the family analysis.

CARRIER STATUS CONFIRMED
Pathogenic variant identified

INTERPRETATION

A heterozygous expanded allele in the premutation range was detected in the *FMR1* gene by WGS-based analysis. This result was confirmed by repeat expansion analysis.

Alleles of this size are not associated with fragile X syndrome but are associated with primary ovarian insufficiency (FXPOI) in females and an increased risk for late onset fragile X-associated tremor/ataxia syndrome (FXTAS) in both males and females.

With each pregnancy there is a 50% risk that the abnormal allele (premutation) will be transmitted to the offspring. Please also note that due the potential repeat instability upon transmission of premutation alleles, women with alleles in this range are at risk of having children with fragile X syndrome (Saul et al., 2012 - PMID: 20301558).

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

RECOMMENDATIONS

- Clinical follow-up for primary ovarian insufficiency and late onset fragile X- associated tremor/ataxia syndrome (FXTAS) 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)					**Heterozygous expanded allele in premutation range (92 ± 1 CGG repeats) with 2 interruptions**			
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	**Allele 1: 31 ± 1 Allele 2: 92 ± 1 with 2 interruptions**	<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 a heterozygous allele in the premutation range (see the table above). The CGG repeats in this patient were found to be interrupted by two AGG repeats. In stable normal alleles these AGG triplets are thought to anchor the region during replication and prevent strand slippage (Monaghan et al., 2013 - PMID: 23765048). Alleles with 55 to ~200 CGG repeats correspond to the premutation range, without abnormal methylation of the neighboring CpG island and promoter, these alleles associate to the fragile X- associated primary ovarian insufficiency (FXPOI) in females and the fragile X-associated tremor/ataxia syndrome (FXTAS) in males and females. It has been shown that the premutation leads to overexpression and toxicity as well as a non-AUG translation of the *FMR1* mRNA in FXTAS (Biancalana et al., 2015 - PMID: 25227148). Alleles with more than 200 CGG are causative for the fragile X syndrome. The detected expansion is classified as pathogenic according to the recommendations of CENTOGENE, ACMG/AMP and ClinGen SVI general recommendations (please, see additional information below).

The *FMR1*-related disorders are inherited in an X-linked manner and include fragile X syndrome (FXS), FXTAS, and FXPOI (PMID: 20301558). 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.

FXTAS occurs in individuals who have an *FMR1* premutation and is characterized by late-onset, progressive cerebellar ataxia and intention tremor followed by cognitive impairment. Psychiatric disorders are common. Age of onset is typically between 60 and 65 years and is more common among males who are hemizygous for the premutation (40%) than among females who are heterozygous for the premutation (16%-20%). FXPOI, defined as hypergonadotropic hypogonadism before age 40 years, has been observed in 20% of women who carry a premutation allele compared to 1% in the general population.

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.

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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 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.

ANALYSIS STATISTICS

CentoXome® Trio

Targeted nucleotides covered	≥ 10x	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 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,

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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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