CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE GmbH • Am Strande 7 • 18055 Rostock • Germany

XXX

Order no.: xxx
Order received: xxx
Sample type / Sample collection date:
blood, CentoCard® / xxx
Report date: xxx
Report type: Final Report

Patient no.: **xxx**, First Name: **xxx**, Last Name: **xxx**
DOB: **xxx**, Sex: female, Your ref.: **xxx**

Additional report recipient(s): xxx

Test(s) requested: CentoGenome® Trio (including mitochondrial genome analysis)

CLINICAL INFORMATION

Unaffected.
(Clinical information indicated above follows HPO nomenclature.)

Family history: Yes.
Consanguineous parents: Yes.

We performed whole genome analysis for the child of the proband. Please refer to our report [Order: xxx, Name: xxx].

This report reflects exclusively the segregation information for the proband in the context of the family analysis.

CARRIER STATUS CONFIRMED

Likely pathogenic variant identified

INTERPRETATION

A heterozygous likely pathogenic variant was identified in the *WRN* gene. Considering the recessive mode of inheritance of the *WRN*-related Werner syndrome, **the proband is not at risk of developing the phenotype. The proband is a carrier of the *WRN* variant.**

This result, together with the result of the proband´s partner, confirms homozygosity of the detected variant in their child (index patient). The proband and the partner have a 25% chance of having a child affected by Werner syndrome.

As a secondary (incidental) finding, a heterozygous pathogenic variant was identified in the *BRCA1* gene. **This result is consistent with an increased genetic susceptibility to the development of *BRCA1*-related malignancies.**

RECOMMENDATIONS

- Genetic counselling and oncogenic surveillance are recommended.

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com

RESULT SUMMARY

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
WRN	NM_000553.5:c.1822C>T	p.(Gln608*)	N/A	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: Disease causing Conservation_nt: high Conservation_aa: N/A	gnomAD: - ESP: - 1000 G: - CentoMD®: -	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.

VARIANT INTERPRETATION

WRN, c.1822C>T p.(Gln608*)

The *WRN* variant c.1822C>T p.(Gln608*) creates a premature stop codon. It is classified as likely pathogenic (class 2) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *WRN* gene are associated with Werner syndrome (WRN), a rare autosomal recessive disorder (OMIM®: 277700).

SECONDARY (INCIDENTAL) FINDINGS

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BRCA1	NM_007300.3:c.3627dup	p.(Glu1210Argfs*9)	rs80357729	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: Conservation_nt: N/A Conservation_aa: Conservation_aa: N/A	gnomAD: 0.0000080 ESP: - 1000 G: 0.0000081 CentoMD®: 0.000018	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.

VARIANT INTERPRETATION

BRCA1, c.3627dup p.(Glu1210Argfs*9)

The *BRCA1* variant c.3627dup p.(Glu1210Argfs*9) creates a shift in the reading frame starting at codon 1210. The new reading frame ends in a stop codon 8 positions downstream. According to HGMD Professional 2020.3, this variant has previously been described as disease causing for Breast cancer by Kim et al., 2006 (PMID: 16949048), Schneegans et al., 2012 (PMID: 22160602), George et al., 2013 (PMID: 23633455). ClinVar lists this variant as pathogenic (clinical testing/research, Variation ID: 37534). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com

Pathogenic germline variants in the *BRCA1* gene are associated with familial breast-ovarian cancer type 1, also known as hereditary breast and ovarian cancer syndrome (HBOC), an autosomal dominant disorder. It is characterized with an increased lifetime risk for breast cancer (46%-87%), ovarian cancer (39%-63%), prostate cancer (9%), and pancreatic cancer (1%-3%), and possibly also melanoma. Breast cancer is one of the most common forms of cancer, accounting for about 25% of all cancers in women. It is 100 times more common in women than in men, although men tend to have poorer outcomes due to delays in diagnosis. About 5 to 10% of all breast cancers are inherited, and most of them are associated with *BRCA1* and *BRCA2* genes. *BRCA1/BRCA2* germline mutations might also have implications in cancer therapy which should be discussed with the oncologist.

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 libraries are generated by PCR-mediated addition of Illumina compatible adapters. The libraries are paired end sequenced on an Illumina platform to yield an average coverage depth of ~30x. An in-house bioinformatics pipeline including read alignment to GRCh37/hg19 genome assembly, variant calling and annotation is used. Structural variant (SV) calling is based on the DRAGEN pipeline from Illumina. 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 considered. While the evaluation is focused on coding exons and flanking +/-20 intronic bases, the complete gene region is interrogated for candidate variants with plausible association to the phenotype. All potential modes of inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and causality. Variants are categorized into five classes (pathogenic; likely pathogenic; VUS; likely benign; benign). All variants related to the phenotype of the patient are reported. SVs of unknown significance are not reported. 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.

For the mitochondrial genome, sequence reads are aligned to the Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920) and variant calling is performed using validated in-house software. The pipeline confidently detects heteroplasmy levels down to 15%. Structural variant (SV) calling is based on the DRAGEN pipeline from Illumina. All identified variants are evaluated with respect to their pathogenicity and causality. Variants are categorized into five classes (pathogenic; likely pathogenic; VUS; likely benign; benign). All 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 quality are confirmed by orthogonal methods. Consequently, a specificity of >99.9% for all reported variants is warranted.

ANALYSIS STATISTICS

CentoGenome® Trio (including mitochondrial genome analysis)

Targeted nucleotides covered	≥ 10x	99.61%

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 information is inaccurate and/or incomplete. If the obtained genetic results do not concur with the clinical findings, additional testing should be considered.

Due to technical limitations, repeat expansions cannot be assessed with the applied method. 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.

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com

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.

If consent is provided, in line with ACMG recommendations for reporting of secondary (incidental) findings in clinical exome and genome sequencing (Genetics in Medicine, 2021; PMID: 34012068), we report secondary (incidental) findings, i.e. pathogenic variants (class 1) and likely pathogenic variants (class 2) in the recommended genes for the indicated phenotypes.

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.

COPYRIGHT NOTICE

Prof Dr XXX, MD
Chief Genomic Officer
Human Geneticist

Dr XXX, MD
Senior Medical Director
Human Geneticist

Dr XXX, PhD
Clinical Scientist