CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE Am Strande 7 • 18055 Rostock • Germany

XXX

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

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

Test(s) requested: *BRCA1, BRCA2* panel

CLINICAL INFORMATION

Breast carcinoma; Family history of cancer
(Clinical information indicated above follows HPO nomenclature.)

Diagnosed condition(s): locally advanced breast cancer (triple negative).

Family history: Yes.
Siblings affected.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A heterozygous pathogenic variant was identified in the *BRCA1* gene.

A genetic diagnosis of autosomal dominant hereditary breast and ovarian cancer syndrome type 1 is confirmed.

No other clinically relevant variant was identified by sequencing analysis of the *BRCA1* and *BRCA2* gene.

RECOMMENDATIONS

- Genetic counselling and oncology counselling are recommended for the index patient, as well as for first-degree family members (**especially affected siblings**).

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RESULT SUMMARY

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BRCA1	NM_007300.3:c.4587G>A	p.(Trp1529*)	rs80356885	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: - Conservation_nt: no Conservation_aa: N/A	gnomAD: - ESP: - 1000 G: 0.00028 CentoMD: 0.00023	Nonsense Pathogenic (class 1)

Variant annotation based on OTFA (using VEP v93). * 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.4587G>A p.(Trp1529*)

The *BRCA1* variant c.4587G>A p.(Trp1529*) creates a premature stop codon. According to HGMD Professional 2019.4, this variant has previously been described as disease causing for Breast and/or ovarian cancer by Laitman et al., 2011 (PMID: 20960228), Walsh et al., 2011 (PMID: 22006311), Bayraktar et al., 2012 (PMID: 22009639). ClinVar lists this variant as pathogenic (clinical testing/research, Variation ID: 55221). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

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/gynecologist.

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).

METHODS

Genomic DNA is enzymatically fragmented and regions of interest are selectively enriched using capture probes targeted against coding regions of selected gene/s. Libraries are generated with Illumina compatible adaptors and sequenced on an Illumina platform.
Evaluation is focused on coding exons along with flanking +/-10 intronic bases of the *BRCA1, BRCA2* gene/s. Raw sequence data analysis, including base calling, demultiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37) and variant calling (single nucleotide variants, InDels and copy number variations (CNVs)) is performed using validated in-house software. Relevant variants reported in HGMD®, in ClinVar or in CentoMD® as well as all variants with minor allele frequency (MAF) of less than 1% in gnomAD database are considered. All pertinent inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate eventually identified variants. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Low quality variants are confirmed by Sanger sequencing. As a result, we warrant specificity of >99.9% for all reported variants.

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ANALYSIS STATISTICS

***BRCA1, BRCA2* panel**

Targeted nucleotides covered	≥ 20x	99.67%

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, and high-CG-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 exons-intron boundaries are not considered for aberrant splicing analysis. However, pathogenic splicing variants evidenced by external sources will be reported.

ADDITIONAL INFORMATION

This test was developed and its performance validated by CENTOGENE GmbH. 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 also 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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Signature **Signature**

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