CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE • Am Strande 7 • 18055 Rostock • Germany

Order no.: **documented by:**
Order received: DD/MM/YYYY
Sample type: blood, CentoCard®
Sample collection date: DD/MM/YYYY
Report date: DD/MM/YYYY
Report type: Final Report

Patient no.: , First Name: , Last Name:
DOB: **DD/MM/YYYY**, Sex: , Your ref.:

Additional report recipient(s):

Test(s) requested: CentoBreast® (sequencing including NGS-based CNV analysis)

CLINICAL INFORMATION

Breast carcinoma; Ductal carcinoma in situ
(Clinical information indicated above follows HPO nomenclature.)

Age of onset: 58 year(s). Diagnosed condition(s): invasive ductal carcinoma. Previous external genetic testing, results negative: immunohistochemistry right breast (PR, HER2). Previous external genetic testing, results positive: immunohistochemistry right breast (ER), immunohistochemistry left breast (ER, PR, HER2).

Family history: Yes.
Father : Brain neoplasm; Maternal aunt 1: Breast carcinoma; Maternal aunt 2: Uterine neoplasm; Maternal male cousin : Neoplasm of the lung; Niece : Breast carcinoma; Paternal aunt : Breast carcinoma; Paternal female cousin 1: Breast carcinoma; Paternal female cousin 2: Breast carcinoma; Paternal female cousin 3: Breast carcinoma; Paternal male cousin : Sudden death; Paternal uncle : Neoplasm of the lung; Sister : Neoplasm
Consanguineous parents: Yes, first cousins.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A heterozygous pathogenic variant was identified in the *BRCA2* gene. **This finding is consistent with the increased genetic risk to autosomal dominant familial breast-ovarian cancer type 2 associated cancers.**

No further clinically relevant variants were detected.

RECOMMENDATIONS

- If possible, parental targeted testing is recommended as establishing the origin of the variant, inherited or de novo, is important for familial genetic counselling. Additionally, targeted testing for all affected and adult at-risk family members, if any, is recommended.
- Genetic and oncological counselling is recommended.

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RESULT SUMMARY

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BRCA2	NM_000059.3:c.755_758del	p.(Asp252Valfs*24)	N/A	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: 0.0000081 ESP: - 1000 G: 0.000022 CentoMD: 0.000018	Frameshift 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

BRCA2, c.755_758del p.(Asp252Valfs*24)

The *BRCA2* variant c.755_758del p.(Asp252Valfs*24) creates a shift in the reading frame starting at codon 252. The new reading frame ends in a stop codon 23 positions downstream. According to HGMD Professional 2019.4, this variant has previously been described as disease causing for Breast cancer by Tavtigian et al., 1996 (PMID: 8589730), Schrader et al., 2016 (PMID: 26556299), Park et al., 2017 (PMID: 28111427). ClinVar lists this variant as pathogenic (clinical testing/research, Variation ID: 38103). 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 *BRCA2* gene are associated with familial breast-ovarian cancer type 2, also known as hereditary breast and ovarian cancer syndrome (HBOC), an autosomal dominant disorder. It is characterized with an increased life-time risk for breast cancer (38%-84%), ovarian cancer (16.5%-27%), prostate cancer (15%), and pancreatic cancer (2%-7%), 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 (OMIM®:612555).

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 regions of interest are enriched using DNA capture probes. The final indexed libraries are sequenced on an Illumina platform. For the CentoBreast® (sequencing including NGS-based CNV analysis), the coding regions of the panel genes, 10 bp of flanking intronic sequences, and known pathogenic/likely pathogenic variants within these genes included in the enrichment design (coding and non-coding), are targeted for analysis. The panel gene list can be obtained in the appendix of this report or at www.centogene.com/ngspanels-medical-reporting as part of our panel portfolio (please contact CENTOGENE customer support if the gene list has been updated after this report was issued). Data analysis, including alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37), variant calling, and annotation is performed using validated in-house software. All identified variants are evaluated with respect to their pathogenicity and disease causality, and are categorized into five classes (pathogenic, likely pathogenic, VUS, likely benign, and benign) along ACMG guidelines for classification of variants. All potentially clinically relevant variants that may explain or contribute to the phenotype are reported. VUSs are not reported in the following cases: the described phenotype(s) is already explained by a detected pathogenic or likely pathogenic variant(s); the detected VUSs are not related to the described phenotype(s); lack of clinical information; for oncogenetic panels. 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. The copy number variation (CNV) detection software has a sensitivity of more than 95% for all homozygous/hemizygous deletions, as well as heterozygous deletions/duplications and homozygous/hemizygous duplications spanning at least three consecutive exons.

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ANALYSIS STATISTICS

CentoBreast® (sequencing including NGS-based CNV analysis)

Targeted nucleotides covered	≥ 20x	99.66%

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 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.
More complex genetic events such as inversions, translocations, and repeat expansions, 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 (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. 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 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.

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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Chief Genomic Officer **Human Geneticist**	**Human Geneticist**	**Clinical Scientist**

APPENDIX

CentoBreast® (sequencing including NGS-based CNV analysis)

ABRAXAS1, ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER1, EPCAM, FANCC, MEN1, MLH1, MRE11, MSH2, MSH6, MUTYH, NBN, PALB2, PMS1, PMS2, PTEN, RAD50, RAD51C, RAD51D, RECQL, SMARCA4, STK11, TP53, XRCC2

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