xxx

Order no.: xxx
Order received: xxx
Sample type: blood, filter card
Sample collection date: xxx
Report date: xxx
Report type: Final Report

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

Test(s) requested: CentoCancer® panel (sequencing)

CLINICAL INFORMATION

Patient with breast cancer. Positive family history of breast cancer.

UNCLEAR RESULT
Variants of uncertain significance (VUS) identified

INTERPRETATION

A heterozygous variant of uncertain significance was identified in the *BRCA2* gene, and a heterozygous variant of uncertain significance was identified in the *PALB2* gene.

Thus, a genetic diagnosis of susceptibility to hereditary breast cancer is possible.

RECOMMENDATIONS

- Parental carrier testing is recommended in the first step to establish whether the variants were inherited or of *de novo* nature. If inherited, segregation analysis in additional relevant family members (affected and unaffected in an appropriate age range) is recommended to obtain further information on the variants' clinical significance.
- Onco-genetic counselling is recommended to explain and discuss the results.

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

RESULT SUMMARY

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BRCA2	Chr13(GRCh37):g.32944589C>G NM_000059.3:c.8382C>G p.(Phe2794Leu) Exon 19	Het	PolyPhen: Probably damaging Align-GVGD: C0 SIFT: Deleterious MutationTaster: Disease causing Conservation: nt weak/aa high	gnomAD: - ESP: - 1000 G: - CentoMD: 0.000039	Missense Uncertain significance (class 3)
PALB2	Chr16(GRCh37):g.23634417T>G NM_024675.3:c.2869A>C p.(Lys957Gln) Exon 9	Het	PolyPhen: Possibly damaging Align-GVGD: C0 SIFT: Tolerated MutationTaster: Polymorphism Conservation: nt weak/aa moderate	gnomAD: 0.000016 ESP: - 1000 G: 0.00020 CentoMD: 0.000068	Missense Uncertain significance (class 3)

Variant description based on Alamut Batch (latest database available). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations

VARIANT INTERPRETATION

BRCA2, c.8382C>G p.(Phe2794Leu)

The *BRCA2* variant c.8382C>G p.(Phe2794Leu) causes an amino acid change from Phe to Leu at position 2794. ClinVar lists this variant as uncertain (clinical testing, Variation ID: 52570). It is classified as variant of uncertain significance (class 3) 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.

PALB2, c.2869A>C p.(Lys957Gln)

The *PALB2* variant c.2869A>C p.(Lys957Gln) causes an amino acid change from Lys to Gln at position 957. ClinVar lists this variant as uncertain (clinical testing, Variation ID: 126694). It is classified as variant of uncertain significance (class 3) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic *PALB2* variants have been described as associated to autosomal dominant susceptibility to breast cancer (OMIM® 114480), susceptibility to pancreatic cancer (OMIM® 613348) and autosomal recessive Fanconi anemia of complementation group N (OMIM® 610832).
PALB2 colocalizes with *BRCA2* in nuclear foci, promotes its localization and stability in nuclear structures, and enables its recombinational repair and checkpoint functions (PMID: 16793542). Male breast cancer has also been observed in families with molecularly confirmed *PALB2*-associated breast cancer (PMID: 21285249).
Pathogenic variants in many genes are associated with a high risk of developing familial breast cancer, an autosomal dominant disorder. Breast cancer is one of the most common form of cancers, accounting for ~23% of

all cancers in women, and it is more than 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 one or both of these following genes: *BRCA1* (BReast CAncer gene one) and *BRCA2* (BReast CAncer gene two). The penetrance of *BRCA1* and *BRCA2* germline mutations is the most significant clinical aspect of hereditary breast ovarian cancers. Mutations in dozens of other genes have been studied as possible risk factors for breast cancer. These genes are described as "low penetrance" or "moderate penetrance" because changes in each of these genes appear to make only a small or moderate contribution to overall breast cancer risk.

ANALYSIS STATISTICS

Please be aware that NGS panels do not give full coverage for all genes. An overall coverage of 99.98% was achieved, with 36 missing base pairs (coding region including +/- 10bp). At your request, it is possible to test for every fragment with missing bases.

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 panel genes. Libraries are generated with Illumina compatible adaptors and sequenced on an Illumina platform.
For the CentoCancer® panel, the entire coding region of the *APC, ATM, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, FH, FLCN, HNF1A, HNF1B, HOXB13, MC1R, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS1, PMS2, POLD1, POLE, POT1, PRSS1, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, STK11, TP53, TSC1, TSC2, VHL, WT1, XRCC2, XRCC3* genes including 10 bp of flanking intronic sequences are targeted. Due to limitations of the NGS analysis alone, the targeted region within the requested panel may not reach 100% coverage. Raw sequence data analysis, including base calling, demultiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37) and variant calling is performed using validated in-house software. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized into classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported.

ADDITIONAL INFORMATION

This test was developed and its performance validated by CENTOGENE AG. 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.

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

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xxx

Chief Scientific Officer
Human Geneticist

xxx

Clinical Scientist

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