CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany

Dr. med. John Doe
Paul-Ehrlich-Str. 23
72076 Tübingen

Patient	Doe, Jane
ID 123456	Female (*DD.MM.YYYY)
Sample receipt	DD.MM.YYYY
Material	EDTA blood
Report date	DD.MM.YYYY
Report-ID	R7891011

Genetic Report – Doe, Jane (*DD.MM.YYYY)

Indication	Suspected hereditary breast cancer, patient is affected herself
Order	Panel Diagnostics: Gynecologic Cancer (whole exome enrichment)

Result: Report with Significant Findings

- **Detection of a pathogenic variant in gene *BRCA2*, which is most likely causative for your patient's breast cancer.**
- Based upon current scientific knowledge, we did not identify any reportable copy number variants which are likely to be causative for your proband's suspected familial predisposition of breast cancer.

Gene	Variant	Zygosity	Heredity	MAF (%)	Classification
BRCA2	**c.8517C>A;p.Tyr2839*** **chr13:32945122C>A (hg19)**	**het.**	**AD, AR**	**-**	**pathogenic**

Information for the interpretation of this table can be found in section *Additional Information*.

Recommendation

We recommend further clinical management and surveillance according to the current guidelines for *BRCA2*-associated disease (Petrucelli et al., last revision: 09/2023, PMID: 20301425, GeneReviews).

It is possible to investigate further affected family members regarding the variant identified in gene *BRCA2*.

Testing of adult asymptomatic family members regarding the variant c.8517C>A;p.Tyr2839* identified in gene *BRCA2* may only be performed following genetic counseling.

Genetic Relevance

Your proband is heterozygous for a pathogenic variant in gene *BRCA2*. This may be of relevance for family planning and at-risk family members.

Individual variants have a 50% probability of being passed on to each respective offspring.

Clinical Information and Variant Interpretation

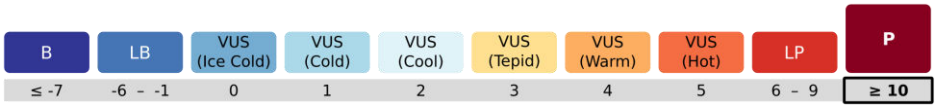
BRCA2, NM_000059.4

OMIM / Reference	Phenotype	Heredity
612555	Breast-ovarian cancer, familial, 2	AD
114480	Breast cancer, male, susceptibility to	AD
176807	Prostate cancer	AD
613347	Pancreatic cancer 2	AD
194070	Wilms tumor	AD
605724	Fanconi anemia, complementation group D1	AR

BRCA2 is a tumor suppressor gene and is involved in repairing damaged DNA (Powell and Kachnic, 2003, PMID: 12947386). Carriers of pathogenic germline variants in *BRCA2* have an increased risk of breast and ovarian carcinoma. Additionally, an increased risk of pancreatic cancer and in men of prostate and breast cancer was observed. Current studies indicate a possible increased risk of gastrointestinal tumors, especially gastric, esophageal, and colon cancer (Maccaroni et al., 2021, PMID: 34367929).

The lifetime risk for these cancers in individuals with a pathogenic variant in *BRCA2* is 45-69% for breast cancer in females, 11-17% for ovarian cancer, 27% by age of 75 years and 60% by age of 85 years for prostate cancer, 6-8% for male breast cancer and 3-5% by age of 70 years for pancreatic cancer. Additionally, carriers of pathogenic *BRCA2* variants may have an increased risk of developing other cancers (Petrucelli et al., updated 09/2023, PMID: 20301425, GeneReviews). Pathogenic biallelic *BRCA2* variants are the cause of Fanconi anemia in complement group D1 (Alter et al., 2007, PMID: 16825431). Patients with Fanconi anemia in complementation group D1 can develop various tumors in early childhood (Alter et al., 2007, PMID: 16825431; Degrolard-Courcet et al., 2014, PMID: 24301060; Trejo Bittar et al., 2014, PMID: 24735155; Malric et al., 2015, PMID: 25381700).

BRCA2, c.8517C>A;p.Tyr2839* (het.), ClinVar ID: 267094

ACMG/ACGS Criterion	Points	Description
PVS1	+8	The variant likely results in a loss (or truncation) of the protein, which is a known pathomechanism for *BRCA2*-associated disease.
PS4 (supporting)	+1	The variant was detected in one, or several, further affected unrelated individuals who show a phenotype consistent with the associated disease. Rebbeck et al., 2018, PMID: 29446198; Yang et al., 2015, PMID: 25927356
PM2	+2	This variant is absent from the gnomAD global population dataset.
ACMG/ACGS Classification: pathogenic	+11	

Genetic counseling should be offered with all diagnostic genetic testing, especially following the identification of the molecular cause of a genetic disease.

Medical report written by:

Proofread by:

Validated by:

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup

Consultant for Human Genetics

Additional Information

Requested Regions

The whole exome of the individual above was targeted and sequenced. Analysis was restricted to the following gene regions:

ATM, ***BARD1***, ***BRCA1***, ***BRCA2***, ***BRIP1***, ***CDH1***, ***CHEK2***, ***EPCAM***, ***MLH1***, ***MSH2***, ***MSH6***, ***NF1***, ***PALB2***, ***PMS2***, ***POLD1***, ***PTEN***, ***RAD51C***, ***RAD51D***, ***STK11***, ***TP53*** (Gynecologic Cancer)

General Remarks

Additional variants may be present within regions which were not analyzed (e.g. introns, promoter and enhancer regions and long repeats). A lower specificity enrichment and/or inaccurate variant calling cannot be ruled out for homologous regions with multiple genomic copies. The occurrence of low frequency somatic mosaicism cannot be reliably assessed using a pipeline optimized for germline variant detection, and may therefore remain undetected. Moreover, detection of large deletions and duplications is not guaranteed by next-generation high-throughput sequencing. The classification of variants may change in the future due to new evidence or improvements in scientific understanding.

Information for the interpretation of the tables

Heredity: AD – autosomal dominant, AR – autosomal recessive, XL – X-linked, mito – mitochondrial

MAF: The ***minor allele frequency*** describes the least frequent allele at a specific locus in a given population. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

Classification: Variant classification is based on ACMG, ACGS-2020v4.01, and ClinGen SVI WG guidelines (Richards et al., 2015, PMID: 25741868; Ellard et al., 2020, Association for Clinical Genomic Science; https://clinicalgenome.org/working-groups/sequence-variant-interpretation/). The weighting of criteria and their modification follows the current ACGS guidelines in the strength levels *very strong* (+ 8), *strong* (+/- 4), *moderate* (+/- 2), and *supporting* (+/- 1). According to the respective category (pathogenic or benign) and criterion strength, positive or negative points are assigned as mentioned above (Tavtigian et al., 2020, PMID: 32720330). Variants of uncertain significance (VUS) are subcategorized into *hot*, *warm*, *tepid*, *cool*, *cold*, and *ice cold* VUS according to their likelihood of reaching a pathogenic classification in the future. Posterior probability decreases from 90% to 10% in this order (Ellard et al., 2020, Association for Clinical Genomic Science). If a variant reaches the classification pathogenic, after checking of all benign criteria, not necessarily all other applicable criteria are listed.

The chromosomal positions of variants listed in the report refer to the human reference genome hg19.

Methods

Sequencing: Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq 6000/NovaSeq X Plus system.

NGS based CNV-Calling: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth (only applicable for nuclear encoded genes). Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. *CNV calling* was performed by computing the sample's normalized coverage profile and its deviation from the expected

coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage. Copy number variants are named according to current ISCN guidelines. NGS based CNV-Calling will not detect copy number neutral structural variants such as balanced translocations, inversions, uniparental heterodisomy or low-level mosaicism. Aberrations within the pseudoautosomal region (PAR) cannot be detected with high accuracy. The integration site of duplications cannot be determined by NGS based CNV-Calling.

Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g. MLPA. Copy-neutral structural aberrations cannot be detected using this method (e.g. balanced translocations and balanced inversions). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

Computational Analysis: Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). The variants were annotated based on several internal as well as external databases.

Diagnostic data analysis: Variants were classified and reported based on ACMG/ACGS-2020v4.01 guidelines (Richards et al., 2015, PMID: 25741868, Ellard et al., 2020, Association for Clinical Genomic Science).

Only variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Minor allele frequencies are taken from public databases (e.g. gnomAD) and an in-house database. If an acceptable sequencing-depth per base is not achieved by high-throughput sequencing, our quality guidelines demand local re-sequencing using classical Sanger-technology. Candidate CNV calls are evaluated manually. Potentially pathogenic findings are validated with a second method, like MLPA, on a case-by-case basis.

In this case, 97.63% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base. **The evaluation of variants is dependent on available clinical information at the time of analysis.** The medical report contains all variants not classified as uncertain, benign or likely benign according to current literature. Synonymous variants in mitochondrially encoded genes are classified as benign. In silico predictions were performed using the programs MetaLR (Dong et al., 2015, PMID: 25552646), PrimateAI (Sundaram et al., 2018, PMID: 30038395), and SpliceAI (Jaganathan et al., 2019, PMID: 30661751). This prediction can be complemented with additional *in silico* predictions in individual cases.

Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT).