CeGaT
Center for Genomics
and Transcriptomics

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

Mr.
Xxxxxx, MD

Name	Xxxxx
Forename	Xxxxx
Date of birth	XX.XX.XXXX
Sex	XXX
CeGaT-Pat. No.	XXXXX
Sample receipt	XX.XX.XXXX
Material	EDTA blood
Report date	XX.XX.XXXX

E-Mail: xxx

Genetic analysis report –Xxxxx, Xxxxx | XX.XX.XXXX

Indication Hereditary breast- and ovarian cancer, patient affected by breast cancer.
Order *BRCA1* and *BRCA2* sequencing and deletion/duplication analysis

Dear Dr. Xxxxx,

Thank you for requesting a molecular genetic analysis.

RESULTS:

- **Detection of a mutation in gene *BRCA1*, which is most likely causative for your patient's disease.**

Gene	Variant	Zygosity	Heredity	MAF (%)	*in silico* Prediction	Classification
BRCA1	**c.1674delA; p.Gly559Val*fs**13**	**het.**	**AD, AR**	**-**	**-**	**pathogenic**

Information for table interpretation:
AD: Describes a trait or disorder requiring only one copy of a gene mutation at a particular locus in order to express an observable phenotype. Single heterozygous gene variants can only be causative for the phenotype if the disorder follows an **autosomal dominant** mode of inheritance.
AR: Describes a trait or disorder requiring the presence of two copies of a gene mutation at a particular locus in order to express an observable phenotype. In **autosomal recessive** disorders, a single heterozygous variant in one gene cannot by itself be causative for the observable phenotype.
XL: X-linked mode of inheritance
MAF: The **minor allele frequency** describes the least frequent allele at a specific locus in a given population. The less frequently a variant occurs, the more likely it is pathogenic; however, the prevalence and mode of inheritance of any particular disease must be considered.
***in silico* Prediction:** The ACMG (American College of Medical Genetics) guidelines recommend using several prediction programs to assess the possible pathogenicity of variants of unknown clinical significance (VUS). Each program calculates its predictions based upon different criteria, and the correspondence between a prediction and the actual functional effect of a variant ranges between 60-80 %. **These predictions may therefore not serve as the sole basis for the evaluation of pathogenicity.**
Classification: Refers to the possible pathogenicity of a variant, but does not necessarily provide clear evidence of clinical significance. Variants are evaluated based upon current data and specific criteria, and are then classified as follows: known pathogenic, probably pathogenic, variant of unknown clinical significance, or probably benign. **All variants for which clinical relevance cannot be conclusively confirmed or excluded are referred as variants of unknown clinical significance.**

INTERPRETATION:

***BRCA1*, c.1674delA; p.Gly559Val*fs**13 (het.), NM_007294.3, rs80357600:**

OMIM / Reference	Phenotype	Heredity
604370	Breast and ovarian cancer, familial, 1	AD
614320	Pancreatic cancer, susceptibility to	AD
Sawyer; PMID 25472942	Fanconi anemia, complementation group S	AR

BRCA1 is a tumor suppressor gene and is involved in repair of damaged DNA, therefore, it plays an important role in ensuring the stability of the cell's genetic material (Wu et al., 2010; PMID 21203981). Carriers of pathogenic germ line mutations in *BRCA1* 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. The lifetime risk for these cancers in individuals with a pathogenic variant in *BRCA1* is 50-80% for breast cancer among females, 24-40% for ovarian cancer, up to 30% for prostate cancer, 1-2% for breast cancer in men and 1-3% for pancreatic cancer (GeneReviews).

The identified mutation creates a shift in the reading frame, which will result in either a truncated protein or a nonsense-mediated decay of mRNA. This mutation was previously found in families with hereditary breast and ovarian cancer and evaluated as pathogenic (CLINVAR, June 2016).

Based upon available data, the detected mutation is most likely causative for the phenotype observed in your patient.

Mutations may also be present within regions which were not analyzed (e.g. introns, promoter and enhancer regions and long repeats). Due to the rapid growth of scientific knowledge, the interpretation of variant pathogenicity may change in the future.

CLINICAL SIGNIFICANCE:

Individual variants have a probability of 50 % to be passed on to each offspring, respectively.
Your patient is a carrier of a mutation in the *BRCA1* gene, which can be relevant for at-risk family members.

RECOMMENDATION:

For carriers of a pathogenic mutation in the gene *BRCA1* there is an increased risk to develop further tumors. We recommend intensive preventive-check up examinations.

BRCA1 deficient tumor cells are sensitive to DNA damaging drugs like Cisplatin and Carboplatin (Robson, 2011, PMID: 21900108). These tumors are also sensitive to PARP inhibitors. Some of these drugs are tested in clinical trials (Leung et al., 2011, PMID: 21424107; Ledermann et al., 2014, PMID: 24882434).

Lynparza (Olaparib) is approved in Europe as a monotherapy for the maintenance treatment of adult patients with platinum-sensitive relapsed *BRCA*-mutated (germline and/or somatic) high grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in response (complete response or partial response) to platinum-based chemotherapy (European Medical Agency, WC500176336-1). Clinical studies of PARP inhibitors in breast cancer are ongoing (e. g. NCT02163694).

These results should be communicated by a human geneticist or by a genetic counselor. If you have any further questions please do not hesitate to contact us at any time.

CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany
Tel: + 49 7071 565 44 55 | Fax: + 49 7071 565 44 56 | info@cegat.de | www.cegat.de
Court District Stuttgart - HRB 729958 | VAT No: DE265504070
Volksbank Tübingen | IBAN: DE14 6419 0110 0021 2890 00 | SWIFT / BIC: GENODES1TUE
Managing Directors: Dr. Dr. Saskia Biskup, Dr. Dirk Biskup

With kind regards

Dr. med. Dr. rer. nat. Saskia Biskup	Dr. med. Dennis Döcker	Dr. biol. hum. Antje Rinckleb	PD Dr. rer. nat. Sorin Armeanu-Ebinger
Consultant for Human Genetics	Diagnostics	Diagnostics	Diagnostics

ADDITIONAL INFORMATION:

Requested Genes ***BRCA1*** (NM_007294.3, incl. Del/Dup)***, BRCA2*** (NM_000059.3, incl. Del/Dup)

Methods

Sequencing: The coding and flanking intronic regions were amplified using Multiplex-PCR and were sequenced using the Illumina HiSeq2500/4000 system.

Copy Number Analysis: Deletion and duplication analysis of the genes *BRCA1*, *BRCA2* was performed using MLPA (MRC Holland P002-D1-V02, and P090-B1-V19) as relative quantification in comparison to reference sample DNA.

Computational Analysis: Illumina CASAVA 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. Variants were called using samtools and varscan. Technical artifacts were removed and the remaining variants were annotated based on several internal as well as external databases.

Diagnostic data analysis: 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 the following databases: 1000Genomes, dbSNP, Exome Variant Server, ExAC 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.

The medical report contains all variants with a MAF < 1 % (in genes with autosomal recessive heredity) or < 0.1 % (in genes with dominant heredity), not including variants classified as benign according to current literature. In silico prediction of variants listed in the chart above is calculated on the basis of the output of the programs Mutation Taster, fathmm, Mutation Assessor, SIFT, fathmm-MKL coding, LRT, and PROVEAN according to the following criteria: 100 % consensus = pathogenic/benign, ≥ 75 % consensus = mostly pathogenic/benign, consensus < 75 % or no prediction possible = inconsistent. For assessing the consequences of variants on splicing, two algorithms are applied (Jian et al., 2014, PMID: 25416802) which are complemented with additional in silico predictions in individual cases.

A list of all analyzed variants is available upon request. 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).