CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE AG Am Strande 7 • 18055 Rostock • Germany

Dr. Gulshan Yadav
Molecular Quest Healthcare
Laboratory
28-29, Sector 18, Electronic City Udyog Vihar
122001 Gurgaon
India

Order no.: 62460138
Order received: 09 Apr. 2018
Sample type: blood, filter card
Sample collection date: 03 Apr. 2018
Report date: 05 May 2018
Report type: Final Report

Patient no.: **1276988**, First Name: **Priyanka**, Last Name: **Garg**
DOB: **06 Dec. 1985**, Sex: **female**, Your ref.: **-**

Test(s) requested: Clinical Exome Sequencing (CentoDX™)

CLINICAL INFORMATION

The patient has been diagnosed with triple negative breast cancer at the age of 32 years. Her mother (deceased) was also diagnosed with triple negative breast cancer. No other family members are affected. Specific focus has been requested on all genes related to breast and ovarian cancers (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CHEK2, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS1, PMS2, RAD50, RAD51C, RAD51D, XRCC2*).

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A pathogenic variant was identified in the *TP53* gene. This gene is associated with germline and somatic forms of cancer. Given the fact that the mother of this patient was also affected, we consider that **the result is consistent with the genetic diagnosis of autosomal dominant hereditary breast cancer associated to *TP53*.**

RECOMMENDATIONS

- Oncologic evaluation of the patient and other relatives at risk is recommended, as the gene has been associated with different types of tumors, including Li-Fraumeni syndrome.
- Genetic counselling is recommended.

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

RESULT SUMMARY

GENE	VARIANT COORDINATES	ZYGOSITY	*IN SILICO* PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
TP53	Chr17(GRCh37):g.7578406C>T NM_000546.4:c.524G>A p.(Arg175His) Exon 5	Het	PolyPhen: Possibly damaging Align-GVGD: C25 SIFT: Deleterious MutationTaster: Disease causing Conservation: nt high/aa high	gnomAD: 0.0000040 ESP: - 1000 G: - CentoMD: 0.000050	Missense Pathogenic (class 1)

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

Based on the clinical information provided, we have paid specific attention to the following genes: *BRCA1, BRCA2, ATM, BARD1, BRIP1, CHEK2, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS1, PMS2, RAD50, RAD51C, RAD51D, XRCC2*. We did not detect any relevant variant in these genes. However, pathogenic variants cannot be completely excluded since not all exons were fully covered due to limitations of the method. For these genes, an overall coverage of 98.54% was achieved (>20x), with 1067 missing base pairs (coding region including +/- 10bp). Note that clinical exome sequencing for diagnostic purposes does not provide full coverage for all genes and cannot detect large deletions/duplications. If needed, it is possible to test for every single gene that might likely explain the given phenotype.

VARIANT INTERPRETATION

TP53, c.524G>A p.(Arg175His)

The *TP53* variant c.524G>A p.(Arg175His) causes an amino acid change from Arg to His at position 175. This variant has been confirmed by Sanger sequencing. According to HGMD Professional 2018.1, this variant has previously been described as disease causing for osteosarcoma by McIntyre *et al*., 1994 (PMID: 8164043) and other authors have associated it with further types of cancer, including breast cancer (Melhem-Bertrandt *et al*., 2012 - PMID: 21761402) and Li-Fraumeni syndrome (Hwang *et al*., 2008 - PMID: 19127115) . ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 12374). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic variants in the *TP53* gene are associated with autosomal dominant forms of hereditary cancer, such as breast cancer, colorrectal cancer, and Li-Fraumeni syndrome. Li-Fraumeni syndrome is characterized by early onset of tumors, multiple tumors within an individual, and multiple affected family members. The *TP53* gene is also associated with somatic cancer, such as hepatocellular carcinoma, pancreatic cancer or susceptibility to glioma (OMIM®: 191170).

INCIDENTAL FINDINGS

We did not detect any class 1 or 2 variants in the genes for which incidental findings are reported based on the ACMG guidelines, apart from the *TP53* variant mentioned above.

ANALYSIS STATISTICS for the offered genes

AVERAGE COVERAGE (X)	% TARGET BP COVERED					
	0X	≥ 1X	≥ 5X	≥ 10X	≥ 20X	≥ 50X
190.35	0.33	99.67	99.41	99.20	98.53	93.20

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

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 ~6700 genes with known clinical significance. 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 within the captured region. Due to limitations of the method, the target region is not covered 100%. 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. 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. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized in classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported. Any relevant variant(s) identified by NGS is(are) Sanger sequenced to exclude NGS artefacts. In case, Sanger confirmation for the reported variant is still ongoing, we will only contact you if the results are inconsistent.
Exon 5 of the *TP53* gene was analysed by PCR and sequencing of both DNA strands of the entire coding region and the highly conserved exon-intron splice junctions. The reference sequence is: NM_000546.4.

LIMITATIONS

Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical findings, additional testing should be considered.
Due to limited read length and other contributing technical limitations, repeat expansions (i.e. in the Huntington gene, the SCA-genes, the myotonic dystrophy repeat region, and other similar regions) cannot be assessed with the applied method. Of note, CNV calls from Whole Genome Sequencing have a limited accuracy and sensitivity, and structural changes below 2 kb at a genome-wide level are not called by our pipeline.

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.

In line with ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (Genetics in Medicine, 2016), we report incidental findings, i.e. pathogenic variants (class 1) and likely pathogenic variants (class 2) only in the recommended genes for the recommended phenotypes.

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 (dmqc@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.

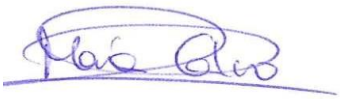
COPYRIGHT NOTICE

This document contains information from the Online Mendelian Inheritance in Man® (OMIM®) database, which has been obtained under a

> Contact Details
Tel.: +49 (0)381 80113416
Fax: +49 (0)381 80113 401
dmqc@centogene.com
www.centogene.com

Prof. Dr. Peter Bauer, MD

Chief Scientific Officer
Human Geneticist



Dr. Maria Calvo, PhD

Clinical Scientist

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