Patient Name	Mrs. Indu Andelkar	Ref. Doctor	Self
Age/Gender	59Y / FEMALE	-	Sage Path Labs Pvt Ltd.
Barcode	23765224	Sample Type	Whole Blood EDTA

Clinical History:

Mrs. Indu Andelkar was diagnosed with high-grade serous adenocarcinoma of the right ovary. A multinodular cystic lesion was observed in the pelvis and lower abdomen in the midline anterior to the uterus and superior to the urinary bladder with signs of cystic neoplasm of ovarian origin. ?Mucinous cystadenocarcinoma. Bilateral salpingo-oophorectomy omentectomy was performed. She also had a history of left breast cancer and hence referred for *BRCA1* and 2 germline sequencing.

Results: Heterozygous Likely Pathogenic (LP) has been identified in the *BRCA1* gene.

Variants in genes known to be associated with the provided phenotype

Gene and Transcript	Exon	Variant Nomenclature	Zygosity	Classification	Disease	Inheritance
BRCA1 NM_007294.4	14	c.4654del, p.Tyr1552Thrfs*7 chr17:43074351TA>T	Heterozygous	Likely Pathogenic (LP)	Susceptibility to familial breast-ovarian cancer-1 (OMIM# 604370)	Autosomal Dominant

Variant Interpretation:

The exome data analysis identified a heterozygous frameshift deletion **c.4654del, p.Tyr1552Thrfs*7 (chr17:43074351TA>T)** which causes termination 7 amino acids downstream at position 1552 in ***BRCA1*** gene. The observed variant was not reported in the gnomAD database. The p.Tyr1552fs variant was reported as pathogenic in ClinVar [1437343]. This frameshift variant is predicted to result in protein truncation, in a gene for which loss-of-function is a known mechanism of disease. Hence the observed variant has been classified as a **Likely Pathogenic (P).**

References:

1. Claus, E. B., Risch, N., Thompson, W. D. Genetic analysis of breast cancer in the cancer and steroid hormone study. Am. J. Hum. Genet. 48: 232-242, 1991.[PubMed: 1990835]
2. Hall, J. M., *et al.,* Closing in on a breast cancer gene on chromosome 17q. Am. J. Hum. Genet. 50: 1235-1242, 1992.[PubMed: 1598904]

Recommendations:

- Genetic counseling and clinical correlation are recommended for accurate test results interpretation.
- If the above result does not correlate completely with the patient phenotype, additional testing is advised based on the clinician's discretion.

- Based on genetic testing on the parents and other family members, the significance/ classification of the variant(s) may alter.

Test Information

The total genomic DNA was extracted from the biological sample using the column-based method and DNA quality and quantity were assessed using electrophoretic and Qubit methods. The QC-qualified genomic DNAwas randomly fragmented and ligating sequencing adapters were added to both ends of DNA fragments. Sequencing libraries were size-selected using beads to optimal template size and amplified by polymerase chain reaction. The regions of interest (exons and flanking intronic targets) are targeted by a hybridization-based target capture method. Sequencing libraries that passed the quality control were sequenced on the MGI platform using paired-end chemistry. Reads were assembled and are aligned to reference sequences based on NCBI Ref Seq transcripts and human genome build GRCh38. Data was filtered and analyzed to identify variants of interest related to patients' clinical phenotype.

Tools and databases used for data analysis:

We followed the Genome Analysis Toolkit (GATK) best practices framework for the identification of variants in the sample. The sequences obtained were subjected to quality assessment and pre-processing. The pre-processed sequences were aligned with human reference genome sequence (assembly GRch38) by Burrows-Wheeler Aligner and post-alignment processing like read duplicate removal and base quality score recalibration (BQSR) was carried out by using GATK (v4.2.5.0). Variant calling was done by using the GATK Haplotype Caller. Each called variant is annotated using different clinical and population databases. Common variants were filtered out based on minor allele frequency (MAF) in 1000Genome Phase 3[4], gnomAD (v3), ExAC [3], and dbSNP (v155). Non-synonymous variants effect is calculated using multiple in-silico algorithms. Only non-synonymous and splice site variants with clinical relevance were selected using published literature and a set of disease databases -ClinVar, OMIM, GWAS, and SwissVar. The classification of the variant is done based on American College of Medical Genetics guidelines.

QC METRICS

Total data generated	5.72 Gb
Total reads aligned reads (%)	92.85%
Data ≥Q30(%)	90.67%
Total data which passed mapping quality cut-off	5.18 Gb

Variant or Mutation	The change(s) in a gene. This could be disease-causing (pathogenic) or non-disease-causing (benign).
Pathogenic	A disease-causing mutation in a gene has been identified, which may explain or correlate with the patient's symptoms. This usually denotes the confirmation of a suspected condition for which testing was requested.
Likely Pathogenic	A variant that is very likely to play a role in the development of disease, but currently scientific evidence is inadequate, additional evidence in the future may declare the pathogenicity of this variant.
Variant of Uncertain Significance	A variant has been identified, but existing scientific information makes it impossible to define as pathogenic (disease-causing) or benign (non-disease-causing) and further required functional studies. The clinician may recommend additional tests for the patient or family members. It is likely that their relevance will only be determined over time, depending on the availability of scientific information.

Test Limitations:

A negative or normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate/incomplete information may lead to misinterpretation of the results.

Test Attributes:

- It is presumed that the specimen used to perform the test belongs to the patient specified above, such verification having been carried out at the collection level of the sample.
- The current results are based on analysis of coding regions (exons) as well as certain intron padding regions on the patient's genomic DNA with respect to patient phenotype as defined in the target regions (link available below). However, due to inherent technology limitations, coverage is not uniform across all regions. Hence pathogenic variants of insufficient coverage, as well as those variants that currently do not correlate with the provided phenotype may not be analysed/ reported. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity.
- The reported variants have not been Sanger confirmed. Sanger confirmation is recommended for the same.
- The test methodology currently does not detect large deletions/duplications, triplet repeat expansions, and epigenetic changes. The test also does not include an analysis of predictors for multifactorial, polygenic, and/or complex diseases. Novel synonymous changes as well as intronic mutations (excluding those affecting invariant splice nucleotides) are not routinely reported.
- CNV analysis is not included.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity & specificity for variant detection, analysis, and interpretation due to the inability of the data tools to unambiguously determine the origin of the sequence data. The mutations have not been validated by Sanger sequencing unless specified.
- Regions other than the targeted are not covered and hence cannot be reported.
- Phenotype variability may be due to modifying genetic/non-genetic factors and is not a part of the current

analysis.

- This test is not FDA approved/CE marked.
- In some instances, the classification and interpretation of variants (VUS) may change as new scientific information comes to light. We recommend a re-analysis of this report yearly. Please contact the laboratory in case a re-analysis of the report is desired. It is the lab's policy to perform re-analysis once on a complimentary basis. However, this re-analysis is performed only when requested.

References:

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