Name: Mrs. Vanitha Reddy
Barcode: 24339420

Patient Name	Mrs. Vanitha Reddy	Ref. Doctor	---
Age/Gender	44Y/ Female	Ref. Hospital	-----
Test Name	*BRCA1&2* Germline	Reported Date	04/03/2024

Clinical History:

Mrs. Vanitha Reddy I/V/O left triple negative breast cancer; she was referred for *BRCA1&2* germline sequencing.

Results: No Clinically Relevant Pathogenic Mutations Identified in the BRCA1 or BRCA2 genes

Interpretation:

The sequence analysis revealed no significant pathogenic or likely pathogenic variants associated to the patient's clinical phenotype. The absence of pathogenic mutations does not rule out the patient's disease condition. Hence, additional testing is recommended.

Recommendations:

- Genetic counselling and clinical correlation for accurate interpretation of test results are recommended.
- It is recommended to test for tumor/somatic BRCA1 and BRCA2 mutations when germline BRCA1/2 variant is absent.
- Copy number variation (CNV)/ large genomic rearrangement in the BRCA1/2 genes account for approximately 4-28% of inherited BRCA variants. Testing for CNV is advised by MLPA.

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

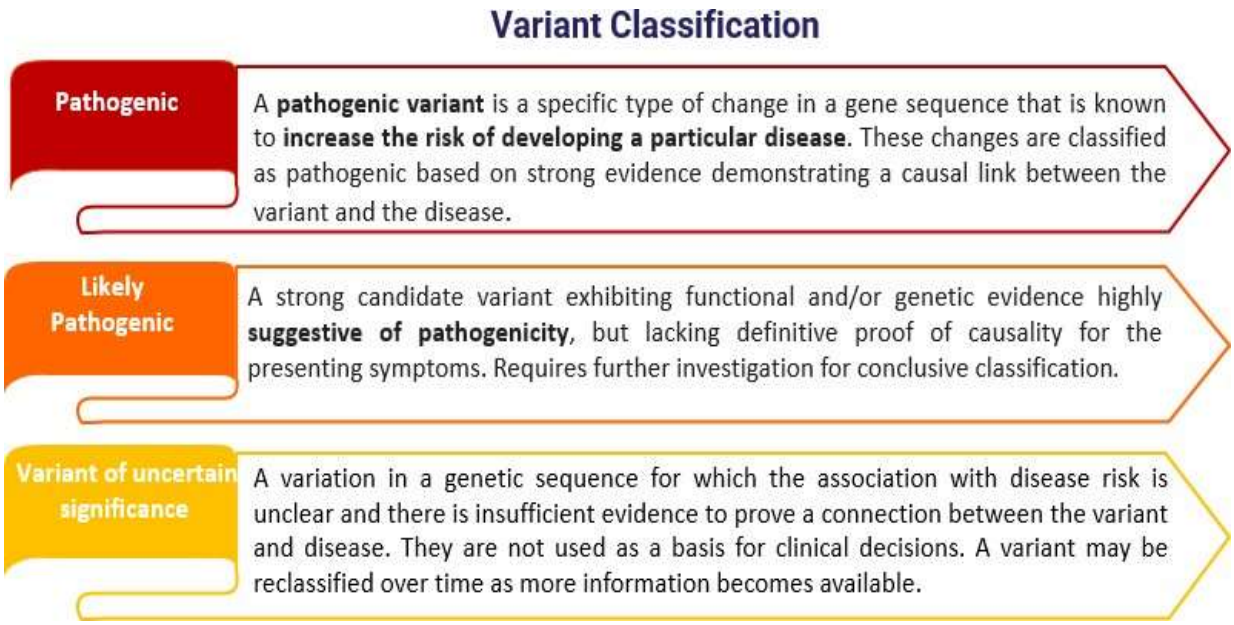
out by using GATK (v4.2.5.0). Variant calling was done by using the GATK Haplotype Caller. Each called variant is annotated using different clinical and population databases. Common variants were filtered out based on minor allele frequency (MAF) in 1000Genome Phase 3[4], gnomAD (v4), ExAC [3], and dbSNP (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 reads aligned reads (%)	99.52%
Data ≥Q30(%)	92.97%

Gene Coverage:

Gene	% Covered (30X)
BRCA1	100%
BRCA2	100%



Test Limitations:

A negative result does not exclude a heritable form of cancer. This test only detects variants within the coding regions and intron-exon boundaries of the *BRCA1* and *BRCA2* genes. Regulatory region variants and deep intronic variants will not be identified. Large deletions/duplications/insertions of any size may not be detected by massively parallel sequencing as the precise breakpoints for large deletions or duplications are not determined in this assay. Single exon deletions/duplications may not be detected due to its lower sensitivity and the actual breakpoints for the deletion or duplication may extend beyond or be within the exon(s). Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the

presence of pseudogenes, repetitive, or homologous regions. This test is not intended to detect low-level mosaic or somatic variants, gene conversion events, complex inversions, translocations, mitochondrial DNA (mtDNA) variants, or repeat expansions. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding transcripts were not analyzed.

This test was developed and its performance characteristics determined by Yoda Diagnostics. It has not been cleared or approved by the US Food and Drug Administration.

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 has not been validated by the FDA, NABL, or CAP, and it has been determined by the accrediting bodies that such validation is not required atthis time.
- 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:

1. Clarke L, Fairley S, Zheng-Bradley X, Streeter I, Perry E, Lowy E, et al. The International Genome Sample Resource (IGSR): A worldwide collection of genome variation incorporating the 1000 Genomes Project data. Nucleic Acids Research. 2016 Sep 15;45(D1):D854–9.
2. Jiaxin Wu and Rui Jiang., Prediction of Deleterious Nonsynonymous Single-Nucleotide Polymorphism for Human Diseases; The Scientific World Journal, Volume 2013, Article ID 675851, 10 pages; http://dx.doi.org/10.1155/2013/675851

3. Karczewski KJ, Weisburd B, Thomas B, Solomonson M, Ruderfer DM, Kavanagh D, et al. The ExAC browser: displaying reference data information from over 60,000 exomes. Nucleic Acids Research. 2016 Nov 28;45(D1):D840–5.
4. Karczewski, K.J., Francioli, L.C., Tiao, G. et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581, 434–443 (2020). https://doi.org/10.1038/s41586-020-2308
5. Landrum M. J. et al., ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res., 44(D1): D862-8, 2015. 1000 Genomes Project Consortium et al., A global reference for human genetic variation. Nature, 526(7571): 68-74, 2015.
6. Li H, Durbin R. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics [Internet].2010Jan15;26(5):589–95.
7. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res, 20:1297-303. DOI: 10.1101/gr.107524.110.
8. McKusick V.A., Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press (12th edition), 1998.
9. McLaren W. et al., Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics, 26(16):2069-70, 2010.
10. Miller DT, Lee K, Abul-Husn NS, Amendola LM, Brothers K, Chung WK, Gollob MH, Gordon AS, Harrison SM, Hershberger RE, Klein TE, Richards CS, Stewart DR, Martin CL; ACMG Secondary Findings Working Group. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2022 Jul;24(7):1407-1414. doi: 10.1016/j.gim.2022.04.006. Epub 2022 Jun 17. PMID: 35802134.
11. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003 Jul 1;31(13):3812-4. doi: 10.1093/nar/gkg509. PMID: 12824425; PMCID: PMC168916.
12. Richards S. et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet Med., 17(5):405-24, 2015.
13. Sherry ST. dbSNP: the NCBI database of genetic variation. Nucleic Acids Research. 2001 Jan 1;29(1):308–11.
14. Welter D. et al., The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res., 42: D1001-1006, 2014.

--------- End of the Report ---------

Dr.S.M. Naushad
Chief Scientific Officer

Dr. S. Sudha Murthy
MD(PATH) DNB, AMPH(ISB)
Lab Director