Germline Cancer Predisposition Panel-Focussed

Test Description

The MolQ BRCA Germline mutation test helps assess your risk of developing cancer by detecting a potentially harmful change (mutation) in BRCA1 and BRCA2 genes.

Patient Demographic

Name: Ms. Kusum Lata
Sex: Female
Date of Birth/Age: 38 years
Disease: Ovarian Carcinoma

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Dr AV Institute of Personalized Cancer Therapy and Research
Pathologist: Not Provided

Specimen

Booking ID: 012209070075
Site: NA
Sample Type: Blood
Date of Collection: 07-09-2022
Date of Booking: 07-09-2022

CLINICAL SYNOPSIS

Kusum Lata, is a case of stage IIIC, carcinoma ovary. She has been evaluated for pathogenic variations in the *BRCA1* and *BRCA2* genes.

RESULTS

Pathogenic variant causative of the reported phenotype was detected.

Gene (Transcript)#	Location	Variant	Zygosity	Disease (OMIM/Ref$)	Inheritance	Classification$
BRCA1 (ENST00000357654.9)	Exon 10	c.3008_3009del; (p.Phe1003Ter)	Heterozygous	Breast-ovarian cancer, familial[1]	Autosomal dominant	Pathogenic

$Genetic test results are reported based on the recommendations of American College of Medical Genetics.[1]

ADDITIONAL FINDINGS: NO VARIANT(S) OF UNCERTAIN SIGNIFICANCE (VUS) DETECTED

No significant variant(s) that warrants to be reported was detected. Variations with high minor allele frequencies which are likely to be benign will be given upon requests.

The *BRCA1* and *BRCA2* genes are 100% covered in this assay.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

BRCA1 **p.Phe1003ter**
Overall depth: 198X

Variant description: A heterozygous two base pair deletion in exon 10 of the ***BRCA1*** gene **chr17: g.43092523_43092524del; Depth: 198x**) that results in a stop codon and premature truncation of the protein at codon 1003 (**p.Phe1003Ter; ENST00000357654.9**) was detected (Table). The observed variation is documented as pathogenic in hereditary breast and ovarian cancer syndrome in the ClinVar database[2]. The p.Phe1003Ter variant has not been reported in the 1000 genomes and gnomAD databases and has a minor allele frequency of 0.003% in reference laboratory internal database. The *in silico* prediction# of the variant is damaging by Mutation Taster2 tool. The reference region is conserved in mammals. The observed variation (**also referred to as 3127del2**) has been previously reported in patients with breast cancer[3,4].

OMIM phenotype: Susceptibility to familial breast-ovarian cancer-1 (OMIM#604370) is caused by mutations in the ***BRCA1*** gene (OMIM*113705)[5].

Based on the above evidence[$], ***this BRCA1 variation is classified as a pathogenic variant and has to be carefully correlated with the clinical symptoms.***

RECOMMENDATION

- The *BRCA1* gene has a pseudogene in the human genome. Validation of the variant(s) by Sanger sequencing is recommended to rule out false positives.
- Targeted testing of at-risk family member especially first-degree relatives (children, siblings and parents) is recommended, as it could benefit other carriers in risk assessment and constituting measures for early detection, prevention and better management of disease.
- Genetic counselling is advised for interpretation on the consequences of the variant(s).

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APPENDIX 1: TEST METHODOLOGY

Method

Targeted gene sequencing: Selective capture and sequencing of the protein coding regions of the genome/genes is performed. Mutations identified in the exonic regions are generally actionable compared to variations that occur in non-coding regions. Targeted sequencing represents a cost-effective approach to detect variants present in multiple/large genes in an individual.

DNA extracted from sample was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80- 100X coverage on Illumina sequencing platform. We follow the GATK best practices framework for identification of variants in the sample using Sentieon (v201808.01)[1]. The sequences obtained are aligned to human reference genome (GRCh38/hg38) using Sentieon aligner[1,2] and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon haplotype caller has been used to identify variants which are relevant to the clinical indication. Gene annotation of the variants is performed using VEP program[3] against the Ensemble release 99 human gene model[4]. In addition to SNVs and small indels, copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth (v1.1.10) method[5]. This algorithm detects rare CNVs based on comparison of the read-depths of the test data with the matched aggregate reference dataset.

Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM (updated on 11th May 2020), GWAS, HGMD (v2020.2) and SwissVar[6-10]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v3.1), EVS, dbSNP (v151), 1000 Japanese Genome and reference laboratory internal Indian population database[11-15]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Only non-synonymous, splice site variants found in the coding regions in the *BRCA1* and *BRCA2* genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

$The classification of the variations is done based on American College of Medical Genetics as described below[16]

Variant	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).
Pathogenic	A disease causing variation in a gene which can explain the patient's symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed.
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non-disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

#The transcript used for clinical reporting generally represents the canonical transcript, which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.
Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.
#The *in-silico* predictions are based on Variant Effect Predictor, Ensembl release 99 (SIFT version - 5.2.2; PolyPhen - 2.2.2); bdNSFPv4.0 (LRT version – December 5, 2019) and Mutation Taster2 (MT2). MutationTaster2 predictions are based on NCBI/Ensembl 66 build (GRCh38 genomic coordinates are converted to hg19 using UCSC LiftOver and mapped to MT2).

For any further technical queries please contact contact@molq.in.

DISCLAIMER

- Interpretation of variants in this report is performed to the best knowledge of the laboratory based on the information available at the time of reporting. The classification of variants can change over time and MolQ cannot be held responsible for this. Please feel free to contact MolQ Laboratory (contact@molq.in) in the future to determine if there have been any changes in the classification of any variations. Re-analysis of variants in previously issued reports in light of new evidence is not routinely performed, but may be considered upon request, provided the variant is covered in the current panel.
- The sensitivity of this assay to detect large deletions/duplications of more than 10 bp or copy number variations (CNV) is

80-90%. The CNVs detected have to be confirmed by alternate method.

- Due to inherent technology limitations of the assay, not all bases of the exome can be covered by this test. Accordingly, variants in regions of insufficient coverage may not be identified and/or interpreted. Therefore, it is possible that certain variants are present in one or more of the genes analyzed, but have not been detected. The variants not detected by the assay that was performed may/ may not impact the phenotype.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- The mutations have not been validated/confirmed by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines[17] can be given upon request.
- The report shall be generated within turnaround time (TAT), however, such TAT may vary depending upon the complexity of test(s) requested. MolQ Laboratory under no circumstances will be liable for any delay beyond afore mentioned TAT.
- It is hereby clarified that the report(s) generated from the test(s) do not provide any diagnosis or opinion or recommends any cure in any manner. MolQ Laboratory hereby recommends the patient and/or the guardians of the patients, as the case may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. MolQ Laboratory hereby disclaims all liability arising in connection with the report(s).
- In a very few cases genetic test may not show the correct results, e.g. because of the quality of the material provided to MolQ Laboratory. In case where any test provided by MolQ Laboratory fails for unforeseeable or unknown reasons that cannot be influenced by MolQ Laboratory in advance, MolQ Laboratory shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by MolQ Laboratory in advance.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by reference laboratory.

LIMITATIONS

- Genetic testing is an important part of the diagnostic process. However, genetic tests may not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in current medical knowledge or testing technology. Accurate interpretation of test results may require knowing the true biological relationships in a family. Failing to accurately state the biological relationships in {my/my child's} family may result in incorrect interpretation of results, incorrect diagnoses, and/or inconclusive test results.
- 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.
- Structural variants such as deletions/duplications (CNVs) reported through NGS assay needs to be confirmed by orthogonal method to rule out the possibility of false positives. Translocations, repeat expansions and chromosomal rearrangements are not detected through this assay.
- Genetic testing is highly accurate. Rarely, inaccurate results may occur for various reasons. These reasons include, but are not limited to mislabelled samples, inaccurate reporting of clinical/medical information, rare technical errors or unusual circumstances such as bone marrow transplantation, blood transfusion; or the presence of change(s) in such a small percentage of cells that may not be detectable by the test (mosaicism).
- The variant population allele frequencies and *in silico* predictions for GRCh38 version of the Human genome is obtained after lifting over the coordinates from hg19 genome build. The existing population allele frequencies (1000Genome, gnomAD-Exome) are currently available for hg19 genome version only. This might result in some discrepancies in variant annotation due to the complex changes in some regions of the genome.

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