Germline Cancer Predisposition Panel

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. Sangeeta Das
Sex: Female
Date of Birth/Age: 45 years
Disease: Ovarian Cancer

Clinician

Clinician Name: Dr Amish Vora
Medical Facility: HOPE Clinic
Pathologist: Not Provided

Specimen

Booking ID: 011906140255
Site: NA
Sample Type: Blood
Date of Collection: 14-06-2019
Date of Booking: 14-06-2019

CLINICAL SYNOPSIS

Sangeeta Das, is diagnosed with ovarian cancer and has been evaluated for pathogenic variations in the *BRCA1* and *BRCA2* genes.

RESULTS

Pathogenic variant causative of the reported phenotype is detected

Gene (Transcript) #	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
BRCA1 (-) (ENST00000471181.2)	Exon 11	c.4120_4121del (p.Ser1374Ter)	Heterozygous	Breast-Ovarian Cancer, familial, 1	Autosomal dominant	Pathogenic

ADDITIONAL FINDINGS: VARIANT(S) OF UNCERTAIN SIGNIFICANCE (VUS) DETECTED

No other variant that warrants to be reported was detected. Variations with high minor allele frequencies which are likely to be benign will be given upon request.

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

CLINICAL CORRELATION AND VARIANT INTERPRETATION

BRCA1 **p.Ser1374Ter**
Overall depth: 580X
Variant description: A heterozygous 2 base pair deletion in the exon 11 of the ***BRCA1*** gene (**chr17:g.41243028_41243029del**) that results in a frameshift stop codon and premature truncation of the protein at codon 1374 (**p.Ser1374Ter; ENST00000471181.2**) was detected (Table). The **p.Ser1374Ter** variant has not been reported in the 1000 genomes and ExAC databases and has a minor allele frequency of 0.001% in reference laboratory internal database. It is documented as a pathogenic variant in ClinVar[1] and BRCA Exchange database[2]. The *in-silico* prediction of the variant is damaging by Mutation Taster-2 tool. The observed variation has been previously reported in patients affected with breast and/or ovarian cancer in Italian and Indian population[3,4]. The reference codon is conserved in primates.

Clinical Significance: *BRCA1* (OMIM #113705) gene plays critical roles in DNA repair, cell cycle checkpoint control, maintenance of genomic stability and therefore, functions as a tumor suppressor. Susceptibility to hereditary breast-ovarian cancer-1 (HBOC) (OMIM #604370) is caused by heterozygous germline mutations in the *BRCA1* gene[5].

BRCA1 associated HBOC syndrome shows autosomal dominant mode of inheritance and each first degree relative (children, siblings and parents) of an individual has a 50% chance of having this variation. A tested individual carry one copy (heterozygous) of a loss-of-function variant in *BRCA1* gene.

Based on the above details, ***this BRCA1 variation is classified as a pathogenic variant and needs to be carefully correlated with the clinical symptoms***.

ADDITIONAL INFORMATION

1. This patient has been found to have a mutation in *BRCA1* or *BRCA2* gene. Individuals with mutations in these genes have a risk of developing a condition called Hereditary Breast and Ovarian Cancer Syndrome (HBOC).
2. The risk of developing a malignancy in individuals with a germline *BRCA1* or *BRCA2* pathogenic variant is mentioned below in a table[6].

		Risk for Malignancy	
Cancer Type	**General Population Risk**	***BRCA1***	***BRCA2***
Breast	12%	46%-87%	38%-84%
Second primary breast	2% within 5 years	21.2% within 10 years 83% by age 70	10.8% within 10 years 62% by age 70
Ovarian	1%-2%	39%-63%	16.5%-27%
Male Breast	0.1%	1.2%	Up to 8.9%
Prostate	6% through age 69	8.6% by age 65	15% by age 65 20% lifetime
Pancreatic	0.50%	1%-3%	2%-7%
Melanoma (Cutaneous & Ocular)	1.6%		Elevated risk

3. Women with HBOC also have high risk for fallopian tube and primary peritoneal cancer[5].
4. Based on the recent clinical trials (Study-19 and SOLO-2), PARP-inhibitor (Olaparib) has been approved by FDA for the treatment of patients with deleterious or suspected deleterious germline *BRCA1/2*-mutated high grade serous epithelial ovarian cancer who have been treated with three or more prior lines of chemotherapy[7,8].
5. Similarly, another clinical trial (OlympiAD), showed Olaparib treatment to improve progression free survival (PFS) and overall survival (OS) in patients with loss-of-function germline BRCA1/2-mutated, HER2-negative metastatic breast cancer who have been treated with chemotherapy either in the neoadjuvant, adjuvant, or metastatic setting and has been approved by FDA[9].

RECOMMENDATIONS

- 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. For more details on who should be tested, kindly consult your clinician.
- Genetic counselling is advised to discuss the consequences of the variation for this individual. Kindly email us at contact@molq.in for post-test counselling.
- Kindly consult with your clinician to discuss the surveillance measures and disease management options.
- In case of reported gene variations falling in repeat or pseudogene region or data coverage and depth is not satisfactory or, alternate allele percentage is less than 20%, confirmation of the variant by Sanger sequencing is recommended.

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APPENDIX 1: TEST METHODOLOGY

Method

Library Preparation, **Targeted Enrichment and 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 noncoding regions. Targeted sequencing represents a cost-effective approach to detect variants present in multiple/large genes in an individual. DNA extracted from blood was used to perform targeted gene capture using a custom capture kit for *BRCA1* and *BRCA2* genes (for complete coding region). The libraries were sequenced to mean >80-100X coverage on Illumina sequencing platform.

Analysis: 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 (GRCh37.p13/hg19)[2] using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels[4]. 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 91 human gene model[4].

Databases and *in-silico* Prediction Softwares: Clinically relevant mutations are annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM, GWAS, HGMD and SwissVar[5-11]. Common variants are filtered based on minor allele frequency (MAF) in 1000 Genome Phase 3, ExAC, gnomAD, EVS, dbSNP141, 1000 Japanese Genome and ref lab internal Indian population database[12-17]. The biological effect of non-synonymous variant is calculated using multiple algorithms such as PolyPhen, SIFT, Mutation Taster2, and LRT[18].

Only non-synonymous and splice site variants found in the coding regions of *BRCA1* and *BRCA2* genes are used for clinical interpretation. Silent variations that do not result in any amino acid change in the coding region are not reported. Variants annotated on incomplete and nonsense mediated decay transcripts are not reported.

***In-silico* CNV analysis**: In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth (V1.1.10) method[19]. This is a custom read-depth based algorithm that detects rare CNVs based on the comparison of the read-depths of the test data with the matched aggregate reference dataset. We evaluated the performance of our model using simulation. The sensitivity of 73% is achieved for heterozygous deletion events whereas for homozygous deletion events, it exceeds 90%. The sensitivity exceeds 80% when the target length was greater than 1Kb.

The sensitivity and specificity of NGS based assays to detect large heterozygous deletions/duplications is low and an alternate method such as MLPA is recommended for confirmation.

Analytical performance: Analytical validation of this test in our laboratory has shown sensitivity, specificity and reproducibility of 100%. Our validation study also showed 100% concordance between the results obtained by NGS data and Sanger sequencing (reflex testing method).

The coverage stats for *BRCA1* and *BRCA2* genes at greater than 10X and 20X depth in this sample is given below in the table.

Gene Name	Coverage at ≥ 10X	Coverage at ≥ 20X
BRCA1	100%	100%
BRCA2	100%	100%

Genetic test results are reported based on the variant classification recommendations of American College of Medical Genetics[20], as described in the table below:

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 (according to Ensembl release 87 gene model), 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 91 (SIFT version - 5.2.2; PolyPhen - 2.2.2); LRT version - November, 2009 release from dbNSFPv3.1 and Mutation Taster2 based on build NCBI 37 / Ensembl 69.

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

DISCLAIMER

- Large heterozygous deletions/duplications, complex rearrangements (>10 bp) and copy number variations (CNVs) are not detected in this test. An alternate method such as Multiplex Ligation-dependent Probe Amplification (MLPA) is recommended to detect such structural variants.
- The variants in this report are interpreted based on information available in scientific literature at the time of reporting, therefore, an impact and classification of gene variation might change over time with respect to clinical indication. MolQ Laboratory cannot be held responsible for this, the clinician can request reanalysis of data on an annual basis at an additional cost.
- Variants in untranslated region, promoters and deep intronic regions are not analyzed in this test.
- The reported gene variations have not been confirmed by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines[21] can also be given upon request.
- Test results are interpreted in the context of clinical findings, family history and other laboratory data. In the absence of detailed accurate clinical or family history of the patient, MolQ Laboratory cannot guarantee the accuracy of the interpretation of results.
- The results may be inaccurate in rare circumstances if the individual tested has undergone bone marrow transplantation or blood transfusion.
- MolQ Laboratory is not liable to provide diagnosis, opinion or recommendation related to disease, in any manner. MolQ Laboratory hereby recommends the Patient and/or the guardians of the Patient to contact clinician for further interpretation of the test results.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by the reference laboratory.

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