Booked on	08/06/2018	**Patient Id**	011806080266	**Printed on**	16/07/2018
Name	Mrs Amiri Munira	**Age**	37 Years	**Sex**	F
Ordering Physician	Dr Amit Verma Max Hospital				

Germline Mutation Analysis Panel for Cancer Predisposition

Sample Information

Sample Type: Blood

Clinical Indications

Diagnosed with triple-negative breast cancer (age at diagnosis: 35 years).

Test Details

Inherited cancer: All 86 genes in the germline cancer predisposition panel (listed in attached Appendix) were assessed for any key and incidental findings in the genes recommended by ACMG (American College of Medical Genetics and Genomics).

Results

A heterozygous '**pathogenic**' variant was detected in exon 10 of the *BRCA1* gene.

Gene	Variation	Zygosity	Inheritance	Clinical significance
BRCA1	chr17:41243482_41243485delGATT c.4065_4068delTCAA p.Asn1355LysfsTer10	Heterozygous	Dominant	Pathogenic

Disclaimer:
• Quality: The coverage was >20X with a read quality >Q30, variants with confidence score greater than 50 were considered for SNV (single nucleotide variant) calling.
• For the deletion/duplication nomenclature, the most 3' position possible in the sequence is arbitrarily assigned to have been changed. (Human Genome Variation Society guidelines).

Interpretation

- The individual carries one copy (heterozygous) of a 'pathogenic' variant in the *BRCA1* gene, which has been shown to be associated with hereditary breast and ovarian cancer syndrome (HBOC) predisposition.
- HBOC is characterized by an increased risk for female and male breast cancer, ovarian cancer (includes fallopian tube and primary peritoneal cancers), and to some lesser extent other cancers, such as prostate and pancreatic cancer. Women with a *BRCA1* germline pathogenic variation (in heterozygous state) are at an increased lifetime risk for breast cancer (46%-87%) and ovarian cancer (39%-63%) and to a lesser extent other cancers[1].
- HBOC, caused due to variations in the *BRCA1* gene, shows- an autosomal dominant mode of inheritance, which means one copy of the altered gene in an individual is sufficient to increase the risk of developing cancer. If the

variant is not a new variant (*de novo*) in this individual, then each first degree relative (siblings and parents) has 50% chance of having this variation.

Recommendations

Genetic counseling is recommended for the patient and other family members. Genetic testing for the identified BRCA1 variant in adult family members by a Mutation Specific Test (Sanger) is recommended. Their carrier status could benefit in risk estimation and instituting measures for early detection and prevention of cancers.

The physician can request reanalysis of the data, and this is recommended on an annual basis. Data from this test can be reassessed for the presence of any variants that may be newly linked to established genes or to newly identified disorders since the date of this report that could be associated with the patient's phenotype, based on currently available scientific information. A charge may apply for reanalysis.

For further details, kindly contact: contact@molq.in

Gene and Variant Interpretation - Key Findings

Gene: *BRCA1* (Entrez: 672) (-ve Strand)

The *BRCA1* gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability and DNA repair by facilitating cellular responses to DNA damage. It functions as a tumor suppressor, which means that it keeps cells from growing and dividing too fast or in an uncontrolled way. Approximately 10% of breast cancer cases are thought to be hereditary and about 25% of these are caused by germline pathogenic variations (in heterozygous state) in the tumor suppressor genes, *BRCA1* and *BRCA2*[2]. In addition, pathogenic variations (in heterozygous state) in *BRCA1* and *BRCA2* account for around 15 percent of ovarian cancers overall[3]. The majority of *BRCA1* variations occur in three domains or regions of the protein: the RING domain, exons 11-13 and the BRCT domain[4].

Variant 1

Transcript: NM_007294 chr17:41243482_41243485delGATT, c.4065_4068delTCAA, p.Asn1355LysfsTer10

The identified heterozygous deletion (c.4065_4068delTCAA) lies in exon 10 of the *BRCA1* gene and is predicted to cause a frameshift and consequent premature termination of the protein (p.Asn1355LysfsTer10). The truncated protein is predicted to have a length of 1363 amino acids (aa) as opposed to the original length of 1863 aa. The resultant protein is likely to lack the major functional domains of the protein, such as the serine cluster domain and the BRCT domains[5]; this will likely result in loss-of-function. Moreover, due to introduction of a premature stop codon, this aberrant transcript will likely be targeted by the nonsense mediated mRNA decay (NMD) mechanism[6].
The identified variant has been reported in the dbSNP database with identification number rs80357508 and in the genome Aggregation Database (gnomAD), as a rare variant (allele frequency: <0.01%). In the ClinVar database, the clinical significance of this variant has been reported as 'pathogenic' (RCV000048431.11) with respect to HBOC.
The identified variant (represented as 4184del4, in the articles) has been previously reported in patients of Indian and Pakistani origin affected with breast cancer[7,8] and the variant was absent in healthy individuals tested in the study[7]. In another study, the identified variant (represented as 4184del4, in the article) has been reported in patients of English origin affected with breast and/or ovarian cancer and the variant has been suggested to be associated with significantly higher risk of developing ovarian cancer[9].
The identified variant is predicted to produce a truncated protein, which might result in loss-of-function. It has been previously reported in patients affected with breast and/or ovarian cancer. Thus, the variant has been labelled as '**pathogenic**'.

Limitations of Gene Coverage

For each test gene, all gene target regions were adequately covered by greater than 20 reads.

Gene	HGMD variants not covered	% of Coding Region under covered

MSH2	3/686	0.25%
MSH6	0/363	0.17%

References

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4. Clark SL *et al.* 2012. Structure-Function Of The Tumor Suppressor BRCA1. *Comput Struct Biotechnol J* **1**(1) [PMID: 22737296].
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9. Evans DG *et al.* 2004. Haplotype and cancer risk analysis of two common mutations, BRCA1 4184del4 and BRCA2 2157delG, in high risk northwest England breast/ovarian families. *J. Med. Genet.* **41**(2):e21 [PMID: 14757871].

******End of the report******

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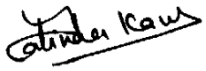

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Appendix

Test Description

The MolQ Germline Cancer Predisposition Panel include genes associated with both common (e.g., breast, colorectal) and rare cancers. The test involves preparation of a target sequence enrichment based library from the patient's genomic DNA (gDNA) using Illumina's Nextera's technology. The panel covers 86 unique genes and includes variation coverage for all coding exons (>97%) and essential splice sites.

Genes evaluated: 86 genes

AIP, ALK, APC, ATM, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1B, CDC73, CDH1, CDK4, CDKN2A, CEP57, CHEK2, CYLD, DDB2, DICER1, DIS3L2, EGFR, EPCAM, ERCC2, ERCC3, ERCC4, ERCC5, EXT1, EXT2, EZH2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FH, FLCN, GPC3, HNF1A, HRAS, KIT, MAX, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, NF2, NSD1, PALB2, PMS1, PMS2, PRF1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RECQL4, RET, SBDS, SDHAF2, SDHB, SDHC, SDHD, SLX4, SMAD4, SMARCB1, STK11, SUFU, TP53, TSC1, TSC2, VHL, WRN, WT1, XPA, XPC.

Methodology

Sample preparation: Genomic DNA isolated from saliva, blood or any other standard tissue source is used for preparation of the 'DNA sequencing ready' library. The DNA is quantified using Qubit Fluorometer and 50 ng is taken for library preparation. "Nextera" library preparation uses transposon-based shearing of the genomic DNA. The Nextera protocol allows the DNA to be "tagmented" (fragmented and tagged simultaneously in the same tube). A limited cycle PCR step allows the incorporation of adaptors, platform-specific tags and barcodes to prepare the DNA sequencing libraries.

Target Enrichment: The tagged and amplified sample libraries are checked for quality and quantified. Approximately 500 ng of each library is used in this step and any deviation from the recommended amount results in over or under representation of the sample and greater or less mean coverage. For this panel, up to 12 libraries can be combined into a single tube and set up for enrichment, to generate adequate coverage total sequencing for each sample on a MiSeq sequencer. Two simultaneous enrichment steps are performed to optimize the pull down of the regions of interest using biotinylated target specific probes. Target libraries are amplified using limited PCR steps and 6-10 pM are loaded for sequencing on the MiSeq.

Sequencing Details: Sequencing is performed using a standard v2 kit on Illumina Miseq with the expected data output of 4.0-5 GB. For this panel, the output size is 0.253 MB, optimum cluster density to be achieved during sequencing is 750-1000 clusters/mm^2.

Analysis

The trimmed fastq files were generated using MiSeq Reporter from Illumina. The reads were aligned against the whole genome build hg19. Five bas e-pairs from the 3' end of the reads were trimmed, as were 3' end bases with quality below 10. Reads which had length less than 25bp after trimming were not considered for alignment. A maximum of 5 matches of alignment score at least 90% were computed. The reads were realigned using the local realignment tool. Reads that failed vendor QC, reads with average quality less than 20, reads with ambiguous characters were all filtered out. The NGS variant caller was used to detect variants at locations in the target regions covered by a minimum of 10 reads with at least 2 variants reads. Variants with a decibel score of at least 50 were reported.

Limitations

This test is based on a research protocol. At present FDA has determined that clearance or approval is not necessary for this test. Variants that have not been confirmed by an independent analysis could represent technical artifacts. Some types of genetic abnormalities may not be detectable by this test. It is possible that the genomic region where a disease causing variation exists in the proband was not captured using the current technologies and therefore was not detected. Additionally, it is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the function of all genes in

the human genome and the impact of variants on those genes. The quality of sequencing varies between regions. Many factors such as homopolymers, GC-rich regions etc. influence the quality of sequencing. This may result in an occasional error in sequence reads.