Germline Cancer Predisposition Panel

Test Description

The MolQ Germline Cancer Predisposition Panel include genes associated with both common and rare hereditary cancers.

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Max Hospital
Pathologist: Not Provided

Patient Demographic

Name: Ms. Sangita Saboo
Sex: Female
Date of Birth/Age: 55 years
Disease: Breast Cancer Triple Negative

Specimen

Booking ID: 012001120045
Site: NA
Sample Type: Blood
Date of Collection: 12-01-2020
Date of Booking: 12-01-2020

CLINICAL SYNOPSIS

Sangita Saboo, is a case of triple negative carcinoma breast. She has a family history of cancer with her mother succumbed to carcinoma breast, one of her maternal uncles succumbed to carcinoma stomach and another succumbed to carcinoma oesophagus and her maternal aunt diagnosed with carcinoma carcinoma ovary. Sangita Saboo has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULTS

Pathogenic variant causative of the reported phenotype is detected

Gene (Transcript) #	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
BRCA1 (-) (ENST00000471181.2)	Intron 17	c.5137+1G>A (5' Splice site)	Heterozygous	Breast-Ovarian Cancer, familial	Autosomal dominant	Pathogenic

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

Gene (Transcript) #	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
PTEN (+) ENST00000371953.3)	Exon 9	c.1130A>T (p.Tyr377Phe)	Heterozygous	Cowden syndrome 1	Autosomal dominant	Uncertain Significance
ERCC5 (+) (ENST00000355739.4	Exon 13	c.2831C>T (p.Ser944Leu)	Heterozygous	Increased risk of breast cancer[1]	-	Uncertain Significance

Genetic test results are reported based on the recommendations of American College of Medical Genetics[2].

CLINICAL CORRELATION AND VARIANT INTERPRETATION

BRCA1 5' Splice Site

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)
Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact @molq.in
P a g e | 1

Germline Cancer Predisposition Panel

Overall depth: 243X

Variant description: A heterozygous 5' splice site variation in intron 17 of the **BRCA1** gene (**chr17:g.41219624C>T; Depth: 243x**) that affects the invariant GT donor splice site of exon 17 (**c.5137+1G>A; ENST00000471181.2**) was detected (Table). The observed variation (also referred to as c.5074+1G>A or IVS17+1G>A) has previously been reported in patients affected with triple-negative breast cancer[3,4]. This splice variation has been strongly predicted to be deleterious based on tumor pathology, clinical histories, and segregation studies[5]. It is documented as pathogenic in Clinvar database[6]. The variant has not been reported in the 1000 genomes and ExAC databases and has a minor allele frequency of 0.03% in reference laboratory internal database. The *in-silico* prediction# of the variant is damaging by Mutation Taster-2 tool. The reference base is conserved in mammals.

OMIM phenotype: Susceptibility to familial breast-ovarian cancer-1 (OMIM#604370) is caused by mutations in the **BRCA1** gene (OMIM*113705)[7].

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

PTEN   p.Tyr377Phe
Overall depth: 201X

Variant description: A heterozygous missense variation in exon 9 of the **PTEN** gene (**chr10:g.89725147A>T; Depth: 201x**) that results in the amino acid substitution of Phenylalanine for Tyrosine at codon 377 (**p.Tyr377Phe; ENST00000371953.3**) was detected (Table). The observed variation is documented as variant of uncertain significance in Clinvar database[8]. The p.Tyr377Phe variant has not been reported in the 1000 genomes databases and has a minor allele frequency of 0.006% (non-pass variant based on Variant Quality Score Recalibration[9]), 0.02% in the ExAC, reference laboratory internal database, respectively. The *in-silico* predictions# of the variant are probably damaging by PolyPhen-2 (HumDiv) and damaging by LRT. The reference codon is conserved across species.

OMIM phenotype: Cowden syndrome 1 (OMIM#158350) is caused by mutations in the **PTEN** gene (OMIM*601728). This disorder is primarily characterized by neoplasia including breast cancer, breast fibroadenomas, cervical carcinoma etc[7].

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

ERCC5   p.Ser944Leu
Overall depth: 280X

Variant description: A heterozygous missense variation in exon 13 of the **ERCC5** gene (**chr13:g.103524700C>T; Depth: 280x**) that results in the amino acid substitution of Leucine for Serine at codon 944 (**p.Ser944Leu; ENST00000355739**.4) was detected (Table). The p.Ser944Leu variant has not been reported in the reference laboratory internal databases and has a minor allele frequency of 0.02% and 0.002% in the 1000 genomes and ExAC databases, respectively. The *in-silico* predictions# of the variant are probably damaging by PolyPhen-2 (HumDiv), damaging by SIFT and LRT. The reference codon is conserved across species.

Phenotype: Germline mutation in **ERCC5** gene are found to be associated with increased risk of breast cancer[1].

Based on the above evidence[$], **this ERCC5 variation is classified as a variant of uncertain significance and has to be carefully correlated with the clinical symptoms**.

RECOMMENDATIONS

- The **BRCA1** and **PTEN** genes have pseudogene in the human genome. 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 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.

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact @molq.in

P a g e | **2**

REFERENCES

1. Bonache S. et al., Multigene panel testing beyond BRCA1/2 in breast/ovarian cancer Spanish families and clinical actionability of findings. Journal of cancer research and clinical oncology (2018): 1432-1335
2. 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.
3. Easton D.F. et al., A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. American journal of human genetics (2007): 0002-9297.
4. Rashid M.U. et al., High prevalence and predominance of BRCA1 germline mutations in Pakistani triple-negative breast cancer patients. BMC cancer (2016): 1471-2407.
5. Lindor N.M. et al., A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). Human mutation (2012): 1098-1004.
6. https://www.ncbi.nlm.nih.gov/clinvar/variation/37629/.
7. McKusick V.A., Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press (12th edition), 1998.
8. https://www.ncbi.nlm.nih.gov/clinvar/variation/186450/.
9. Lek M. et al., Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616):285-91, 2016.

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact @molq.in

P a g e | 3

Germline Cancer Predisposition Panel

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 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 (GRCh37/hg19) using Sentieon aligner[1,2] and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels[1]. Sentieon haplotypecaller 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]. 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 21st November 2018), GWAS, HGMD (v2018.3) and SwissVar[6-9]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, ExAC (v1.0), gnomAD(v2.1), EVS, dbSNP(v151), 1000 Japanese Genome and reference lab internal Indian population database[10-14]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Only non-synonymous and splice site variants found in the germline cancer predisposition panel 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[15]

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

- 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 available upon request.
- The sensitivity of this assay to detect large deletions/duplications of more than 10 bp or copy number variations (CNV) is 70-75%. 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 pathogenic

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in

P a g e | 4

Germline Cancer Predisposition Panel

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 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[16] 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.
- Specific events like copy number variations, translocations, repeat expansions and chromosomal rearrangements may not be reliably detected with targeted clinical exome sequencing. Variants in untranslated region, promoters and intronic variants are not assessed using this method.
- 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).

REFERENCES

1. Freed D. *et al.*, The Sentieon Genomics Tools-A fast and accurate solution to variant calling from next-generation sequence data. BioRxiv:115717, 2017.
2. Li H. *et al.*, Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics, 26(5):589-95, 2010.
3. McLaren W. *et al.*, Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics, 26(16):2069-70, 2010.
4. Zerbino D. R. *et al.*, Ensembl 2018. Nucleic Acids Res., 46(D1): D754-D761, 2018.
5. Plagnol V. *et al.*, A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. Bioinformatics, 28:2747-2754, 2012.
6. Landrum M. J. *et al.*, ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res., 44 Project (D1):D862-8, 2015.
7. Welter D. *et al.*, The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res., 42:D1001-1006, 2014.
8. Stenson P. D. *et al.*, The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Human Genet., 136(6): 665-677, 201.
9. Mottaz A. *et al.*, Easy retrieval of single amino-acid polymorphisms and phenotype information using SwissVar. Bioinformatics, 26(6): 851-852, 2010.
10. 1000 Genomes Project Consortium *et al.*, A global reference for human genetic variation. Nature, 526(7571): 68-74, 2015.

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact @molq.in

P a g e | 5

11. Lek M. *et al.*, Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616):285-91, 2016.
12. Exome Variant Server, NHLBI GO Exome Sequencing (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/).
13. Nagasaki, M. *et al.*, Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. Nat. Commun., 6:8018, 2015.
14. Sherry S. T. *et al.*, dbSNP: the NCBI database of genetic variation. Nucleic Acids Res., 29(1):308-11, 2001.
15. 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.
16. Kalia S.S. *et al.*, Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact @molq.in

P a g e | **6**

Germline Cancer Predisposition Panel

APPENDIX 2: GENE LIST WITH COVERAGE

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
AIP	100.00	ALK	100.00	APC	100.00
AR	100.00	ATM	100.00	BAP1	100.00
BARD1	100.00	BLM	100.00	BMPR1A	100.00
BRCA1	100.00	BRCA2	100.00	BRIP1	100.00
BUB1B	100.00	CD82	100.00	CDC73	100.00
CDH1	100.00	CDK4	100.00	CDKN1C	100.00
CDKN2A	100.00	CEBPA	100.00	CEP57	100.00
CHEK2	100.00	CYLD	100.00	DDB2	100.00
DICER1	100.00	DIS3L2	100.00	EGFR	100.00
ELAC2	100.00	ENG	100.00	EPCAM	100.00
ERCC2	100.00	ERCC3	100.00	ERCC4	100.00
ERCC5	100.00	EXT1	100.00	EXT2	100.00
EZH2	100.00	FANCA	100.00	FANCB	100.00
FANCC	100.00	FANCD2	100.00	FANCE	100.00
FANCF	100.00	FANCG	100.00	FANCI	100.00
FANCL	100.00	FANCM	100.00	FH	100.00
FLCN	100.00	GATA2	100.00	GPC3	100.00
HRAS	100.00	KIT	100.00	MAX	100.00
MEN1	100.00	MET	100.00	MLH1	100.00
MLH3	100.00	MRE11A	100.00	MSH2	100.00
MSH3	100.00	MSH6	100.00	MSR1	100.00
MUTYH	100.00	MXI1	100.00	NBN	100.00
NF1	100.00	NF2	100.00	NSD1	100.00
PALB2	100.00	PHOX2B	100.00	PMS1	100.00
PMS2	100.00	PRF1	100.00	PRKAR1A	100.00
PTCH1	100.00	PTEN	100.00	RAD50	100.00
RAD51C	100.00	RAD51D	100.00	RAD54L	100.00
RB1	100.00	RECQL4	100.00	RET	100.00
RHBDF2	100.00	RNASEL	100.00	RUNX1	100.00
SBDS	100.00	SDHAF2	100.00	SDHB	100.00
SDHC	100.00	SDHD	100.00	SLX4	100.00
SMAD4	100.00	SMARCB1	100.00	STK11	100.00
SUFU	100.00	TGFBR2	100.00	TMEM127	100.00
TP53	100.00	TSC1	100.00	TSC2	100.00
VHL	100.00	WRN	100.00	WT1	100.00
XPA	100.00	XPC	100.00		

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact @molq.in

P a g e | 7