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CIN: U85191MH2011PTC217513

Full Name	SANHITA LOBO	Sample ID	2300086830
Gender	Female	Patient ID	1002338942
Age	48 Years	Collected on	13-06-2023
Referring Clinician	Dr. Ashish Bakshi	Received on	14-06-2023
Referring Centre	Bethany Hospital	Reported on	11-07-2023
Sample Type	Peripheral Blood	Provisional Diagnosis	Ca breast
Test Requested	Germline Panel		

CLINICAL DIAGNOSIS/ SYMPTOMS/ HISTORY

Operated case of mixed mucinous and infiltrating ductal carcinoma (not otherwise specified), left breast, grade II with axillary lymph nodes metastasis

RESULTS

VARIANT OF UNCERTAIN SIGNIFICANCE RELATED TO THE GIVEN PHENOTYPE WAS DETECTED

Gene (Transcript) #	Location	Variant	Zygosity	Disease (OMIM/‡REF)	Inheritance	Classification$
BAP1 (-) (ENST00000460680.6)	Exon 13	c.1271G>A (p.Gly424Glu)	Heterozygous	Tumor predisposition syndrome 1	Autosomal dominant	Uncertain Significance

$Genetic test results are reported based on the recommendations of American College of Medical Genetics PMID: 25741868]

The genes included in this assay and their coverage are listed in Appendix 1.

VARIANT INTERPRETATION AND CLINICAL CORRELATION

Variant description: A heterozygous missense variation in exon 13 of the ***BAP1*** gene (**chr3:g.52403874C>T; Depth: 310x**) that results in the amino acid substitution of Glutamic Acid for Glycine at codon 424 (**p.Gly424Glu; ENST00000460680.6**) was detected (Table). The observed variation is documented as variant of uncertain significance in BAP1-related tumor predisposition syndrome in the ClinVar database [VCV002156960.1]. The p.Gly424Glu variant has not been reported in the 1000 genomes and gnomAD databases and has a minor allele frequency of 0.001% in our internal database. The insilico prediction# of the variant is damaging by Mutation Taster2 tool. The reference codon is conserved across mammals.

OMIM phenotype: Tumor predisposition syndrome 1 (OMIM#614327) is caused by mutations in the *BAP1* gene (OMIM*603089). Patients with tumor predisposition syndrome are at increased risk of developing uveal melanoma, cutaneous melanoma, **breast cancer** and lung adenocarcinoma etc.

Germline variants in the *BAP1* gene have previously been reported in patients with breast cancer [PMID: 21941004, 24243779, 26096145, 26748926].

Based on the above evidence$, **this *BAP1* variation is classified as a variant of uncertain significance and has to be carefully correlated with the clinical symptoms.**

RECOMMENDATIONS

- Validation of the variant(s) by Sanger sequencing is recommended to rule out false positives.
- **Sequencing the variant(s) in the other affected and unaffected members of the family is recommended to confirm the significance.**
- Genetic counseling is advised.
- If results obtained do not match the clinical findings, additional testing should be considered as per referring clinician's recommendations.

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.

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. 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 >10 bp or copy number variations (CNV) is 80- 90%. The CNVs detected are recommended 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 that are not covered may not be identified and/or interpreted. Therefore, it is possible that certain variants are present in one or more of the genes analysed 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 [PMID: 27854360] 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. Lab 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. 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.
- In a very few cases genetic test may not show the correct results, e.g. because of the quality of the material provided. In case where any test fails for unforeseeable or unknown reasons that cannot be influenced by lab in advance, lab shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by lab in advance.

Dr. Madhavi Pusalkar, Ph.D.
GM-Genomics Operation

TEST METHODOLOGY

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 of clinical relevance 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. In this hereditary cancer panel, in addition to complete Coding segment (CDS) of 127 genes, promoter regions of relevant genes and critical other non-coding / coding pathogenic variants <100 bp documented in the ClinVar, HGMD, BRCA Exchange and LOVD databases mapping to the targeted genes are also included [PMID: 26582918, PMID: 28349240, PMID: 30586411, PMID: 21520333]. Additionally, there is an enhanced CNV coverage for better detection of CNVs. This panel provides a comprehensive and robust approach to identify SNV's, Indels and CNVs through single test.

DNA extracted from blood 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 (v201808.07) [https://europepmc.org/article/PPR/PPR28504]. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner [PMID: 20080505] 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 [PMID: 20562413] against the Ensembl release 99 human gene model [PMID: 29155950]. Copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth (v1.1.10) method, a coverage-based approach [PMID: 22942019]. This algorithm detects rare CNVs based on comparison of the read-depths of the test data with the matched aggregate reference dataset and the overall sensitivity of CNV surveillance through ExomeDepth was found to be 97% [PMID: 28378820]. In our internal validation experiments on MLPA verified samples, >80-90% sensitivity was achieved for detecting CNVs.

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), LOVD, BRCA Exchange and SwissVar [PMID: 26582918, PMID: 28349240, PMID: 30586411, PMID: 21520333, PMID: 17357067, PMID: 24316577, PMID: 20106818]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v2.1), EVS, dbSNP (v151), 1000 Japanese Genome and our internal Indian population database [PMID: 26432245, PMID: 11125122, PMID: 32461654, PMID: 26292667, https://esp.gs.washington.edu/drupal/,https://www.nature.com/articles/ncomms9018]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Only non- synonymous, splice site and critical non-coding variants found in the hereditary cancer 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.

Total data generated (Gb)	0.5573
Total reads aligned (%)	99.99
Reads that passed alignment (%)	93.09
Data ≥ Q30 (%)	96.77

$The classification of the variations is done based on American College of Medical Genetics as described below [PMID: 25741868]

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), dbNSFPv4.0 (LRT version - December 5, 2019) and MutationTaster2 (MT2). MutationTaster2 predictions are based on NCBI/Ensembl 66 build (GRCh38 genomic coordinates are converted to hg19 using UCSC LiftOver and mapped to MT2).

APPENDIX 1: COVERAGE OF HEREDITARY CANCER PANEL GENES ^

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
ABRAXAS1	100.00	*AIP*	100.00	*ALK*	100.00
*APC**	100.00	*AR*	100.00	*ATM*	100.00
AXIN2	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
CBL	100.00	*CD82*	100.00	*CDC73*	96.19
CDH1	100.00	*CDK12*	100.00	*CDK4*	100.00
CDKN1B	100.00	*CDKN1C*	100.00	*CDKN2A*	100.00
CEBPA	100.00	*CEP57*	100.00	*CHEK1*	100.00
CHEK2	100.00	*CTNNA1*	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	*FAN1*	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
GALNT12	100.00	*GATA2*	100.00	*GPC3*	100.00
HOXB13	100.00	*HRAS*	100.0	*KIF1B*	100.00
KIT	100.00	*LZTR1*	100.0	*MAX*	100.00
MEN1	100.00	*MET*	100.0	*MITF*	100.00
MLH1	100.00	*MLH3*	100.0	*MRE11*	100.00
MSH2	100.00	*MSH3*	100.0	*MSH6*	100.00
MSR1	100.00	*MUTYH*	100.0	*MXI1*	100.00
NBN	100.00	*NF1*	100.0	*NF2*	100.00
NSD1	100.00	*NTHL1*	100.0	*PALB2*	100.00
PALLD	100.00	*PAX5*	100.0	*PDGFRA*	100.00
PHOX2B	100.00	*PMS1*	100.0	*PMS2*	100.00
POLD1	100.00	*POLE*	100.0	*POT1*	100.00
PPP2R2A	100.00	*PRF1*	100.0	*PRKAR1A*	100.00
PRSS1	100.00	*PTCH1*	100.0	*PTCH2*	100.00
PTEN	100.00	*RAD50*	100.0	*RAD51B*	100.00
RAD51C	100.00	*RAD51D*	100.0	*RAD54L*	100.00
RB1	100.00	*RECQL*	100.0	*RECQL4*	100.00
RET	100.00	*RHBDF2*	100.0	*RINT1*	100.00
RNASEL	100.00	*RNF43*	100.0	*RUNX1*	100.00
SBDS	100.00	*SDHA*	100.0	*SDHAF2*	100.00
SDHB	100.00	*SDHC*	100.0	*SDHD*	100.00
SLC45A2	100.00	*SLX4*	100.0	*SMAD4*	100.00
SMARCA4	100.00	*SMARCB 1*	100.0	*SMARCE1*	100.00
SRGAP1	100.00	*STK11*	100.0	*SUFU*	100.00
*TERT**	100.00	*TGFBR2*	100.0	*TMEM127*	100.00
TP53	100.00	*TSC1*	100.0	*TSC2*	100.00
TYR	100.00	*VHL*	100.0	*WRN*	100.00
WT1	100.00	*XPA*	100.0	*XPC*	100.00
XRCC2	100.00	*XRCC3*	100.0		

* Promoter regions of these genes are also analysed.

^ In addition to complete CDS coverage in these genes, critical non-coding variants reported as pathogenic in clinical databases are also analysed in this assay.

END OF REPORT