PATIENT	REPORT DATE	BOOKING ID
Supratin Das	19 March 2024	#012402150093

Germline Cancer Predisposition

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: Dr AV Institute of Personalized Therapy and Cancer Research (IPTCR)
Pathologist: Not Provided

Patient Demographic

Name: Mr Supratin Das
Sex: Male
Date of Birth/Age: years
Disease: Renal cell carcinoma- metastatic

Specimen

Booking ID: 012402150093
Site: NA
Sample Type: Blood
Date of Collection:15-02-2023
Date of Booking:15-02-2023

CLINICAL SYNOPSIS

Supratin Das is a known case of metastatic renal cell carcinoma. He has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULTS

Variant of uncertain significance related to the given phenotype was detected.

Gene (Transcript)#	Location	Variant	Zygosity	Disease (OMIM/Ref[1])	Inheritance	Classification$
APC (+) (NM_000038.6)	Exon 16	c.3648G>T (p.Glu1216Asp)	Heterozygous	Cancer susceptibility: Adenomatous polyposis coli/ Hereditary desmoid disease/ Gardner syndrome/ Colorectal cancer/ Gastric cancer/ Hepatoblastoma, somatic/ Renal cell carcinoma, somatic/ Brain tumour	Autosomal Dominant	Uncertain significance (PM2)

$Genetic test results are reported based on the recommendations of American College of Medical Genetics. #Due to lack of clinical evidence for this variant, it is classified as variant of uncertain significance.

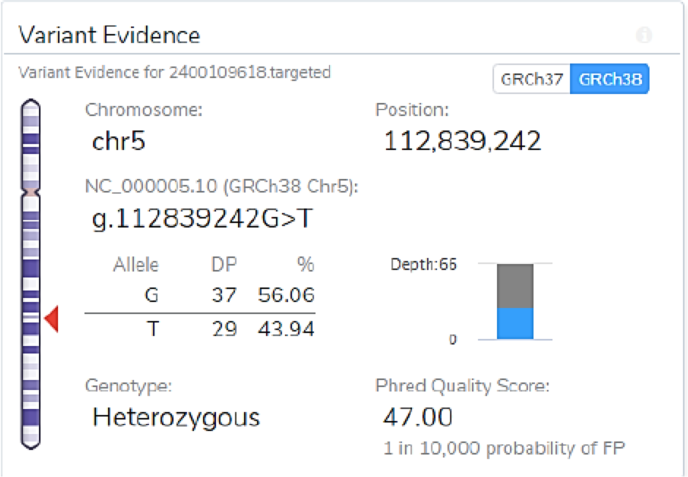
CLINICAL CORRELATION AND VARIANT INTERPRETATION

APC p.Glu1216Asp

Variant description: A heterozygous variant in Exon 14 of gene ***MSH2*** (chr2: g.47478436A>G) was observed in the submitted sample. A missense variant "A" to "G" detected at nucleotide position 2375A leading to change in amino acid sequences from Asparagine to Serine at codon 792. *In silico predictions*: The p.Asn792Ser missense variant is predicted to be tolerated by both SIFT or PolyPhen2. The serine residue at codon 792 of *MSH2* is present in Lesser Egyptian jerboa and 10 other mammalian species. The nucleotide c.2375 in *MSH2* is not conserved according to a GERP++ and PhyloP analysis of 100 vertebrates. *Population frequency and Internal database*: The p.Asn792Ser variant is observed in 4/30,782 (0.013%) alleles from individuals of gnomAD South Asian background in gnomAD All.The p.Asn792Ser variant is novel (not in any individuals) in 1kG All. This variant is absent in reference laboratory's internal database.

A heterozygous variant in Exon 16 of gene ***APC*** (chr5: g.112839242G>T) was observed in the submitted sample. A missense variant "G" to "T" detected at nucleotide position 3648 leading to change in amino acid sequences from Glutamic acid to Aspartic acid at codon 1216. *In silico predictions*: The p.Glu1216Asp missense variant is predicted to be tolerated by both SIFT or PolyPhen2. The nucleotide c.3648 in *APC* is not conserved according to a GERP++ and PhyloP analysis of 100 vertebrates. *Population frequency and Internal database*: The p.Glu1216Asp variant is novel (not in any individuals) in gnomAD All. The p.Glu1216Asp variant is novel (not in any individuals) in 1kG All. . This variant is absent in reference laboratory's internal database.

OMIM Phenotype: Renal cell carcinoma (RCC) is the most common type of kidney cancer in adults. In a study from Taiwan, to elucidate the molecular pathogenesis of sporadic RCC, fifteen patients with RCC were screened for mutations in the von Hippel-Lindau (VHL) gene by PCR and Sanger sequencing along with the methylation status of promoters of 24 tumor suppressor genes by methylation sensitive multiplex ligation-dependent probe amplification analysis. Study demonstrated inactivation of the *VHL* gene in 5 cases: three missense somatic mutations, one promoter methylation, and one small deletion. In RCCs, methylation was most frequently observed in APC (100%), CDKN2B (92.9%), CASP8, MLH1_167, and KLLN (85.7.4%), but not in FHIT, MLH1_463, DAPK1, or HIC1 (0%). Study concluded that in addition to *VHL* inactivation, promoter methylation of *APC* may be a universal pathognomonic event in the tumorigenesis of RCC and a candidate diagnostic and therapeutic biomarker.


Variant Evidence

Variant Evidence for 2400109618.targeted

Chromosome: chr5

Position: 112,839,242

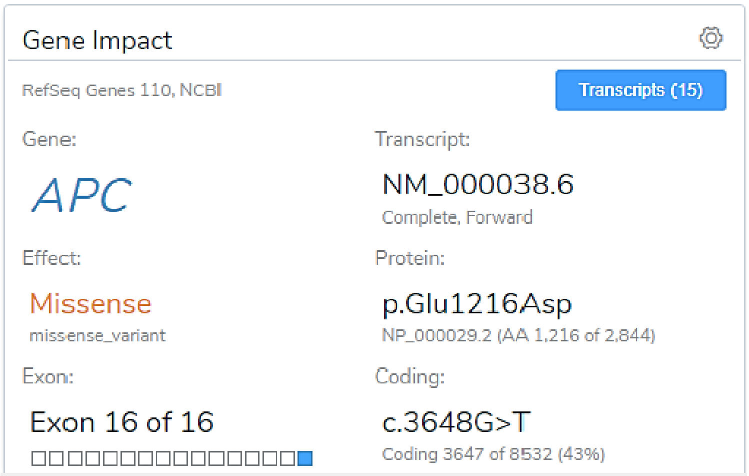
NC_000005.10 (GRCh38 Chr5): g.112839242G>T

Allele	DP	%
G	37	56.06
T	29	43.94

Genotype: Heterozygous

Phred Quality Score: 47.00

1 in 10,000 probability of FP


Gene Impact

RefSeq Genes 110, NCBI

Gene: *APC*

Transcript: NM_000038.6

Complete, Forward

Effect: Missense

missense_variant

Protein: p.Glu1216Asp

NP_000029.2 (AA 1,216 of 2,844)

Exon: Exon 16 of 16

Coding: c.3648G>T

Coding 3647 of 8532 (43%)

Based on the above evidence$, ***<u>this variant (APC: c.3648G>T) is classified as Uncertain significance variant</u>***

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 counselling is advised for interpretation on the consequences of the variant(s).
- If results obtained do not match the clinical findings, additional testing should be considered as per referring clinician's recommendations.

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

REFERENCES

1. Lai YC, Wang WC. Genetic Analysis Reveals the Important Role of the APC Gene in Clear Cell Renal Cell Carcinoma. Anticancer Res. 2021 Sep;41(9):4295-4304. doi: 10.21873/anticanres.15234. PMID: 34475049.

APPENDIX 1: TEST METHODOLOGY

Method

Next Generation Sequencing:[1-5] DNA extracted from blood, saliva, amniotic fluid, CVS or any other standard source is used for targeted capture-based Library preparation. Targeted capture provides an efficient and sensitive means for sequencing specific genomic regions in a high-throughput manner. The libraries were sequenced to mean >85-100x coverage on Illumina Novaseq 6000 sequencing platform with Paired End 2x150 chemistry.

Illumina DRAGEN Bio-IT Platform was followed for identification of variants in the sample. The sequences obtained are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build (GRCh38). Haplotype caller has been used to identify variants which are relevant to the clinical indication.

In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the commercially available algorithm. 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, Commercial datasets and a set of diseases databases.

Common variants are filtered based on allele frequency in 1000Genome, ExAC, gnomAD, dbSNP and reference laboratory's internal database.

Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2. Based on annotations data, ACMG rules-based classification performed for classification of variants identified through next generation sequencing study.

Average Sequencing Depth (x)	Percentage Target Base Pairs Covered		
	1x	≥5x	≥20x
88.50	98.99	98.81	98.44

Title	Data
Total data generated (Gb)	8.16
Total reads aligned (%)	100.00
Reads that passed alignment (%)	99.95
Data ≥ Q30 (%)	93.69

$The classification of the variations is done based on American College of Medical Genetics as described below[6]

Variant	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).
Benign	A variant which is known not to be responsible for disease has been detected. Generally, no further action is warranted on such variants when detected.
Likely Benign	A variant which is very unlikely 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.
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.

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.

- 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 with this assay have to be confirmed by alternate method such as MLPA & Microarray.
- Due to inherent technology limitations, coverage is not uniform across all regions. Hence pathogenic variants present in areas of insufficient coverage as well as those variants which currently do not corelate with the provided phenotype may not be analyzed/ reported. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity.
- 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[2] can be given upon request.
- Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS).
- 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.
- 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.
- Triplet repeat expansions, translocations, large deletion or duplications and chromosomal rearrangements events are currently not reliably detected by next generation sequencing.
- This assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, other gene rearrangements like inversion or translocation and does not detect single or multiexon deletions or duplications.
- 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).
- It should be noted that this test does not sequence all bases in a human genome, not all variants have been identified or interpreted, and this report is limited only to variants with evidence for causing or contributing to disease/clinical details provided to MolQ Laboratory.
- Testing has been performed assuming that the sample received belongs to the above named individual and any stated relationships between individuals are accepted as true.
- The results should be interpreted in the context of the patient's medical evaluation, family history and racial/ethnic

background. Please note that variant classification and/or interpretation may change over time if more information available. Reinterpretation of multi gene next generation sequencing data is recommended on an annual basis and may be requested by a medical provider.

REFERENCES

1. Meyer L.R. et al., The UCSC Genome Browser database: extensions and updates 2013. Nucleic Acids Res., 41(D1):D64-9, 2013.
2. McKenna, A., et al., The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res., 20(9): 1297-303, 2010.
3. 1000 Genomes Project Consortium et al., A global reference for human genetic variation. Nature, 526(7571): 68-74, 2015.
4. Lek M. et al., Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616):285-91, 2016.
5. McLaren W. et al., Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics., 26(16):2069-70, 2010.
6. 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.
7. 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.

APPENDIX 2: GENE LIST WITH COVERAGE

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
ABRAXAS1	100.00	*FANCG*	100.00	*PTCH1*	100.00
ACVRL1	100.00	*FANCI*	100.00	*PTCH2*	100.00
AIP	100.00	*FANCL*	100.00	*PTEN*	100.00
AKT1	100.00	*FANCM*	100.00	*PTPN11*	100.00
ALK	100.00	*FGFR2*	100.00	*RAD50*	100.00
ANKRD26	100.00	*FH*	100.00	*RAD51*	100.00
APC	100.00	*FLCN*	100.00	*RAD51C*	100.00
AR	100.00	*GALNT12*	100.00	*RAD51D*	100.00
ATM	100.00	*GATA2*	100.00	*RAF1*	100.00
ATR	100.00	*GPC3*	100.00	*RASA2*	100.00
AXIN1	100.00	*GPR101*	100.00	*RASAL1*	100.00
AXIN2	100.00	*GREM1*	100.00	*RB1*	100.00
BAP1	100.00	*HAVCR2*	100.00	*RECQL*	100.00
BARD1	100.00	*HNF1A*	100.00	*RECQL4*	100.00
BLM	100.00	*HNF1B*	100.00	*REST*	100.00
BMPR1A	100.00	*HOXB13*	100.00	*RET*	100.00
BRAF	100.00	*HRAS*	100.00	*RFWD3*	100.00
BRCA1	100.00	*IKZF1*	100.00	*RHBDF2*	100.00
BRCA2	100.00	*KIF1B*	100.00	*RINT1*	100.00
BRIP1	100.00	*KIT*	100.00	*RIT1*	100.00
BTNL2	100.00	*KITLG*	100.00	*RNASEL*	100.00
BUB1B	100.00	*KRAS*	100.00	*RNF43*	100.00
CASR	100.00	*LIG4*	100.00	*RPS20*	100.00
CBL	100.00	*LSP1*	79.12	*RRAS*	100.00
CD70	100.00	*LZTR1*	100.00	*RUNX1*	100.00
CD82	100.00	*MAD2L2*	100.00	*SAMD9*	100.00
CDC73	100.00	*MAP2K1*	100.00	*SAMD9L*	100.00
CDH1	100.00	*MAP2K2*	100.00	*SBDS*	100.00
CDK4	100.00	*MAP3K1*	100.00	*SDHA*	100.00
CDKN1B	100.00	*MAX*	100.00	*SDHAF2*	100.00
CDKN1C	100.00	*MC1R*	100.00	*SDHB*	100.00
CDKN2A	100.00	*MEN1*	100.00	*SDHC*	100.00
CDKN2B	100.00	*MET*	100.00	*SDHD*	100.00
CEBPA	100.00	*MITF*	100.00	*SHOC2*	100.00
CEP57	100.00	*MLH1*	100.00	*SLX4*	100.00
CFTR	100.00	*MLH3*	100.00	*SMAD4*	100.00
CHEK2	100.00	*MRE11*	100.00	*SMARCA2*	100.00
CPA1	100.00	*MSH2*	100.00	*SMARCA4*	100.00
CTNNA1	100.00	*MSH3*	100.00	*SMARCB1*	100.00
CYLD	100.00	*MSH6*	100.00	*SMARCE1*	100.00
DDB2	100.00	*MSR1*	100.00	*SOS1*	100.00
DDX41	100.00	*MUTYH*	100.00	*SOS2*	100.00
DICER1	100.00	*MXI1*	100.00	*SPINK1*	100.00
DIS3L2	100.00	*MYSM1*	100.00	*SPRED1*	100.00
DKC1	100.00	*NBN*	100.00	*SRP72*	96.83

EFL1	100.00	*NF1*	100.00	*STK11*	100.00
EGFR	100.00	*NF2*	100.00	*SUFU*	100.00
ELAC2	100.00	*NHEJ1*	100.00	*TERC*	0.00
ELANE	100.00	*NRAS*	100.00	*TERT*	100.00
ENG	100.00	*NSD1*	100.00	*TGFB1*	100.00
EPCAM	100.00	*NSUN2*	100.00	*TGFBR2*	100.00
ERCC1	100.00	*NTHL1*	100.00	*TINF2*	100.00
ERCC2	100.00	*PALB2*	100.00	*TMEM127*	100.00
ERCC3	100.00	*PALLD*	100.00	*TOX3*	96.65
ERCC4	100.00	*PAX5*	100.00	*TP53*	100.00
ERCC5	100.00	*PDGFRA*	100.00	*TRIP13*	100.00
ETV6	100.00	*PHOX2B*	100.00	*TSC1*	100.00
EXO1	100.00	*PIK3CA*	100.00	*TSC2*	100.00
EXT1	100.00	*PMS1*	100.00	*UBE2T*	100.00
EXT2	100.00	*PMS2*	100.00	*VHL*	100.00
EZH2	100.00	*POLD1*	100.00	*VPS13B*	100.00
FAM111B	100.00	*POLE*	100.00	*WRN*	100.00
FANCA	100.00	*POLH*	100.00	*WT1*	100.00
FANCB	100.00	*POT1*	100.00	*XPA*	100.00
FANCC	100.00	*PPM1D*	100.00	*XPC*	100.00
FANCD2	100.00	*PRF1*	100.00	*XRCC2*	100.00
FANCE	100.00	*PRKAR1A*	100.00	*XRCC3*	100.00
FANCF	100.00	*PRSS1*	100.00	*ZFHX3*	100.00

MTDNA GENES LIST WITH COVERAGE

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
MT-ATP6	100.00	*MT-ND6*	100.00	*MT-TL1*	100.00
MT-ATP8	100.00	*MT-RNR1*	100.00	*MT-TL2*	100.00
MT-CO1	100.00	*MT-RNR2*	100.00	*MT-TM*	100.00
MT-CO2	100.00	*MT-TA*	100.00	*MT-TN*	100.00
MT-CO3	100.00	*MT-TC*	100.00	*MT-TP*	100.00
MT-CYB	100.00	*MT-TD*	100.00	*MT-TQ*	100.00
MT-ND1	100.00	*MT-TE*	100.00	*MT-TR*	100.00
MT-ND2	100.00	*MT-TF*	100.00	*MT-TS1*	100.00
MT-ND3	100.00	*MT-TG*	100.00	*MT-TS2*	100.00
MT-ND4	100.00	*MT-TH*	100.00	*MT-TT*	100.00
MT-ND4L	100.00	*MT-TI*	100.00	*MT-TV*	100.00
MT-ND5	100.00	*MT-TK*	100.00	*MT-TW*	100.00
MT-TY	100.00				