Booked on	24/02/2018	**Patient Id**	011802240003	**Printed on**	27/03/2018
Name	Mr Vikas Khosla	**Age**	46 Years	**Sex**	M
Ordering Physician	Dr Amit Verma Max Hospital				

Germline Mutation Analysis Panel for Cancer Predisposition

Sample Information

Sample Type: Blood

Clinical Indications

Diagnosed with colon cancer (age at diagnosis: 46 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 16 of the *APC* gene.

Gene	Variation	Zygosity	Inheritance	Clinical significance
APC	chr5:112175218_112175222delAAAGA c.3927_3931delAAAGA p.Glu1309AspfsTer4	Heterozygous	Dominant	Pathogenic

Disclaimer:
• Quality: The coverage was >20X with a read quality >Q20, 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 *APC* gene, which has been shown to be associated with familial adenomatous polyposis (FAP) predisposition.
- Germline pathogenic variations in heterozygous state in the *APC* gene predisposes an individual to FAP, which is a colon cancer predisposition syndrome. Individuals affected with FAP may begin to develop multiple adenomatous polyps in the colon as early as their teenage years; without colectomy, these polyps will become malignant[1].
- FAP, caused due to variations in the *APC* 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 (children, siblings and parents) has 50% chance of having this variation.

Recommendations

Genetic counseling is recommended for the patient and other family members. 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: *APC* (Entrez: 324) (+ve Strand)

The *APC* gene encodes a protein that functions as a tumor suppressor, which means that it keeps cells from growing and dividing too fast or in an uncontrolled way[2]. APC has been reported to be constitutively expressed in the epithelium of the colon and is suggested to play a key role in the regulation of cell growth in colonic as well as in extra-colonic tissues[3]. Germline pathogenic variations in heterozygous state in the *APC* gene have been shown to be associated with APC-associated polyposis conditions, which include: FAP, attenuated FAP, and gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS)[1].

Variant 1

Transcript: NM_000038 chr5:112175218_112175222delAAAGA, c.3927_3931delAAAGA, p.Glu1309AspfsTer4

The identified heterozygous deletion (c.3927_3931delAAAGA) lies in exon 16 of the *APC* gene and is predicted to cause a frameshift, and consequent premature termination of the protein (p.Glu1309AspfsTer4). The truncated protein is predicted to have a length of 1311 amino acids (aa) as opposed to the original length of 2843 aa. The resultant protein is likely to lack the major functional domains of the protein[4]; 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[5].
The identified variant has been reported in the dbSNP database with identification number rs121913224 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' (RCV000000856.7) with respect to FAP. The identified variant has been previously reported as a hot spot mutation in patients of various ethnicity affected with FAP[6-8]. The variant was found to co-segregate with the disease phenotype and has been associated with more severe phenotypes, such as presence of thousand polyps, early age of onset and advanced neoplastic disease[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 FAP. Thus, the variant has been labelled as '**pathogenic**'.

Limitations of Gene Coverage

For each test gene, the fraction of the gene covered by less than 20 reads is indicated below.

Gene	HGMD variants not covered	% of Coding Region under covered
MSH6	0/363	0.71%

References

1. Jasperson KW *et al.* 2017. Gene Reviews: APC-Associated Polyposis Conditions. [PMID: 20301519].
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5. Maquat LE 2004. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nat. Rev. Mol. Cell Biol.* **5**(2):89-99 [PMID: 15040442].
6. Kim DW *et al.* 2005. Mutation spectrum of the APC gene in 83 Korean FAP families. *Hum. Mutat.* **26**(3):281 [PMID: 16088911].
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8. Torrezan GT *et al.* 2013. Mutational spectrum of the APC and MUTYH genes and genotype-phenotype correlations in Brazilian FAP, AFAP, and MAP patients. *Orphanet J Rare Dis* **8**:54 [PMID: 23561487].
9. Distante S *et al.* 1996. Familial adenomatous polyposis in a 5 year old child: a clinical, pathological, and molecular genetic study. *J. Med. Genet.* **33**(2):157-60 [PMID: 8929955].

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

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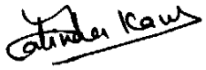

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Appendix

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 from saliva, blood or any other standard tissue source is used to make the library. "Nextera" library preparation uses a transposon based shearing of the genomic DNA and allows the DNA to be "tagmented" (fragmented and tagged simultaneously in the same tube). Limited cycle PCR step allows the incorporation of adaptors, platform-specific tags and barcodes to prepare the DNA sequencing libraries.

Target Enrichment: Approximately 500 ng of each library is used for target enrichment. For this panel, up to 12 libraries can be combined into a single tube and set up for enrichment, to generate adequate coverage on a Miseq sequencer. Two simultaneous enrichment steps are performed to optimize the pull down of regions of interest using target specific probes.

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.