Booked on	10/09/2017	**Patient Id**	11709100003	**Printed on**	24/10/2017
Name	Mrs. Deepa Sharma	**Age**	30 Years	**Sex**	F
Ordering Physician	Dr Amit Verma Max Hospital				

Germline Mutation Analysis Panel for Cancer Predisposition

Sample Information

Sample Type: Blood

Clinical Indications

Risk prediction. Family history of cancer: Father and paternal grandmother affected with colon cancer.

Test Details

This test analyzes 98 genes (see Appendix for full list of genes) associated with hereditary cancer predisposition.

Results

A heterozygous '**pathogenic**' variant was detected in the essential splice acceptor site, in intron 5 of the *MLH1*gene.

Please refer to the 'limitations of gene coverage' section for the list of genes showing low coverage (<20 reads) in this sample.

Gene	Variation	Zygosity	Inheritance	Clinical significance
MLH1	chr3:37050303A>G c.454-2A>G	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 *MLH1* gene, which has been shown to be associated with Lynch syndrome.

• Germline pathogenic variations in heterozygous state in the *MLH1* gene have been shown to be associated with Lynch syndrome, which increases risk of colorectal (52%-82%), endometrial (25%-60%), gastric (6%-13%) and ovarian (4%-12%) cancers[1].

• Lynch syndrome, caused due to variations in the *MLH1* gene, is inherited in 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. Each first

degree relative (children, siblings and parents) of the individual has a 50% chance of having this variation.

Recommendations

Genetic counseling is recommended to discuss the implications of this test result for this individual. Test results should be interpreted in the context of this individual's clinical and family history of cancer. Kindly consult with the referring physician to discuss about surveillance measures. Genetic testing for the identified *MLH1* variant in adult family members by 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 is based on currently available scientific information. This data can be re-assessed for the presence of any variants that may be newly linked to established genes associated with the patient's phenotype or to newly identified disorders since the date of this report. A charge may apply for reanalysis.

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

Gene and Variant Interpretation - Key Findings

Gene: *MLH1* (Entrez: 4292) (+ve Strand)

The MLH1 gene is one of the seven known genes of the DNA mismatch repair (MMR) system. MLH1 plays an essential role in DNA repair. This gene was identified as a locus frequently mutated in Lynch syndrome. Lynch syndrome increases the risk of colorectal cancer. Additionally, it increases risk for endometrial, gastric and ovarian cancers. Germline pathogenic variations in heterozygous state in the *MLH1* and *MSH2* genes account for about 90% of Lynch syndrome cases[1].

Variant 1

Transcript: NM_000249 | chr3:37050303A>G, c.454-2A>G

The identified heterozygous variant (c.454-2A>G) lies in the essential splice acceptor site, in intron 5 of the *MLH1* gene. In silico splice prediction tools (SplicePort and NNSPLICE) predict that this variant is likely to disrupt the splice site at the junction of intron 5 and exon 6 of the MLH1 gene. This could lead to a frameshift, which will probably result in premature termination of the protein. Moreover, due to introduction of a premature stop codon, this aberrant transcript will likely be targeted by the nonsense mediated mRNA decay (NMD) mechanism[2].

The identified variant has been reported in the dbSNP database with identification number rs267607753. In the ClinVar database, the clinical significance of this variant has been reported as 'likely pathogenic' with respect to Lynch syndrome (RCV000075721.2) and hereditary cancer-predisposing syndrome (RCV000218165.1).

The identified variant has been previously reported in a patient affected with Lynch syndrome and the variant has been classified as 'disease-causing'. Analysis on the RNA isolated from the patient's blood sample revealed skipping of exon 6, which was predicted to cause frameshift and premature termination of the protein[3].

The identified variant has been shown to disrupt an essential splice site, which might result in loss-of-function. It has been previously reported in a patient affected with Lynch syndrome. 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.

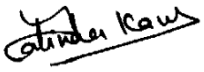
Gene	HGMD variants not covered	% of Coding Region under covered
MSH6	2/363	1.89%

References

1. Kohlmann W et al. 2014. Gene Reviews: Lynch Syndrome. [PMID: 20301390].
2. Maquat LE 2004. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. Nat. Rev. Mol. Cell Biol. 5(2):89-99 [PMID: 15040442].
3. Auclair J et al. 2006. Systematic mRNA analysis for the effect of MLH1 and MSH2 missense and silent mutations on aberrant splicing. Hum. Mutat. 27(2):145-54 [PMID: 16395668].

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

Report Released by:



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Appendix

Test Description

The MolQ Germline Cancer Panel includes genes associated with commonly inherited cancers (breast, gastrointestinal, gynaecological, lung, prostate, renal, endocrine, melanoma and nervous system) as well as several rare cancers. The test involves preparation of a target sequence enrichment based library from the patient's genomic deoxyribonucleic acid (gDNA) using Illumina's Nextera's technology. The panel covers 98 unique genes and includes variation coverage for all coding exons (>97%) and essential splice sites. The generated library is subjected to next generation sequencing (NGS) on the Illumina NGS platform (MiSeq). Genetic variations are identified by using the NGS software and interpreted. The Germline Cancer Test is a Laboratory Developed Test (LDT) that was developed and its performance characteristics determined by the reference laboratory.

Genes evaluated: 98 genes

AIP, ALK, APC, ATM, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1B, CDC73, CDH1, CDK4,CDKN1C, CDKN2A, CEBPA, 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, GATA2, GPC3, HNF1A, HOXB13, HRAS, KIT, MAX, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, NF2, NSD1, PALB2, PJOX2B, PMS1, PMS2, PPM1D, PRF1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RECQL4, RET, RHBDF2, RUNX1, SBDS, SDHAF2, SDHB, SDHC, SDHD, SLX4, SMAD4, SMARCA4, SMARCB1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, WRN, WT1, XPA, XPC.

Methodology

Library 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 test, 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 4-5 GB. For this test, the output size is 0.253 MB, optimum cluster density to be achieved during sequencing is 750-1000 clusters/sqmm.

Analysis

The trimmed FASTQ files were generated using MiSeq Reporter from Illumina. The reads were aligned against the whole genome build hg19 using NGS. Five base 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 25 bp 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 in NGS. 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 variant reads. Variants with a decibel score of at least 50 were reported. Variants were then imported into Omics software. Annotation and prioritization of variants with suggestive ACMG (American College of Medical Genetics and Genomics) labels was done by automated pipelines in Omics software. The Omics user interface was then used for identifying variants of interest and for reporting these variants. All variants reported were verified to have good raw read quality using the NGS genome browser.

Analytical performance: Analytical validation (Document No.: VR0002v2) of this test in our laboratory has shown sensitivity of 98.2%, specificity of 100% and reproducibility of 99.5%.

NGS analysis platform comprises algorithms for alignment, variant calling, exon deletion/duplication analysis, and structural variant calling. A built in genome browser enables inspection of read level data. Several QC steps enable inspection of read quality. Software includes algorithms to identify variant impact from both public content (HGMD, ClinVar, OMIM, HPO, links to dbSNP, 1000 Genomes, Exome Variant Server, and built-in algorithms SIFT, PolyPhen HVAR/HDIV, Mutation Taster and LRT) and proprietary content (curated

variant records). The interpretation interface in Omics software allows quick filtering and evaluation of variants along with capture of justification for inclusion/exclusion.

CNV Analysis: In addition to SNVs and small Indels, copy number analysis was performed using normalized coverage and Z-scores of the genomic regions against a profile of multiple samples run on the NGS panel.
Secondary genes evaluated: In addition to the main genes, 'pathogenic' and 'likely pathogenic' variants are investigated for secondary findings in the genes present in the Germline Cancer Test. However, in case of autosomal recessive disorders, carrier status is not reported for secondary findings.

Data Versions: Data Annotations are updated periodically. Data version or date of download used for annotations are as mentioned: Human Genome (hg19), RefSeq (Dec,2013), NCBI Gene (Jan 2015), ClinVar (Dec 2016), HGMD (Oct 2016), UniProt (Nov 2016), GWAS (Mar 2015), OMIM (Feb 2015), dbSNP (v149), Exome Variant Server (Jan 2017), ExAC (v0.3.1), 1000Genomes (Jan 2017), dbNSFP (v2.8), HPO (Apr 2015).

Limitations and Disclaimer

As with any laboratory test, there is a small chance that this result may be inaccurate for a procedural reason, such as an error during specimen collection and labeling (incorrect patient identification), an error in processing, data collection, or interpretation. Currently available data indicates that technical error rate for analysis involving DNA tests is anywhere between 1-3%. Variants that have not been confirmed by an independent analysis could represent technical artifacts. However, our validation study showed 100% concordance between the results obtained by NGS data and Sanger sequencing (confirmation of the variant), when the supporting read fraction of the variant with atleast 20 reads was >30%. Large insertions, deletions, duplications, inversions and complex rearrangements cannot be characterized accurately by NGS as it uses short-read sequencing data. Such structural variants have a much higher false positive and false-negative rate than seen for SNVs (single nucleotide variant). 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. Not all variations detected may be listed in the report. Inclusion of variations is dependent upon our assessment of their significance. The quality of sequencing and coverage varies between regions. Many factors such as homopolymers, GC-rich regions etc. influence the quality of sequencing and coverage. This may result in an occasional error in sequence reads or lack of detection of a particular genetic lesion. Accurate interpretation of this report is dependent on detailed clinical history of the patient. In the event of unavailability of detailed clinical history, the lab cannot guarantee the accuracy of the interpretation.

Compliance Statement

This assay was developed and its performance characteristics determined by the reference laboratory. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This laboratory is following the regulatory requirements and guidelines of Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) and College of American Pathologists (CAP), National Accreditation Board for Testing and Calibration Laboratories (NABL) and ISO (International Organization for Standardization) 15189. Genetic counselling is recommended for all patients undergoing genetic testing. We follow the American College of Medical Genetics and Genomics (ACMG) guidelines regarding guidelines for test validation, variant classification and reporting.