Lynch Syndrome/ HNPCC Gene Panel

PATIENT	REPORT DATE	BOOKING ID
Ronaq Taneja	11 October 2019	#011909120138

Test Description

Lynch syndrome is a hereditary cancer arising from loss of function mutations in DNA mismatch repair genes, such as *MLH1*, *MSH2*, *MSH3*, *MSH6*, *PMS2*, and *EPCAM.* MolQ Lynch Syndrome panel includes next-generation sequencing of these genes for mutations and large deletions/duplications.

Clinician

Clinician Name: Dr Archit Pandit
Medical Facility: Max Hospital
Pathologist: Not Provided

Patient Demographic

Name: Mr. Ronaq Taneja
Sex: Male
Date of Birth/Age: 27 years
Disease: Colon cancer

Specimen

Booking ID: 011909120138
Site: NA
Sample Type: Blood
Date of Collection: 12-09-2019
Date of Booking: 12-09-2019

CLINICAL SYNOPSIS

Ronaq Taneja, is a case of colon cancer. He 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
MLH1 (+) (ENST00000231790.2)	Exon 3	c.306G>T (p.Glu102Asp)	Heterozygous	Colorectal cancer, hereditary nonpolyposis, type 2	-	Pathogenic

Genetic test results are reported based on the recommendations of American College of Medical Genetics[1]

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

No other variant that warrants to be reported was detected. Variations with high minor allele frequencies which are likely to be benign will be given upon request.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

Variant description: A heterozygous splice site proximal missense variation in exon 3 of the *MLH1* gene (chr3:g.37042544G>T; Depth: 45x) that results in the amino acid substitution of Aspartic Acid for Glutamic Acid at codon 102 (p.Glu102Asp; ENST00000231790.2) was detected (Table). The observed variation (also referred to as E102D) has previously been reported in patients affected with colorectal cancer[2,3]. Another missense variation at same position (c.306G>C) has been functionally characterized as pathogenic resulting in exon 3 skipping[3] and it lies in the Histidine kinase-, DNA gyrase B-, and HSP90-like ATPase domain of the MLH1_HUMAN protein[4]. The p.Glu102Asp variant has not been reported in the 1000 genomes databases and has a minor allele frequency of 0.002% and 0.01% in the ExAC and our internal database, respectively. The *in-silico* predictions# of the variant are probably damaging by PolyPhen-2 (HumDiv), damaging by SIFT, LRT and Mutation Taster 2 tools. The reference codon is conserved across species.

OMIM phenotype: Hereditary nonpolyposis colorectal cancer-2 (OMIM#609310) is caused by mutations in the *MLH1* gene (OMIM*120436)[5].

Based on the above evidence$, ***this MLH1 variation is classified as a pathogenic variant and has to be carefully correlated with the clinical symptoms***.

RECOMMENDATIONS

- **Validation of the variant by Sanger sequencing is recommended to rule out false positives.**
- Sequencing the variant(s) in the parents and 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).

REFERENCES

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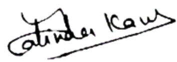

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

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 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. The sequences obtained are aligned to human reference genome (GRCh37/hg19) using BWA program[1,2] and analyzed using Picard and GATK version 3.6[3,4] to identify variants relevant to the clinical indication. We follow the GATK best practices framework for identification of variants in the sample. Gene annotation of the variants is performed using VEP program[5] against the Ensembl release 87 human gene model[6]. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM, GWAS, HGMD and SwissVar[7-14]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, ExAC, EVS, dbSNP147, 1000 Japanese Genome and our internal Indian population database[15-19]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, Mutation Taster2, Mutation Assessor, and LRT. Only non-synonymous and splice site variants found in the lynch syndrome/HNPCC gene 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[20]

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[21].
For any further technical queries please contact contact@molq.in.

DISCLAIMER

- The classification of variants of unknown significance can change over time and MolQ Laboratory cannot be held responsible for this. Please contact MolQ Laboratory at a later date to inquire about any changes.
- Intronic variants are not assessed using this method.
- Large deletions of more than 10 bp or copy number variations /chromosomal rearrangements cannot be assessed using this method.
- Certain genes may not be covered completely and few mutations could be missed. Variants not detected by the assay that was performed may impact the phenotype.
- The mutations have not been validated by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines[22] can also be given upon request.

- 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).

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APPENDIX 2: COVERAGE OF LYNCH SYNDROME/ HNPCC GENE PANEL GENES

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
EPCAM	100.00	*MLH1*	100.00	*MLH3*	100.00
MSH2	99.07	*MSH6*	100.00	*PMS1*	100.00
PMS2	100.00				