240111105360433

Mrs. REETA GUPTA
..

PID NO: P33724531504691
Age: 65 Year(s) Sex: Female

Reference: DR.VINAY KUMAR CHOPRA

Sample Collected At:
Dr Vinay Kumar Chopra
Dr Vinay Kumar Chopra Kos Diagnostic Lab 6349/i Nicholson Road Ambala Cantt Hry 133001. 06-hr 13
Processing Location:- Metropolis Healthcare Ltd,Unit No409-416,4th Floor,Commercial Building-1,Kohinoor Mall,Mumbai-70

VID: 240111105360433
Registered On:
02/09/2024 06:42 PM
Collected On:
30/08/2024 6:40PM
Reported On:
08/10/2024 6:40PM

Test Requested: - Hereditary Lynch Syndrome/HNPCC gene Panel **

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

Endometrioid carcinoma, endometrium, stage I, grade I. IHC results suggestive of loss of expression of MLH1 and PMS2 proteins.

RESULTS

NO PATHOGENIC OR LIKELY PATHOGENIC VARIANTS CAUSATIVE OF THE REPORTED PHENOTYPE WERE DETECTED

VARIANT INTERPRETATION AND CLINICAL CORRELATION

No significant variant(s) that warrant to be reported were detected.

The coverage of genes tested in this assay is listed in Appendix 1.

COPY NUMBER VARIANTS CNV(s)

No significant CNVs for the given clinical indications that warrant to be reported were detected.

ADDITIONAL INFORMATION

- No SNV(s)/INDELS or CNV(s) that warrants to be reported was detected. All the genes covered in this assay have been screened for the given clinical indications. NGS test methodology details are given in the appendix.
- $Genetic test results are reported based on the recommendations of American College of Medical Genetics and Genomics (ACMG) [PMID: 25741868, 31690835, 32906214].

RECOMMENDATIONS

- **The sensitivity of NGS based assays to detect large heterozygous deletions/duplications is low and an alternate method is recommended.**
- No significant variants that may cause loss of expression of MLH1 and PMS2 proteins were detected. Hence, additional testing for somatic variations or promoter methylation in the *MLH1* and *PMS2* genes are recommended.
- Genetic counselling is advised.

LIMITATIONS

Dr. Kirti Chadha
Chief Scientific Officer
Senior Oncopathologist

Dr. MONISHA BANERJEE
Ph.D Senior Consultant Molecular Pathology

METROPOLIS
The Pathology Specialist

INNER HEALTH REVEALED

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- 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 and Laboratory cannot be held responsible for this.
  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, 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. Laboratory under no circumstances will be liable for any delay beyond afore mentioned TAT.

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- 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. 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. 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 Laboratory. In case where any test provided by Laboratory fails for unforeseeable or unknown reasons that cannot be influenced by Laboratory in advance, Laboratory shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognised by Laboratory in advance.

Principal Scientist

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 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. 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.

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: 265829183, 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 (v3.1), 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 and splice site variants found in the coding regions in the Lynch Syndrome/HNPCC 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)	0.9493
Total reads aligned (%)	99.96
Reads that passed alignment (%)	88.44
Data ≥ Q30 (%)	97.31

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$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 LYNCH SYNDROME/ HNPCC 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	100.00	*MSH3*	100.00	*MSH6*	100.00
PMS1	100.00	*PMS2*	100.00		

END OF REPORT

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