MedGenome Labs Ltd. Sy. Nos. 94/1C and 94/2, Tower 1, Ground Floor, Veerasandra Village, Attibele Hobli, Electronic City Phase-1, Electronics City, Bangalore, Bangalore South, Karnataka, India, 560100 Tel: 1800 296 9696, Web: www.medgenome.com.

DNA TEST REPORT - MEDGENOME LABORATORIES

Full Name / Ref No:	**ANOMA JAYAWARDHANA**	Order ID/Sample ID:	**1019265/8648450**
Gender:	Female	Sample Type:	Peripheral Blood (EDTA)
Date of Birth / Age:	64 years	Date of Sample Collection:	8th August 2024
Referring Clinician:	Dr. Mahendra Perera, Aegle Omics Private Limited, Colombo	Date of Sample Receipt:	9th August 2024
		Date of Order Booking:	10th August 2024
		Date of Report:	4th September 2024
Test Requested:	**MGM171 - Adenomatous polyposis coli (APC) gene analysis**		

‡CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

Subject has been evaluated for pathogenic/ likely pathogenic variations in the *APC* gene.

RESULTS

NO PATHOGENIC OR LIKELY PATHOGENIC VARIANTS WERE DETECTED IN THE *APC* GENE

VARIANT INTERPRETATION AND CLINICAL CORRELATION

No significant variant(s) that warrant to be reported were detected in the *APC* gene.

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

‡**NOTE**: Clinical indications, diagnosis, family history details and other relevant medical information pertaining to the patient were not available at the time of report generation. Hence, the results need to be carefully interpreted. The report can be modified based on additional details, if and when received

RECOMMENDATIONS

- **The sensitivity of NGS based assays to detect large heterozygous deletions/duplications is low and an alternate method is recommended.**
- Genetic counselling is advised.

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.

- 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 MedGenome cannot be held responsible for this. Please feel free to contact MedGenome Labs (techsupport@medgenome.com) 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. MedGenome 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. MedGenome 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. MedGenome 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 MedGenome. In case where any test provided by MedGenome fails for unforeseeable or unknown reasons that cannot be influenced by MedGenome in advance, MedGenome shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognised by MedGenome in advance.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by MedGenome.

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Principal Scientist

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Molecular Pathologist Reg Number: KMC 87877

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 *APC* gene 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 [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).

For any further technical queries please contact techsupport@medgenome.com**.**

APPENDIX 1: COVERAGE OF ADENOMATOUS POLYPOSIS COLI (*APC*) GENE ANALYSIS ^

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
APC	100.00				

END OF REPORT