REFERENCE LABORATORY GENETICS

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Pablo Iglesias, 57 – Polígono Gran Via Sur
08908 L'Hospitalet de Llobregat (Barcelona)
Tel. 932 593 700 – Fax. 932 845 000

GENETIC STUDY OF HEREDITARY NON-POLYPOSIS COLON CANCER BY MASSIVE SEQUENCING (NGS)

Request No.:	000		
Client:	-		
Analysis code:	5593		
Patient Name:	xxx		
Date of Birth:	N/A	**Patient Ref.:**	xxx
Gender:	Male	**Sample Type:**	Whole blood
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: 32-year-old patient with a diagnosis of descending colon cancer. Family history by paternal lineage: Two uncles with colon cancer and pancreatic cancer, an aunt with colon cancer and another aunt with breast cancer.

RESULT AND INTERPRETATION

The presence of a heterozygous pathogenic variant in the *MLH1* gene has been identified. It is associated with a predisposition to hereditary colon cancer.

The complete list of studied genes and coverage details is available in Table 1 (Methodology)

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
MLH1	NM_000249.3: **c.2202del** NP_000240.1: **p.(Phe734Leufs*49)**	Heterozygosis	-	Pathogenic

* Nomenclature according to HGVS v15.11
^ Based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)*

The ***MLH1*** variant **c.2202del p.(Phe734Leufs*49)** is a *frameshift* that predicts the loss of a nucleotide and an amino acid change from Phenylalanine to Leucine at position 734 of the protein and causes a premature STOP codon 49 amino acids downstream. It is not described in clinical databases or bibliography consulted to date. It is also not observed in the dbSNP database or the gnomAD population frequency database. The bioinformatic predictor Mutation Taster estimates that the change would have a pathogenic effect.

Based on these data, the variant is classified as a **Pathogenic variant.**

The *MLH1* gene (OMIM: 120436) is associated with a hereditary non-polyposis colorectal cancer type 2 (OMIM: 609310) without an inheritance pattern; a Muir-Torre syndrome (OMIM: 158320) with an autosomal dominant inheritance pattern; and a constitutional mismatch repair deficiency syndrome (OMIM: 276300), that has an autosomal recessive inheritance pattern.

RECOMMENDATIONS

It is recommended that the patient and his relatives make an appointment at a hereditary cancer / genetic oncology unit if it has not yet been performed.

Genetic counselling should be offered to the patient by the prescriber physician. If additional information regarding the results or genetic counselling is required, the physician can contact our team at genetics@referencelaboratory.es.

METHODOLOGY

DNA extraction and quantitative and qualitative evaluation of the DNA obtained.

Capture and enrichment of exonic regions and flanking intronic areas of genes contained in the REFLAB Cancer (Roche) sequencing panel with the Roche NimbleGen SeqCap EZ HyperCap Library™ technology.

Massive sequencing with the NextSeq™(Illumina) sequencer.

Identification of the variants of interest in regard to the reference genome (hg19) after filtering, according to specific quality criteria. Annotation of the obtained variants with a specific bioinformatic software: Alamut Visual™ (Interactive Biosoftware), Ingenuity Variant Analysis™ (QIAGEN), Variant interpreter™ (Illumina) and VarAFT™. The used reference databases have been the population databases dbSNP, 1000genomes, EXAC and gnomAD; the clinical databases Human Gene Mutation Database (HGMD version 2019.3), ClinVar and LOVD; the disease specific databases, if applicable, and Reference Laboratory Genetics´own databases. The bioinformatic analysis to evaluate the possible impact of the variants of interest on the structure and functionality of the protein has been carried out with the bioinformatic programs Mutation Taster, SIFT and PolyPhen-2. These analyses are only a predictive tool; they were not experimentally proven.

The nomenclature used to define the variants follows the criteria of the *Human Genome Variation Society (HGVS)* (http://www.HGVS.org/varnomen).

Classification of variants based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)* (Richards S. *et al*., 2015). Only those variants that, based on current information, are considered pathogenic, likely pathogenic or of uncertain clinical significance, are reported. (The complete list of identified variants is available upon request).

The obtained average reading depth was 108,00x being > 20x in 99,30% of the regions analysed.

The reported INDEL variants are confirmed by Sanger sequencing.

LIMITATIONS: The results obtained do not exclude variants outside the analysed regions of the genome or genetic anomalies not detectable by massive sequencing such as large rearrangements, large deletions/duplications (Copy Number Variant; CNV), insertions / deletions of> = 10 nucleotides, variants in repetitive regions or with a high percentage of GC, and variants in genes with pseudogenes with highly homologous sequences.
It is not possible to rule out the presence of variants in other unanalysed genes.

Table 1. STUDIED GENES AND COVERAGE DETAILS

Gene	NM	10x %	Exons with coverage < 100%*
EPCAM	NM_002354	100,00	-
MLH1	NM_000249	100,00	-
MSH2	NM_000251	100,00	-
MSH6	NM_000179	100,00	-
PMS2	NM_000535	100,00	-

*Due to the current intrinsic limitations associated with massive sequencing technology, some gene exons analysed may be insufficiently covered. If it is considered appropriated by a medical specialist, it would be possible to sequence exons with coverage below 100% using the Sanger method or other alternative molecular technique.

IMPORTANT NOTE

The information contained in this report is based on current scientific knowledge and the results obtained from the application of the technology in this report, are detailed. Due to continuous advances, the documented information may be modified in the future as a result of the emergence of new scientific evidence.
The genetic/genomic studies carried out by Reference Laboratory S.A. are exclusively intended for qualified health professionals for their interpretation. The results obtained are not, per se, a medical consultation, diagnosis or treatment, nor should they be interpreted as such. Only a specialized professional can correctly interpret the results and offer a diagnosis or prescribe a treatment to a patient based on these. Consequently, no information obtained from our studies can be used to replace the advice and diagnosis of a specialized professional.

Signed: Cristina Camprubí, PhD

Head of Diagnosis and Genetic Counseling

License number 21841-C

College of Biologists of Catalonia

Accredited by AEGH

Signed: Irina Royo, MSc

Head of Molecular Genetics

License number 22078-C

College of Biologists of Catalonia

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