HerediGENE

Genekor Medical S.A. | 52, Spaton Ave., 15344, Gerakas, Athens, Greece
email: info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
Scientific Director: George Nasioulas PhD

SAMPLE INFORMATION

Name :	FIRST NAME SURNAME	**Date Received :**	-
Medical ID :	-	**Date of Report :**	-
Date of Birth :	-	**Req. Physician :**	FIRST NAME SURNAME DR.
Location :	-	**Barcode :**	20XXXXXXEN
Material :	WHOLE PERIPHERAL BLOOD	**Sample acceptability :**	Pass

HerediGENE: ***Hereditary Cancer Panel by Next Generation Sequencing***

Result

NO PATHOGENIC VARIANT IDENTIFIED

Gene	Variant	Clinical Significance	Zygosity
APC	NM_000038:c.4073C>T, p.(Ala1358Val)	Variant of Uncertain Significance (VUS)	Heterozygous
BRCA2	NM_000059:c.8825C>T, p.(Ala2942Val)	Variant of Uncertain Significance (VUS)	Heterozygous

Interpretation

The clinical significance of the variants identified is unclear at the time. Until this uncertainty is resolved, this result should not be used in clinical management decisions, or in predictive testing of at risk relatives. In the absence of a molecular explanation for this family's condition, this result does not necessarily inform this individual's risk. However, some mutations in genes of this panel may not be detected by the used methodology. Genes not included in this panel, may also contribute to increased cancer risk. Increased clinical surveillance of this individual may still be warranted. Molecular genetic testing results should always be co-evaluated with the clinical symptoms and family history of the patient.

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Variants Details

***APC*, Exon 16, NM_000038:c.4073C>T, p.(Ala1358Val)** ClinVar

This sequence change replaces Alanine with Valine at codon 1358 of the APC protein. The alanine residue is highly conserved among species in a domain of the protein that is known to be functionally important. There is a moderate physiochemical difference between alanine and valine (Grantham Score 64).This variant is listed in population databases at a very low frequency (rs730881249, ExAC <0.01%) and the mutation database ClinVar contains entries for this variant (Variation ID:181805).Algorithms developed to predict the effect of missense changes on protein structure and function suggest that this variant may be damaging to the protein, but these predictions have not been confirmed by published functional studies. In summary, this is a rare missense change that may affect protein function and cause disease. However, the evidence is insufficient at this time to prove that conclusively. Therefore, it has been classified as a Variant of Uncertain Significance.

Germline mutations in the tumor suppressor gene Adenomatous Polyposis Coli (APC) are responsible for FAP. In approximately 70% of cases such mutations are inherited from an affected parent, while in the remaining 30% they arise in the affected individual (de novo). In attenuated FAP (AAFAP) mutations in the APC gene are restricted to the 5' or the 3' ends of the protein (PMID: 11257105, 8252630) thought to affect protein stability resulting in later age at onset and milder clinical symptoms, such as fewer polyps in patients at the age of 50-60 years. To date, pathogenic mutations in this gene are identified in approximately 50-80% of patients with the FAP phenotype.

The patient must be referred for genetic counseling for adequate interpretation of the study and post-genetic support.

***BRCA2*, Exon 22, NM_000059:c.8825C>T, p.(Ala2942Val)** ClinVar

This sequence change replaces Alanine with Valine at codon 2942 of the BRCA2 protein. The alanine residue is moderately conserved among species in a domain of the protein that is not known to be functionally important. There is a moderate physiochemical difference between alanine and valine (Grantham Score 64).This variant is listed in population databases at a very low frequency (rs373227180,gnomAD_genome <0.01%) and the mutation database ClinVar contains entries for this variant (Variation ID:141807).Algorithms developed to predict the effect of missense changes on protein structure and function all suggest that this variant is likely to be tolerated, but these predictions have not been confirmed by published functional studies. In summary, this is a rare missense change that is not predicted to affect protein function and cause disease. However, the evidence is insufficient at this time to prove that conclusively. Therefore, it has been classified as a Variant of Uncertain Significance.

The BRCA2 gene involved in the homologous recombination complex (HR) and is associated with autosomal dominant hereditary breast and ovarian cancer (HBOC) syndrome and autosomal recessive Fanconi anemia. The lifetime risk for

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contralateral breast cancer in individuals with a single pathogenic BRCA2 variant is 23% within 5 years of the primary breast cancer (PMID: 24764694, 14576434, 10498392). The lifetime risk for ovarian, fallopian tube, or peritoneal cancer is 16-27% (PMID: 9497246, 9145676). The risk for male breast cancer in individuals with a pathogenic BRCA2 mutation is 7-8% (PMID: 27144062). There are also increased risks for melanoma, prostate cancer (20%), and pancreatic cancer (2-3%) (PMID: 10433620). Clinical management guidelines for individuals carrying pathogenic variants in the BRCA2 gene can be found at www.nccn.org.

The patient must be referred for genetic counseling for adequate interpretation of the study and post-genetic support.

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Methodology

Genomic DNA was extracted from the sample under investigation and was analysed by a solution based capture approach using a custom target enrichment panel containing 36 genes involved in hereditary predisposition to cancer, of which 15 genes involved in the homologous recombination (HR) complex (SeqCap EZ Choice, NimbleGen, Roche) (see table). Sequencing was carried out using Illumina technology. Reads were aligned to the reference sequence (GRCh37), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. All clinically significant observations were confirmed by Sanger Sequencing. All targeted regions were sequenced with ≥60x depth. Unless otherwise stated, this assay targets all coding regions of the indicated transcripts and 20 base pairs of flanking intronic sequences. For the *HOXB13*, *POLE* and *POLD1* genes, distinct genomic regions have been associated with increased cancer risk. Consequently, the reported regions for the aforementioned genes are: *HOXB13* - rs138213197, *POLE* - Exons 35-48 (NM_006231), *POLD1*- Exons 7-12 (NM_001256849).

The presence of large genomic rearrangements (LGRs), is investigated using the commercial computational algorithm SeqPilot Version 4.4 Build 505 (JSI Medical System). In addition, the computational algorithm panelcn.MOPS (Hum Mutat. 2017, 38:889-897) was also used in the *BRCA1* and *BRCA2* genes .The presence of LGRs is verified by use the MLPA method (Multiplex Ligation-dependent Probe Amplification, MRC Holland; AJHG 67:841-50, 2000).

*Notes:

Every molecular test has an internal 0,5-1% chance of failure. This is due to rare molecular events and factors related to the preparation and analysis of the samples.

The variants reported in *PMS2* gene are detected with coverage >25%. The method used cannot detect low-level mosaicism (with coverage <25%).

The method used achieves 99% sensitivity and specificity for single nucleotide variants and insertions and deletions <15bp. Sensitivity to detect genomic rearrangements larger than 15bp but smaller than a full exon may be reduced. Balanced genomic rearrangements cannot be detected.

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Details about non-pathogenic variants

All individuals carry DNA changes (i.e., variants), and most variants do not increase an individual's risk of cancer or other diseases. When identified, variants of uncertain significance (VUS) are reported. Benign variants (Polymorphisms) are not reported and available data indicate that these variants most likely do not cause increased cancer risk. Present evidence does not suggest that non-clinically significant variant findings be used to modify patient medical management beyond what is indicated by the personal and family history and any other clinically significant findings.

Genes Analyzed

Gene	Reference sequence	Gene	Reference sequence
APC	NM_000038	*MSH2**	NM_000251
ATM	NM_000051	*MSH6**	NM_000179
BARD1	NM_000465	*MUTYH**	NM_001128425
BMPR1A	NM_004329	*NBN*	NM_002485
*BRCA1**	NM_007294	*NF1*	NM_000267
*BRCA2**	NM_000059	*PALB2**	NM_024675
BRIP1	NM_032043	*PMS2*	NM_000535
CDH1	NM_004360	*POLD1* (Exons 7-12)	NM_001256849
CDK4	NM_000075	*POLE* (Exons 35-48)	NM_006231
CDKN2A (p14ARF, p16INK4a)	NM_000077	*PTEN*	NM_000314
*CHEK2**	NM_007194	*RAD50**	NM_005732
*EPCAM**	NM_002354	*RAD51C**	NM_058216
FANCA	NM_000135	*RAD51D**	NM_002878
FANCM	NM_020937	*RET*	NM_020975
HOXB13:c.251G>A p.(G84E)	NM_006361	*SMAD4*	NM_005359
MEN1	NM_000244	*STK11*	NM_000455
*MLH1**	NM_000249	*TP53**	NM_000546
MRE11A	NM_005591	*VHL*	NM_000551

Genes of the homologous recombination (HR) complex are labelled **blue**

***** Unless otherwise noted analysis of large rearrangement was performed on the following genes:
BRCA1, BRCA2, CHEK2, EPCAM (Εξώνια 8, 9), MLH1, MSH2, MSH6, MUTYH, PALB2, RAD50 (Exons 1, 2, 4, 10, 14, 21, 23 and 25), RAD51C, RAD51D, and TP53.

Electronically Signed by **- Georgia Pepe, MSc Molecular Biologist, AMKA:09038403029**
- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

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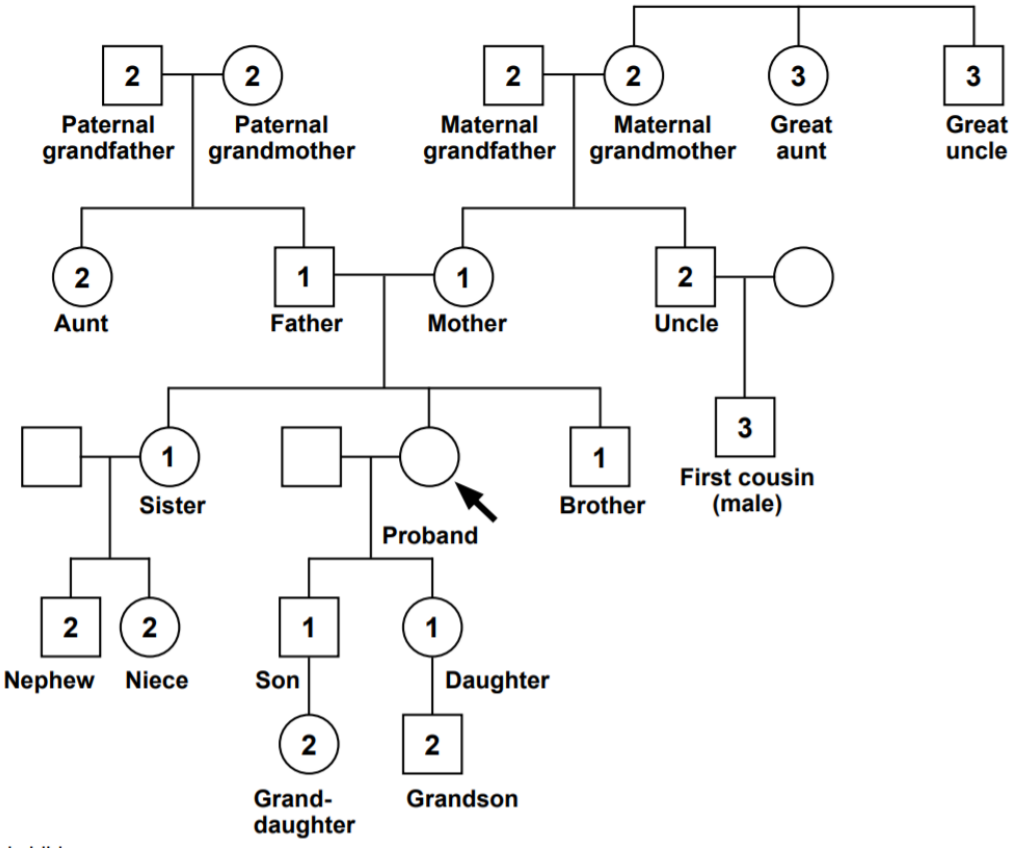
Family tree



Note: **The information shown on the family tree has been provided by the patient and not by medical records.**

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