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

PATHOGENIC VARIANTS IDENTIFIED

Gene	Variant	Clinical Significance	Zygosity
BRCA1	NM_007294:c.181T>G, p.(Cys61Gly)	Pathogenic-Clinically significant variant	Heterozygous
CHEK2	NM_007194:c.470T>C, p.(Ile157Thr)	Pathogenic variant in a low/unspecified risk cancer gene	Heterozygous
FANCA	NM_000135:c.1874G>C, p.(Cys625Ser)	Variant of Uncertain Significance (VUS)	Heterozygous
POLE	NM_006231:c.139C>T, p.(Arg47Trp)	Variant of Uncertain Significance (VUS)	Heterozygous

Note: "CLINICALLY SIGNIFICANT" as defined in this report, is a genetic change that is associated with the potential to alter medical intervention

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Variants Details

***BRCA1*, Exon 4, NM_007294:c.181T>G, p.(Cys61Gly)** ClinVar

This sequence change replaces cysteine with glycine at codon 61 of the BRCA1 protein (p.Cys61Gly). The cysteine residue is highly conserved and there is a large physiochemical difference between cysteine and glycine. This variant is present in population databases (rs28897672, 0.01%) and has been reported in the literature. ClinVar contains an entry for this variant (RCV000019229). This variant is a common cause of breast and ovarian cancer in individuals of Eastern European ancestry (PMID: 20345474, 20507347, 20569256, 19594371). It has been reported in hundreds of individuals with these cancers (PMID: 7894493, 10788334, 21324516, 20180014). It is also known as 300T>G in the literature. This variant affects a highly conserved Cys61 residue within the N-terminal RING domain of the BRCA1 protein (PMID: 22843421). Experimental studies have shown that this variant disrupts several aspects of BRCA1 function (PMID: 11278247, 9525870, 22172724, 23161852, 23867111). For these reasons this variant has been classified as pathogenic. According to international guidelines it is recommended that relatives of the patient are tested for the above mutation.

The BRCA1 gene involved in the homologous recombination complex (HR) and is associated with autosomal dominant hereditary breast and ovarian cancer (HBOC) syndrome. This result is consistent with a predisposition to, or diagnosis of, BRCA1-related conditions. HBOC syndrome is characterized by an increased lifetime risk for breast cancer, contralateral breast cancer, male breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, and other cancers (PMID: 12237281). The lifetime risk for female breast cancer in individuals with a pathogenic BRCA1 sequence change is 40-87% (PMID: 10498392). The risk for contralateral breast cancer in these individuals is up to 43% within ten years of the initial breast cancer diagnosis (PMID: 15197194). The lifetime risk for male breast cancer in individuals with a pathogenic BRCA1 sequence change is 1.2% (PMID: 18042939). The lifetime risk for ovarian cancer, fallopian tube, or peritoneal cancer in females is 16-44% (PMID: 23628597). Clinical management guidelines for HBOC syndrome 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. Relatives of this individual have up to 50% risk of having the same mutation. Predictive testing of this mutation should be offered to all at-risk adult relatives after receiving genetic counseling.

Patients with germline mutations in HR genes may benefit from platinum-based therapies (PMID: 20406929) and treatment with PARP inhibitors (PMID: 31218365).

***CHEK2*, Exon 4, NM_007194:c.470T>C, p.(Ile157Thr)** ClinVar

This sequence change replaces isoleucine with threonine at codon 157 of the CHEK2 protein (p.Ile157Thr). The isoleucine residue is moderately conserved and there is a moderate physiochemical difference between isoleucine and

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threonine. This variant is present in population databases (rs17879961, 2.6%) and has been reported in the literature. ClinVar contains entries for this variant (RC000116018, RC000144596). In large meta-analyses involving several thousand cases and controls, patients who carried this variant had a slightly increased risk of breast cancer (OR=1.48-1.58) (PMID: 22799331, 23713947), and colorectal cancer (OR=1.48-1.67) (PMID: 22901170, 23713947). The risk was found to be more pronounced for lobular breast tumors (OR=4.17) (PMID: 22799331). Smaller case-control studies suggest this variant may also lead to increased risk of additional cancers, including kidney, prostate, thyroid, and gastric cancer (PMID: 15492928, 23296741, 24599715). Experimental studies find that this missense change reduces the binding of the CHEK2 protein to Cdc25A, BRCA1 and p53 proteins in vitro and may have a dominant-negative effect in cells, although it does not have an effect on CHEK2 protein kinase activity (PMID: 11298456, 11571648, 15239132, 12049740, 22419737). The relationship between these experimental findings and the cancer risk is unclear. In summary, this variant is reported to cause an increased risk for cancer, however, since this variant is associated with a much lower risk than other Pathogenic alleles in the CHEK2 gene, it has been classified as Pathogenic (low penetrance).

The CHEK2 gene involved in the homologous recombination complex (HR) and is associated with an increased risk for autosomal dominant breast and possibly other cancers in individuals who carry a single pathogenic CHEK2 variant (PMID: 20597918, 21876083). CHEK2 is associated with the DNA damage repair response Fanconi anemia (FA)-BRCA pathway (PMID :19686080). Carriers of pathogenic CHEK2 variants have up to 2- to 5-fold increased risk for breast cancer above the general population (PMID: 23652375, 21876083).

The patient must be referred for genetic counseling for adequate interpretation of the study and post-genetic support. Relatives of this individual have up to 50% risk of having the same mutation. Predictive testing of this mutation should be offered to all at-risk adult relatives after receiving genetic counseling.

Patients with germline mutations in HR genes may benefit from platinum-based therapies (PMID: 20406929) and treatment with PARP inhibitors (PMID: 31218365).

***FANCA*, Exon 21, NM_000135:c.1874G>C, p.(Cys625Ser)** ClinVar

This sequence change replaces Cysteine with Serine at codon 625 of the FANCA protein. The cysteine residue is highly conserved among species in a domain of the protein that is not known to be functionally important. There is a large physiochemical difference between cysteine and serine (Grantham Score 112). This variant is present in population databases at a very low frequency (rs139235751, 0.1%) and the mutation database ClinVar contains entries for this variant (https://www.ncbi.nlm.nih.gov/clinvar/variation/265136/). 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

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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 FANCA gene involved in the homologous recombination complex (HR) and is associated with autosomal recessive Fanconi anemia type A. Additionally, FANCA gene is associated with increased risk for prostate cancer in individuals who carry a single pathogenic FANCA variant (PMID: 28864460, 27701467, 26181256).

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

***POLE*, Exon 2, NM_006231:c.139C>T, p.(Arg47Trp)** ClinVar

This sequence change replaces Arginine with Tryptophan at codon 47 of the POLE protein. The Arginine residue is weakly conserved among species in a domain of the protein that is not known to be functionally important. There is a big physiochemical difference between arginine and tryptophan (Grantham Score: 101). This variant is present in population databases at a very low frequency (rs143626223, ExAC 0.09%) and is listed in the mutation database ClinVar (Variation ID: 240391). Algorithms developed to predict the effect of missense changes on protein structure and function 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 POLE gene is associated with an increased risk for autosomal dominant colonic adenomatous polyps and colon cancer (PMID: 23263490, 26133394, 23585368, 24501277, 24788313)

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 - **Konstantinos Agiannitopoulos, PhD Molecular Biologist, AMKA:16058503091**
- **George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255**

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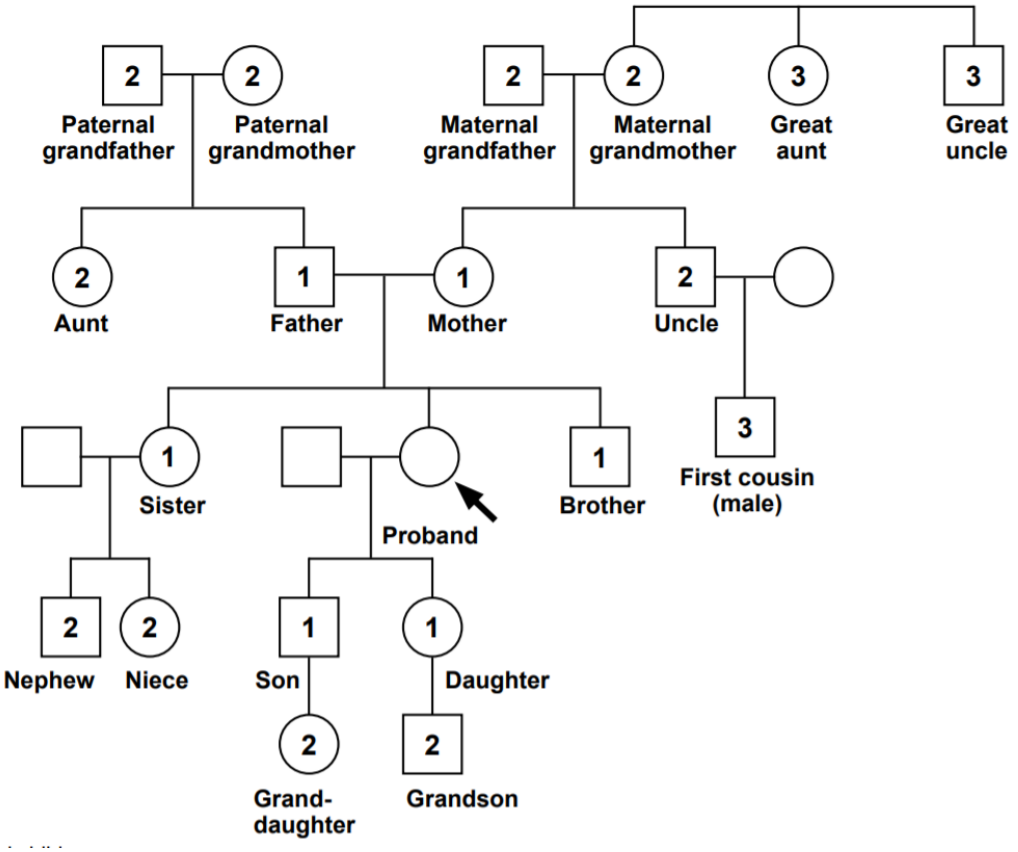
Family tree



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

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