Genesys Diagnostics Inc.
8 Enterprise Lane Oakdale, CT, 06370
Phone: (860) 574-9172 / Fax: (860) 574-9264
http://gdilabs.com

Name: **Smith, John**

Accession ID: **20092932**

DOB: **1/15/1945**
Sex: **Male**
Race/Ethnicity:
Family #:

MRN:
Referring facility: **Office, Doctor's**
Referring physician: **Physician, Ordering**
Copies to:

Specimen: **Buccal Swab**
Date of Collection: **11/4/2020**
Date of Receipt: **11/17/2020**
Date of Report: **4/14/2021**

Test(s) Performed: **Comprehensive Hereditary Cancel Panel**
Indication for test: **Family history of malignant neoplasm of prostate, Family history of malignant neoplasm of digestive organs, Family history of leukemia**

RESULT: Positive
One pathogenic variant and one variant of uncertain clinical significance were identified

APPROACH

Sequencing of select genes was done using Next Generation Sequencing and the data was analyzed to identify both previously classified and novel variants in targeted genes. A total of 22 genes with previous implications in various mendelian disorders (see Supplement for a list of genes and coverage information) were covered with minimum read depth of 20X. Note that this test cannot exclude the possibility of variants in genes not analyzed or assayed with incomplete coverage.

VARIANTS RELEVANT TO INDICATION FOR TESTING

DNA sequencing identified two variants: one pathogenic variant in BRCA2 and one variant of uncertain clinical significance in ATM. No other variants of clinical significance relevant to the patient's phenotype or family history were identified. It is recommended that this individual and any 1st degree relative receive continued clinical evaluation. Please see below for more detailed variant information. The mean depth coverage is 716x with a quality threshold of 99.9%.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
ATM NM_000051.3	c.251C>T p.Ala84Val	Het.	Exon 4	Ataxia-telangiectasia Syndrome	Autosomal Recessive / Heterozygous	Uncertain Significance
BRCA2 NM_000059.3	c.6724_6725delGA p.Asp2242Phefs*2	Het.	Exon 11	Breast and other cancers	Autosomal Dominant / Heterozygous	Pathogenic

OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)

No incidental findings are possible due to targeted sequencing.

Monogenic Disease Risk

There were NO monogenic disease risk variants identified in this individual in genes unrelated to this individual's clinical presentation. Please see limitations for more detail.

Carrier Status

There were NO variants inferring a carrier status of a recessive disorder identified in this individual in genes unrelated to this individual's clinical presentation. Please see limitations for more detail.

RECOMMENDATIONS

Genetic counseling is recommended for this individual and their family. Genesys Diagnostics provides assistance in genetic counseling services, please contact the laboratory at (860) 574-9172 for further information. A medical provider can request reanalysis of the variants, and this is recommended on an annual basis. Data from this next generation sequencing analysis can be reassesed for the presence of any new variants that may be newly linked to established genes or to newly characterized genes and/or disorders identified since the data used in this report was generated. A charge may be applied for reanalysis. Please contact the laboratory for more information at the time reanalysis is requested.

DETAILED VARIANT INFORMATION (VARIANTS RELEVANT TO INDICATION FOR TESTING)

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Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
ATM NM_000051.3	c.251C>T p.Ala84Val	Het.	Exon 4	Ataxia-telangiectasia Syndrome	Autosomal Recessive / Heterozygous	Uncertain Significance
Genomic Position			**Variant Frequency**			
Chr11:NC_000011.9:g.108099970C>T			0.006% max frequency observed in gnomad East Asian			

VARIANT INTERPRETATION: The missense variant p.A84V in ATM (NM_000051.3) has been identified in 1/250950 alleles in gnomAD and has been reported to ClinVar as a variant of Uncertain Significance (Variation ID: 141686). To the best of our knowledge, this variant has not been reported in the literature in individuals with ATM-related disease. There is a small physicochemical difference between alanine and valine, which is not likely to impact secondary protein structure as these residues share similar properties. Algorithms developed to predict the effect of missense changes on protein structure and function output the following: SIFT: "Deleterious"; PolyPhen-2: "Benign". The valine amino acid residue is found in multiple mammalian species, suggesting that this missense change does not adversely affect protein function. These predictions have not been confirmed by published functional studies and their clinical significance is uncertain. In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance. ATM is believed to play a major role in maintaining the integrity of the genome and alterations in these proteins is characterized by defects in DNA damage responses making the individual cancer prone (PMID: 18383876). Germ line mutations in ATM result in the well characterized ataxia telangiectasia syndrome, which shows an autosomal recessive inheritance pattern and manifests with an increased cancer predisposition, including a 20% to 30% lifetime risk of lymphoid, gastric, breast, central nervous system, skin, and other cancers (PMID: 27413114). Germ line ATM heterozygosity occurs in about 1% of the population. Family members heterozygous for ATM gene mutations showed an approximate 2- to 3- fold risk of cancer, and a 5- to 9-fold risk of breast cancer in women, with the relative risk increased in women less than 50 years of age. Clinical correlation and genetic counseling are recommended.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
BRCA2 NM_000059.3	c.6724_6725delGA p.Asp2242Phefs*2	Het.	Exon 11	Breast and other cancers	Autosomal Dominant / Heterozygous	Pathogenic
Genomic Position			**Variant Frequency**			
Chr13:NC_000013.10:g.32915216_32915217delGA			0.003% max frequency observed in gnomnAD Latino			

VARIANT INTERPRETATION: The frameshift variant p.D2242Ffs*2 (NM_000059.3) has been identified in 1/251336 alleles in gnomAD and has been reported to Clinvar as Pathogenic (Variant ID: 38062). The two nucleotide deletion results in a substitution of Aspartate with Phenylalanine and a stop codon at the second amino acid position of the new reading frame, leading to loss of function by premature protein truncation or nonsense-mediated mRNA decay. This variant has been reported in multiple Korean patients and families with breast and/or ovarian cancer (PMID:19656164, 22798144, 25863477, 27836010, 29446198). Therefore, it has been classified as a Pathogenic Variant. BRCA2 is a tumor suppressor gene inherited in an autosomal dominant pattern. Mutations in this highly penetrant gene increase the chance of cancer of the breast, ovaries (including primary peritoneal and fallopian tube), pancreas, and prostate. Studies suggest female BRCA2 mutation carriers have a 45-81% lifetime risk to develop breast cancer and an 11-18% risk to develop ovarian cancer by age 70. Male BRCA2 mutation carriers have up a 15% lifetime prostate cancer risk and a cumulative lifetime breast cancer risk of 6.8% by ages 65 and 70, respectively. BRCA2 mutation carriers may also be at an increased risk for melanoma, pancreatic cancer, and potentially other cancers. BRCA2 is also known as FANCD1. Individuals who inherit a BRCA2/FANCD1 mutation from each parent may have a rare autosomal recessive condition called Fanconi anemia type D1 (FAD1), which affects multiple bodysystems. Clinical correlation and genetic counseling are recommended.

METHODOLOGY

Genomic DNA was extracted from the patient's sample submitted to our laboratory using QIAamp mini kit. The TruSight Rapid Capture Kit (Illumina) with Illumina Canadian Consortia Inherited Cancer Probes was used to target the requested genes of tagmented genomic DNA. These targeted regions were then sequenced using Illumina MiSeq with paired end reads. The DNA sequenced was mapped to, and analyzed in comparison with, the published human genome build (UCSC hg19 reference sequence). The targeted coding genes and splice junctions of the known coding RefSeq genes were assessed for the average depth of coverage and the data quality threshold values. All pathogenic, likely pathogenic, and indel variants are confirmed by Sanger sequence analysis using a separate DNA preparation.
Mean depth of coverage refers to the sequence mean read depth across the targeted region.
The quality threshold refers to the percentage of the defined target region where read depth was at least 20x coverage to permit high quality exome variant base calling, annotation and evaluation. Average quality thresholds may range from >85% of the targeted region, indicating a small portion of the target region may not be covered with sufficient depth or quality to confidently call variant positions. All reported pathogenic, likely pathogenic, and indel variants are confirmed by independent sanger sequencing (SNVs) or Microarray/MLPA(CNVs).
Variant classified as likely benign or benign are not confirmed by Sanger sequencing and are not reported but are available upon request.
The genes covered in this panel and the accession numbers of the reference are: APC (NM_000038.5), ATM (NM_000051.3), BARD1 (NM_000465.3), BRCA1 (NM_007294.3), BRCA2 (NM_000059.3), BRIP1 (NM_032043.2), CDH1 (NM_004360.4), CHEK2 (NM_007194.3), EPCAM* (NM_002354.2), FANCC (NM_000136.2), MLH1 (NM_000249.3), MSH2 (NM_000251.2), MSH6 (NM_000179.2), MUTYH (NM_00128425.1), NBN (NM_002485.4), PALB2 (NM_024675.3), PMS2 (NM_000535.6), PTEN (NM_000314.6), RAD51C (NM_058216.2), RAD51D (NM_002878.3), STK11 (NM_000455.4), TP53 (NM_000546.5).

VARIANT ASSESSMENT PROCESS

The following databases and insilico algorithms are used to annotate and evaluate the impact of the variant in the context of human disease: 1000 genomes, gnomAD, ClinVar, OMIM, dbSNP, NCIB RefSeq Genes, ExAC Gene Constraints, VSSIFT, VSPolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Analysis was reported using the to HGVS nomenclature (www.hgvs.org/mutnomen) as implemented by the VarSeq transcript annotation algorithm. The reported transcript matches that used most frequently by the clinical labs submitting to ClinVar. The following variants are not reported but are available upon request: (1) variants classified as likely benign or benign; (2) synonymous or non coding variants which are predicted not to affect splicing by in silico algorithms; (3) variants of uncertain clinical significance in a gene associated with autosomal recessive diseases without a second hit.

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LIMITATIONS

APC Promoter Region 1A and 1B are not covered by the Illumina Canadian Consortia Inherited Cancer (20011891), and therefore, are not included in the panel. All genes that have pseudogenes will have poorer performance on the MiSeq instrument. PMS2 Exon 15, specifically, does not have consistent coverage that meets our minimum threshold and therefore, is not included in this panel. Variants in pseudogene regions may not be reliably detected. DNA alterations not in regions covered by this test will not be detected using Next Generation Sequencing analysis. There are technical limitations on the ability of Next Generation Sequencing to detect small insertions and deletions and these types of alterations are not detected as reliably as single nucleotide variants. This assay is not designed or validated for the detection of low level mosaicism or somatic mutations. This assay will not detect certain types of genomic aberrations such as translocations, inversions, or repeat expansions. Large deletions or duplications may be identified, but not guaranteed to be detected by this test. Rare diagnostic errors can result from incorrectly assigned family relationships (e.g. nonpaternity) or DNA alterations (e.g. DNA variant under a primer or probe binding site and the presence of pseudogene artifacts). Additionally, it is possible that a particular genetic abnormality may not be recognized as the underlying cause of genetic disorder dur to incomplete scientific knowledge about the function of all genes in the human genome and the impact of variants in those genes.
Disclaimer:
This test was developed and its performance characteristics determined by Genesys Diagnostics Inc (CLIA# 07D2046796). The U.S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

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Approved by:
Jun Liao (5/4/2021)