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Lab Director: Neil B Quigley, PhD, HCLD(ABB)
CLIA License ID: 36D2162956

Germline Variant Report

Patient

First name: Jane
Last name: Doe
Patient ID: SG10000003
DOB: 12 Aug 1973
Gender: Male

Specimen

Date collected: 23 Aug 2019
Date received: 26 Aug 2019
Date tested: 03 Sept 2019
Date of report: 06 Sept 2019

INTERPRETATION

POSITIVE FOR CLINICALLY SIGNIFICANT MUTATION IN *BRCA2* GENE

Interpretation:

The patient is positive for pathogenic variant in *BRCA2* gene. This variant has been associated with increased risk for breast and ovarian cancer.

BRCA2 (Breast Cancer 2) gene encodes a protein that serves in DNA repair mechanism. The BRCA2 protein is involved in repairing damaged DNA. In the nucleus of many types of normal cells, the BRCA2 protein interacts with several other proteins to mend breaks in DNA. These breaks can be caused by natural and medical radiation or other environmental exposures, and they also occur when chromosomes exchange genetic material in preparation for cell division. By helping to repair DNA, the BRCA2 protein plays a critical role in maintaining the stability of a cell's genetic information.

Patient has a deletion of Adenine nucleotide at position of 4631 in exon 11 of *BRCA2* gene. This deletion causes a frame shift in amino acid reading frame and results in the termination of protein synthesis at amino acid 1568, resulting in non-functional protein being produced.

The absence of functional BRCA2 protein results in increased DNA damage and risk of cancer. With clinically significant mutation in *BRCA2* gene the risk of breast cancer in females increases to up to 84%.

Recommendation:

Genetic counseling is recommended to discuss the implication of this test result.

Medical management:

Medical management of unaffected females with positive results: NCCN guidelines version 3.2019:

Breast Cancer

- Breast awareness starting at age 18 years
- Clinical breast exam every 6 – 12 months, starting at age 25 years
- Annual breast screening, MRI or mammogram with consideration for tomosynthesis

Ovarian Cancer

- Transvaginal ultrasound may be considered at the clinician's discretion.
- Serum CA-125 is an additional ovarian screening test with limited benefits

Analyzed by: Neil Quigley, PhD

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RESULTS

SNVs/INDELs

Gene Transcript	Exon	c.DNA Protein alteration	Variant Fraction Coverage (ref / alt)	Coding consequence	Pathogenicity
BRCA2 NM_000059	11	c.4631delA p.= (p.Asn1544fs)	54.32 % (291 / 346)	frame shift	Flagged Pathogenicity 5 | Definitely Pathogenic
comment: The BRCA2 is a tumor suppressor protein involved in DNA repair mechanism. The absence of this protein results in increased DNA damage and increased risk for cancer. The patient has a single nucleotide deleted at position 4631 in *BRCA2* gene. This mutation causes a frame-shift resulting in synthesis of non-functional protein					
ATM NM_000051	31	c.4709T>C p.Val1570Ala	99.76 % (2 / 826)	missense	Flagged Pathogenicity 3 | Uncertain
comment: The *ATM* gene encodes a serine/threonine kinase that is involved in cell signaling and DNA repair. Certain mutations in *ATM* gene are known to increase cancer risk. The patient has a single nucleotide polymorphism at 4709. The functional consequence of this variant is unknown					

METHODOLOGY AND DISCLAMER

Clariti NGS Methodology

Clariti Diagnostic Laboratories LLC uses Next Generation Sequencing (NGS) technology to analyze coding regions and exon / intron boundaries of selected genes. Nextera Flex For Enrichment chemistry from Illumina is utilized to prepare genomic DNA for sequencing. Genomic DNA is fragmented using Transposomes. Each patient sample is labelled with a unique DNA index to allow libraries to be developed and sequenced simultaneously. Hybrid capture chemistry is utilized to target and then amplify specific regions of interest. The target regions are sequenced with the Illumina MiniSeq instrument using either, a mid-output or high-output kit. At least 99.0 % of target regions are covered at 20X or higher.

FASTQ files are analyzed by SOPHiA GENETICS for secondary and tertiary analysis. DNA sequences are assembled and aligned against human genome build GRCh37. The genomic variants are identified and evaluated for clinical relevance using ACMG guidelines. Bioinformatics analysis is provided by SOPHiA GENETICS, Inc (Boston, MA).

This test was developed and tested by Clariti Diagnostics Laboratories, LLC. Performance parameters were characterized by running extensive set of control samples, patient samples and samples from normal volunteers. The test is not currently FDA cleared. The FDA has determined such clearance for this test is not necessary for laboratories with good standing with CLIA (Clinical Laboratory Improvement Amendments of 1988).

Test performed:HCS_v1_1 **Reference Genome:**GRCh37/hg19 **SOPHiA DDM:**5.3-SNAPSHOT

Pipeline ID / Revision Number / Splitting ID:ILL1XG1G2_CNV / v5.3.4 /GEN1GN1FSQ2

Test Limitations and Disclaimer

NGS based sequencing cannot detect genetic mosaicism. Bone marrow transplantation and blood transfusion may affect the test outcome. Some gene regions inherently have low complexity or other properties that make accurate detection of variant difficult. A list of gene regions with low coverage or low sequence complexity in this patient DNA can be obtained by submitting a written request. Clariti Diagnostics Laboratories LLC technology attempts to cover 99.0% of target genes at 20X or higher. PMS2 gene is complicated by the presence of pseudogene. Presence of a pseudogene may affect test results.

Negative result on this test does not rule out the diagnosis of cancer as some genetic mutations may be undetectable with this test. This test is expected to produce highly accurate results. However, the possibility of false negative or false positive due to laboratory errors and data analysis during any step of the testing process cannot be completely ruled out. This test was developed and validated by Clariti Diagnostics Laboratories, LLC. Performance parameters were characterized by running extensive set of control samples, patient samples and samples from normal volunteers. The test is not currently FDA cleared. The FDA has determined such clearance for this test is not necessary for laboratories with good standing with CLIA (Clinical Laboratory Improvement Amendments of 1988).

TEST PERFORMED

Gene Panel The patient gave thier consent to analyse the following genes:*APC , ATM , BARD1 , BMPR1A , BRCA1 , BRCA2 , BRIP1 , CDH1 , CDK4 , CDKN2A , CHEK2 , DICER1 , EPCAM , GREM1 , HOXB13 , MEN1 , MLH1 , MSH2 , MSH3 , MSH6 , MUTYH , NBN , NF1 , NF2 , NTHL1 , PALB2 , PMS2 , POLD1 , POLE , PTEN , RAD50 , RAD51C , RAD51D , SMAD4 , SMARCA4 , STK11 , TP53*

ANNEXES

CNVs

No CNVs detected.

Glossary

ATM	Ataxia Telangiectasia gene
BRCA1	Breast Cancer 1 gene
BRCA2	Breast Cancer 2 gene
Exon	Segment of gene encoding protein sequence
Heterozygous	Variant found in one copy of the gene
Homozygous	Variant found in both copies of the gene
MRI	Magnetic Resonance Imaging
NCCN	National Comprehensive Cancer Networ
Tomosynthesis	3-D imaging of breast tissue

References

- ClinVar, US National Library of Medicine (https://www.ncbi.nlm.nih.gov/clinvar/variation/37913/)
- Breast Cancer Information Core – BIC (https://research.nhgri.nih.gov/bic/)