Blueprint Genetics

Hereditary Breast Cancer High Risk Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		55	Female	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood		

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *BRCA2* c.3847_3848del, p.(Val1283Lysfs*2), which is pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
BRCA2	13:32912337	NM_000059.3	c.3847_3848del, p.(Val1283Lysfs*2)	frameshift_variant	HET	**Pathogenic**

ID	gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER
rs80359405	10/232146	N/A	N/A	N/A

OMIM	PHENOTYPE	INHERITANCE	COMMENT
OMIM	Breast-ovarian cancer familial	AD	-

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Hereditary Breast Cancer High Risk Panel	7	246	32719	32703	330	99.9

TARGET REGION AND GENE LIST

The Blueprint Genetics Hereditary Breast Cancer High Risk Panel (version 2, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: BRCA1*, BRCA2, CDH1, PALB2, PTEN*, STK11 and TP53. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel should be used to detect single nucleotide variants and small insertions and deletions (INDELs) up to 220 bps and copy number variations defined as single exon or larger deletions and duplications. This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/

The sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 55year-old female with breast cancer.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Hereditary Breast Cancer High Risk Panel identified a heterozygous frameshift variant c.3847_3848del, p.(Val1283Lysfs*2) in *BRCA2*.

There are 10 individuals heterozygous for this variant in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). The two base pair deletion generates a frameshift leading to a premature stop codon in the new reading frame (at codon 1284 out of 3418 aa). It is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay.

The *BRCA2* c.3847_3848del, p.(Val1283Lysfs*2) variant is a well-recognized pathogenic mutation listed in several public databases (ClinVar ID 37859, HGMD, LOVD, BRCA Exchange). The exact number of patients with this mutation is unclear, but is has been reported in over 200 patients in clinical testing (ClinVar ID 37859) and the variant is listed >70 times in the LOVD database. It has been classified as pathogenic by the ENIGMA consortium. In the literature the variant has been identified in several individuals and families affected with breast, ovarian, and prostate cancer (PMID: 8589730, 21324516, 22752604, 23199084, 21952622). It was also shown to segregate with disease in a family with colorectal and other cancers (PMID: 24814045). This variant is also known as 4075delGT in the literature.

BRCA2

BRCA2 (MIM *600185) gene encodes breast cancer type 2 susceptibility protein involved in double-strand break repair and homologous recombination. BRCA2 acts as a tumor suppressor. Germline mutations in *BRCA2* cause hereditary breast and ovarian cancer syndrome (HBOC; GeneReviews: NBK1247). HBOC is an autosomal dominant cancer predisposition syndrome caused by germline mutations in *BRCA1* or *BRCA2*. The lifetime risk for breast cancer in individuals with a pathogenic variant in *BRCA2* is estimated at 40-70%, and individuals have increased risk of ovarian, prostate and pancreatic cancer, and melanoma. Male breast cancer is more commonly associated with mutation of *BRCA2* than *BRCA1*. Most pathogenic mutations in *BRCA2* are truncating (frameshift, nonsense, splice site altogether >95%of those reported in the ClinVar as pathogenic). The clinical significance of rare missense variants remain often uncertain, especially without family segregation data. Recessive mutations of *BRCA2* cause Fanconi anemia (FA) complementation group D1 (MIM #605724). Some 3% of all FA cases are attributed to pathogenic variants in *BRCA2* (GeneReviews NBK1401).

The treatment of both breast and ovarian cancer in individuals with *BRCA1*- or *BRCA2*-related tumors is similar to that in sporadic forms of these diseases. National Comprehensive Cancer Network (NCCN) guidelines do suggest that for primary surgical treatment of breast cancer, women with a *BRCA1* or *BRCA2* pathogenic variant could consider bilateral mastectomy because of their elevated rate of ipsilateral and contralateral breast cancer (GeneReviews: NBK1247). Breast cancer screening in women and men relies on a combination of monthly breast self-examination, annual or semiannual clinical breast examination, annual mammography, and breast MRI. In 2007, the American Cancer Society (ACS) added annual breast MRI to their screening recommendations for individuals with a germline pathogenic variant in *BRCA1* or *BRCA2*. Prophylactic surgery (mastectomy and oophorectomy) has been proposed as a means of reducing cancer risk in people with genetic susceptibility to breast and ovarian cancer. Annual pelvic ultrasound and/or CA-125 concentration has not been effective in detecting early-stage ovarian cancer, either in high-risk or average-risk women. Prostate cancer screening relies on annual digital rectal examination and prostate-specific antigen (PSA) testing. Screening of asymptomatic individuals for pancreatic cancer is not generally recommended.

Mutation nomenclature is based on GenBank accession NM_000059.3 (*BRCA2*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature, the well-established role of *BRCA2* c.3847_3848del, p.(Val1283Lysfs*2) as a disease causing mutation, we classify it as pathogenic. Genetic counseling and family member testing is recommended. Disease caused by *BRCA2* mutations is inherited in an autosomal dominant manner, thus each child of an affected individual has a 50% chance of inheriting the mutation. BpG offers mutation testing for the family if requested.

CONFIRMATION

BRCA2 c.3847_3848del, p.(Val1283Lysfs*2) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	Feb 19, 2018

STEP	DATE
Sample received	Mar 14, 2018
Reported	Apr 20, 2018

On Apr 20, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

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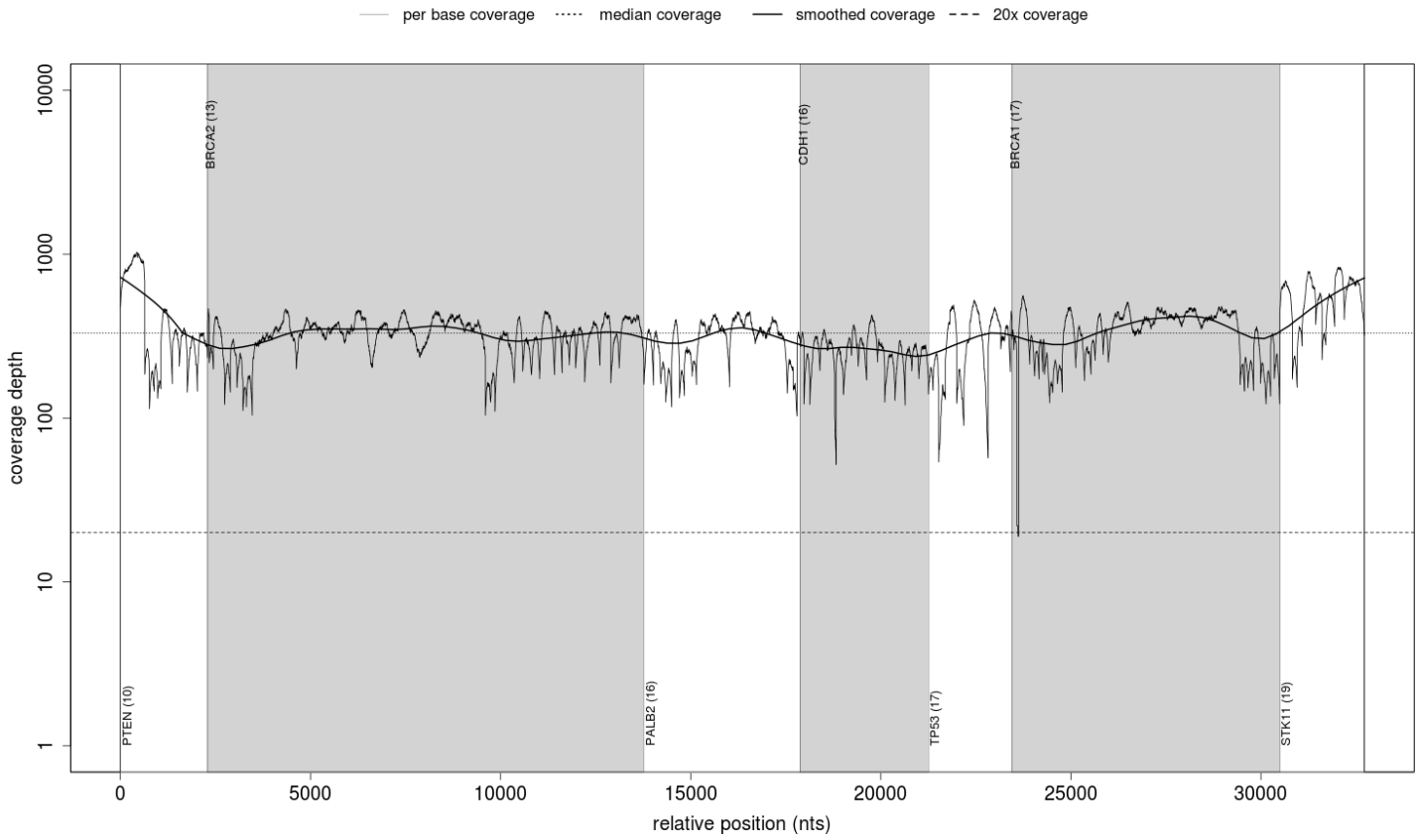
per base coverage
median coverage
smoothed coverage
20x coverage
coverage depth
10000
1000
100
10
1
BRCA2 (13)
CDH1 (16)
BRCA1 (17)
PTEN (10)
PALB2 (16)
TP53 (17)
STK11 (19)
0
5000
10000
15000
20000
25000
30000
relative position (nts)

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.
Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample was calculated based on the alignments. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content.
Interpretation: Our variant classification follows the Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Likely benign and benign variants were not reported. The pathogenicity potential of the identified variants were assessed by considering the predicted consequence, the biochemical properties of the codon change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited, to the 1000 Genomes Project, gnomAD, ClinVar and HGMD. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster were used to assist with variant classification. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as 1000 Genomes Project, Database of Genomic Variants, ExAC, DECIPHER. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score for a true positive call. Pathogenic and likely pathogenic CNVs (Dels/Dups) that established a molecular diagnosis were confirmed using a quantitative-PCR assay if they covered less than 10 target exons or were not confirmed at least three times previously at our laboratory.
Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, and specificity >99.9% for most variant types). A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.
Regulation and accreditations: This test has not been cleared or approved by the FDA. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation.

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_007294.3(BRCA1):c.*1271T>C
NM_007294.3(BRCA1):c.*800T>C
NM_007294.3(BRCA1):c.*718A>G
NM_007294.3(BRCA1):c.*103_*106delTGTC
NM_007294.3(BRCA1):c.*58C>T
NM_007294.3(BRCA1):c.5468-40T>A
NM_007294.3(BRCA1):c.5407-25T>A
NM_007294.3(BRCA1):c.5277+2916_5277+2946delAAATTCTAGTGCTTTGGATTTTTTCCTCCATinsGG
NM_007294.3(BRCA1):c.5194-12G>A
NM_007294.3(BRCA1):c.213-11T>G
NM_007294.3(BRCA1):c.213-12A>G
NM_007294.3(BRCA1):c.213-15A>G
NM_007294.3(BRCA1):c.-19-2A>G
NM_000059.3(BRCA2):c.-40+1G>A
NM_000059.3(BRCA2):c.8954-15T>G
NM_000059.3(BRCA2):c.9502-28A>G
NM_004360.3(CDH1):c.687+92T>A

NM_024675.3(PALB2):c.109-12T>A
chr10:g.89622883-89623482
NM_000314.6(PTEN):c.-1239A>G
NM_000314.6(PTEN):c.-1178C>T
NM_000314.6(PTEN):c.-1171C>T
NM_000314.6(PTEN):c.-1111A>G
NM_000314.4(PTEN):c.-1001T>C
NM_000314.4(PTEN):c.-931G>A
NM_000314.4(PTEN):c.-921G>T
NM_000314.4(PTEN):c.-896T>C
NM_000314.4(PTEN):c.-862G>T
NM_000314.4(PTEN):c.-854C>G
NM_000314.4(PTEN):c.-765G>A
NM_000314.4(PTEN):c.254-21G>C
NM_000546.5(TP53):c.-29+1G>T

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
CNV = Copy Number Variation e.g. one exon or multiexon deletion or duplication
gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)
gnomAD AC/AN = allele count/allele number in the genome Aggregation Database (gnomAD)
HEM = hemizygous
HET = heterozygous
HOM = homozygous
ID = rsID in dbSNP
MutationTaster = in silico prediction tools used to evaluate the significance of identified amino acid changes.
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
OMIM = Online Mendelian Inheritance in Man®
PolyPhen = *in silico* prediction tool used to evaluate the significance of amino acid changes.
POS = genomic position of the variant in the format of chromosome:position
SIFT = *in silico* prediction tool used to evaluate the significance of amino acid changes.