PATHWAY GENOMICS®

BRCATRUE™ SAMPLE REPORT

Protected Health Information

PERSONAL DETAILS

NAME	Joan Smith
DOB	Jul 29, 1980
GENDER	F
ETHNICITY	Caucasian

ORDERING HEALTHCARE PROFESSIONAL

Michael Nova M.D.
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TEST METHODOLOGY

DNA Sequencing, qPCR and/or aCGH

LABORATORY INFO

ACCESSION NUMBER	E3515741
ACTIVATION CODE	JPTST-DZMNQ
SPECIMEN TYPE	SALIVA
COLLECTED DATE	Apr 15, 2014
RECEIVED DATE	May 1, 2014
REPORT DATE	N/A

For Review Only

MUTATION DETECTED

RESULTS

Gene	Mutation	Variant	Classification
BRCA1	c.68_69delAG		Pathogenic

NO MUTATIONS OR VARIANTS WERE DETECTED IN THE FOLLOWING:

BRCA2

COMMENTS

This patient has a pathogenic mutation in the *BRCA1* gene. Pathogenic germline mutations in the *BRCA1* gene result in hereditary breast and ovarian cancer (HBOC) syndrome. The primary risks associated with HBOC syndrome are early-onset female breast cancer and ovarian cancer, and there is also increased risk for other cancers. Although the exact cancer risk for this patient is not known, in a combined analysis of families from 22 population-based studies, individuals with this mutation had increased cumulative risks of female breast cancer to 64% (95% confidence interval=34-80%) and ovarian cancer to 14% (95% confidence interval=2-24%) by age 70[1].

RECOMMENDATIONS

Consultation with a health care professional who has training and experience in cancer genetics is strongly recommended for this patient in order to discuss cancer risks and other disease risks associated with this genetic test result. The type and frequency of cancer surveillance, cancer prevention options and strategies, and the impact of this result on the cancer risks for members of the patient's family are also recommended topics of discussion with a health care professional.

If you have any questions about this report or wish to speak with one of Pathway Genomics' genetic counselors, please call (877) 505.7374. In addition, information about cancer surveillance and prevention options that can be recommended to an individual with a genetic risk for cancer can be found at the following websites:

- National Society of Genetic Counselors: http://www.nsgc.org
- American Cancer Society: http://www.cancer.org
- National Comprehensive Cancer Network: http://www.nccn.org
- American Society of Clinical Oncology: http://www.asco.org/practice-research/familial-risk-assessment-management
- National Cancer Institute: http://www.cancer.gov/cancertopics/prevention

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Interpretation of Mutation(s)

BRCA1

Mutation: c.68_69delAG
Transcript ID: NM_007294.3
Classification: Pathogenic

Estimated Risk:

The BRCA1 c.68_69delAG (p.E23Vfs) mutation, also known as 185delAG and 187delAG, is a founder mutation in the Ashkenazi Jewish population that results in hereditary breast and ovarian cancer (HBOC) syndrome. In a combined analysis of families from 22 population-based studies, the c.68_69delAG mutation increased cumulative risks of female breast cancer to 64% (95% confidence interval=34-80%) and ovarian cancer to 14% (95% confidence interval=2-24%) by age 70[1]. Risk of contralateral breast cancer conferred by this specific mutation has not been reported, however, similar mutations increase risk to 27% within 5 years of initial cancer diagnosis[2]. BRCA1 mutations may also increase risk of other types of cancer, including but not limited to fallopian tube[3], peritoneal[4,5], pancreatic[6], prostate[7,8,9], and male breast cancers[10].

Gene Summary:

The BRCA1 (breast cancer 1, early onset) gene encodes a multifunctional protein that interacts with tumor suppressors, DNA repair proteins, cell cycle regulators, RNA polymerase II holoenzyme, transcription factors, corepressors, chromatin remodeling enzymes, and RNA processing factors. BRCA1, therefore, has a critical role in maintaining genomic stability and is involved in many cellular processes important in tumor biology, including DNA repair, cell cycle progression, and transcriptional regulation[11,12,13,14,15,16,17]. Loss or inactivation of one copy of BRCA1 is thought to result in accumulation of mutations and structural changes in the genome, thereby increasing risk of cancer[18].

Hereditary breast and ovarian cancer (HBOC) syndrome is an autosomal dominant disorder caused primarily by germline mutations in the BRCA1 or BRCA2 gene. Mutations in the BRCA1 gene primarily increase the risk of breast and ovarian cancer; however, mutations in BRCA1 also increase risk of other cancer types, including but not limited to fallopian tube[3], peritoneal[4,5], pancreatic[6], prostate[7,8,9], and male breast cancers[10].

SUPPLEMENTAL INFORMATION

ABOUT THE DISEASE

Inheritance of a pathogenic mutation in the *BRCA1* or *BRCA2* gene results in hereditary breast and ovarian cancer (HBOC) syndrome, an autosomal dominant disorder associated primarily with increased risk of early-onset, breast and ovarian cancer in females. In the U.S., breast cancer and ovarian cancer account for approximately 29% (232,670) and 3% (21,980) of new cases of cancer reported each year in women[19]. Breast and ovarian cancer contribute 15% and 5% respectively of all cancer-related deaths in females[19]. HBOC syndrome accounts for 5-7% of all breast cancer cases[16]; the average cumulative risks in *BRCA1* mutation carriers by age 70 are approximately 65% for breast cancer and 39% for ovarian cancer. The estimates for *BRCA2* mutation carriers are 45% for breast cancer and 11% for ovarian cancer[20,21,22]. However, mutations in *BRCA1* and *BRCA2* are uncommon in sporadic breast cancer[16].

The HBOC syndrome is also associated with increased risk of other types of cancer, including but not limited to fallopian tube[23,3,24], peritoneal[4,25,5], pancreatic[26,6,27], prostate[7,26,8,9], and male breast cancer[10,28].

TECHNICAL INFORMATION

Assay Method

The *BRCA*True™ test is a next-generation sequencing-based (NGS) test to assess the presence of mutations in the *BRCA1* and *BRCA2* genes associated with hereditary breast and ovarian cancer (HBOC) syndrome. Genomic DNA (gDNA) is extracted from the patient's specimen and evaluated for quality and quantity using standard methodology and procedures. The gDNA is then processed to enrich the targeted gene regions (exons and exon flanking regions) in a PCR-based reaction with target-specific primers. Massively parallel sequencing is carried out on the enriched target DNA to detect mutations in these regions. Additional Sanger DNA sequencing (capillary electrophoresis) is carried out in cases where targeted gene regions are insufficiently covered for variant detection. Sanger DNA sequencing is also carried out to confirm specific findings when mutations are detected. Large gene rearrangements (large deletions or duplications) within the *BRCA1* and *BRCA2* genes are detected using quantitative PCR (qPCR); positive results are confirmed by array comparative genomic hybridization (aCGH).

Reportable Range

The panel detects mutations and variants of unknown significance within exons and exon flanking regions of the genes listed using NGS and/or Sanger sequencing methods. Large gene rearrangements are detected using a qPCR method; positive results are confirmed by aCGH. Mutations are classified according to the American College of Medical Genetics (ACMG) guidelines, using as references the Human Genome Mutation Database (HGMD), Catalogue of Somatic Mutations in Cancer (COSMIC), National Center for Biotechnology Information (NCBI) and other public databases, as well as protein function prediction and classification algorithms SIFT and PolyPhen-2.

Expected Values

0, 1 or more mutations or variants of unknown significance (single-nucleotide substitutions and small insertions/deletions with known or uncertain functional consequences).

0, 1 or more large gene rearrangements (large deletions or duplications).

SIFT

SIFT predicts the effect of non-synonymous single nucleotide polymorphisms (SNPs), resulting in an amino acid substitution, on protein function. SIFT prediction is based on the degree of conservation of amino acid residues determined by comparing the human gene under consideration to similar amino acid sequences (homologs) from all domains of life. Potential homologs are gathered from the non-redundant (nr) NCBI database. Alignments constructed from homologous sequences are used to assess the probability of observing the sequenced variant and used to derive a score. In addition to scoring potentially damaging variants, SIFT (in combination with PolyPhen-2) is used to filter out likely benign variants, which have not been shown to have clinical significance. In some cases, limited homology or a lack of sequence diversity may prohibit evaluation of a variant. SIFT scores below 0.05 are predicted to be damaging, while scores above 0.05 are tolerated polymorphisms[29]. In some cases results are

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TECHNICAL INFORMATION (Continued)

incalculable, which indicates that there is not enough homology data for predictive purposes. Homology data limitations can be due to either a lack of homologous sequence diversity or too few homologs.

PolyPhen-2

PolyPhen-2 predicts the possible impact of amino acid substitutions on the stability and function of human proteins using structural and comparative evolutionary considerations. It performs functional annotation of single-nucleotide polymorphisms (SNPs), maps coding SNPs to gene transcripts, extracts protein sequence annotations and structural attributes, and builds conservation profiles. It then estimates the probability of the missense mutation being damaging based on a combination of all these properties. The output of the PolyPhen-2 prediction pipeline is a prediction of probably damaging, possibly damaging, or benign, along with a numerical score ranging from 0.0 (benign) to 1.0 (damaging).

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DISCLAIMER

This report is not intended to be used solely by the patient without the consultation of a licensed health care professional. This test was developed and its performance characteristics determined by the testing laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA).

If you have any questions about this report or wish to speak with one of Pathway Genomics' genetic counselors, please call (877) 505.7374.

RISKS AND LIMITATIONS

Risks and Limitations

The laboratory of Pathway Genomics Corporation has developed this *BRCA*True™ DNA sequencing test, determined the performance characteristics and validated the test to meet regulatory requirements. Pathway Genomics' laboratory is accredited by the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) and the College of American Pathologists (CAP) to perform high complexity testing. This test is designed to detect nucleotide substitutions, small insertions and deletions and large gene rearrangements. The test is not designed to detect chromosomal rearrangements, genetic changes located within introns or other unknown abnormalities.

The *BRCA*True test is clinically useful to detect mutations, variants, small insertions and deletions as well as large gene rearrangements occurring within the *BRCA1* and *BRCA2* genes. The mutations or variants detected in the *BRCA*True test are classified as one of the following: **Pathogenic mutations** are genetic changes that have sufficient evidence to be classified as capable of causing disease thus increasing the risk for cancer. **Likely pathogenic** variants are genetic changes with strong but limited evidence to be classified as pathogenic and are likely to increase the risk for cancer. **Uncertain pathogenicity variants (VUS)** are genetic changes that are either previously not reported or have inadequate/conflicting evidence to determine clinical relevance and cancer risk. **Benign** variants are genetic changes that have sufficient evidence to be considered of no clinical significance and do not increase the risk for cancer. **Likely benign** variants are genetic changes with strong but limited evidence to be classified as benign and are unlikely to increase the risk for cancer. Pathogenic mutations, likely pathogenic variants and VUS(s) are always reported. Likely benign and benign variants are not reported.

The etiology of cancer is multifactorial and can occur as a result of various factors, including both inherited and acquired genetic mutations, diet, lifestyle choices and age. Pathway Genomics' genetic test evaluates only inherited genetic mutations. It is possible that mutations in genes and genetic regions not tested in Pathway Genomics' *BRCA*True test may contribute to an individual's risk for cancer. Therefore, a negative test result, where no mutations are detected, does not eliminate the individual's cancer risk.

Pathway Genomics' laboratory maintains a high regulatory standard for quality in genetic testing to protect against technical and operational errors. However, in rare instances errors can occur in molecular diagnostic assays. Such errors may include, but are not limited to, sample mislabeling, DNA contamination, uninterpretable results, and human and/or testing system errors.

RESULT STATUS DEFINITIONS

Amended 	Test results and/or patient information that have been revised in a way that does not impact the clinical significance of the result(s) and/or patient diagnosis, treatment or management.
Corrected 	Test results and/or patient information that have been revised in a way that may impact the clinical significance of the result(s) and/or patient diagnosis, treatment or management.
Final 	Test results that are available at the time of report issue or have been revised from pending status to final status.
Pending 	Test results that are not available at the time of report issue. All pending results will be specified in the report.