OncoGeneDx: BRCA1/2 Sequencing and Deletion/Duplication Analysis
In Hereditary Breast and Ovarian Cancer (HBOC)

Gene List: BRCA1, BRCA2

Clinical Features:
In the general population, approximately 1 in 8 women (12%) will develop breast cancer in their lifetime, and 1 in 75 women (1.4%) will be diagnosed with ovarian cancer in their lifetime.1 Most cases of breast or ovarian cancers develop sporadically with no family history of the cancer. Individual risk factors and exposures, such as age, pregnancy history, menstrual history, benign breast disease, radiation exposure, and alcohol intake, are known to modify a woman’s chance of developing these types of cancers. However, 5-10% of breast cancer cases and 15-20% of ovarian cancer cases are thought to be due to a hereditary predisposition. The features suggestive of a hereditary cancer predisposition include: young age at diagnosis, multiple primary cancers in a single individual, diagnosis of a cancer type that is not common in general population (such as ovarian cancer, male breast cancer, or pancreatic cancer), and several relatives affected with related cancers spanning multiple generations.

Pathogenic BRCA1 and BRCA2 variants increase the lifetime risk for breast and ovarian cancer significantly over the general population risk. The chances to develop breast cancer begin increasing when a woman is in her mid-20s.2 Women with pathogenic BRCA1 or BRCA2 variants have between a 41-87% lifetime risk to develop breast cancer and up to a 63% risk for a contralateral breast cancer.2–8 This risk depends on the age at which the first breast cancer was detected.7 The lifetime risk for breast cancer in males with a pathogenic BRCA2 variant is approximately 7%, and slightly increased for those with a pathogenic BRCA1 variant.9,10

The risk of ovarian cancer begins to increase in the mid-30s, but becomes most significant in the 50s and beyond. The lifetime risk to develop ovarian cancer is between 24-54% for pathogenic BRCA1 variant carriers and 11-27% for pathogenic BRCA2 variant carriers.2–4,6,8 Other associated cancers in women include fallopian tube carcinoma, primary peritoneal carcinoma, and uterine serous carcinoma.11–13

The risk for other malignancies has been reported in families with pathogenic variants in BRCA1 or BRCA2 including prostate cancer in men as well as pancreatic cancer and melanoma in both men and women. Male and female pathogenic BRCA2 variant carriers are estimated to have up to a 7% risk for pancreatic cancer while male carriers are estimated to have up to a 34% risk for prostate cancer.14,15 Male pathogenic BRCA1 variant carriers have
been shown to have a slightly increased risk for prostate cancer before age 65 while pancreatic cancer have been suggested to also be slightly increased in both men and women.\(^9,16–18\)

Two pathogenic variants in the BRCA2 gene, one in each copy of the gene (biallelic pathogenic variants), are associated with an extremely rare autosomal recessive syndrome called Fanconi anemia. This condition is characterized by an increased risk for malignancy in children including leukemia and certain solid tumors as well as physical abnormalities and bone marrow failure. Therefore, if both mother and father are carriers of a pathogenic BRCA2 variant, each of their children would have a 25% chance to inherit both variants, a 50% chance to inherit one of the variants, and a 25% chance to inherit neither variant.

**Inheritance Pattern:**

BRCA1 and BRCA2 are associated with an autosomal dominant cancer risk. BRCA2 is also associated with Fanconi anemia when inherited in an autosomal recessive fashion. The specifics of this inheritance are outlined above.

**Test Methods:**

Genomic DNA from the submitted specimen is enriched for the complete coding region and splice site junctions of the genes on the panel using a proprietary targeted capture system developed by GeneDx. The products are sequenced on an Illumina HiSeq instrument with 2x100 paired-end reads. The sequence is aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Capillary sequencing is used to confirm all variants with clinical or uncertain significance and to analyze regions with inadequate coverage by Next Generation sequencing (NGS). If present, apparently homozygous variants are confirmed using alternate primer pairs to significantly reduce the possibility of allele drop-out. All sequence alterations are described according to the Human Genome Variation Society (HGVS) nomenclature guidelines. Concurrent deletion/duplication analysis from NGS data is performed for all relevant genes on the panel to detect multi-exonic and most single-exon deletions and duplications. For specimens with insufficient copy number data and for confirmation of identified copy number changes, exon-level array CGH, MLPA or other appropriate methods are used. Copy-number alterations are reported according to the International System for Human Cytogenetic Nomenclature (ISCN) guidelines. Benign and likely benign variants, if present, are not reported.

**Test Sensitivity:**

Regarding clinical sensitivity, approximately 20-25% of familial breast cancer risk and 75% of hereditary ovarian cancer risk are thought to be attributed to pathogenic variants in the BRCA1 or BRCA2 genes.\(^19–22\) The test is expected to be greater than 99% sensitive in detecting
variants identifiable by sequencing and will detect most single exon deletions and duplications. The likelihood of a false positive result is expected to be <1%.

Technical Limitations: Neither sequencing, exon-level array CGH nor MLPA can reliably detect mosaicism, and cannot detect chromosomal aberrations. Deletions involving more than 20bp and insertions involving more than 10bp are not reliably detected by the sequencing methodology, and deletions or duplications of less than 250bp are not reliably detected by NGS-CNV analysis or array CGH. Regions of certain genes have inherent sequence properties that yield suboptimal data, potentially impairing accuracy of the results. In the absence of mRNA/cDNA studies, we cannot completely exclude the possibility of undetectable clinically significant variants in certain regions of these genes. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In individuals with active leukemia or lymphoma or with known chronic myeloid or lymphoid neoplasms (such as low grade MDS, CML, ET, P. vera, PMF, CLL), there is a possibility that testing of specimens containing leukocytes may detect an acquired somatic variant, resulting in a false positive result. In this situation, please contact one of our genetic counselors to discuss the utility of submitting an alternate specimen. The ability to detect genetic variants and naming conventions can differ among laboratories. Rare false negatives, therefore, may occur when testing for a specific variant identified at a laboratory other than GeneDx, if a positive control is not provided. Based on the specific array design and technology used, the reported coordinates of duplications and deletions at the exon or gene level can slightly differ among family members tested but, in general, relatives are expected to have the same copy number variant.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Inheritance</th>
<th>Disease Associations</th>
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<tbody>
<tr>
<td>BRCA1</td>
<td>BREAST CANCER TYPE 1 SUSCEPTIBILITY PROTEIN</td>
<td>AD</td>
<td>Hereditary Breast and Ovarian Cancer (HBOC) syndrome: breast, ovarian, pancreatic, prostate &amp; endometrial serous cancer</td>
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<tr>
<td>BRCA2</td>
<td>BREAST CANCER TYPE 2 SUSCEPTIBILITY PROTEIN</td>
<td>AD</td>
<td>Hereditary Breast and Ovarian Cancer (HBOC) syndrome: breast, ovarian, pancreatic, prostate, melanoma &amp; endometrial serous cancer</td>
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<tr>
<td></td>
<td></td>
<td>AR</td>
<td>Fanconi anemia</td>
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</tbody>
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Because evolving and expanding phenotypes, this list of cancer/tumor types is not exhaustive. Gene-specific risk for some of the cancers and other features listed are not well-defined.

Abbreviations:
AD – Autosomal Dominant
AR – Autosomal Recessive
CGH – Comparative genomic hybridization
MLPA – Multiplex ligation-dependent probe amplification
References: