OncogeneDx: BRCA1 and BRCA2 Deletion and Duplication Analysis
In Hereditary Breast and Ovarian Cancer (HBOC)

Mendelian Inheritance in Man:
113705 – Breast Cancer Gene 1 (BRCA1); 600185 – Breast Cancer Gene 2 (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 (SEER). 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 20’s (King 2003). 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 (Antoniou 2003, Chen 2007, Claus 1996, Ford 1998, Graeser 2009, King 2003, Risch 2006). This risk depends on the age at which the first breast cancer was detected (Graeser 2009). The lifetime risk for breast cancer in males with a pathogenic BRCA variant approximately 7%, and slightly increased for those with a pathogenic BRCA1 variant (Liede 2004, Tai 2007).

The risk of ovarian cancer begins to increase in the mid 30’s, but becomes most significant in the 50’s 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 (Antoniou 2003, Chen 2007, Ford 1998, King 2003, Risch 2006). Other associated cancers in women include fallopian tube carcinoma, primary peritoneal carcinoma, and uterine serous carcinoma (Levine 2003, Biron-Shental 2006, Pennington 2013).

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 (Ozcelik 1997, The Breast Cancer Linkage Consortium 1999). 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 (Brose 2002, Leongamornlert 2012, Liede 2004, Thompson 2002).

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.
Specific cancer screening and prevention recommendations for those who carry a pathogenic BRCA1/2 variant can be found in the NCCN Clinical Practice Guidelines in Oncology Genetic/Familial High-Risk Assessment: Breast and Ovarian at www.nccn.org.

For additional clinical information on the BRCA1/2 genes and management options, please refer to the OncogeneDx BRCA1/2 Physician Guide.

**Inheritance Pattern:**
Pathogenic variants in the BRCA1 and BRCA2 genes are inherited in an autosomal dominant manner; de novo pathogenic variants are uncommon.

**Reason for referral:**
Deletion and duplication analysis of the BRCA 1 and BRCA2 genes may be indicated when sequencing of the genes is negative. Approximately 6-12% of individuals with Hereditary Breast and Ovarian Cancer syndrome carry whole or partial gene deletions or duplications in the BRCA1 or BRCA2 genes (Judkins 2012, Walsh 2006). Large deletions or duplications, like other pathogenic variants in BRCA1 and BRCA2, increase the lifetime risk for breast and ovarian cancer significantly over the general population risk. This additional testing may be helpful for the following reasons:

1) Verification of a genetic basis for cancer in families indicative/suggestive of hereditary breast and ovarian cancer.
2) Determination of appropriate screening and treatment.
3) Identification of at-risk family members.

The NCCN Clinical Practice Guidelines in Oncology Genetic/Familial High-Risk Assessment: Breast and Ovarian include specific testing criteria for BRCA1/2. Information about risk assessment and genetic counseling for hereditary breast and ovarian cancer can be found at www.nchpeg.org/hboc

**Methods:**
Genomic DNA from the submitted specimen is obtained and Deletion/Duplication testing was performed on both of the genes using either exon-level array CGH or MLPA based on DNA quality and quantity. Data analysis was performed using gene-specific filtering. Probe sequences and locations were based on human genome build GRCh37/UCSC hg19. Confirmation of copy number changes was performed by MLPA, qPCR, or repeat array CGH analysis. The test will detect most single exon deletions and duplications.

GeneDx offers BRCA1 and BRCA2 sequencing as an additional test. The BRCA1 and BRCA2 genes are also included on our OncogeneDx next generation sequencing panels for hereditary cancer.

**Test Performance:**
This test will detect most single exon deletions and duplications.

Technical Limitations: Exon-level aCGH cannot reliably detect mosaicism and cannot detect chromosomal aberrations. Deletions or duplications of less than 250bp are not reliably detected by array CGH. False negatives may 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. Additionally, rare false negatives 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. The ability to detect genetic variants and naming conventions can differ among laboratories.

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 (Easton 1999, Pharoah 2002, van der Groep 2011, Walsh 2011). Approximately 6-12% of Hereditary Breast and Ovarian Cancer syndrome cases are due to large genomic deletions or duplications in the BRCA1 or BRCA2 genes (Judkins 2012, Walsh 2006).

**Reporting of Results:**
Results will be interpreted and reported following recommendations of the American College of Medical Genetics as a guideline (www.acmg.net). Variations detected by sequencing or deletion/duplication analysis will be analyzed and classified into the following categories based on current scientific knowledge. Our analysis includes a comprehensive assessment of the variation on a molecular and clinical level in order to determine its clinical significance and classification. Trained PhD analysts perform a detailed review of the variation on the molecular level, including exhaustive searches of gene- and locus-specific databases and the Human Gene Mutation Database (HGMD), and genetic counselors and clinical molecular geneticists carefully review literature reports.

**Pathogenic Variant**
Examples of variations detected by our exon-level array that may be reported as pathogenic may include large exon-level deletions and exon-level duplications that result in a frameshift.

**Likely Pathogenic Variant**
Variations for which there is significant, but not conclusive, evidence supporting pathogenicity will be classified as Likely Pathogenic.

**Variant of Uncertain Significance**
Variations for which there is not sufficient evidence for classification will be classified as Variant of Uncertain Significance.

**Negative**
No variation of clinical or uncertain significance was detected. Any variation detected and classified as a likely benign or benign variant based on population data, review of the literature, Human Gene Mutation Database (HGMD), and appropriate locus-specific databases will not be reported.

**Specimen Requirements and Shipping/Handling:**
- Blood: Two EDTA (lavender top) tubes containing 4 ml each whole sterile blood
- Oral Rinse: Saliva collected in at least 30mL of mouthwash using our GeneDx collection kit
- Extracted DNA: >20 ug

**Test Codes and Turnaround Times**  –  Please contact us for price information:

<table>
<thead>
<tr>
<th>Test Code</th>
<th>Description</th>
<th>Turnaround Time</th>
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<tbody>
<tr>
<td>B501-8</td>
<td>BRCA1/2 Deletion Duplication Analysis</td>
<td>8-10 days</td>
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</tbody>
</table>
References


Walsh T et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. JAMA. 2006 Mar 22;295(12):1379-88. (PMID: 16551799)


<table>
<thead>
<tr>
<th>Gene</th>
<th>Most commonly associated cancers</th>
<th>Associated recessive syndrome</th>
<th>Management Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>Breast, Ovarian, Pancreatic, Prostate, Endometrial serous carcinoma*</td>
<td></td>
<td>NCCN-BR/OV</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Breast, Ovarian, Pancreatic, Prostate, Endometrial serous carcinoma*</td>
<td>Fanconi anemia</td>
<td>NCCN-BR/OV, CAPS*</td>
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Key

Red font denotes significantly increased cancer risk. We consider significantly increased risk to be a relative risk of 4 or higher in relation to the general population risk. This translates to the following lifetime cancer risks: ≥50% breast cancer, ≥26% ovarian cancer.

Blue font denotes moderately increased cancer risk. We consider moderately increased risk to be a relative risk between 2 and 4 in relation to the general population risk. This translates to the following lifetime cancer risks: 32-60% prostate cancer, 3-6% pancreatic cancer.

* Gene-specific risk for this cancer type is not well-defined.

# CAPS - International Cancer of the Pancreas Screening (CAPS) Consortium report on the management of patients with increased risk for familial pancreatic cancer (Canto 2013).