Hereditary Breast Cancer: Genes, Associated Syndromes and Testing Options

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Introduction
Cancer can occur as a result of various factors, including inherited and acquired genetic mutations, diet, lifestyle choices and age. It has long been recognized that cancer risk is higher in some families and ethnic groups as compared to the population at large, suggesting a genetic component to the overall breast cancer risk. Studies of high risk breast and ovarian cancer families led to the discovery of the \textit{BRCA1} and \textit{BRCA2} genes in the mid 1990s. \textit{BRCA1} and \textit{BRCA2} are the two genes with the strongest association with increased risk for breast cancer accounting for approximately 5-7\% of all breast cancer cases \cite{1}. In addition, mutations in \textit{BRCA1} and \textit{BRCA2} greatly increase the risk for ovarian cancer \cite{2}; mutations in \textit{BRCA1/2} lead to the so called hereditary breast and ovarian cancer syndrome (HBOC). Although \textit{BRCA1} and \textit{BRCA2} are the most recognized genes with an association with breast cancer, mutations in other genes need to be considered in non-\textit{BRCA1/2} high risk families. Most notably amongst these are genes previously associated with well-studied genetic disease syndromes, i.e., \textit{PTEN} (Cowden), \textit{TP53} (Li-Fraumeni), \textit{CDH1} (hereditary diffuse gastric cancer), and \textit{STK11} (Peutz-Jehgers) \cite{3}. Remarkably, a recent (August, 2014), large study of hereditary breast cancer patients established the \textit{PALB2} gene as a key contributor to hereditary breast cancer \cite{4-6}. Given the complexities of testing for a predisposition for breast cancer, Pathway Genomics offers a suite of testing options to best accommodate the personal and family history of each patient. These testing options include single mutation testing, an Ashkenazi Jewish mutation panel, a \textit{BRCA1/2} test, and, importantly, a high-risk breast cancer panel that includes the \textit{PALB2} gene. To further add to the flexibility of testing, several reflex testing options are also available.
Breast and Ovarian Cancer
Breast cancer is defined as a malignant tumor in the breast caused by uncontrolled division of abnormal cells. The disease occurs in men and in women, though male breast cancer is rare. Women in the general population have a 12.3% lifetime risk of breast cancer; 1 in 8 women will develop the disease [7]. In contrast, men have a lifetime risk of 0.13%, or about 1 in 1,000 [8].

Ovarian cancer is a malignancy in the ovaries caused by uncontrolled division of abnormal cells. The lifetime risk of developing ovarian cancer in the general population is 1.4%, or about 1 in 71 [7].

Hereditary Breast and Ovarian Cancer (HBOC)
DNA changes (mutations) in the BRCA1 and BRCA2 genes can lead to significantly increased lifetime risks for breast and ovarian cancer (Figure 1). Mutations in BRCA1 and BRCA2 lead to a condition known as hereditary breast and ovarian cancer syndrome or HBOC. HBOC accounts for 5-7% of breast cancer cases [1] and 8–13% of epithelial ovarian cancer cases [2]; however, BRCA1 and BRCA2 mutations account for up to 80% of breast and ovarian cancer in families with multiple cases of either disease [3, 9, 10]. Schematic representations of family pedigrees diagnosed with HBOC are depicted in figure 2. Importantly, mutations in either BRCA1 or BRCA2 can be inherited from the maternal as well as the paternal side of the family. HBOC syndrome is also associated with increased risk of other types of cancer, including but not limited to fallopian tube [11-13], peritoneal [14-16], pancreatic [17-19], prostate [18, 20-22], and male breast cancers [23, 24].

Figure 1: The cumulative lifetime risk to age 70 years for breast or ovarian cancer. [22]
Figure 2: Schematic representations of family pedigrees diagnosed with pathogenic mutations in either the BRCA1 or BRCA2 gene. Interpretation guide: (1) circles represent females while squares represent males, (2) shading indicates an affected individual, (3) a diagonal line indicates the individual is deceased and (4) each level indicates a generation. Age of diagnosis is indicated as “dx”. Adapted from [25].

**BRCA1/2 and Ethnicity**

Prevalence of BRCA1/2 in the general population has been reported to be approximately 1/400 [22]. Large population studies [8, 26] of breast cancer survivors under the age of 65 revealed strong ethnic differences in the prevalence of pathogenic mutations in BRCA1 or BRCA2 gene (Table 1).

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>BRCA1</th>
<th>BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi Jewish</td>
<td>8.3-10.2%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3.5%</td>
<td>Data not available</td>
</tr>
<tr>
<td>Caucasian (non-Ashkenazi Jewish)</td>
<td>2.2-2.4%</td>
<td>2.2%</td>
</tr>
<tr>
<td>African-American</td>
<td>1.3-1.4%</td>
<td>2.6%</td>
</tr>
<tr>
<td>Asian-American</td>
<td>0.5%</td>
<td>Data not available</td>
</tr>
</tbody>
</table>

Table 1: Prevalence of BRCA1 and BRCA2 mutations in women with breast cancer by ethnic group within U.S. Adapted from [8, 26].

**BRCA1/2 Ashkenazi Jewish founder mutations**

Inherited predisposition for breast and ovarian cancer among people of Ashkenazi (Central and Eastern European) Jewish ethnic background has long been recognized. Approximately one in forty Ashkenazim carry one of three founder mutations in BRCA1 (185delAG or 5382insC) or BRCA2 (6174delT) [27-29]. Recent studies show that 29% of Jewish women with ovarian cancer carry one of these three founder mutations [30]. Data from a recent study [31] indicate that a BRCA1/2 mutation was detected in 44% of a group of 220 high risk Ashkenazi breast cancer families. This number increased to 73% if ovarian cancer was present in the kindred.
**BRCA1/2 founder mutations in other ethnic groups**

Although, as discussed above, the prevalence of founder mutations in BRCA1 and BRCA2 is higher in Ashkenazim compared to the general population, there are also some well-characterized BRCA1/2 founder mutations in other ethnic groups. For example, in the Mexican population, two mutations in BRCA1 represent about 45% of the observed BRCA1/2 mutations (185delAG and ex9-12 del) in that population. A partial list of well-documented founder mutations is presented in Table 2.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Ethnicity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2</td>
<td>4265delCT</td>
<td>Filipino</td>
<td>[34]</td>
</tr>
<tr>
<td>BRCA2</td>
<td>c.9345+1G&gt;A</td>
<td>Finns</td>
<td>[35]</td>
</tr>
<tr>
<td>BRCA1</td>
<td>3600del11</td>
<td>French</td>
<td>[36]</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Y978X</td>
<td>Iranian Jews</td>
<td>[37]</td>
</tr>
<tr>
<td>BRCA1</td>
<td>5083del19</td>
<td>Italian</td>
<td>[38, 39]</td>
</tr>
<tr>
<td>BRCA1</td>
<td>c.188T&gt;A</td>
<td>Japanese</td>
<td>[40]</td>
</tr>
<tr>
<td>BRCA1</td>
<td>185delAG</td>
<td>Mexican</td>
<td>[32, 33]</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Exon9-12del</td>
<td>Mexican</td>
<td>[32, 33]</td>
</tr>
<tr>
<td>BRCA2</td>
<td>3492insT</td>
<td>Mexican</td>
<td>[32, 33]</td>
</tr>
<tr>
<td>BRCA2</td>
<td>c.145G&gt;T</td>
<td>Mexican</td>
<td>[32, 33]</td>
</tr>
<tr>
<td>BRCA1</td>
<td>c.1556delA</td>
<td>Norway</td>
<td>[41]</td>
</tr>
<tr>
<td>BRCA1</td>
<td>c.3048_3052dupTGAGA</td>
<td>Swedish/Danish</td>
<td>[42]</td>
</tr>
</tbody>
</table>

**Table 1:** Partial list of BRCA1/2 founder mutations by ethnic group.
**Other High Penetrance Genes**

Cases of familial breast cancer not related to **BRCA1** or **BRCA2** are thought to be caused by other genes, each one accounting for a fraction of the total cases of familial breast cancer. Some of these additional high risk susceptibility genes include **CDH1**, **PALB2**, **PTEN**, **STK11**, and **TP53** [3]. As can be appreciated from Figure 3, extremely rare, high-penetrance mutations in a handful of genes are responsible for the largest contribution to breast cancer risk.

![BreastTrue® High Risk Panel](image)

**BreastTrue® High Risk Panel**

Syndromes such as Li-Fraumeni, Cowden, Peutz-Jeghers, and Hereditary Diffuse Gastric Cancer (HDGC) have been known to significantly increase the risk for breast cancer [41-48]. Genetic testing of the genes associated with these conditions (i.e., **TP53**, **PTEN**, **STK11** and **CDH1**) has been part of the clinical management of patients with a family history of breast cancer. Recently, **PALB2** has been identified as an important contributor to breast cancer risk [4-6]. Table 3 summarizes the risk contributions from the top breast-cancer related genes.

**Figure 3:** Rare mutations in a few highly penetrant genes are the largest contributors to breast cancer risk.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Syndrome</th>
<th>Neoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>HBOC</td>
<td>Breast (female), ovarian cancer</td>
</tr>
<tr>
<td>BRCA2</td>
<td>HBOC</td>
<td>Breast (male and female), prostate, pancreatic</td>
</tr>
<tr>
<td>TP53</td>
<td>Li-Fraumeni</td>
<td>Breast cancer, sarcomas, leukemia, brain tumors, lung cancer, adrenocortical carcinoma</td>
</tr>
<tr>
<td>PTEN</td>
<td>Cowden</td>
<td>Breast, thyroid, endometrial, kidney cancer</td>
</tr>
<tr>
<td>STK11/LK81</td>
<td>Peutz-Jeghers</td>
<td>Breast, ovarian, cervical, uterine, testicular, small bowel, colon cancer</td>
</tr>
<tr>
<td>CDH1</td>
<td>HDGC</td>
<td>Hereditary diffuse gastric, lobular breast</td>
</tr>
<tr>
<td>PALB2</td>
<td>PALB2-related</td>
<td>Breast, pancreatic, ovarian, male breast cancer</td>
</tr>
</tbody>
</table>

**Table 3:** high penetrance breast cancer genes and other associated cancer risks [3,6,23,24,43-56].

**Breast Cancer High Risk Genes**

**BRCA1**

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 [1, 57-62]. 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 [63].

**BRCA2**

The **BRCA2** (breast cancer 2, early onset) gene encodes a protein with important roles in the DNA damage response and DNA repair pathways [1]. **BRCA2** is a tumor suppressor that mediates recruitment of the RAD51 recombinase protein to DNA double-strand breaks [1]. The primary function of the **BRCA2** protein is to facilitate homologous recombination, an important DNA repair mechanism in maintenance of genomic integrity [1, 63]. Loss or inactivation of one copy of **BRCA2** is thought to result in accumulation of mutations and structural changes in the genome, thereby increasing risk of cancer [63].

**CDH1**

The **CDH1** gene encodes the E-cadherin protein, a member of the trans-membrane glycoprotein family. E-cadherin is expressed on epithelial tissues and is responsible for calcium-dependent cell-cell adhesion. Loss of **CDH1** expression is associated with cancer cell invasiveness [64]. Cancers develop in individuals with a **CDH1** germline mutation when the second copy of the **CDH1** gene is somatically inactivated or down regulated [64-67]. Germline mutations in **CDH1** have been shown to segregate in families with HDGC. It has been estimated that individuals who are heterozygous for mutations in the **CDH1** gene have a cumulative risk of diffuse gastric cancer of over 80% in both men and women by age 80 years, with a mean age at diagnosis of 40 years. In addition, female heterozygotes have a cumulative risk of lobular breast cancer of 60% by age 80 years [60].
**PALB2**

The PALB2 (partner and localizer of BRCA2) gene encodes a protein that plays essential roles in homologous recombination (HR)-mediated DNA repair by interacting with breast cancer 1, early onset (BRCA1), breast cancer 2, early onset (BRCA2) and other proteins involved in the HR DNA repair mechanism [68, 69]. Pathogenic PALB2 mutations have been detected in 1-3% of BRCA1/2 mutation-negative hereditary breast cancer patients [68-71]. Although PALB2 mutations are relatively rare, some of the PALB2 mutations have been shown to confer a breast cancer risk that is comparable to pathogenic mutations in BRCA1 and BRCA2 genes [72-74]. Clinical data suggest that PALB2 mutations may also increase the risk for pancreatic cancer [75].

Fanconi anemia (FA) is a rare, recessive chromosome instability syndrome caused by mutations in at least one of the several genes that encode FA pathway components [76]. FA is characterized by congenital abnormalities, bone marrow failure, hypersensitivity to DNA crosslinking agents, defective DNA repair and cancer susceptibility [77, 78]. The PALB2 protein has been shown to be one of the component of FA pathway; certain biallelic mutations in PALB2 gene can cause a subtype of FA known as FA complementation group N (FANCN) [79, 80].

**PTEN**

The PTEN (phosphatase and tensin homolog) gene encodes a dual-specificity phosphatase that acts on both lipid and protein substrates. The lipid phosphatase activity of PTEN suppresses the PI3K/AKT/mTOR signaling pathway, which regulates cell growth and survival [81]. The importance of PTEN as a tumor suppressor gene is supported by the high frequency of somatic mutations in PTEN found in a variety of sporadic human cancers [82].

PTEN hamatoma tumor syndrome (PHTS) is a collection of rare autosomal dominant disorders found in individuals with deleterious germline mutations in the PTEN gene. Individuals with PHTS exhibit a spectrum of autosomal dominant disorders involving disorganized growth of benign tumors called hamartomas in multiple organ systems [83, 84]. The two most common inherited PHTS disorders are the adult Cowden Syndrome (CS) and the pediatric Bannayan-Riley-Ruvalcaba syndrome (BRRS) [84]. The PTEN-related Proteus syndrome (PS) and Proteus-like syndrome are the two other types of PHTS.

**STK11**

The STK11 (also known as LKB1) gene encodes a serine-threonine kinase that is involved in the regulation of metabolism, cell differentiation, proliferation, polarity and apoptosis [85, 86]. STK11 is a tumor suppressor, and mutations in the gene have been associated with Peutz-Jehgers syndrome (PJS). PJS has been associated with mutations in tumor suppressor gene STK11. PJS is an autosomal dominantly inherited disorder that is characterized by hamartomatous polyps in the gastrointestinal tract, pigmented mucocutaneous lesions and cancer predisposition. The hamartomatous polyps of PJS are most common in the small intestine but can also occur in the stomach, large bowel and extraintestinal sites [48]. Gastrointestinal polyps can lead to chronic bleeding resulting in anemia and increased risk of malignant transformation [48].

PJS is associated with increased risk for various malignancies, including colorectal, gastric, breast, gynecologic, pancreatic and lung cancers, as well as tumors of the testes [48, 87, 88]. The risks among PJS patients for developing any first cancer by ages 20, 30, 40, 50, 60 and 70 years are 2%, 5%, 17%, 31%, 60% and 85%, respectively [89]. In PJS, malignancies appear at a younger age compared to the general population, i.e., an average age of 42 years [87]. Most patients who are clinically diagnosed with PJS have a causative mutation in STK11 [90]. Loss of kinase activity is likely to be responsible for the development of this condition [91]. The clinical diagnosis of PJS is made when a patient meets at least 2 of the following criteria: 2 or more Peutz-Jeghers polyps of the small intestine; typical mucocutaneous hyper pigmentation; and a family history of PJS.
**TP53**

The TP53 gene encodes a transcription factor that is involved in cellular responses to environmental and genotoxic stress [92]. This tumor suppressor binds consensus DNA in the responsive elements of several hundreds of genes [93]. TP53 mutations are the most frequent genetic alterations in human cancers, with greater than 35,000 mutations described in different types of cancer [94]. Approximately 95% of the mutations are localized in the DNA-binding domain of TP53 [93].

Li-Fraumeni (LFS) and its variant, Li-Fraumeni-like (LFL) syndrome are autosomal dominant disorders caused by germline mutations in TP53 (tumor protein p53) gene and are characterized by predisposition to multiple early onset cancers [95]. LFS has high variability in penetrance, age of cancer onset and tumor spectrum [96]. LFL syndrome is associated with incomplete LFS features [97]. The most common cancers associated with LFS are sarcoma, breast carcinoma, brain tumors and adrenocortical carcinoma. Other cancers include leukemia, choroid plexus papilloma, Wilms tumors, and gastric, colorectal and pancreatic cancer [98].

Clinically, LFS is diagnosed in individuals who have a germline mutation in TP53 gene, or if they meet the established clinical criteria for LFS. At least 70% of clinically diagnosed LFS cases are associated with identifiable germline mutations in TP53 gene.

**Pathway Genomics Tests for Hereditary Breast and Ovarian Cancer**

Given the diversity in patient’s medical and family histories, Pathway Genomics offers a wide range of testing options to help determine the hereditary risk of breast and/or ovarian cancer. The testing options are summarized in table 4.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Site</th>
<th>BRCA1 True® Ashkenazi Jewish (3-site)</th>
<th>BRCA1 True®</th>
<th>BreastTrue® High Risk Panel</th>
<th>BRCA1 True® with reflex to BreastTrue®* High Risk Panel</th>
<th>BRCA1 True® with reflex to BRCA1 True® Ashkenazi Jewish (3-site) with reflex to BRCA1 True®</th>
<th>Single Site</th>
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<tbody>
<tr>
<td>BRCA1</td>
<td>185delAG</td>
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<td>BRCA1</td>
<td>5382insC</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BRCA2</td>
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<td>X</td>
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<tr>
<td>BRCA2</td>
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<td>YES</td>
</tr>
</tbody>
</table>

**Table 4:** Summary of Pathway Genomics genetic testing options for hereditary breast and or ovarian cancer. The asterisk (*) indicates that reflex testing is triggered if a VUS or no reportable variant is found in the initial test.
Potential Indications
The BRCATrue® and BreastTrue® line of tests is best suited for individuals with either a history of early onset breast or ovarian cancer or a strong family history of breast and/or ovarian cancer. Analysis of personal and family medical histories by medical professionals will help identify best tests to perform. The following are some of the medical history details that may result in a recommendation for genetic testing:

- Early onset breast cancer (especially if under 50 years of age)
- Bilateral or multiple breast cancers
- Diagnosed with both breast and ovarian cancer
- Family history of breast and/or ovarian cancer
- Two or more BRCA1 or BRCA2-related cancers in a single family member
- Male breast cancer within family
- Ashkenazi Jewish ethnic background

Test Technology
Pathway Genomics uses the most advanced Next Generation Sequencing (NGS) technology to search for mutations in the coding regions of BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11 and TP53; regions of insufficient coverage by NGS are sequenced using Sanger chemistry. Mutations found by NGS are confirmed using Sanger sequencing technology. Sanger sequencing is also used for single-site detection including mutations in the BRCATrue® Ashkenazi Jewish (3-site) test. Large gene deletions and duplications are detected using array comparative genomic hybridization (aCGH). Pathway’s sequencing tests display outstanding performance characteristics compatible with the highly demanding genetic diagnostic applications (sensitivity: 99.99%, specificity: 99.997%, reproducibility: >99.99%).

Possible Outcomes
Pathway Genomics classifies variants using a 5-tier system based on the American College of Medical Genetics (ACMG) guidelines. According to this system, variants are classified as either “Pathogenic”, “ Likely Pathogenic”, “Uncertain Pathogenicity (VUS)” , “Likely Benign” or “Benign”. Pathogenic, Likely Pathogenic and Uncertain Pathogenicity (VUS) are always reported in the BRCATrue® test. Likely Benign and Benign variants are not reported.

- **Pathogenic:** Mutations with known clinical significance and demonstrated to increase the risk of cancer pathogenesis.
- ** Likely Pathogenic:** Genetic changes that have some preliminary clinical data suggesting an association with cancer but not sufficient to make a definitive determination of pathogenicity and associated cancer risk.
- **Uncertain Pathogenicity (VUS):** Genetic changes with either no supporting data or the data are conflicting, thus a determination of pathogenicity cannot be made.
- **Likely Benign:** Likely benign variants are genetic changes with strong but limited evidence to be classified as benign and are not likely to increase the risk for cancer.
- **Benign:** Benign variants are genetic changes that are previously reported and have sufficient evidence to be classified as benign with no clinical relevance.

Limitations and Warnings
The etiologies of cancer are multifactorial, that is cancer can occur as a result of various factors, including inherited and acquired genetic mutations, diet, lifestyle choices and age. The BRCATrue® and BreastTrue® line of tests evaluates only inherited genetic mutations. It is possible that mutations in genes and genetic regions not tested in Pathway Genomics’ DNA sequencing 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.
References


For More Information

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