BreastTrue® High Risk Panel

Technical Bulletin
Hereditary Breast Cancer

1. 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; that is 1 in 8 women will develop the disease.\textsuperscript{1} In contrast, men have a lifetime risk of 0.13%, or about 1 in 1000.\textsuperscript{1} The most common type of breast cancer is ductal carcinoma, occurring in about 7 of 10 women with the disease.\textsuperscript{2} Approximately 1 in 10 women with breast cancer has lobular cancer.\textsuperscript{2} A mixture between ductal and lobular, and other less common types account for the remainder of breast cancer cases.\textsuperscript{2} Ovarian cancer is a malignancy in the ovaries caused by uncontrolled division of abnormal cells. The lifetime risk of developing ovarian cancer is 1.4%, or about 1 in 72.\textsuperscript{1} The most common types of ovarian cancer are ovarian epithelial carcinoma and malignant germ cell tumors.\textsuperscript{3}

2. Hereditary Breast and Ovarian Cancer (HBOC) Syndrome

Increased incidence of breast and ovarian cancer is observed among women with a family history of the disease.\textsuperscript{4} Studies have demonstrated that highly penetrant genetic variations in a small number of genes are responsible for the increased risk of breast and ovarian cancer in families.\textsuperscript{1} In the early 1990s, \textit{BRCA1} and \textit{BRCA2} were identified as the two predominant genes harboring these highly penetrant pathogenic variants (Table 1). Pathogenic variants in either \textit{BRCA1} or \textit{BRCA2} results in an up to 85% life-time risk of breast and ovarian cancer in high-risk families.\textsuperscript{5,6,7}
Table 1: Proportion of cancer attributable to \textit{BRCA1}, \textit{BRCA2}, or other loci in high-risk families.\textsuperscript{6}

<table>
<thead>
<tr>
<th>High-Risk Family Type</th>
<th>\textit{BRCA1}</th>
<th>\textit{BRCA2}</th>
<th>Other Loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Families with female breast cancer only</td>
<td>0.36</td>
<td>0.08</td>
<td>0.56</td>
</tr>
<tr>
<td>Four or five breast cancers</td>
<td>0.29</td>
<td>0.66</td>
<td>0.05</td>
</tr>
<tr>
<td>Six or more breast cancers</td>
<td>0.35</td>
<td>0.36</td>
<td>0.29</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Families with breast and ovarian cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One ovarian cancer</td>
<td>0.67</td>
<td>0.16</td>
<td>0.17</td>
</tr>
<tr>
<td>Two or more ovarian cancers</td>
<td>0.80</td>
<td>0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Overall</td>
<td>0.74</td>
<td>0.16</td>
<td>0.10</td>
</tr>
<tr>
<td>All families (female breast cancer only families + breast-ovarian cancer families)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Four or five breast cancers</td>
<td>0.56</td>
<td>0.05</td>
<td>0.39</td>
</tr>
<tr>
<td>Six or more breast cancers</td>
<td>0.50</td>
<td>0.42</td>
<td>0.08</td>
</tr>
<tr>
<td>Overall</td>
<td>0.54</td>
<td>0.27</td>
<td>0.19</td>
</tr>
</tbody>
</table>

HBOC syndrome is an autosomal dominant disorder caused by germline pathogenic variants in the \textit{BRCA1} and \textit{BRCA2} genes. Inheritance of one pathogenic variant in either gene is sufficient to cause the syndrome. HBOC syndrome accounts for 5-7\% of breast cancer cases\textsuperscript{8} and 8-13\% of epithelial ovarian cancer cases;\textsuperscript{9} however, germline \textit{BRCA1} and \textit{BRCA2} pathogenic variants account for 80\% of breast and ovarian cancer in families with multiple cases of either disease.\textsuperscript{5,6,7} The primary risks associated with HBOC syndrome are early-onset, female breast cancer and ovarian cancer (Table 2).

Pathogenic variants in \textit{BRCA1} and \textit{BRCA2} are uncommon in sporadic breast cancer\textsuperscript{8}.

HBOC syndrome is also associated with increased risk of other types of cancer, including but not limited to fallopian tube,\textsuperscript{10,11,12} peritoneal,\textsuperscript{13,14,15} pancreatic,\textsuperscript{16,17,18} prostate,\textsuperscript{16,19,20,21} and male breast cancers.\textsuperscript{22,23}

Table 2: HBOC syndrome cancer risk associated with \textit{BRCA1} and \textit{BRCA2} pathogenic variants.\textsuperscript{5,11,24,25,26,27,28}

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>\textit{BRCA1}</th>
<th>\textit{BRCA2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female breast cancer</td>
<td>Cumulative risk of approx. 57-73% by age 70 years.</td>
<td>Cumulative risk of approx. 45-55% by age 70 years.</td>
</tr>
<tr>
<td>Female breast cancer in high-risk families</td>
<td>Cumulative risk up to 85% by age 70 years.</td>
<td>Cumulative risk up to 84% by age 70 years.</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Cumulative risk up to 41% by age 70 years.</td>
<td>Cumulative risk of approx. 11-18% by age 70 years.</td>
</tr>
<tr>
<td>Ovarian cancer in high-risk families</td>
<td>Cumulative risk up to 63% by age 70 years.</td>
<td>Cumulative risk up to 27% by age 70 years.</td>
</tr>
</tbody>
</table>
2.1. Ashkenazi Jewish founder mutations

Inherited predisposition for breast and ovarian cancer among people of Ashkenazi (Central and Eastern European) ethnc background has long been recognized. Approximately one in forty Ashkenazim carry one of the three founder mutations in \textit{BRCA1} (c.68_69delAG or c.5266dupC) or \textit{BRCA2} (c.5946delT) genes.\textsuperscript{29,30,31} A population based study demonstrated that 29\% of Jewish women with ovarian cancer carry one of these three founder mutations.\textsuperscript{32} Similarly, a study further demonstrated that one of these three founder mutations was present in 45.5\% of a group of 220 high-risk Ashkenazi breast cancer families.\textsuperscript{33} This number increased to 73\% if ovarian cancer was present in the kindred.\textsuperscript{33,34}

2.2. Hispanic founder mutations

Inherited predisposition for breast and ovarian cancer among people of Hispanic descent (Mexican, Central American, Caribbean, South American, Spanish) has been reported. Eight pathogenic variants in \textit{BRCA1} and \textit{BRCA2} are highly recurrent in the Hispanic population: \textit{BRCA1} c.68_69delAG (also known as 185delAG and 187delAG), c.212+1G>A (IVS5+1G>A), exon8-11del (previous nomenclature: exon9-12del), c.2433delC (2552delC), c.2864C>A (p.S955X), c.4327C>T (p.R1443X), c.5266dupC (5382insC) and \textit{BRCA2} c.3264dupT (3492insT).\textsuperscript{35,36,37,38,39,40,41,42,43}

The prevalence of \textit{BRCA1} pathogenic variants among breast cancer survivors is estimated to be 3.5\% in Hispanic women compared to 2.2\% in non-Hispanic white women.\textsuperscript{35} A study of 746 Hispanics (primarily of Mexican descent) with personal or family history of breast and/or ovarian cancer revealed that two pathogenic variants in \textit{BRCA1} (c.68_69delAG and exon8-11del) and \textit{BRCA2} c.3264dupT account for 21.7\% of the observed \textit{BRCA1}/2 pathogenic variants.\textsuperscript{37} The study also identified each of the c.212+1G>A, c.2433delC, c.2864C>A and c.4327C>T pathogenic variants in multiple breast and/or ovarian cancer patients from Mexico, Guatemala and Colombia.

The \textit{BRCA1} c.4327C>T pathogenic variant was originally reported as a founder mutation in French-Canadian (Quebec) population.\textsuperscript{44,45} The \textit{BRCA1} c.5266dupC pathogenic variant found in many European populations also has a high prevalence in Brazilians among Latin America countries.\textsuperscript{42,43}

3. Other breast cancer susceptibility genes

Cases of familial breast cancer not related to \textit{BRCA1} or \textit{BRCA2} are thought to be caused by other genes, each one accounting for a fraction of the total risk.\textsuperscript{46} Some of these additional high risk susceptibility genes include \textit{CDH1}, \textit{CHEK2}, \textit{PALB2}, \textit{PTEN}, \textit{STK11}, and \textit{TP53}.\textsuperscript{7} 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.\textsuperscript{47,48,49,50,51,52,53,54,55} Genetic testing of the genes associated with these conditions (i.e., \textit{TP53}, \textit{PTEN}, \textit{STK11} and \textit{CDH1}) have been part of the clinical management of patients with a family history of breast cancer. Recently, \textit{CHEK2} and \textit{PALB2} have been identified as important contributors to breast cancer risk.\textsuperscript{56,57,58,59,60,61} Table 3 summarizes the risk contributions from the top breast-cancer related genes.

\begin{table}
\begin{center}
\textbf{Table 3} High Risk breast cancer genes.
\end{center}
\end{table}
<table>
<thead>
<tr>
<th>Gene</th>
<th>Syndrome</th>
<th>Neoplasm</th>
<th>Lifetime Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>HBOC</td>
<td>Breast (female), ovarian cancer</td>
<td>40-85%</td>
</tr>
<tr>
<td>BRCA2</td>
<td>HBOC</td>
<td>Breast (male and female), ovarian, prostate, pancreatic</td>
<td>9-84%</td>
</tr>
<tr>
<td>TP53</td>
<td>Li-Fraumeni</td>
<td>Breast cancer, sarcomas, leukemia, brain tumors, lung cancer, adrenocortical carcinoma</td>
<td>56-90%</td>
</tr>
<tr>
<td>PTEN</td>
<td>Cowden</td>
<td>Breast, thyroid, endometrial, kidney cancer</td>
<td>25-85%</td>
</tr>
<tr>
<td>STK11</td>
<td>Peutz-Jeghers</td>
<td>Breast, ovarian, cervical, uterine, testicular, small bowel, colon cancer</td>
<td>32-54%</td>
</tr>
<tr>
<td>CDH1</td>
<td>HDGC</td>
<td>Hereditary diffuse gastric, lobular breast cancer</td>
<td>60-80%</td>
</tr>
<tr>
<td>PALB2</td>
<td>PALB2-related</td>
<td>Breast, pancreatic, ovarian, male breast cancer</td>
<td>35-58%</td>
</tr>
<tr>
<td>CHEK2</td>
<td>CHEK2-related</td>
<td>Breast, prostate, colon cancer</td>
<td>20-44%</td>
</tr>
</tbody>
</table>

4. Reported 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. The BRCA1 protein, 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. Loss or inactivation of one copy of BRCA1 is thought to result in accumulation of variants and structural changes in the genome, thereby increasing risk of cancer.

**BRCA2**

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

**CDH1**

The CDH1 (cadherin 1, type 1, E-cadherin (epithelial)) gene encodes the E-cadherin protein, a member of the transmembrane 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. Cancers develop in individuals with a CDH1 germline pathogenic variant when the second copy of the CDH1 gene is somatically inactivated or down
Germline pathogenic variants in \textit{CDH1} have been shown to segregate in families with hereditary diffuse gastric cancer (HDGC) syndrome.

\textbf{CHEK2}

The \textit{CHEK2} (checkpoint kinase 2) gene encodes a checkpoint protein kinase important for maintaining genome integrity.\cite{75} The \textit{CHEK2} protein transduces DNA damage response signals detected by the ATM protein kinase.\cite{76,77} The ATM-CHEK2 DNA damage response pathway halts cell divisions to provide time for DNA repair, or to initiate programmed cell death (apoptosis) if the damage is irreversible. The \textit{CHEK2} protein interacts with other cancer susceptibility gene products such as the p53 and BRCA1 tumor suppressor proteins.\cite{78,79}

Pathogenic variants in the \textit{CHEK2} gene increases the susceptibility of developing certain cancers such as breast cancer,\cite{59,60,61} prostate cancer\cite{80,81,82} and colon cancer.\cite{81,83}

\textbf{PALB2}

The \textit{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.\cite{84,85} Pathogenic \textit{PALB2} variants have been detected in 1-3\% of \textit{BRCA1/2} mutation-negative hereditary breast cancer patients.\cite{84,85,86,87} Although \textit{PALB2} pathogenic variants are relatively rare, some of the \textit{PALB2} pathogenic variants have been shown to confer a breast cancer risk that is comparable to pathogenic variants in \textit{BRCA1} and \textit{BRCA2} genes.\cite{88,89,90} Clinical data suggest that \textit{PALB2} pathogenic variants may also increase the risk for pancreatic cancer.\cite{91}

Fanconi anemia (FA) is a rare, recessive chromosome instability syndrome caused by pathogenic variants in at least one of the several genes that encode FA pathway components.\cite{92} FA is characterized by congenital abnormalities, bone marrow failure, hypersensitivity to DNA crosslinking agents, defective DNA repair and cancer susceptibility.\cite{93,94} The \textit{PALB2} protein has been shown to be one of the component of FA pathway; certain biallelic pathogenic variants in \textit{PALB2} gene can cause a subtype of FA known as FA complementation group N (FANCN).\cite{95,96}

\textbf{PTEN}

The \textit{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.\cite{97} The importance of \textit{PTEN} as a tumor suppressor gene is supported by the high frequency of somatic variants in \textit{PTEN} found in a variety of sporadic human cancers.\cite{98}

PTEN hamatoma tumor syndrome (PHTS) is a collection of rare autosomal dominant disorders and found in individuals with deleterious germline pathogenic variants in the \textit{PTEN} gene. Individuals with PHTS exhibit a spectrum of disorders involving disorganized growth of benign tumors called hamartomas in multiple organ systems.\cite{99,100} The two most common inherited PHTS disorders are the adult Cowden syndrome (CS) and the pediatric Bannayan-Riley-Ruvalcaba syndrome (BRRS).\cite{100} The \textit{PTEN}-related Proteus syndrome (PS) and Proteus-like syndrome are the two other types of PHTS.
• CS is a multiple hamartoma syndrome with a high risk of benign and malignant tumors of multiple organ systems. Up to 85% of CS individuals are found to carry a detectable PTEN pathogenic variant.\textsuperscript{100} A large prospective study of individuals meeting relaxed International Cowden Consortium PHTS criteria found that the presence of a pathogenic variant in the PTEN gene gave a lifetime risk of 85% for breast cancer, 35% for thyroid cancer, 28% for endometrial cancer, 9% for CRC, 34% for kidney cancer and 6% for melanoma.\textsuperscript{54} In contrast, the lifetime cancer risk for the general population was 12% for breast cancer, 1% for thyroid cancer, 2.6% for endometrial cancer, 5% for CRC, 1.6% for kidney cancer and 2% for melanoma.\textsuperscript{101}

• BRRS is a congenital syndrome characterized by macrocephaly, intestinal hamartomatous polyposis, lipomas, and pigmented macules of the glanspenis. Up to 65% of BRR individuals are found to carry a PTEN pathogenic variant.\textsuperscript{100,102}

\textit{STK11}

The \textit{STK11} (also known as \textit{LKB1}) gene encodes a serine-threonine kinase that is involved in the regulation of metabolism, cell differentiation, proliferation, polarity and apoptosis.\textsuperscript{103,104} STK11 is a tumor suppressor, and pathogenic variants in the gene have been associated with Peutz-Jehgers syndrome (PJS).

PJS is an autosomal dominant 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.\textsuperscript{55} Gastrointestinal polyps can lead to chronic bleeding resulting in anemia and increased risk of malignant transformation.\textsuperscript{55}

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.\textsuperscript{55,105,106} 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.\textsuperscript{107} In PJS, malignancies appear at a younger age compared to the general population, i.e., an average age of 42 years.\textsuperscript{105}

Most patients who are clinically diagnosed with PJS have a pathogenic variant in \textit{STK11}.\textsuperscript{108} Loss of kinase activity is likely to be responsible for the development of this condition.\textsuperscript{109} The clinical diagnosis of PJS is made when a patient meets at least 2 of the following criteria: 2 or more Peutz-Jehgers polyps of the small intestine; typical mucocutaneous hyperpigmentation; and a family history of PJS.

\textit{TP53}

The \textit{TP53} gene encodes a transcription factor that is involved in cellular responses to environmental and genotoxic stress.\textsuperscript{110} This tumor suppressor binds consensus DNA in the responsive elements of several hundreds of genes.\textsuperscript{111} \textit{TP53} pathogenic variants are the most frequently acquired genetic alteration in human cancers, with greater than 35,000 pathogenic variants described in different types of cancer.\textsuperscript{112} Approximately 95% of the pathogenic variants are localized in the DNA-binding domain of p53.\textsuperscript{111}

LFS and its variant, Li-Fraumeni-like (LFL) syndrome are autosomal dominant disorders caused by germline pathogenic variants in the \textit{TP53} gene and are characterized by predisposition to multiple early onset cancers.\textsuperscript{113} LFS has high
variability in penetrance, age of cancer onset and tumor spectrum.\textsuperscript{114} LFL syndrome is associated with incomplete LFS features.\textsuperscript{115} The most common cancers associated with LFS are sarcoma, breast cancer, brain tumors and adrenocortical carcinoma. Other cancers include leukemia, choroid plexus papilloma, Wilms tumors, and gastric, colorectal and pancreatic cancer.\textsuperscript{116}

Clinically, LFS is diagnosed in individuals who have a germline pathogenic variant in the \textit{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 pathogenic variants in the \textit{TP53} gene.\textsuperscript{113}

5. 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. Likely Benign and Benign variants are not reported.

- **Pathogenic**: Variants 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.

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

6. Limitations and Warnings

Pathway Genomics offers next generation sequencing (NGS) based single or multiple-gene panel tests. Pre-specified single or multiple-site Sanger DNA sequencing tests are also offered.

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

References


70. Petrucelli N, Daly MB, Pal T. BRCA1- and BRCA2-Associated Hereditary Breast and Ovarian Cancer *GeneReviews®*. 1993;


100. Eng C. *PTEN Hamartoma Tumor Syndrome GeneReviews.* 1993;


