OncoGeneDx: Breast Cancer High Risk Panel and \textit{PALB2}

\textbf{Panel Gene List:} \textit{BRCA1, BRCA2, CDH1, PALB2, PTEN, TP53}

\textbf{Clinical Features:}
In the general population, approximately 1 in 8 women (12\%) will develop breast cancer in their lifetime (SEER). Most cases of breast cancer develop sporadically with no family history of the cancer; however, 5-10\% of cases are thought to be due to a hereditary predisposition. The features suggestive of a hereditary cancer predisposition include: young age at diagnosis (before age 50), multiple primary cancers in a single individual, diagnosis of a cancer type that is not common in the general population (such as ovarian cancer, male breast cancer, or pancreatic cancer), and several relatives affected with related cancers spanning multiple generations.

The OncoGeneDx Breast Cancer High Risk Panel and \textit{PALB2} includes genes associated with a defined increased level of breast cancer risk including those associated with Hereditary Breast and Ovarian Cancer syndrome (\textit{BRCA1} and \textit{BRCA2}), Hereditary Diffuse Gastric Cancer syndrome (\textit{CDH1}), \textit{PTEN} hamartoma tumor syndrome (\textit{PTEN}), Li-Fraumeni syndrome (\textit{TP53}) as well as \textit{PALB2}.

It is estimated that 20-25\% of familial breast cancer risk can be attributed to pathogenic variants in the \textit{BRCA1} and \textit{BRCA2} genes (Easton 1999, Pharoah 2002, van der Groep 2011). The contribution of pathogenic variants in the \textit{CDH1}, \textit{PTEN}, \textit{TP53} and \textit{PALB2} genes to familial breast cancer risk overall is less well-characterized but is considerably lower than the contribution of \textit{BRCA1} and \textit{BRCA2} pathogenic variants.

\textbf{Hereditary Breast and Ovarian Cancer syndrome (\textit{BRCA1} and \textit{BRCA2}):} Women with pathogenic variants in \textit{BRCA1} or \textit{BRCA2} have a 41-87\% lifetime risk to develop breast cancer and an up to 63\% risk for contralateral breast cancer (Antoniou 2003, Chen 2007, Claus 1996, Ford 1998, King 2003, Graeser 2009, Risch 2006). Studies have shown that the lifetime risk to develop ovarian cancer is between 24-54\% for \textit{BRCA1} pathogenic variant carriers and 11-27\% for \textit{BRCA2} pathogenic variant carriers (Antoniou 2003, Chen 2007, Ford 1998, King 2003, Risch 2006). Other cancers associated with pathogenic variants in \textit{BRCA1} and \textit{BRCA2} in women include fallopian tube carcinoma, primary peritoneal carcinoma, and uterine serous carcinoma (Levine 2003, Biron-Shental 2006, Pennington 2013). The lifetime risk for breast cancer in male \textit{BRCA1/2} pathogenic variant carriers is approximately 7\% with a pathogenic variant in \textit{BRCA2} and slightly increased with a pathogenic variant in \textit{BRCA1} (Liede 2004, Tai 2007). Other malignancies reported in families with pathogenic variants in \textit{BRCA1} or \textit{BRCA2}
include prostate cancer in men, as well as pancreatic cancer and melanoma in both men and women.

**Hereditary Diffuse Gastric Cancer syndrome (CDH1):** Women with a pathogenic variant in CDH1 have a 39-52% lifetime risk for lobular breast cancer. The lifetime risk of diffuse gastric cancer has been estimated to be 40-67% for men and 63-83% for women (Kaurah 2007, Pharoah 2001). Diffuse gastric cancer generally occurs before age 50 in carriers of CDH1 pathogenic variants, and even cases under the age of 18 have been reported in families with hereditary diffuse gastric cancer (Guilford 1998). Signet ring cell cancer of the colon has also been reported in individuals with CDH1 pathogenic variants (Brooks-Wilson 2004).

**PTEN hamartoma tumor syndrome (PTEN):** Cowden Syndrome (CS) and Bannayan-Riley-Ruvalcaba (BRRS) are two conditions belonging to the spectrum of PTEN hamartoma tumor syndrome (PHTS) and are associated with an increased risk of developing cancer. There is an approximate 25-45% risk for breast cancer and 5-10% risk for endometrial cancer in women, and 10% risk for non-medullary thyroid cancer in women and men (Hobert 2009). Additional clinical features in CS include increased head circumference ≥97th percentile (macrocephaly), trichilemmomas, papillomatous papules, and the pathognomonic finding of cerebellar dysplastic gangliocytoma (Lhermitte-Duclos disease). CS may be also associated with benign breast disease, thyroid goiters, benign gastrointestinal polyps, and uterine fibroids. BRRS is associated with macrocephaly, intestinal hamartomas, pigmented macules of the glans penis, and can be associated with developmental delay or autism. Vascular abnormalities, such as hemangiomas and arterio-venous malformations, have also been reported in individuals with pathogenic variants in PTEN.

**PALB2:** Women with a pathogenic variant in PALB2 have been estimated to have a 2 to 3-fold increased risk of breast cancer over the general population (Erkko 2008, Rahman 2007) resulting in a lifetime risk of approximately 25% to 40%. More recent data has suggested a lifetime risk (up to age 70) ranging from 33% to 58% depending on the individual’s family history of breast cancer (Antoniou 2014). Women with a pathogenic variant in PALB2 who have a family history of early-onset breast cancer may have a lifetime risk up to 58% (Byrnes 2008, Antoniou 2014). Casadei et al. (2011) found that PALB2 pathogenic variant carriers are 6-fold more likely to have a family history of pancreatic cancer, 1.3-fold more likely to have a family history of ovarian cancer and 4-fold more likely to have a family history of male breast cancer. Although the association of pathogenic variants in PALB2 and pancreatic cancer has been established, the exact risks are not yet well-understood (Jones 2009, Slater 2010).

**Li-Fraumeni syndrome (TP53):** The following core cancer types account for 70-77% of LFS-associated tumors (in order of frequency): breast cancer, soft tissue sarcoma, brain tumors, osteosarcoma, and adrenocortical carcinoma (Gonzalez 2009, Olivier 2003, Ruijs 2010).
Other types of cancer that may be associated with LFS include ovarian, gastrointestinal, pancreatic, genitourinary, skin, thyroid and lung cancers as well as leukemia, lymphoma, and neuroblastomas. Age-related and sex-specific cancer risks have been reported. Individuals with LFS who have been diagnosed with cancer have up to a 57% risk of developing a second primary cancer within 30 years of the first diagnosis and up to a 38% risk of a third primary diagnosis (Hisada 1998). Several studies have demonstrated that subsequent tumors often develop in the radiation field of the previously treated cancer (Chompret 2000, Hisada 1998).

Inheritance Pattern:
All of the genes on this panel are associated with an autosomal dominant cancer risk. Some of the genes on this panel are also associated with extremely rare conditions when inherited in an autosomal recessive fashion. The specifics of this inheritance are outlined in the table below.

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. (For PTEN, nucleotides c.-700 through c.-1300 in the promoter region are also captured.) 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 but are available upon request. Data analysis is performed using gene-specific filtering; the genes evaluated by this test are listed on the first page of the report.

Test Sensitivity:
The clinical sensitivity of sequencing and deletion/duplication analysis of the 6 genes included in the OncoGeneDx Breast Cancer High Risk Panel and PALB2 depends in part on the patient’s clinical phenotype and family history. In general, the sensitivity is highest for individuals with features suggestive of hereditary predisposition to cancer as outlined above. DNA sequencing will detect nucleotide substitutions and small insertions and deletions, while
NGS-CNV analysis, array CGH, or MLPA will detect exon-level deletions and duplications. These methods are expected to be greater than 99% sensitive in detecting pathogenic variants identifiable by sequencing or CNV technology. 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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</thead>
<tbody>
<tr>
<td><strong>BRCA1</strong></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>
</tr>
<tr>
<td><strong>BRCA2</strong></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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<td></td>
<td>AR</td>
<td>Fanconi Anemia</td>
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<tr>
<td><strong>CDH1</strong></td>
<td>CADHERIN 1</td>
<td>AD</td>
<td>Hereditary Diffuse Gastric Cancer (HDGC) syndrome: gastric-diffuse, breast &amp; colon (signet ring) cancer</td>
</tr>
<tr>
<td><strong>PALB2</strong></td>
<td>PARTNER AND LOCALIZER OF BRCA2</td>
<td>AD</td>
<td>Breast, pancreatic &amp; ovarian cancer</td>
</tr>
<tr>
<td>Gene</td>
<td>Cancer Type</td>
<td>Inheritance Pattern</td>
<td>Genetic Abnormality</td>
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<tr>
<td>PTEN</td>
<td>BRCA1 and BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies.</td>
<td>AD</td>
<td>TP53, PTEN, MLPA, CGH</td>
</tr>
<tr>
<td>TP53</td>
<td>BrCa1 and BrCa2 penetrance.</td>
<td>AD</td>
<td>TP53</td>
</tr>
</tbody>
</table>

**PTEN**

**Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN**

**AR**

**Fanconi anemia**

**TP53**

**Cellular Tumor Antigen P53**

**AD**

**PTEN hamartoma tumor syndrome (PHTS): breast, thyroid, endometrial, colon, melanoma & renal cancer, gastrointestinal polyps, Lhermitte-Duclos Disease**

**Li-Fraumeni syndrome (LFS): breast cancer, sarcoma, brain cancer, hematologic malignancies, adrenocortical carcinoma, among others**

Because of 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.

*High overall risk of cancer: 75% lifetime risk for males to develop cancer, nearly 100% risk for females.*

**Abbreviations:**

AD – Autosomal Dominant

AR – Autosomal Recessive

CGH – Comparative genomic hybridization

MLPA – Multiplex ligation-dependent probe amplification

**References:**


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