

OncoGeneDx: Breast Cancer Management Panel

Panel Gene List: *ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, STK11, TP53*

Clinical Features:

In the general population, approximately 1 in 8 women (12%) will develop breast cancer in their lifetime.¹ 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 Management Panel includes genes associated with an increased lifetime risk for breast cancer of 20% or higher.

It is estimated that 20-25% of familial breast cancer risk can be attributed to pathogenic variants in the *BRCA1* and *BRCA2* genes.²⁻⁴ The contribution of pathogenic variants in the *ATM, BARD1, CDH1, CHEK2, PALB2, PTEN, STK11,* and *TP53* genes to familial breast cancer risk overall is less well-characterized but is considerably lower than the contribution of *BRCA1* and *BRCA2* pathogenic variants.

***ATM*:** Women with a pathogenic variant in *ATM* have approximately a two-fold increase risk for breast cancer (RR = 2.2-2.4).⁵⁻⁷ Thompson et al. studied 1160 *ATM* pathogenic variant carriers and concluded that female heterozygous *ATM* pathogenic variant carriers who are less than 50 years of age had a significantly increased risk for breast cancer (RR = 4.9) compared to women over 50 years of age where a statistically significant risk could not be identified.⁵ This same study suggests an increased risk for colon cancer, but the confidence intervals are wide.⁵ Roberts et al. reported an association with pancreatic cancer showing that 2.4% of familial pancreatic cancer patients were found to carry a pathogenic variant in *ATM*, and 4.6% of families with 3 or more cases of pancreatic cancer carried a pathogenic variant in *ATM*.⁸ Of note, certain missense pathogenic variants in the *ATM* gene may confer a higher breast cancer risk.⁷

***BARD1*:** Pathogenic variants in *BARD1* are associated with an increased risk for female breast cancer, particularly triple negative breast cancer (TNBC).⁹⁻¹¹ Studies have shown a significant association with odds ratios (OR) of 2.16-5.35 for unselected breast cancer and 4.35-11.27 for TNBC.^{9,10,12} Other cancers, including ovarian and neuroblastoma, have also been seen in individuals with *BARD1* variants.^{11,13-15}

BRCA1 and BRCA2 (Hereditary Breast and Ovarian Cancer syndrome): Women with pathogenic variants in *BRCA1* or *BRCA2* have a 41-87% lifetime risk to develop breast cancer and an up to 63% risk for contralateral breast cancer.¹⁶⁻²² Studies have shown that the lifetime risk to develop ovarian cancer is between 24-54% for carriers of pathogenic variants in *BRCA1* and 11-27% for *BRCA2* pathogenic variant carriers.^{16,17,19,20,22} Other cancers associated with pathogenic variants in *BRCA1* and *BRCA2* in women include fallopian tube carcinoma, primary peritoneal carcinoma, and uterine serous carcinoma.²³⁻²⁵ The lifetime risk for breast cancer in male carriers of a *BRCA1/2* pathogenic variant is approximately 7% with a *BRCA2* pathogenic variant and slightly increased with a *BRCA1* pathogenic variant.^{26,27} Other malignancies reported in families with pathogenic variants in *BRCA1* or *BRCA2* include prostate cancer in men, as well as pancreatic cancer and melanoma in both men and women.

CDH1 (Hereditary Diffuse Gastric Cancer syndrome): 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.^{28,29} Diffuse gastric cancer generally occurs before age 50 in *CDH1* pathogenic variant carriers, and even cases under the age of 18 have been reported in families with hereditary diffuse gastric cancer.³⁰ Signet ring cell cancer of the colon has also been reported in individuals with a pathogenic variant in *CDH1*.³¹ More recently, *CDH1* pathogenic variants have also been identified in families with lobular carcinoma in situ or invasive lobular carcinoma of the breast (LCIS/ILC) but no history of gastric cancer, suggesting the spectrum may include breast-only families.^{32,33}

CHEK2: Pathogenic variants in *CHEK2* are known to confer an increased risk of breast cancer and have been suggested to also increase the risk of colon and others cancers. While studies have shown that the risk for breast cancer may vary depending on the type of pathogenic variant and family history, in general those found to harbor a pathogenic variant in *CHEK2* are estimated to have a 2-fold increased risk.³⁴⁻³⁷ Additionally, pathogenic variants in *CHEK2* have been reported to be associated with colon, prostate, male breast, thyroid, renal, and gastric; however, the risks are not well defined.^{25,34,36-43}

PTEN (PTEN Hamartoma Tumor syndrome): 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. Individuals with PHTS are at increased risk for benign and malignant tumors as well as neurodevelopmental issues. Breast (77-85% lifetime risk), thyroid (35-38% lifetime risk), and endometrial cancers (21-28% lifetime risk) are most common in individuals with PHTS; however renal, colorectal, and melanoma skin cancers have also been reported.⁴⁴⁻⁴⁶ While most cancers are diagnosed in adulthood, thyroid, genitourinary, and other malignancies have been reported in childhood.⁴⁷⁻⁴⁹ Common benign neoplasias in individuals with PHTS include gastrointestinal polyposis, benign mucocutaneous lesions

of diverse histologies, and other benign lesions affecting the organs at increased cancer risk.^{50–52} *PTEN*-related hamartomas of soft tissue (PHOSTs) and arteriovenous malformations may develop in childhood or adulthood.^{53,54} Dysplastic cerebellar gangliocytoma, also called Lhermitte-Duclos disease, is estimated to occur in less than 10% of individuals with PHTS.⁴⁶

Apart from tumor development, individuals with PHTS often have increased head circumference and are at risk of having autism or neurocognitive delay. Macrocephaly is the most common feature observed, identified in 94% of affected individuals.⁵⁵ In addition, within a series of children with macrocephaly and autism, up to 17% were found to have PHTS.⁵⁶

PALB2: Pathogenic variants in the *PALB2* gene have been estimated to confer a 2 to 3-fold increased risk of breast cancer over the general population resulting in a lifetime risk of approximately 25% to 40%.^{57,58} More recent data have suggested a lifetime risk (up to age 70) ranging from 33% to 58% depending on the individual's family history of breast cancer.⁵⁹ 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%.^{59,60} Casadei et al. found that *PALB2* pathogenic variant carriers are 6-times more likely to have a family history of pancreatic cancer, 1.3- times more likely to have a family history of ovarian cancer and 4 times more likely to have a family history of male breast cancer.⁶¹ Although the association of pathogenic variants in *PALB2* and pancreatic cancer has been established, the exact risks are not yet well-understood.^{62,63}

***STK11* (Peutz-Jeghers syndrome)**: Pathogenic germline variants in *STK11* are associated with Peutz-Jeghers syndrome (PJS), a condition characterized by gastrointestinal hamartomatous polyps, mucocutaneous hyperpigmentation, and an increased risk of breast, colon, stomach, small intestine, pancreatic, and lung cancers, as well as malignant and benign tumors of the reproductive tract.^{64–66} All PJS-associated cancers are associated with an earlier age of onset compared to the general population, including cancer risks in childhood, and certain cancer types carry significant risks into elderly years.⁶⁵ The lifetime risk of breast cancer associated with PJS is as high as 54%, and early-onset breast cancer is sometimes diagnosed prior to the discovery of gastrointestinal polyps.⁶⁵

***TP53* (Li-Fraumeni syndrome)**: Pathogenic germline variants in *TP53* are associated with Li-Fraumeni syndrome (LFS), a cancer predisposition syndrome with a high risk of childhood- and adult-onset cancers. While breast cancer, soft tissue sarcomas, brain tumors, osteosarcomas, and adrenocortical carcinomas account for 70-77% of LFS-associated tumors, other cancers have been reported in association with LFS, including ovarian, gastrointestinal, pancreatic, genitourinary, skin, renal, thyroid, prostate, and lung cancers as well as leukemia, lymphoma, and neuroblastomas.^{67,68} The risk for males and females with a germline *TP53* pathogenic variant to develop cancer by age 60 is estimated to be 88% and 95%, respectively.⁶⁹ The chance of a second primary cancer diagnosis within ten years of the first cancer diagnosis is approximately 50% for both men and women.⁶⁹ Radiation-induced second malignancies have been reported in individuals with LFS, suggesting that

radiation may increase *TP53* pathogenic variant carriers' risk for subsequent cancers within the radiation field.^{70,71}

Genetics:

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.

Test Methods:

Genomic DNA is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured and used for DNA extraction. This DNA is enriched for the complete coding regions and splice site junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next generation sequencing with CNV calling (NGS-CNV). For *PTEN* nucleotides c.-700 through c.-1300 in the promoter region are also captured. The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

Clinical Sensitivity:

The clinical sensitivity of sequencing and deletion/duplication analysis of the 10 genes included in the OncoGeneDx Breast Cancer Management Panel depends in part on the patient's clinical phenotype and family history. In general, the sensitivity is highest for individuals with features suggestive of a hereditary predisposition to breast 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.

Genetic testing using the methods applied at GeneDx is expected to be highly accurate. Normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable by this test. The methods used cannot reliably detect deletions of 20bp to 250bp in size, or insertions of 10bp to 250 bp in size. Sequencing cannot detect low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Rarely, incidental findings of large chromosomal rearrangements outside the gene of interest may be identified. Regions of certain genes have inherent sequence properties (for example: repeat, homology, or pseudogene regions, high GC content, rare polymorphisms) that yield suboptimal data, potentially impairing accuracy of the results. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or

suboptimal DNA quality. In individuals with active or chronic hematologic neoplasms or conditions, there is a possibility that testing may detect an acquired somatic variant, resulting in a false positive result. As the ability to detect genetic variants and naming conventions can differ among laboratories, rare false negative results may occur when no positive control is provided for testing of a specific variant identified at another laboratory. The chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. Consultation with a genetics professional is recommended for interpretation of results.

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