ApolloGen, Inc.
13766 Alton Parkway
Suite 147
Irvine CA 92618
Phone: (949) 916-8886
Fax: (949) 916-8899
inquiries@apollogen.com
www.apollogen.com

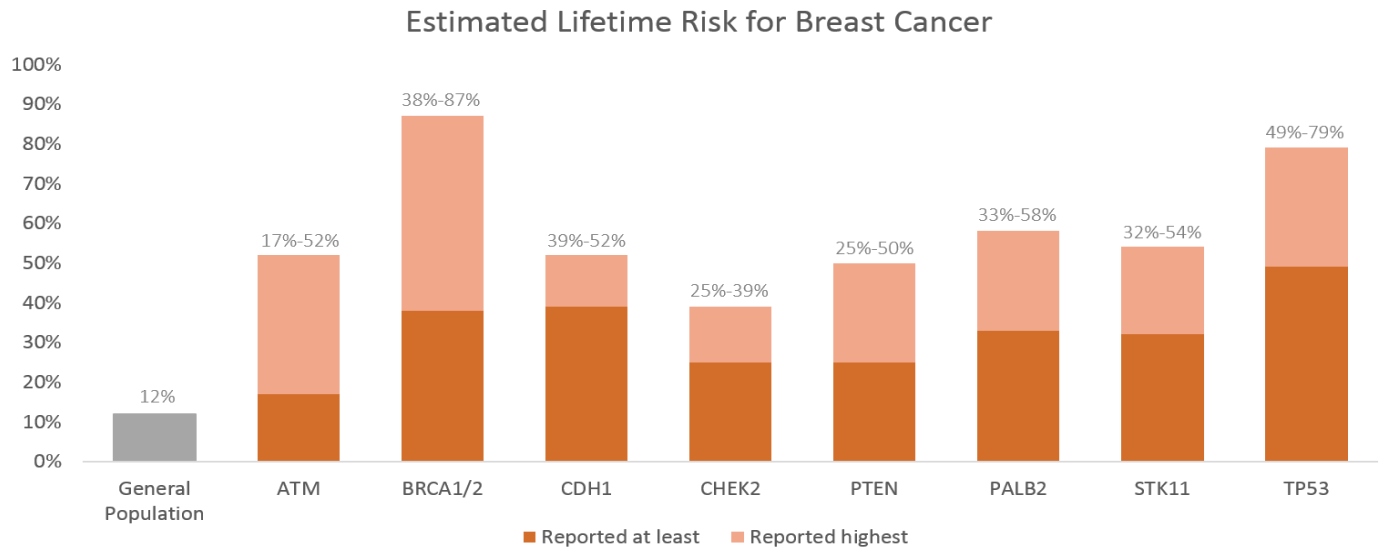
Breast Cancer Panel

Next Gen Sequencing Test Specifications

Disease Overview

Approximately 12.4 percent of women (1 in 8) will be diagnosed with invasive breast cancer over the course of their lifetime. The National Cancer Institute (NCI) estimates that in 2017, approximately 255,180 new cases of invasive breast cancer are expected to be diagnosed in the women in the U.S., along with 63,410 new cases of non-invasive (in situ) breast cancer. Based on 2011-2013 data. In 2013, there were an estimated 3,053,450 women living with female breast cancer in the United States.

Most cases of breast cancer are sporadic with no family history of the cancer; but, 5-10% of cases are understood to be due to a hereditary predisposition. Hereditary breast cancers tend to occur earlier in life than sporadic cases and are more likely to occur in both breasts and frequently associated with other primary cancers. The genes *BRCA1* and *BRCA2* appear to be responsible for a large number of hereditary breast cancer cases. However, apart from these two genes, there are other genes that could lead to significantly increased risks of breast cancer and other types of cancer. ApolloGen's Breast Cancer Panel analyzes 19 genes simultaneously, including both high-risk genes (*BRCA1, BRCA2, CDH1, STK11, PTEN,* and *TP53*) and moderate-to-high-risk genes, which will affect the patients' medical management based on clinical guidelines. The estimated lifetime risk of breast cancer for some of the included genes are shown below.



In addition to the breast cancer risk, these genes very often are associated with increased risks of other primary cancers. *ATM* is associated with risk of pancreatic cancer, *BRIP1, RAD50, RAD51C, STK11* with ovarian cancer, *TP53* with sarcoma and *CHEK2* with colon cancer, etc.

The diagnosis of a certain type of hereditary breast cancer is established by molecular genetic testing. Genetic testing is therefore useful for individuals with a personal and/or family history suggestive of hereditary breast and/or other related cancers. Genetic testing facilitates the identification of hereditary breast cancer, alerts the patient about risks of having other cancers, empowers patients to make their medical decisions and provides information for other at-risk family members. A positive test result will affect medical management of the patient. Physicians and clinicians can provide appropriate screening recommendations and risk-reduction options based on the test results. Sometimes the knowledge of genetic mutation status may qualify patients for gene-specific clinical trials and research studies as well.

According to the National Comprehensive Cancer Network (NCCN) guidelines, breast cancer genetic risk evaluation is suggested for individuals with a personal or family history of any of the following:

- Early on-set breast cancer (diagnosed ≤50 years of age)
- Breast and ovarian cancer or a related cancer in the same individual
- ≥3 cases of breast cancer
- ≥3 cases of breast cancer and/or a related cancer

Indications for Testing

1. Confirmation of diagnosis in patients with personal or family history suggestive of a predisposition to hereditary breast cancer
2. Assessment for at-risk family members

Requisition Form: Cancer Test Requisition Form or General Test Requisition Form (www.apollogen.com)

Genes (19): *BRCA1, BRCA2, TP53, PTEN, CDH1, STK11, ATM, AR, BARD1, BRIP1, CASP8, CHEK2, DIRAS3, ERBB2, NBN, PALB2, RAD50, RAD51C,* and *TGFB1*

CPT Codes: 81211×1, 81213×1, 81321×1, 81405×3, 81406×2, 81408×1, 81479×10
Turnaround Time: 4 weeks
Specimen Requirement: 3-5 mL Blood (EDTA) – Lavender Top Tube (preferred);
Saliva samples are optional
Other Specimen Types: Contact ApolloGen Diagnostic Laboratory
Pricing: Please contact us at (949) 916-8886 or at inquiries@apollogen.com for current pricing

Testing Methodology

Genomic DNA is extracted from the patient's specimen and fragmented via sonication. All of the exons, flanking intronic (at least 10 nucleotides into the introns), and untranslated regions (5' and 3') of the targeted genes are enriched using capture-based hybridization. Massively parallel sequencing is applied to the enriched target DNA regions to detect mutations. Variants with an allele frequency > 1% are considered likely benign polymorphisms, and are not included in the final report. Interpretation of rare alterations with allele frequency <1% is based on ACMG guidelines. All pathogenic and likely pathogenic variants are verified by Sanger Sequencing.

Massively parallel sequencing can reliably detect insertion/deletion mutations smaller than 10 base pairs. However, larger insertion, deletion, duplication due to rearrangement, and mutations in regulatory and deep intronic regions cannot be detected by this technology. Rare primer site variants may lead to erroneous results that may need further investigation.

Analytical Sensitivity:
This test can detect >95% of the small variants in the examined regions. Please contact us for detailed information regarding coverage for specific genes of interest.

Related Test

BRCA1 and BRCA2 Sequencing
Diagnose *BRCA1*- and *BRCA2*-Associated Hereditary Breast and Ovarian Cancer.
iGene Cancer Panel
Simultaneously analyzes 20 critical genes associated with an increased risk for breast, ovarian, endometrial, colorectal, pancreatic and other cancers.

Variant Classification

Sequencing results will be interpreted and reported following the recommendations of the American College of Medical Genetics (www.acmg.net). Sequence variations will be analyzed and classified into the following categories based on current scientific knowledge. Variants found in categories 1-3 (pathogenic, likely pathogenic, and variants of unknown clinical significance) will be reported.

1. **Pathogenic:** Pathogenic variants include nonsense mutations and frame shift mutations that are predicted to result in premature protein truncation, splice site mutations, and previously reported missense mutations that are recognized as disease-causing by databases and the scientific literature.
2. **Likely Pathogenic:** Likely pathogenic variants are those variants that are likely to adversely affect gene function, but for which there is no conclusive evidence to strongly support pathogenicity.
3. **Variant of Unknown Clinical Significance (VUS):** VUSs are sequence variations for which there is insufficient evidence to either confirm or exclude pathogenicity.
4. **Likely Benign**: Likely benign variants are sequence variations for which there is significant, but not conclusive evidence supporting that the variant is not disease-causing.
5. **Negative:** A negative classification is reported when no disease-causing variant is found, or a variant is classified as a benign variant based on the ACMG criteria, the population data, or if there is no clinical significance based on review of the literature and mutation databases.

References

1. Antoniou, A. C. et al. Common breast cancer-predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. Am. J. Hum. Genet. 82, 937–948 (2008).
2. http://www.breastcancer.org/symptoms/understand_bc/statistics
3. Frank, T. S. et al. Sequence analysis of BRCA1 and BRCA2: correlation of mutations with family history and ovarian cancer risk. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 16, 2417–2425 (1998).
4. Loman, N., Johannsson, O., Kristoffersson, U., Olsson, H. & Borg, A. Family history of breast and ovarian cancers and BRCA1 and BRCA2 mutations in a population-based series of early-onset breast cancer. J. Natl. Cancer Inst. 93, 1215–1223 (2001).
5. Edlich, R. F. et al. Breast cancer and ovarian cancer genetics: an update. J. Environ. Pathol. Toxicol. Oncol. Off. Organ Int. Soc. Environ. Toxicol. Cancer 27, 245–256 (2008).
6. Meindl, A., Ditsch, N., Kast, K., Rhiem, K. & Schmutzler, R. K. Hereditary breast and ovarian cancer: new genes, new treatments, new concepts. Dtsch. Arzteblatt Int. 108, 323–330 (2011).
7. Friedenson, B. BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. MedGenMed Medscape Gen. Med. 7, 60 (2005).
8. Walsh, T. & King, M.-C. Ten genes for inherited breast cancer. Cancer Cell 11, 103–105 (2007).

9. Byrnes, G. B., Southey, M. C. & Hopper, J. L. Are the so-called low penetrance breast cancer genes, ATM, BRIP1, PALB2 and CHEK2, high risk for women with strong family histories? Breast Cancer Res. BCR 10, 208 (2008).
10. Wong, M. W. et al. BRIP1, PALB2, and RAD51C mutation analysis reveals their relative importance as genetic susceptibility factors for breast cancer. Breast Cancer Res. Treat. 127, 853–859 (2011).
11. Pennington, K. P. & Swisher, E. M. Hereditary ovarian cancer: beyond the usual suspects. Gynecol. Oncol. 124, 347–353 (2012).
12. Kwong, A., Chen, J. W. & Shin, V. Y. A new paradigm of genetic testing for hereditary breast/ovarian cancers. Hong Kong Med J 22, 171–177 (2016).
13. ACMG, "Statement on Population Screening for BRCA-1 Mutation in Ashkenazi Jewish Women". 2001-2006
14. National Comprehensive Cancer Network., Genetic/Familial High-Risk Assessment: Breast and Ovarian (Version 2.2017). https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf. Accessed January, 2017