

Assay Summary

Breast Cancer Susceptibility

ATM, CDH1, CHEK2, TP53, PALB2, PTEN, and STK11 Gene Mutation Analyses

Synopsis

Inherited breast cancer represents approximately 7% of all breast cancer.¹ Highly penetrant mutations in the two main causal genes, *BRCA1* and *BRCA2*, explain anywhere between 20% to 80% of strongly familial breast cancer cases, with a progressively decreasing contribution in pedigrees with fewer affected family members.^{2,3} Loci with low-to-intermediate rather than high penetrance following a polygenic mode of inheritance are thought to underlie a substantial portion of the remaining cases of inherited breast cancer.⁴⁻⁸ This theory has been supported empirically as most breast cancer associated genes identified since the discovery of *BRCA1* and *BRCA2* appear to confer low to moderate elevation in breast cancer risk.⁷

Our breast cancer test includes seven moderate-to-highly penetrant genes: *TP53*, whose defects increase the risk of breast cancer at least 10 fold over that of the general population, as well as six additional genes whose germline mutations typically confer risk of 2-10 fold: *ATM, CDH1, CHEK2, PALB2, PTEN, and STK11*. Estimates of penetrance (8% to 90%) and combined prevalence (5% to 10%) of mutations in these genes in inherited breast cancer are approximate and vary widely, as they are conditional on the study population, mutation, cancer subtype, and syndromic association.⁸⁻¹¹

Tumor protein p53 (TP53) is a multifunctional tumor suppressor involved in transcriptional regulation, DNA repair, cell cycle arrest, and apoptosis.¹² Germline mutations in *TP53* gene represent the most frequent cause of Li-Fraumeni syndrome (LFS)¹² and account for 2%-7% of early-onset breast cancer (i.e., onset before 30 years of age).¹³⁻¹⁵ Breast cancer is the most frequent type of cancer in female mutation carriers, with a median onset age of 33.^{16,17} The likelihood of finding *TP53* mutations increases from 4%-7% in women with isolated, early-onset breast cancer to between 22% and 38% in women with a personal history of bilateral breast cancer or other LFS tumors, or with a family history of breast cancer or LFS.¹⁸⁻²⁰ According to a recent review, by age 60, breast cancer penetrance of germline mutations in *TP53* is approximately 90%.²¹ The most frequently identified mutations in the context of breast cancer are missense mutations disturbing normal binding and activation of TP53 target genes.²²⁻²⁴

Cadherin 1 (CDH1) is another tumor suppressor and a member of a family of transmembrane glycoproteins participating in cell-cell adhesion, differentiation, cell motility, and signaling.²⁵ Mutations that abolish cellular adhesion are believed to facilitate metastasis.²⁶ In addition to the risk of gastric cancer of 83%, women with *CDH1* germline mutations also have an elevated risk of 39% to 60% for, in particular, lobular breast cancer with a mean onset age of 53.²⁷⁻²⁹ As is the case with *PTEN* and *STK11*, germline mutations in *CDH1* are thought to represent rare causes of inherited breast cancer.⁹

Phosphatase and tensin homolog protein gene (*PTEN*) also encodes a tumor suppressor which initiates cell cycle arrest or apoptosis, depending on its cellular location.³⁰⁻³² Germline mutations in *PTEN* are a major cause of Cowden syndrome - a multiple hamartoma syndrome with elevated cancer risk and a prevalence of one in 200,000.³⁰ Breast cancer is its most common malignancy.³⁰ Female mutation carriers have a lifetime risk of breast cancer of 25%-50%, with an average age of diagnosis between 38 and 46

years.^{21,33} In contrast to *TP53*-associated breast cancer, *PTEN* germline mutations have been reported in both females and males with breast cancer.³⁴

Serine/threonine protein kinase 11 (*STK11*) is involved in the suppression of cellular proliferation and apoptosis.³⁵ Mutations in *STK11* explain 100% of familial and about 90% of nonfamilial cases of Peutz-Jeghers syndrome (PJS), which predisposes to gastrointestinal polyposis, mucocutaneous pigmentation and cancer.³⁵ *STK11* mutations also confer a risk of female breast cancer of 8% by age 40 and of 30% by age 65.^{36,37} Truncating mutations appear to be enriched in patients with PJS that ultimately develop breast cancer.³⁸

Heterozygous, germline mutations in *ATM* (Ataxia-telangiectasia-mutated gene) may be responsible for up to 2% of familial breast cancer.¹⁰ The risk conferred is conditional on the type of mutation and may range from 2 to more than 10 fold.^{9,10,39} Some evidence to date suggests missense mutations⁴⁰ as largely responsible for breast cancer predisposition, although they are less common in patients affected with ataxia telangiectasia, a disorder characterized by biallelic rather than monoallelic *ATM* mutations.⁴¹ Functional assays indicate that selected missense *ATM* mutations exert a dominant negative effect,⁴² interfering with normal ATM functions such as detection of double-stranded DNA breaks, regulation of cell-cycle checkpoints, and coordination of DNA repair.⁴³

Monoallelic *PALB2* (Partner and localizer of *BRCA2* gene) mutations are found in 0.6% to 2.9% of familial breast cancer cases.^{9,11,44,45} An average breast cancer risk associated with a *PALB2* germline mutation is estimated to be around 2.3 fold in families negative for *BRCA1/2* defects.⁴ As is the case with *ATM*, however, the risk may vary depending on the specific alteration: Presence of a highly penetrant mutation (c.3113G>A) in an Australian population, for example, carried a lifetime risk of 90% by age 70,⁴⁶ while the 1592delT mutation studied in a Finish sample conferred a risk increase of 4-fold.⁴⁷ A large majority of the *PALB2* mutations associated with breast cancer susceptibility are truncating and include small insertions, deletions, and nonsense mutations.^{40,44} Truncating mutations that abrogate the WD40-repeats functional domain are thought to disrupt the BRCA1–PALB2–BRCA2 complex and, consequently, the normal repair of double-stranded breaks.^{11,48} Other phenotypes caused by *PALB2* are Fanconi anemia N and prostate cancer. FANCN/*PALB2*-associated tumours are typically ER and HER2 negative.¹¹

Like the majority of proteins encoded by susceptibility genes for inherited breast cancer, the Cell-cycle-checkpoint kinase 2 (*CHEK2*) is involved in the repair of damaged DNA. *CHEK2* germline mutations may account for 3% to 5% of cases of inherited breast cancer,^{6,9} and increase the risk between 2 and 5 fold, with the attendant lifetime risk of 37%-59%, depending on the presence of family history and bilateral disease.^{49,50} While some of these mutations have lower penetrance, the expressivity can be severe.^{49,51} A frequently reported *CHEK2* mutation is the 1100delC mutation, associated with a lifetime risk of breast cancer of 15-20%,⁵² with differences in risk based on sex (risk of 2-3 fold in women and 10 fold in men) and age of onset (higher penetrance in early-onset breast cancer).⁵³⁻⁵⁵

Indications for testing

- Individuals with personal history of breast cancer, and/or family history of breast cancer, who are negative for *BRCA1* and *BRCA2* mutations

Methodology

Full sequencing-based mutation analysis of *ATM*, *CDH1*, *CHEK2*, *TP53*, *PALB2*, *PTEN*, and *STK11* genes: all coding exons and associated splice site junctions of these genes, as well as the promoter region of *PTEN*, are analyzed by direct automated fluorescent sequencing. All sequencing is performed in both the upstream and downstream directions. When a mutation or novel variant is detected, confirmation is carried out on an independent PCR amplification using a second blood prep (when available), and demonstrated in both the upstream and downstream direction.

Performance

Germline mutations in *TP53*, *CHEK2*, *PALB2*, and *ATM* may be responsible for 1%-7%, 3%-5%, 0.6%-2.9%, and 2% of selected subtypes of inherited breast cancer, respectively.^{8-10,20} The contribution of the remaining genes tested in this panel (*PTEN*, *CDH1*, and *STK11*) is thought to be very low.⁹ Sensitivity for heterozygous point mutations or small deletions/insertions within sequenced regions is approximately 99% with a specificity greater than 98%. Once a mutation is found, the sensitivity and specificity for carrier detection in families with identified mutations are estimated to be greater than 99%.

Limitations

The mutation analysis will not detect mutations located in regions of the genes that are not analyzed (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions). The method will also not detect gross genetic alterations, including most duplications, inversions, or deletions. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. As breast cancer is genetically heterogeneous, mutations in genes other than the ones tested in this panel are possible and will not be analyzed by this assay.

Interpretation of test results should be done in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes.

Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

Note: We accept DNA; at least 10 micrograms is required.

Test Request Form (TRF)

- a) A completed MDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
- b) [General Cancer Patient Information Form](#): Include a completed Cancer Patient Information Form for the proband and a complete pedigree (at least four generations).

Order Codes

CPT Codes

TAT

Order Codes	CPT Codes	TAT
Breast_Cancer-SEQ (<i>PTEN</i> , <i>STK11</i> , <i>CHEK2</i> , <i>TP53</i> , <i>PALB2</i> , <i>ATM</i> and <i>CDH1</i> , full gene sequencing)	83890, 83898(x127), 83894(7), 83904(x127), 83912(x7)	5 wks

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NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc.