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.\(^8-11\)

**Synopsis**

**ATM**: A tumor suppressor gene involved in cell cycle arrest and apoptosis. Mutations in *ATM* are associated with Li-Fraumeni syndrome, a familial cancer syndrome with a high risk of breast cancer.

**CDH1**: A member of the cadherin family involved in cell adhesion, differentiation, and signaling. Mutations in *CDH1* are associated with Cowden syndrome, a condition with an increased risk of breast cancer.

**CHEK2**: A checkpoint kinase involved in DNA damage response. Mutations in *CHEK2* are associated with an increased risk of breast cancer.

**TP53**: A tumor suppressor gene involved in transcriptional regulation, DNA repair, cell cycle arrest, and apoptosis. Mutations in *TP53* are associated with Li-Fraumeni syndrome and account for a small percentage of early-onset breast cancer.

**PALB2**: A protein involved in DNA damage repair. Mutations in *PALB2* are associated with an increased risk of breast cancer.

**PTEN**: A tumor suppressor gene involved in cell cycle arrest and apoptosis. Mutations in *PTEN* are associated with Cowden syndrome, a condition with an increased risk of breast cancer.

**STK11**: A protein involved in cell cycle regulation. Mutations in *STK11* are associated with Peutz-Jeghers syndrome, a condition with an increased risk of breast cancer.

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In contrast to TP53-associated breast cancer, PTEN germline mutations have been reported in both females and males with breast cancer. Serine/threonine protein kinase 11 (STK11) is involved in the suppression of cellular proliferation and apoptosis. 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. STK11 mutations also confer a risk of female breast cancer of 8% by age 40 and of 30% by age 65. Truncating mutations appear to be enriched in patients with PJS that ultimately develop breast cancer.

Heterozygous, germline mutations in ATM (Ataxia-telangiectasia-mutated gene) may be responsible for up to 2% of familial breast cancer. The risk conferred is conditional on the type of mutation and may range from 2 to more than 10 fold. Some evidence to date suggests missense mutations 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. Functional assays indicate that selected missense ATM mutations exert a dominant negative effect, interfering with normal ATM functions such as detection of double-stranded DNA breaks, regulation of cell-cycle checkpoints, and coordination of DNA repair.

Monoallelic PALB2 (Partner and localizer of BRCA2 gene) mutations are found in 0.6% to 2.9% of familial breast cancer cases. 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. 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, while the 1592delT mutation studied in a Finish sample conferred a risk increase of 4-fold. A large majority of the PALB2 mutations associated with breast cancer susceptibility are truncating and include small insertions, deletions, and nonsense mutations. 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. Other phenotypes caused by PALB2 are Fanconi anemia N and prostate cancer. FANCN/PALB2-associated tumours are typically ER and HER2 negative.

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, 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. While some of these mutations have lower penetrance, the expressivity can be severe. A frequently reported CHEK2 mutation is the 1100delC mutation, associated with a lifetime risk of breast cancer of 15-20%, 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).

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. The contribution of the remaining genes tested in this panel (PTEN, CDH1, and STK11) is thought to be very low. 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 CMDL 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

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<th>Test Request</th>
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<td>81321, 81405(x2), 81406(x2), 81408, 81479, G0452</td>
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References


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