

Assay Summary

CHEK2 (Checkpoint Kinase 2) Gene Mutation Analysis

Li-Fraumeni like syndrome, Breast cancer, Ovarian Cancer, Prostate cancer, Hereditary breast and colorectal cancer

Synopsis

CHEK2 test encodes a Serine/threonine-protein kinase which plays a critical role in DNA damage signaling pathways¹. CHEK2 directly phosphorylates and regulates the functions of p53 and BRCA1^{2,3}. Most women with breast and/or ovarian cancer are not carriers of mutant BRCA1 or BRCA2. Multiple studies have shown that a CHEK2*1100delC confers about a two-fold increased risk of breast cancer in unselected females and a 10-fold increase in males^{4,5}. Moreover, studies have shown that first-degree relatives of bilateral breast cancer cases who carried the CHEK2*1100delC allele had an eight-fold increased risk of breast cancer⁶. The cumulative risk by age 80 years was 58.8% (95% CI 33.8–85.3) for CHEK2*1100delC carriers with a bilaterally affected first-degree relative⁶. The CHEK2 mutations were identified in women with breast cancer and/or ovarian cancers^{4,13}. A CHEK2 mutation was also reported in 18.2% families with hereditary breast and colorectal cancer (HBCC)¹⁵ and was associated with a two-fold increased risk of colon cancer^{16,17}. It has been suggested that CHEK2 functions as a low-penetrance susceptibility gene for cancers and multiplies the risks associated with other gene(s) to increase cancer risk^{4,5,6,15}. Heterozygous germline mutations in CHEK2 have been identified in about 4% patients with prostate cancer^{7,8,9}. Germline mutations have also been detected in at least one family with classic Li-Fraumeni syndrome (LFS)^{10,11} and in other families with Li-Fraumeni-like syndrome¹² or with a subtype of LFS known as “phenotypically suggestive of LFS”^{10,11,12}.

Indications for testing

- Women with familial breast and ovarian cancer (4 or more cancer cases in the family) with no mutation detected in the BRCA1 and BRCA2 genes¹³
- Women with bilateral breast cancer with no mutation detected in the BRCA1 and BRCA2 genes⁶
- Men with breast cancer with no mutation detected in the BRCA2 and p53 genes⁴
- Hereditary breast and colorectal cancer (HBCC)¹⁵ * with no mutation detected in the BRCA1 and BRCA2 genes
- Individuals with either sporadic or familial prostate cancer⁷;
- If a mutation has been identified in an affected family member, with genetic counseling at-risk relatives may consider CHEK2 known mutation detection analysis.

* HBCC phenotype was defined as a family with breast cancer characterized by the presence of at least two patients with breast cancer who were first- or second-degree relatives and of whom at least one is diagnosed before age 60 years and

1. at least one patient with breast cancer and colorectal cancer diagnosed at any age; or
2. at least one individual with colorectal cancer diagnosed before age 50 years who was a first- or second degree relative of a patient with breast cancer; or
3. at least two patients with colorectal cancer diagnosed at any age of whom at least one was a first or second-degree relative of a patient with breast cancer.

Methodology

CHEK2-SEQ: All of the coding exons and associated intron junctions of the CHEK2 gene are amplified using CHEK2 specific primers, followed by direct DNA sequence analysis using an automated fluorescent sequencer. When a mutation is detected, confirmation is carried out by sequencing an independent PCR amplification in the opposite direction. If no mutation is found, sequence analysis is performed in both directions. Testing of at risk family members can be offered by DNA sequence analysis of only the region of the gene with the previously identified mutation.

CHEK2-MLPA large deletion analysis: We have incorporated the SALSA Multiplex Ligation-Dependent Probe Amplification (MLPA) kit which is a rapid, high-throughput technique for copy number quantification, specifically testing for large deletions for the CHEK2 gene. The P190 probemix contains probes for each of the 15 exons of the CHEK2 gene and the promoter. The deletion of exons 9-10 was identified as founder mutation among Czeck and Slovak population¹³.

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 also will not detect gross genetic alterations including most duplications, inversions or deletions (in females). Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

- (a) 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:

- i) for infants, a minimum of 3 ccs is sufficient.
 - ii) we accept DNA; at least 10 micrograms is required.
- (b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W. A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

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) [Cancer Patient Information Form](#): Include a completed Cancer Patient Information Form for the proband and a complete pedigree (at least four generations).

<i>Order Codes</i>	<i>CPT Codes</i>	<i>TAT</i>
CHEK2-SEQ (CHEK2 gene, full gene sequencing)	83890, 83898(x14), 83904(x14), 83894, 83912	6 wks
CHEK2-CAS (CHEK2 gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	3 wks
CHEK2-DEL (CHEK gene, MLPA analysis)	83890, 83896 (x17), 83909, 83912	3 wks
CHEK2-DEL-CAS (CHEK2 gene, MLPA analysis, known deletions/duplications)	83890, 83896 (x17), 83909, 83912	3 wks
CHEK2-DEL-PD (CHEK2 gene, MLPA analysis, prenatal)	83890, 83896 (x17), 83909, 83912	3 wks

References

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