

Assay Summary

ATM Gene Mutation Analysis Ataxia Telangiectasia Certain Leukemias and Lymphomas (T-PLL, B-CLL, Mantle Cell Lymphomas)

Synopsis

Germline mutations in the ATM gene cause the autosomal recessive neurological disease ataxia telangiectasia (A-T). The prevalence of A-T is ~1/40,000 – 1/100,000 affected individuals, with a carrier frequency of ~1/100. A-T is characterized by progressive cerebellar degeneration, immunodeficiency, radiation sensitivity, and a predisposition to cancer development. Heterozygous ATM mutations have also been found in some patients with T-cell prolymphocytic leukemia (T-PLL)¹, B-cell chronic lymphocytic leukemia (B-CLL)^{2,3} and mantle cell lymphoma⁴. Identification of both ATM gene mutations in A-T patients may permit identification of carriers in these families. Greater than 500 unique mutations have been identified in ATM. Identification of heterozygous ATM mutations may also identify individuals with an increased risk for certain cancers, such as leukemias, lymphomas, and breast.

Indications for testing

Patients with ataxia telangiectasia may consider, with genetic counseling, ATM gene mutation analysis. If a mutation is identified in the patient, other at-risk family members may be tested for carrier status. In patients with certain leukemias and lymphomas, identification of an ATM mutation may suggest an increased risk for these cancers in other family members who carry the mutation.

Methodology

ATM sequence analysis: All coding exons (exons 4-65) and associated intron junctions of the ATM gene are analyzed by direct DNA sequence analysis using an automated fluorescent sequencing machine. When a mutation is detected, confirmation is carried out on an independent amplification of PCR using a second prep (B-prep) by sequencing in the opposite direction. If no mutation is found, sequence analysis is performed in both directions. At-risk family members can be offered DNA sequence analysis of only the region of the gene with the previously identified mutation.

ATM MLPA analysis: ATM large deletions/duplications are found in 2% of A-T patients⁵⁻⁶. We have incorporated the SALSA MLPA (multiplex ligation-dependent probe amplification) kit that is a rapid, high-throughput technique for copy number quantification, specifically testing for large deletions/duplications for the ATM gene. This assay should be considered for patients where full gene sequencing did not detect a mutation or only one mutation identified in patients with A-T.

Performance

ATM sequence analysis: If a point mutation or small deletion/insertion is present within the regions of the ATM gene that are scanned, the sensitivity of mutation detection is approximately 99%. Alterations in the ATM gene are detected in about 90% of A-T cases⁷. However, multiple factors, including genetic heterogeneity, mutations outside of the regions of likely functional significance, or large genomic rearrangements implies that two mutations will not be detected in about 20% of A-T families. Thus, the sensitivity for the detection of two mutations in ataxia telangiectasia patients by direct DNA sequencing is approximately 80%. Once a mutation is found, the sensitivity and specificity for carrier detection for families with identified ATM gene mutations are both estimated to be greater than 99%.

ATM MLPA analysis: Large deletions/duplications in the ATM gene account for approximately 2% of alterations⁵⁻⁶. This assay tests all exons in ATM gene with 3 probes for exon 1. The MLPA method is designed to detect deletions/duplications of one or more exons of the ATM gene. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe need to be confirmed by a second method, whenever it's possible.

Limitations

ATM sequence and MLPA analysis: The mutation analysis will not detect mutations located in regions of the ATM gene 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 duplications, inversions, or deletions (other than those regions set-up for MLPA analysis). 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.

Note: Prenatal diagnosis is available once both parents have been established as carriers for a mutation in ATM.

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 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.

| Order Codes | CPT Codes | TAT |
|--|--|------------|
| ATM-SEQ (ATM gene, full gene full gene sequencing) | 83890, 83898(x58), 83904(x58), 83894, 83912 | 8 wks |
| ATM-CAS (ATM gene, targeted mutation analysis, known mutation) | 83890, 83898, 83904, 83894, 83912 | 3 wks |
| ATM-PD (ATM gene, known mutation detection, prenatal) | 83890, 83898, 83894, 83904, 83912 | 2 wks |
| ATM-DEL (ATM gene, MLPA analysis, all exons) | 83890, 83896(x66), 83909, 83912 | 3 wks |
| ATM-DEL-CAS (ATM gene, MLPA analysis, known deletions/duplications) | 83890, 83896(x66), 83909, 83912 | 3 wks |

References

1. Vorechovsky, I. et al. (1997). *Nature Genet.* 17: 96-99.
2. Bullrich, F. et al. (1999). *Cancer Res.* 59: 24-27.
3. Stankovic, T. et al. (1999). *The Lancet* 353: 26-29.
4. Stilgenbauer, S. et al. (1999). *Blood* 94: 3262-3264.
5. Cavalieri S. et al. (2006) *Hum Mutat.* 27: 1061
6. Cavalieri S. et al. (2008). *Ann Hum Genet.* 72: 10–18.
7. Bernstein J.L. et al (2003) *Hum Mutat* 21:542-50.

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