MAYO CLINIC LABORATORIES

Test Definition: TTRX

Familial Amyloidosis Reflex

Overview

Useful For

Diagnosis of adult individuals suspected of having transthyretin-associated familial amyloidosis

Genetics Test Information

Mass spectrometry to evaluate transthyretin (TTR) protein structure is performed first. In all cases demonstrating a structural change, the *TTR* gene will be further analyzed by DNA sequence analysis. If no alterations are detected, the reflex full gene analysis will not be performed unless a specific request for ATTRZ / *TTR* Gene, Full Gene Analysis is submitted by the ordering physician or client.

Testing Algorithm

If familial amyloidosis by liquid chromatography-mass spectrometry is abnormal, DNA sequence will be performed and charged separately.
See Amyloidosis (Familial) Test Algorithm in Special Instructions.

Special Instructions

- Amyloidosis (Familial) Test Algorithm

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
ATTRZ	TTR Gene, Full Gene Analysis	Yes	No

Method Name

Liquid Chromatography-Mass Spectrometry (LC-MS)

NY State Available

Yes

Specimen

Specimen Type

Whole blood

Shipping Instructions

Specimen must arrive within 96 hours of draw.

Specimen Required

Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: ACD
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.

2. Send specimen in original tube.

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-Hematopathology/Cytogenetics Test Request (T726)
-Biochemical Genetics Test Request (T798)

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK
Gross icterus	OK

Specimen Minimum Volume

0.5 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Refrigerated (preferred)	4 days	
	Ambient	4 days	

Clinical & Interpretive

Clinical Information

The amyloidoses are a group of diseases that result from the abnormal deposition of amyloid in various tissues of the body. They have been classified into 3 major types: primary, secondary, and hereditary. The most common form of amyloidosis (AL) is a disease of the bone marrow called primary systemic AL (immunoglobulin light chain). Secondary AL usually occurs in tandem with chronic infectious or inflammatory diseases, such as rheumatoid arthritis, tuberculosis, or osteomyelitis. Familial or hereditary AL is the least common form. Determining the specific type of AL is imperative in order to provide both an accurate prognosis and appropriate therapies.

Familial or hereditary transthyretin AL is an autosomal dominant disorder caused by mutations in the transthyretin gene (*TTR*). The resulting amino acid substitutions lead to a relatively unstable, amyloidogenic transthyretin (TTR) protein. Most individuals begin to exhibit clinical symptoms between the third and seventh decades of life. Affected individuals may present with a variety of symptoms including sensorimotor and autonomic neuropathy, vitreous opacities, cardiomyopathy, nephropathy, and gastrointestinal dysfunction. TTR-associated AL is progressive over a course of 5 to 15 years and usually ends in death from cardiac or renal failure or malnutrition. Orthotopic liver transplantation is a treatment option for some patients who are diagnosed in early stages of the disease.

Mayo Clinic Laboratories recommends a testing strategy that includes both protein analysis by mass spectrometry (MS) and *TTR* gene analysis by DNA sequencing for patients in whom TTR-associated familial AL is suspected. The structure of TTR protein in plasma is first determined by MS. The presence of a pathogenic variant in the *TTR* gene leads to conformational changes in the TTR protein. This ultimately disrupts the stability of the mature TTR protein tetramer, leading to increased amounts of pro-amyloidogenic TTR monomers in the plasma of affected individuals. MS is able to identify mass difference between wild type TTR and variant TTR protein. Only the transthyretin (also known as prealbumin) is analyzed for amino acid substitutions. Other proteins involved in other less common forms of familial amyloidosis are not examined. If no alterations are detected, gene analysis will not be performed unless requested by

the provider (ie, when the diagnosis is still strongly suspected; to rule out the possibility of a false-negative by MS). In all cases demonstrating a structural change by MS, the entire *TTR* gene will be analyzed by DNA sequence analysis to identify and characterize the observed alteration (gene mutation or benign polymorphism). More than 90 mutations that cause TTR-associated familial AL have now been identified within the *TTR* gene. Most of the mutations described to date are single base pair changes that result in an amino acid substitution. Some of these mutations correlate with the clinical presentation of AL.

For predictive testing in cases where a familial mutation is known, testing for the specific mutation by DNA sequence analysis (FMTT / Familial Mutation, Targeted Testing) is recommended. These assays do not detect mutations associated with non-*TTR* forms of familial AL. Therefore, it is important to first test an affected family member to determine if *TTR* is involved and to document a specific mutation in the family before testing at risk individuals.

Reference Values

An interpretive report will be provided.

Interpretation

The presence of a structural change in transthyretin (TTR) is suggestive of a gene mutation that requires confirmation by DNA sequence analysis. A negative result by mass spectrometry does not rule out a *TTR* mutation. Mass spectrometric (MS) results are falsely negative if the amino acid substitution does not produce a measurable mass shift for the transthyretin mutation. Approximately 90% of the *TTR* mutations are positive by MS (see Cautions).

After identification of the mutation at the DNA level, predictive testing for at-risk family members can be performed by molecular analysis (FMTT / Familial Mutation, Targeted Testing).

Cautions

There are 3 circumstances where testing by mass spectrometry will not identify amyloid-causing mutations:

-If the amino acid change results in a protein different by less than 10 atomic mass units (amu), the mutation will not be reliably detected.

-If an amino acid change results from a frequent nondisease-causing mutation (+30 amu). Since over 12% of the population has this innocuous polymorphism, it is an instance in which molecular testing must be done.

-Coinheritance of the polymorphism with a -30 amu mutation would result in a transthyretin mass indistinguishable from normal.

Clinical Reference

1. Shimizu A, Nakanishi T, Kishikawa M, et al: Detection and identification of protein variants and adducts in blood and tissues: an application of soft ionization mass spectrometry to clinical diagnosis. J Chromatogr B Analyt Technol Biomed Life Sci 2002 Aug 25;776(1):15-30

2. Lim A, Prokaeva T, McComb ME, et al: Characterization of transthyretin variants in familial transthyretin amyloidosis by mass spectrometric peptide mapping and DNA sequence analysis. Anal Chem 2002 Feb 15;74(4):741-751

3. Sekjima Y, Yoshida K, Tokuda T, Ikeda S: Familial Transthyretin Amyloidosis In GeneReviews. Edited by RA Pagon, MP Adam, HH Ardinger, et al: Retrieved February 24, 2019. Available at www.ncbi.nlm.nih.gov/books/NBK1194/

4. Theberge R, Connors LH, Skinner M, Costello CE: Detection of transthyretin variants using immunoprecipitation and matrixassisted laser desorption/ionization bioreactive probes: a clinical application of mass spectrometry. J Am Soc Mass Spectrom. 2000,11:172-175

5. Finsterer J, Iglseder S, Wanschitz J, et al: Hereditary transthyretin-related amyloidosis. Acta Neurol Scand 2019 Feb;139(2):92-105

Performance

Method Description

Familial Amyloidosis, Mass Spectrometry (MS):
Transthyretin (TTR) is purified from plasma using affinity chromatography. The chromatography is done using an antihuman-TTR antibody that has been coupled to POROS-aldehyde media. Plasma is reduced to simplify the mass spectra by removing Cys10 adducted species. The solution is then injected onto the affinity column, which sequesters TTR. TTR is then eluted from the affinity column and concentrated on a C4 column, which is then washed to remove excess components that suppress MS response. TTR is then eluted from the C4 column and introduced to the MS. The acquired ion spectra are deconvoluted and reviewed for TTR variants. After deconvolution, normal patients present with a single peak corresponding to wild-type (wt) TTR, which serves as a reference. When positive, amyloid patients are typically heterozygous and are detected by the presence of 2 peaks (ie, wt TTR and mutant *TTR*) differing in mass.(Bergen HR 3rd, Zeldenrust SR, Butz ML, et al: Identification of transthyretin variants by sequential proteomic and genomic analysis. Clin Chem 2004 Sep;50[9]:1544-1552)
TTR Gene, Full Gene Analysis:
All 4 exons of the *TTR* gene are amplified by PCR and then subjected to direct DNA sequence analysis.(Bergen RH 3rd, Zeldenrust SR, Butz ML, et al: Identification of transthyretin variants by sequential proteomic and genomic analysis. Clin Chem 2004;50:1544-1552)

PDF Report

No

Specimen Retention Time

2 months

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

82542 LC-MS
81404 TTR gene (if appropriate)