

Ordered By	Contact ID:1212036 Org ID:10197	Patient Name: Test, TTR Analysis 4.2.2.3
Physician: Unknown, Unknown, MD		Accession #: 00-081817 Specimen #:
Ph:N/A Fx:N/A		AP2 Order #: 556106 Specimen: Blood EDTA (Purple top)
Client: Unknown (12966)		Birthdate: 09/08/9999 Age: y m
CA US		Gender: U
		MRN #: N/A Collected: N/A
		Indication: Internal Testing Received: 05/03/2018
		Ethnicity: N/A

Transthyretin Amyloidosis: Gene Sequence & Deletion/Duplication Analyses of TTR

RESULTS

TTR **Pathogenic Mutation: p.S70R**

SUMMARY

POSITIVE: Pathogenic Mutation Detected

INTERPRETATION

- This individual is heterozygous for the **p.S70R** pathogenic mutation in the *TTR* gene.
- This result is consistent with a diagnosis of transthyretin (TTR) amyloidosis.
- The expression and severity of disease for this individual cannot be predicted.
- Genetic testing for pathogenic mutations in family members can be helpful in identifying at-risk individuals.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected in the *TTR* gene.

TTR Additional Information

The **p.S70R** pathogenic mutation (also known as c.210T>A and S50R), located in coding exon 3 of the *TTR* gene, results from a T to A substitution at nucleotide position 210. The serine at codon 70 is replaced by arginine, an amino acid with dissimilar properties. This mutation has been described in numerous unrelated families of Japanese, Caucasian, and Mexican ancestry with familial amyloid polyneuropathy (Ueno S et al. *Biochem. Biophys. Res. Commun.* 1990 Jun;169(3):1117-21; Munar-Qués M et al. *Amyloid* 2007 Jun;14(2):147-52; González-Duarte A et al. *Amyloid* 2013 Dec;20(4):221-5). Of note, the nucleotide changes observed in these studies, which all result in the same p.S70R amino acid substitution, include c.210T>G, c.208A>C, and c.210T>A, respectively. In one Italian study, three of four individuals from two unrelated families with this mutation were observed to have a mixed neurologic and cardiac phenotype, while one individual had a primarily neurologic phenotype (Rapezzi C et al. *Eur. Heart J.* 2013 Feb; 34(7):520-8). Based on the supporting evidence, this alteration is interpreted as a disease-causing mutation.

The transthyretin (*TTR*) gene (NM_000371.3) encodes the transthyretin protein. This protein transports thyroid hormones in the plasma and cerebrospinal fluid, and vitamin A in the plasma. *TTR* is located on chromosome 18q12.1 and contains 4 coding exons. Alterations in this gene are typically inherited in an autosomal dominant fashion in association with TTR amyloidosis. TTR amyloidosis includes three main phenotypes, familial amyloid neuropathy, familial amyloid cardiomyopathy, and familial leptomeningeal amyloidosis, and is characterized by slowly progressive peripheral sensorimotor neuropathy and autonomic neuropathy as well as non-neuropathic changes of cardiomyopathy, nephropathy, vitreous opacities, and central nervous system amyloidosis (Sekijama Y. *J. Neurol. Neurosurg. Psychiatry.* 2015;86:1036-43).

Order Summary: The following products were included in the test order for this individual. Please note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- TTR Analysis (Product Code 1560)

ASSAY INFORMATION

General Information: Transthyretin (TTR) amyloidosis is a hereditary, multisystem disease caused by abnormal formation and extracellular deposit of TTR protein fibrils in various tissues. Inheritance is autosomal dominant, but family history may appear negative as approximately two-thirds of cases arise from new mutations and incomplete penetrance has been described. Additionally, age of onset is typically in the thirties but can vary by decades. Genotypes do not typically predict the onset, severity, or specific symptoms; however, some pathogenic variants tend to associate with one of the three main phenotypes of TTR amyloidosis. TTR amyloid neuropathy is characterized by carpal tunnel syndrome, sensorimotor polyneuropathy of the legs, autonomic dysfunction, constipation, diarrhea, cardiomyopathy, vitreous opacities, and nephropathy. Individuals with TTR cardiac amyloidosis may have cardiomegaly, conduction block, arrhythmia, congestive heart failure and sudden death, but neuropathy is absent or less evident. TTR leptomeningeal/CNS amyloidosis is characterized by dementia, ataxia, spasticity, seizures, intracranial hemorrhage, psychosis, and hydrocephalus. TTR amyloidosis is caused by pathogenic variants in the *TTR* gene, which encodes a protein that transports thyroid hormones in the plasma and cerebrospinal fluid, as well as vitamin A in the plasma.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using standardized methodology and quantified. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes, and is followed by polymerase chain reaction (PCR) and Next-Generation sequencing. Additional Sanger sequencing is performed for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Reportable small insertions and deletions, potentially homozygous variants, variants in regions complicated by pseudogene interference, and single nucleotide variant calls not satisfying 100x depth of coverage and 40% het ratio thresholds are verified by Sanger sequencing (Mu W et al. *J Mol Diagn.* 2016 Oct 4. PubMed PMID: 27720647). Gross deletion/duplication analysis is performed using a custom pipeline based on read-depth from NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable. Sequence analysis is based on the following NCBI reference sequence: *TTR* NM_000371.3.

Analytical Range: This test targets detection of DNA sequence mutations in the *TTR* gene by either Next-Generation or Sanger sequencing of all coding domains and well into the 5' and 3' ends of all the introns and untranslated regions. Gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of *TTR*.

Result Reports: In result reports, alterations in the following classifications are always reported, and are based on the following definitions and clinical recommendations:

- **Pathogenic Mutation:** alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk relatives and appropriate changes in medical management for pathogenic mutation carriers recommended. Previously described pathogenic mutations, including intronic mutations at any position, are always reported when detected.
- **Variant, Likely Pathogenic (VLP):** alterations with strong evidence in favor of pathogenicity. Targeted testing of at-risk relatives and appropriate changes in medical management for VLP carriers typically recommended. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
- **Variant, Unknown Significance (VUS):** alterations with limited and/or conflicting evidence regarding pathogenicity. Familial testing via the Family Studies Program recommended. Medical management to be based on personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 base pairs from the splice junction when detected.

Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not routinely included on results reports. These include findings classified as "likely benign" and "benign" alterations.

Assay Information Continued on Next Page

ASSAY INFORMATION (Supplement to Test Results - Continued)

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med*. 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. <http://www.ambrygen.com/variant-classification>.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol*. 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet*. 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research*. 2003;31(13):3568-3571. <http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home>.
7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.
8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science*. 1974;185(4151):862-864.
9. HGMD® [Internet]; Stenson PD et al. *Genome Med*. 2009;1(1):13. www.hgmd.cf.ac.uk.
10. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: <http://omim.org>.
12. PolyPhen [Internet]; Adzhubei IA, et al. *Nat Methods*. 2010;7(4):248-249. genetics.bwh.harvard.edu/pph2.
13. SIFT [Internet]; Ng PC & Henikoff S. *Hum Genet*. 2006;7:61-80. <http://sift.jcvi.org>.
14. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: <http://exac.broadinstitute.org>.
15. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: <http://gnomad.broadinstitute.org>.
16. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533

Disclaimer: This test was developed and its performance characteristics were determined by Ambry Genetics Corporation. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. The **Transthyretin Amyloidosis** test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels, and gross deletions/duplications. Other than alterations noted in the methodology section above, these assays are not intended to analyze the following types of mutations: gross rearrangements, deep intronic variations, Alu element insertions, and other unknown abnormalities. The pattern of mutation types varies with the gene tested and this test detects a high but variable percentage of known and unknown mutants of the classes stated. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable group. The **Transthyretin Amyloidosis** test is designed and validated to be capable of detecting >99% of described mutations in the gene represented on the test (analytical sensitivity). The clinical sensitivity of the **Transthyretin Amyloidosis** test may vary widely according to the specific clinical and family history. Transthyretin amyloidosis is a complex clinical disorder. Mutations in other genes or the regions not analyzed by the **Transthyretin Amyloidosis** test can also give rise to similar clinical conditions. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, low-level mosaicism, presence of pre-malignant or malignant cells in the sample, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data.