

<b>Ordered By</b> Physician: Unknown, Unknown, MD Ph:N/A Fx:N/A Client: Ambry CA US <b>Additional Authorized Recipient:</b> Thai, Julia Ph:(949) 900-5500 Fx:(949) 900-5501	Contact ID:1524667 Org ID:1	<b>Patient Name: 9570_Test, ABC</b> Accession #: <b>00-125751</b> AP2 Order #: 705073 Birthdate: 01/01/1976 Gender: M MRN #: N/A Indication: Internal Testing Ethnicity: Caucasian	Specimen #: Specimen: Blood EDTA (Purple top) Age: 43y 6m Collected: N/A Received: 07/15/2019
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**NeuropathySelect: Analyses of 81 Genes Associated with Hereditary Neuropathy**

**RESULTS**

**TTR Pathogenic Mutation: p.V50M**

**SUMMARY**

**POSITIVE: Pathogenic Mutation Detected**

**INTERPRETATION**

- This individual is heterozygous for the **p.V50M** 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. Genes Analyzed (81 total): *ATL1, DNAJB2, DST, FAM134B, FGD4, FIG4, FUS, GAN, GDAP1, GJB1, HINT1, HSPB1, IGHMBP2, IKBKAP, KIF1A, LMNA, LRSAM1, MFN2, MPZ, MTMR2, NDRG1, NEFL, NGF, NTRK1, OPTN, PLEKHG5, PMP22, PRDM12, PRX, REEP1, SBF2, SCN9A, SH3TC2, SIGMAR1, SLC25A46, SLC52A2, SLC52A3, SPG11, TTR, VRK1* and *WNK1* (sequencing and deletion/duplication); *AARS, AIFM1, APOA1, ATL3, ATP7A, BICD2, BSCL2, CHCHD10, DCTN1, DNMT2, DNMT1, DYNC1H1, EGR2, FBXO38, GARS, GNB4, GSN, HARS, HSPB8, INF2, LITAF, MARS, MORC2, NEFH, PDK3, PRPS1, RAB7A, SCN10A, SCN11A, SETX, SLC5A7, SPTLC1, SPTLC2, TARDBP, TFG, TRPV4, UBA1, VAPB, VCP* and *YARS* (sequencing only).

**TTR Additional Information**

The **p.V50M** pathogenic mutation (also known as c.148G>A and V30M), located in coding exon 2 of the *TTR* gene, results from a G to A substitution at nucleotide position 148. The valine at codon 50 is replaced by methionine, an amino acid with highly similar properties. This mutation was first reported in TTR-related amyloid protein of tissue samples from Portuguese individuals with familial amyloidotic polyneuropathy (Saraiva MJ et al. *J. Clin. Invest.*, 1984 Jul;74:104-19). This is the most common *TTR* mutation and is associated with the polyneuropathy type of familial TTR amyloidosis; however, age of onset and severity is considered variable (Soares ML et al. *Eur. J. Hum. Genet.*, 2004 Mar;12:225-377). Based on the supporting evidence, this alteration is interpreted as a disease-causing mutation.

The transthyretin (*TTR*) gene (NM\_000371.3) encodes TTR, a serum and cerebrospinal fluid prealbumin protein that transports thyroxine (T4) and holo-retinol-binding protein (OMIM\_176300). *TTR* is located on chromosome 18q12.1 and contains 4 exons. Alterations in this gene have been associated with transthyretin (*TTR*) amyloidosis, which is inherited in an autosomal dominant fashion. TTR-related amyloidosis is a multisystem disease caused by abnormal formation and extracellular deposit of TTR protein fibrils in various tissues including the nerves, heart, brain, eyes, intestines, kidneys, and skin (Finsterer J et al. *Acta. Neurol. Scand.*, 2019;139(2):92-105). Many mutations tend to associate with one of three broad phenotypes of TTR amyloidosis: polyneuropathy (ATTRm-related polyneuropathy), cardiac dominant amyloidosis (TTR-CA), and leptomeningeal amyloidosis. The most common form is ATTRm-related polyneuropathy, a length dependent axonal polyneuropathy with possible involvement of the sensory, motor, and autonomic nerves. Inheritance is autosomal dominant, but family history may appear to be negative in the majority of patients due to incomplete penetrance and the variable age of onset which may differ by decades within and between

populations (Banyersad SM et al. *J. Am. Heart Assoc.*, 2012;1(2):e000364).

**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.

- NeuropathySelect (Product Code 9570)

**ELECTRONICALLY SIGNED BY**

**Negar Ghahramani, PhD, MS, DABMGG, CGMBS, on 07/24/2019 at 07:44:32 am**

**ASSAY INFORMATION**

**General Information:** Peripheral neuropathies (PNP), also known as polyneuropathy disorders affecting a variety of peripheral nerve cells and fibers (motor, sensory, and autonomic), are a relatively common diverse group of diseases with an estimated prevalence of 5-8%. Clinically this group of disorders presents with distal symmetric sensorimotor neuropathy most frequently. Heterogeneous clinical symptoms are observed depending upon involvement of sensory, motor, or autonomic nerve fiber impairment. Symptoms of PNP may include hypalgesia, heat and cold allodynia, dysesthesia, sensory ataxia, paresis, muscle atrophy, hypotonia, hypohidrosis and/or anhidrosis, bladder dysfunction, indigestion, cardiac arrhythmias and tachycardia, gastroparesis, urogenital dysfunction, and periodic pain. Hereditary forms of peripheral neuropathy include but are not limited to hereditary motor and sensory neuropathy (HMSN), often referred to as Charcot-Marie-Tooth disease (CMT), hereditary motor neuropathies (HMN), and small fiber neuropathies (SFN) (Hanewinkel R et al. *Handb Clin Neurol*, 2016;138:263-82; Sommer C et al. *Dtsch Arztebl Int*, 2018 Feb;115:83-90). Specific therapies for PNP are based on the precise etiology diagnosis and it is often hard to distinguish inherited PNP from sporadic or acquired forms of neuropathy without genetic testing. Over 100 genes have been identified as associated with hereditary peripheral neuropathies with autosomal dominant, autosomal recessive, X-linked, and mitochondrial inheritance patterns observed. Given the overlap in genetic causes and variability in clinical symptoms and presentation, one comprehensive inherited neuropathy test may be the most effective way of identifying at-risk individuals, or confirming a diagnosis (England JD et al. *Neurology*, 2005 Jan;64:199-207; Eggermann K et al. *Dtsch Arztebl Int*, 2018 Feb;115:91-97; Mary P et al. *Orthop Traumatol Surg Res*, 2018 Feb;104:S89-S95).

**Methodology:** Ambry's NeuropathySelect™ is a comprehensive screen of 81 genes associated with peripheral neuropathies. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's whole blood. Samples are prepared using the IDT xGen Exome Research Panel V1.0 (Integrated DNA Technologies). Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing. Initial data processing, base calling, alignments and variant calls are generated by a custom bioinformatics pipeline. Additional Sanger sequencing is performed for regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Reportable small insertions and deletions, potentially homozygous variants, and single nucleotide variant calls not satisfying established confidence thresholds based on coverage and alternative read ratios are verified by Sanger sequencing. Gross deletion/duplication analysis is assessed for all genes using a custom pipeline based on coverage and/or breakpoint analysis from NGS data and is followed by a confirmatory orthogonal method, as needed. The pipeline detects deletions and duplications >5 exons in size in sequences with sufficient resolution. Deletions or duplications suspected in the *PMP22* gene are detected by multiplex ligation-dependent probe amplification (MLPA) using SALSA® MLPA® Probemix P033-B4 CMT1. In most cases, phase of reported variants cannot be determined.

Sequence analysis is limited to the following 81 genes and associated NCBI reference sequences: *AARS* (NM\_001605.2), *AIFM1* (NM\_004208.3), *APOA1* (NM\_000039.1), *ATL1* (NM\_015915.4), *ATL3* (NM\_015459.3), *ATP7A* (NM\_000052.4), *BICD2* (NM\_001003800.1), *BSCL2* (NM\_032667.6), *CHCHD10* (NM\_213720.1), *DCTN1* (NM\_004082.4), *DNAJB2* (NM\_001039550.1), *DNM2* (NM\_001005360.2), *DNMT1* (NM\_001379.2), *DST* (NM\_001144769.2), *DYNC1H1* (NM\_001376.4), *EGR2* (NM\_000399.3), *FAM134B* (NM\_001034850.1), *FBXO38* (NM\_205836.1), *FGD4* (NM\_139241.2), *FIG4* (NM\_014845.5), *FUS* (NM\_004960.3), *GAN* (NM\_022041.3), *GARS* (NM\_002047.2), *GDAP1* (NM\_018972.2), *GJB1* (NM\_000166.5), *GNB4* (NM\_021629.3), *GSN* (NM\_000177.4), *HARS* (NM\_002109.3), *HINT1* (NM\_005340.5), *HSPB1* (NM\_001540.3), *HSPB8* (NM\_014365.2), *IGHMBP2* (NM\_002180.2), *IKBKAP* (NM\_003640.3), *INF2* (NM\_022489.3), *KIF1A* (NM\_001244008.1), *LITAF* (NM\_004862.3), *LMNA* (NM\_170707.2), *LRSAM1* (NM\_138361.4), *MARS* (NM\_004990.3), *MFN2* (NM\_014874.3), *MORC2* (NM\_001303256.2), *MPZ* (NM\_000530.6), *MTMR2* (NM\_016156.5), *NDRG1* (NM\_006096.3), *NEFH* (NM\_021076.3), *NEFL* (NM\_006158.3), *NGF* (NM\_002506.2), *NTRK1* (NM\_001012331.1), *OPTN* (NM\_021980.4), *PDK3* (NM\_001142386.2:c.473G>A[p.R158H]), *PLEKHG5* (NM\_020631.3), *PMP22* (NM\_000304.2), *PRDM12* (NM\_021619.2), *PRPS1* (NM\_002764.3), *PRX* (NM\_181882.2), *RAB7A* (NM\_004637.5), *REEP1* (NM\_022912.2), *SBF2* (NM\_030962.3), *SCN10A* (NM\_006514.2), *SCN11A* (NM\_014139.2), *SCN9A* (NM\_002977.3), *SETX* (NM\_015046.5), *SH3TC2* (NM\_024577.3), *SIGMAR1* (NM\_005866.2), *SLC25A46* (NM\_138773.1), *SLC52A2* (NM\_024531.3), *SLC52A3* (NM\_033409.3), *SLC5A7* (NM\_021815.2), *SPG11* (NM\_025137.3), *SPTLC1* (NM\_006415.2), *SPTLC2* (NM\_004863.3), *TARDBP* (NM\_007375.3), *TFG* (NM\_006070.5), *TRPV4* (NM\_021625.4), *TTR* (NM\_000371.3), *UBA1* (NM\_003334.3), *VAPB* (NM\_004738.4), *VCP* (NM\_007126.3), *VRK1* (NM\_003384.2), *WNK1* (NM\_213655.4), and *YARS* (NM\_003680.3).

**Analytical Range:** The NeuropathySelect™ test targets detection of DNA sequence variants in 81 genes by either Next-Generation or Sanger sequencing of all coding domains and plus at least 6 bases into the 5' and 3' ends of all the introns. Gross deletion/duplication analysis determines gene copy number for the exons of 41 genes (specified on 1st page of report) in which copy number variations are clinically relevant.

**Result Reports:** In result reports, sequencing alterations classified as pathogenic mutations, VLPs, or VUS are always reported. Gross deletions and duplications classified as pathogenic mutations or VLPs are reported when confirmed. Classifications 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 may be recommended. Medical management to be based on personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 6 basepairs from the splice junction when detected.

Sequence alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) and gross deletions and duplications classified as VUS and of unlikely clinical significance 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.

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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](http://www.ncbi.nlm.nih.gov/SNP). Accessed Jan 2012).
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14. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: <http://gnomad.broadinstitute.org>.
15. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533

**Test Limitations and Disclaimer:** This test was developed and its performance characteristics were determined by Ambry Genetics. 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 referred to a genetic counselor, medical geneticist, or physician skilled in evaluating 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 following types of mutations are detectable: nucleotide substitutions, deletions, insertions and small indels. Multi-exon deletions smaller than 5 exons will not be identified with this analysis. This test is not intended to systematically analyze the following types of mutations: deep intronic variations, long repeat sequences, portions of genes with highly homologous pseudogenes, trinucleotide repeat sequences, mutations involved in tri-allelic inheritance, mitochondrial genome mutations, epigenetic effects, oligogenic inheritance, and X-linked recessive mutations in females who manifest disease due to skewed X-inactivation and other unknown abnormalities. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the regions outside of the reportable range. This test is designed and validated to be capable of detecting >99% of described abnormalities in the genes and chromosome regions represented on the test (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical and family history. 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 rare genetic variants that may interfere with analysis, or from other sources.