Ambry Genetics

SAMPLE REPORT

Ordered By	Contact ID:281059	Org ID:395	
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Client:	Ambry Genetics (00618) 15 Argonaut Aliso Viejo CA 92656 US		
Additional Authorized Recipient:			
Lindwall, Chelsea QA Ph:949-900-5500 Fx:949-900-5501			

Patient Name:	**15-TAAD-02, N/A**		
Accession #:	**15-277351**	Specimen #:	N/A
AP2 Order #:	145945	Specimen:	DNA
Birthdate:	01/01/2001	Age:	15y 3m
Gender:	U		
MRN #:	N/A	Collected:	09/16/2015
Family #:	254621	Received:	09/22/2015
Indication:	Proficiency Testing	Authorized:	09/22/2015
Ethnicity:	UNKNOWN		

TAADNext: Analyses of 22 Genes Associated with Thoracic Aortic Aneurysms and Dissections

RESULTS

Pathogenic Mutation(s): None Detected
Variant(s) of Unknown Significance: None Detected
Gross Deletion(s)/Duplication(s): None Detected

SUMMARY

NEGATIVE: No Clinically Significant Variants Detected

INTERPRETATION

- No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected.
- **Risk Estimate:** low likelihood of variants in the genes analyzed contributing to this individual's clinical history.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

Genes Analyzed: ***ACTA2, COL3A1, COL5A2, FBN1, FBN2, MED12, MYH11, MYLK, NOTCH1, PLOD1, PRKG1, SKI, SLC2A10, SMAD3, TGFB2, TGFBR1*** **and** ***TGFBR2*** **(sequencing and deletion/duplication);** ***CBS, COL5A1, FLNA, SMAD4*** **and** ***TGFB3*** **(sequencing only).**

COMMENT: You may wish to contact Dr. Dianna M. Milewicz and her research staff of the University of Texas Health Science Center at Houston, regarding a study of the genes that, when altered, lead to aortic aneurysms and dissections and related vascular diseases. Participation in research is optional. You or your patient may contact the study coordinator's office at (713) 500-7072 or email at info@JohnRitterResearchProgram.org.

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.

- TAADNext (Product Code 8789)

ASSAY INFORMATION

General Information: Marfan syndrome (MFS) is an autosomal dominant disorder characterized by cardiovascular, skeletal, and ocular findings. Diagnosis can be challenging due to phenotypic variability and overlap with related disorders. One of the major features of MFS is an increased risk for aortic aneurysms and dissections, which, if untreated, can be fatal. Approximately 13,000 Americans die each year from aortic aneurysms. Identifying at-risk individuals is complicated by the fact that sudden death is often the first major clinical sign. Some conditions overlap clinically with MFS, but do not include the same level of risk for aneurysms. These include Shprintzen-Goldberg syndrome, homocystinuria, and congenital contractural arachnodactyly. MFS is the most common form of syndromic thoracic aortic aneurysms and dissections (TAAD). Other syndromic TAAD conditions include Loeys-Dietz syndrome, Ehlers-Danlos syndrome (EDS), arterial tortuosity syndrome, and Lujan-Fryns syndrome. Familial non-syndromic TAAD is characterized by aneurysms without other manifestations and typically follows an autosomal dominant pattern of inheritance. Up to 20% of individuals with TAAD have a first-degree relative with thoracic aortic disease. Several genes have been associated with familial non-syndromic TAAD or familial aortic valve abnormalities, which lead to an increased risk for TAAD. Sporadic forms of TAAD have also been reported. Early diagnosis of MFS, familial TAAD, and other related syndromes is essential for improved prognosis, management, and genetic counseling. Identifying the specific genetic cause will help stratify risks, direct management options, and dramatically improve outcome.

Methodology: TAADNext is a comprehensive analysis of 22 genes associated with TAAD. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a standardized kit 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. Suspect variant calls other than "likely benign" or "benign" are verified by Sanger sequencing. Gross deletion/duplication analysis for 17 genes (excluding *CBS, COL5A1, FLNA, SMAD4,* and *TGFB3*) is also performed utilizing a targeted chromosomal microarray. Sequence analysis is based on the following NCBI reference sequences: *ACTA2* NM_001613.2, *CBS* NM_000071.2, *COL3A1* NM_000090.3, *COL5A1* NM_000093.4, *COL5A2* NM_ 000393.3, *FBN1* NM_000138.4, *FBN2* NM_001999.3, *FLNA* NM_001456.3, *MED12* NM_005120.2, *MYH11* NM_002474.2, *MYLK* NM_053025.3, *NOTCH1* NM_017617.3, *PLOD1* NM_000302.3, *PRKG1* NM_006258.3, *SKI* NM_003036.3, *SLC2A10* NM_030777.3, *SMAD3* NM_005902.3, *SMAD4* NM_005359.5, *TGFB2* NM_003238.3, *TGFB3* NM_003239.2, *TGFBR1* NM_004612.2, *TGFBR2* NM_003242.5.

Analytical Range: TAADNext targets detection of DNA sequence mutations in 22 genes (*ACTA2, CBS, COL3A1, COL5A1, COL5A2, FBN1, FBN2, FLNA, MED12, MYH11, MYLK, NOTCH1, PLOD1, PRKG1, SKI, SLC2A10, SMAD3, SMAD4, TGFB2, TGFB3, TGFBR1,* and *TGFBR2*) by either Next-Generation or Sanger sequencing of all coding domains and well into the flanking 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 17 genes (excluding *CBS, COL5A1, FLNA, SMAD4,* and *TGFB3*). If *FBN1* or *COL3A1* gene sequence and deletion/duplication analysis is requested, then only the specific gene is analyzed.

Expected (Normal) Value: Diagnostic or Family History: 0, 1, or more mutation(s) detected.

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 carries 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 basepairs 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, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, Maglott DR. 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.

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 *COL3A1, FBN1,* and TAADNext tests analyze 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. These tests 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 *COL3A1, FBN1,* and TAADNext tests are designed and validated to be capable of detecting >99% of described mutations in the genes represented on the tests (analytical sensitivity). The clinical sensitivity of the *COL3A1, FBN1,* and TAADNext tests may vary widely according to the specific clinical and family history. Syndromic thoracic aortic aneurysms and dissections are complex clinical disorders. Mutations in other genes or the regions not analyzed by the *COL3A1, FBN1,* and TAADNext tests 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.