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SAMPLE REPORT

Ordered By	Contact ID:1332827	Org ID:8141	Patient Legal Name:	**8789, Test12**	
Medical Professional:	Rhodarmer, Jake, MA		Accession #:	**00-135408**	Specimen #:
Client:	MOCKORG44 (10829)		AP2 Order #:	614279	Specimen: Blood EDTA (Purple top)
			Birthdate:	09/08/9999	Sex assigned at birth: U
			MRN #:	N/A	Collected: N/A
			Indication:	Internal Testing	Received: 11/20/2018
					Test Started: 11/20/2018

TAADNext®: Analyses of 35 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 (35 total): ***ACTA2, BGN, CHST14, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, EFEMP2, FBN1, FBN2, FKBP14, FLNA, FOXE3, LOX, MAT2A, MED12, MFAP5, MYH11, MYLK, NOTCH1, PLOD1, PRDM5, PRKG1, SKI, SLC2A10, SMAD3, SMAD4, TGFB2, TGFB3, TGFBR1, TGFBR2, TNXB*** **and** ***ZNF469*** **(sequencing and deletion/duplication);** ***CBS*** **(sequencing only)**.

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 35 genes associated with TAAD and related disorders. 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 (excluding *TNXB* exons 32-44) 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. Variants in regions complicated by pseudogene interference, variant calls not satisfying depth of coverage and variant allele frequency quality thresholds, and potentially homozygous variants are verified by Sanger sequencing. Gross deletion/duplication analysis is performed for all genes (excluding *CBS* and *TNXB* exons 32-44) using a custom pipeline based on read-depth from NGS data followed by a confirmatory orthogonal method, as needed. Exon-level resolution may not be achieved for every gene. Sequence analysis is based on the following NCBI reference sequences: *ACTA2* NM_001613.2, *BGN* NM_001711.4, *CBS* NM_000071.2, *CHST14* NM_130468.3, *COL1A1* NM_000088.3, *COL1A2* NM_000089.3, *COL3A1* NM_000090.3, *COL5A1* NM_000093.4, *COL5A2* NM_ 000393.3, *EFEMP2* NM_016938.4, *FBN1* NM_000138.4, *FBN2* NM_001999.3, *FKBP14* NM_017946.2, *FLNA* NM_001456.3, *FOXE3* NM_012186.2, *LOX* NM_002317.5, *MAT2A* NM_005911.5, *MED12* NM_005120.2, *MFAP5* NM_003480.2, *MYH11* NM_002474.2, *MYLK* NM_053025.3, *NOTCH1* NM_017617.3, *PLOD1* NM_000302.3, *PRDM5* NM_018699.2, *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, *TNXB* NM_019105.6, *ZNF469* NM_001127464.1.

Analytical Range: TAADNext® targets detection of DNA sequence mutations in 35 genes (*ACTA2, BGN, CBS, CHST14, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, EFEMP2, FBN1, FBN2, FKBP14, FLNA, FOXE3, LOX, MAT2A, MED12, MFAP5, MYH11, MYLK, NOTCH1, PLOD1, PRDM5, PRKG1, SKI, SLC2A10, SMAD3, SMAD4, TGFB2, TGFB3, TGFBR1, TGFBR2, TNXB* (excluding exons 32-44), and *ZNF469*) 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 all genes (excluding *CBS* and *TNXB* exons 32-44). If *FBN1* gene sequence and deletion/duplication analysis is requested, then only the specific gene is analyzed.

Result Reports: Results reported herein may be of constitutional or somatic origin. This methodology cannot differentiate between these possibilities. 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.
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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.
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12. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat.* 2017 Mar;38(3):243-251.
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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
16. Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647
17. Karczewski KJ et al. *Nature*. 2020 May;581(7809):434-443. PMID: 32461654
18. Splicing Prediction: Jaganathan K et al. *Cell.* 2019 Jan 24; 176(3):535-548.e24. PMID: 30661751

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 *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 *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 *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 *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, germline or somatic mosaicism, active hematologic disease, a history of allogeneic bone marrow or peripheral stem cell transplant, 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.

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Understanding Your Negative Genetic Test Result for Thoracic Aortic Aneurysms/Dissections (TAAD) or Related Conditions

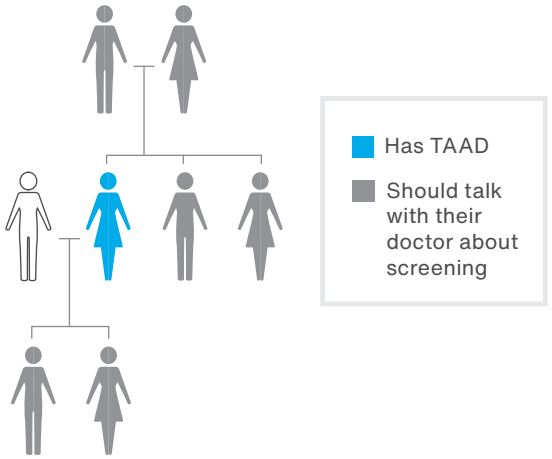
INFORMATION FOR PATIENTS

Result	NEGATIVE	Your (or your family member's) testing did not find any disease-causing mutations. You may have a mutation in a gene that was not included in this test. If someone in your family has a specific mutation in one of these genes, you are not likely at increased risk for TAAD or a related condition.
Diagnosis	NO CHANGE	This testing does not change your diagnosis. If you have been diagnosed with TAAD or a related condition, that remains the same.
Further Testing	DISCUSS	More genetic testing may be right for you. Please talk about this with your doctor or genetic counselor.
Management Options	PATIENTS WITH TAAD OR RELATED CONDITIONS	Treatment options may include: medications, surgery, or avoiding athletic activities. Your doctor may also recommend tests for other medical problems that are sometimes associated with TAAD. Talk to your doctor about which options may be right for you.
Screening Options	FAMILY MEMBERS	Options for screening and early detection include: physical exams, echocardiograms, or cardiac MRI. Talk to your doctor about which, if any, options may be right for you.
Next Steps	DISCUSS	Share this with family members so they can talk with their doctors and learn more.
Reach Out	RESOURCES	▪ Ambry's Cardiology Site for Families patients.ambrygen.com/cardiology ▪ National Society of Genetic Counselors nsgc.org ▪ The Marfan Foundation marfan.org ▪ Loeys-Dietz Syndrome Foundation loeysdietz.org ▪ The Ehlers-Danlos National Foundation ednf.org ▪ Genetic Information Nondiscrimination Act (GINA) ginahelp.org
Participate	RESEARCH OPPORTUNITIES	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 gene changes that can lead to TAAD and related diseases. Participation in research is optional. You may contact the study office at (713) 500-6715 or JRRP.research@uth.tmc.edu. For more information about the John Ritter Foundation, you may visit johnritterfoundation.org.

TAAD and Related Conditions in the Family

Even though your (or your family member's) genetic testing was negative, TAAD could still be running in your family. All close family members of someone with TAAD (like parents, brothers, sisters, children) should talk with their doctor about screening.

Please talk with your doctor or genetic counselor about this. The field of genetics is continuously changing, so updates related to your result, medical recommendations, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or taken as medical advice.



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