Ambry Genetics®

SAMPLE REPORT

Ordered By	Contact ID:1332810	Org ID:8141	Patient Legal Name: **8911, Test08**		
Medical Professional:	Rhodarmer, Jake, MA		Accession #: **00-135397**	Specimen #:	
Client:	MOCKORG44 (10829)		AP2 Order #: 614269	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

CardioNext®: Analyses of 92 Genes Associated with Inherited Cardiomyopathies and Arrhythmias

RESULTS

SCN5A Variant, Unknown Significance: p.M715V

SUMMARY

Variant of Unknown Significance Detected

INTERPRETATION

- **No known clinically actionable alterations were detected.**
- One variant of unknown significance was detected in the *SCN5A* gene.
- **Risk Estimate:** should be based on clinical and family history, as the clinical significance of this result is unknown.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

This individual is heterozygous for the p.M715V (c.2143A>G) variant of unknown significance in the *SCN5A* gene, which may or may not contribute to this individual's clinical history. Refer to the supplementary pages for additional information on this variant. No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected. Genes Analyzed (92 total): ***ABCC9, ACTC1, ACTN2, AKAP9, ALMS1, ALPK3, ANK2, ANKRD1, BAG3, CACNA1C, CACNA2D1, CACNB2, CALM1, CALM2, CALM3, CASQ2, CAV3, CRYAB, CSRP3, DES, DMD, DOLK, DSC2, DSG2, DSP, EMD, EYA4, FHL1, FKRP, FKTN, FLNC, GATAD1, GLA, GPD1L, HCN4, JPH2, JUP, KCND3, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, LAMA4, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, MYPN, NEXN, NKX2-5, PKP2, PLN, PRKAG2, PTPN11, RAF1, RBM20, RIT1, RYR2, SCN10A, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SNTA1, SOS1, TAZ, TBX20, TBX5, TCAP, TECRL, TGFB3, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TRDN, TRPM4, TTN, TTR, TXNRD2*** **and** ***VCL*** **(sequencing and deletion/duplication)**.

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.

- CardioNext® (Product Code 8911)

ASSAY INFORMATION

General Information: CardioNext® is a panel including 92 genes associated with hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), left ventricular non-compaction (LVNC), restrictive cardiomyopathy (RCM), long QT syndrome (LQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and short QT syndrome (SQTS). This panel also includes genes that can cause cardiomyopathy associated with inherited muscular dystrophies, as well as some genes associated with congenital heart defects. Given the overlap in genetic causes and variability in clinical symptoms and presentation, one comprehensive inherited cardiovascular test may be the most effective way of identifying at-risk individuals, or confirming a diagnosis.

Methodology: CardioNext® is a comprehensive analysis of 92 genes associated with inherited cardiomyopathies, arrhythmias, and other cardiovascular diseases. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a standardized methodology and quantified. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides (excluding *FLNC* exons 46-48) 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 *FLNC* exons 46-48) 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: *ABCC9* NM_005691.2, *ACTC1* NM_005159.4, *ACTN2* NM_001103.2, *AKAP9* NM_005751.4, *ALMS1* NM_015120.4, *ALPK3* NM_020778.4, *ANK2* NM_001148.4, *ANKRD1* NM_014391.2, *BAG3* NM_004281.3, *CACNA1C* NM_000719.6, *CACNA2D1* NM_000722.2, *CACNB2* NM_201590.2, *CALM1* NM_006888.4, *CALM2* NM_001743.4, *CALM3* NM_005184.2, *CASQ2* NM_001232.3, *CAV3* NM_033337.2, *CRYAB* NM_001885.1, *CSRP3* NM_003476.3, *DES* NM_001927.3, *DMD* NM_004006.2, *DOLK* NM_014908, *DSC2* NM_024422.3, *DSG2* NM_001943.3, *DSP* NM_004415.2, *EMD* NM_000117.2, *EYA4* NM_004100.4, *FHL1* NM_001449, *FKTN* NM_001079802.1, *FKRP* NM_024301.4, *FLNC* NM_001458.4, *GATAD1* NM_021167.3, *GLA* NM_000169.2, *GPD1L* NM_015141.3, *HCN4* NM_005477.2, *JPH2* NM_020433.4, *JUP* NM_002230.2, *KCND3* NM_004980.4, *KCNE1* NM_000219.3, *KCNE2* NM_172201.1, *KCNE3* NM_005472.4, *KCNH2* NM_000238.3, *KCNJ2* NM_000891.2, *KCNJ5* NM_000890.3, *KCNJ8* NM_004982.2, *KCNQ1* NM_000218.2, *LAMA4* NM_002290.3, *LAMP2* NM_002294.2, *LDB3* NM_007078.2, *LMNA* NM_170707.2, *MYBPC3* NM_000256.3, *MYH6* NM_002471.3, *MYH7* NM_000257.2, *MYL2* NM_000432.3, *MYL3* NM_000258.2, *MYOZ2* NM_016599.4, *MYPN* NM_032578.2, *NEXN* NM_144573.3, *NKX2-5* NM_004387.3, *PKP2* NM_004572.3, *PLN* NM_002667.3, *PRKAG2* NM_016203.3, *PTPN11* NM_002834.3, *RAF1* NM_002880.3, *RBM20* NM_001134363.1, *RIT1* NM_006912.4, *RYR2* NM_001035.2, *SCN10A* NM_006514.3, *SCN1B* NM_001037.4, *SCN2B* NM_004588.4, *SCN3B* NM_018400.3, *SCN4B* NM_174934.3, *SCN5A* NM_198056.2, *SNTA1* NM_003098.2, *SOS1* NM_005633.3, *TAZ* NM_000116.3, *TBX5* NM_000192.3, *TBX20* NM_001077653.2, *TCAP* NM_003673.3, *TECRL* NM_001010874.4, *TGFB3* NM_003239.2, *TMEM43* NM_024334.2, *TNNC1* NM_003280.2, *TNNI3* NM_000363.4, *TNNT2* NM_001001430.1, *TPM1* NM_001018005.1, *TRDN* NM_006073.2, *TRPM4* NM_017636.3, *TTN* NM_003319.4, *TTR* NM_000371.3, *TXNRD2* NM_006440.3, *VCL* NM_014000.2.

Analytical Range: CardioNext® targets detection of DNA sequence mutations in 92 genes (listed above in Methodology, excluding *FLNC* exons 46-48) 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. For *TTN*, only truncating variants are routinely reported. Gross deletion/duplication analysis determines gene copy number for the exons and untranslated regions of 92 genes, excluding *FLNC* exons 46-48.

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 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 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 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. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat.* 2017 Mar;38(3):243-251.
13. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
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 **CardioNext®** 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 **CardioNext®** test is 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 **CardioNext®** test may vary widely according to the specific clinical and family history. Inherited cardiomyopathies and arrhythmias are a complex spectrum of clinical disorders. Mutations in other genes or the regions not analyzed by the **CardioNext®** 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, 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.

SCN5A NM_198056 c.2143A>G p.M715V

VARIANT DETAILS:

The **p.M715V** variant (also known as c.2143A>G), located in coding exon 13 of the *SCN5A* gene, results from an A to G substitution at nucleotide position 2143. The methionine at codon 715 is replaced by valine, an amino acid with highly similar properties. This amino acid position is well conserved in available vertebrate species. In addition, this alteration is predicted to be deleterious by *in silico* analysis. Based on the available evidence, the clinical significance of this variant remains unclear.

FAMILY STUDIES PROGRAM:

Ambry Genetics offers complimentary genetic studies for variants of unknown significance (VUSs) meeting specific criteria in appropriate family members. Review of clinical information is required. Additional information, application instructions and required forms, and patient education materials are available at http://ambrygen.com/family-studies-program. For additional information, please email us at GeneticCounselor@ambrygen.com or call 949-900-5500 and ask to speak with a genetic counselor.

Please note that the classification of variants may change over time as additional information becomes available. Alerts are disseminated via fax and/or AmbryPort email to clinicians upon clinically relevant variant reclassifications. If no updates are received, clinicians are encouraged to contact the laboratory at 949-900-5500 once a year to review the status of previously reported variants.

GENE INFORMATION:

The *SCN5A* gene (NM_198056.2) is located on chromosome 3p22.2, contains 27 coding exons, and encodes the sodium channel protein type 5 subunit alpha. Pathogenic variants in this gene have been associated with a spectrum of *SCN5A*-related arrhythmias and/or cardiomyopathy including Brugada syndrome, long QT syndrome, arrhythmogenic right ventricular cardiomyopathy, cardiac conduction defect, sick sinus syndrome, atrial fibrillation, and dilated cardiomyopathy (DCM), which are inherited in an autosomal dominant fashion. *SCN5A*-related arrhythmias and/or cardiomyopathy disorders are characterized by cardiac arrhythmia and increased risk for sudden death. Brugada syndrome is defined by ECG cardiac conduction abnormalities in the right precordial leads V1 to V3, which occur particularly during rest and sleep in apparently healthy and young individuals. Long QT syndrome is defined by QT prolongation and T-wave abnormalities. Arrhythmogenic right ventricular cardiomyopathy is generally an adult-onset disease defined by fibrofatty replacement of the right ventricle. Cardiac conduction defect (also known as progressive familial heart block or Lenègre/Lev disease) is defined by progressive alteration of cardiac conduction with right or left bundle branch block and widening of QRS complexes, leading to complete atrioventricular block. *SCN5A*-related atrial fibrillation is described as rapid, irregular beating of the atria without causative risk factors such as structural heart disease. Sick sinus syndrome is defined by inappropriate sinus bradycardia, sinus arrest, or chronotropic incompetence related to dysfunction of the sinoatrial (SA) node, typically presenting with syncope, presyncope, dizziness, and fatigue. DCM is defined by ventricular dilation, reduced systolic function, and impaired contractility. Reduced penetrance and variable expressivity, including overlapping phenotypes, are observed (Benson DW et al. *J Clin Invest*, 2003 Oct;112:1019-28; Schwartz PJ. J *Intern Med*, 2006 Jan;259:39-47; Meregalli PG et al. *Heart Rhythm*, 2009 Mar;6:341-8; Remme CA. *J Physiol*, 2013 Sep;591:4099-116; Verkerk AO et al. *Front Cardiovasc Med*, 2018 Oct;5:137; Brugada R et al. *GeneReviews*. 2005 Mar 31 [Updated 2022 Aug 25]; Hershberger RE et al. *GeneReviews*. 2007 Jul 27 [Updated 2023 May 11]; McNally E et al. *GeneReviews*. 2005 Apr 18 [Updated 2023 May 11]; Groffen AJ et al. *GeneReviews*. 2003 Feb 20 [Updated 2024 Mar 21]). Altered channel function and loss of function have been reported as mechanisms of disease for *SCN5A*-related arrhythmias and/or cardiomyopathy.

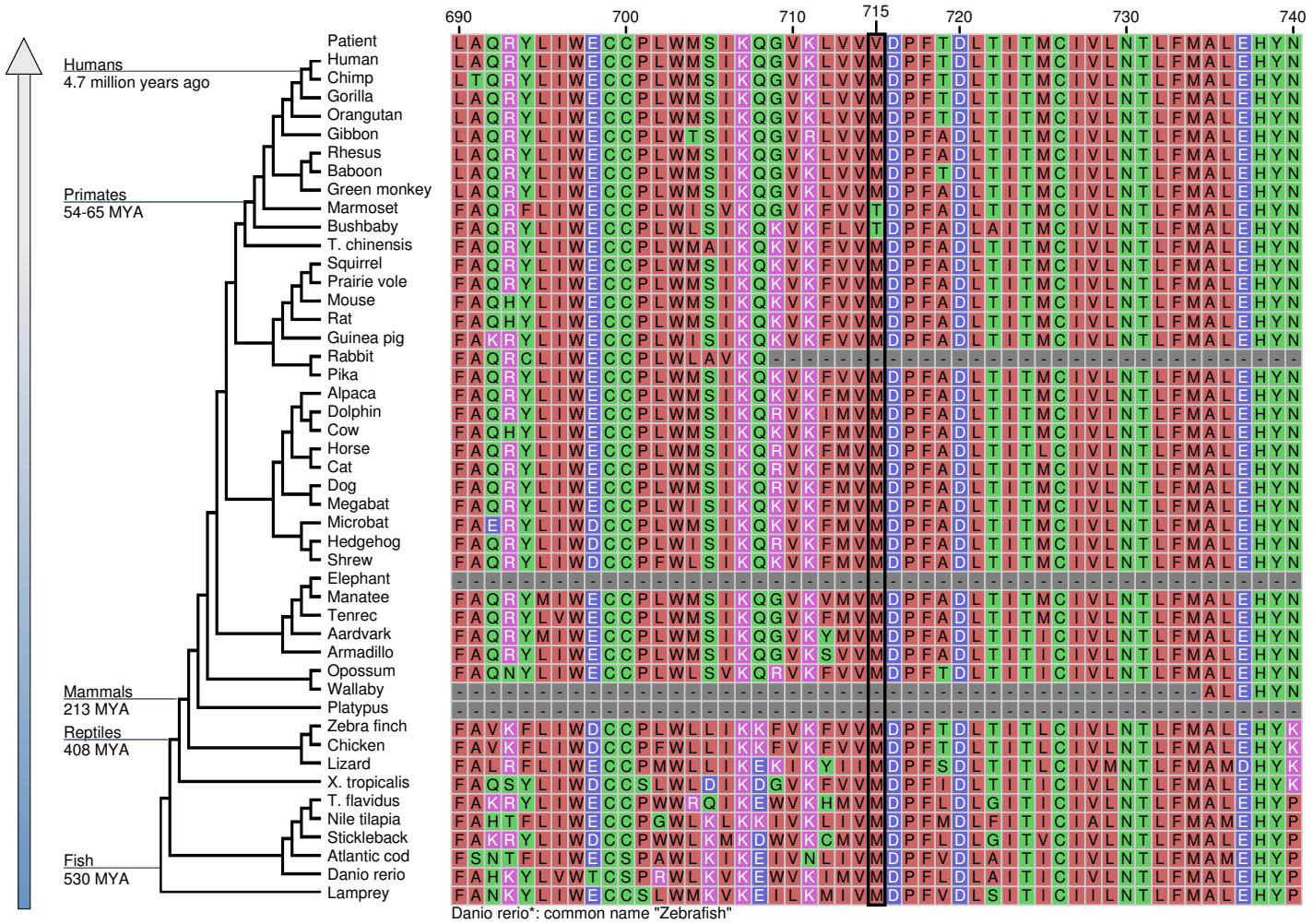
ADDITIONAL SUPPORTING INFORMATION:

Co-Segregation	Co-segregation data for this variant is currently unavailable.
Co-occurrence	No significant co-occurrence data is currently available at our laboratory.
Frequency	No population frequency information could be found.
Grantham Score	21 (highly similar amino acid substitution)
in silico	Deleterious

SCN5A NM_198056 c.2143A>G p.M715V

Evolutionary conservation diagram: Amino Acid Alignment

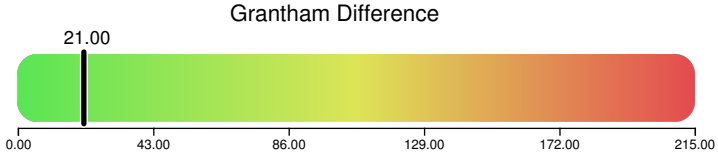
This amino acid position is well conserved in available vertebrate species.



Danio rerio*: common name "Zebrafish"

Amino Acid Change:

Trait	Met (M)	Val (V)
Amino Acid Name	Methionine	Valine
Polarity/Charge	non-polar	non-polar
pH	neutral	neutral
Residue Weight	131	99
Hydrophobicity Score	1.9	4.2
Hydrophilicity Score	-1.3	-1.5
Secondary Structure Propensity	strong α former / β former	α former / strong β former



Ambry Genetics®

Understanding Your VUS Cardiovascular Genetic Test Result

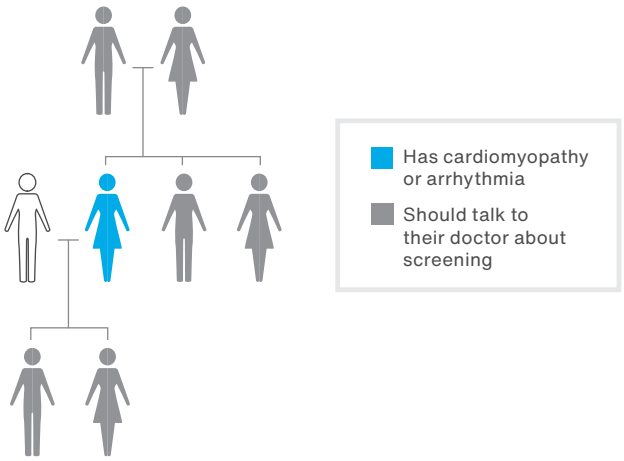
INFORMATION FOR PATIENTS WITH A VARIANT OF UNKNOWN SIGNIFICANCE

Result	VUS	Your test result shows you have a variant of unknown significance (VUS), a change in a gene that can cause an inherited cardiovascular disorder. In this case, we do not know if it is the source or not.
Diagnosis	NO CHANGE	This test result does not change your cardiovascular diagnosis. If you were diagnosed with cardiomyopathy, arrhythmia, or another, that remains the same.
Family Members	POSSIBLE TESTING	Your report will indicate if testing family members may help us learn more about your specific VUS. Please speak with your healthcare provider to determine if they might also benefit from a test to evaluate their personal risk of developing a disease.
Management Options	PATIENTS WITH ARRHYTHMIA OR CARDIOMYOPATHY	Treatment options include medications, surgery, pacemakers, implantable cardioverter defibrillators (ICDs), or avoiding certain athletic activities. Talk to your doctor about which may be right for you.
Screening Options	PATIENTS WITH A FAMILY HISTORY OF CARDIOMYOPATHY OR ARRHYTHMIA, BUT NO PERSONAL SIGNS	Options for screening and early detection include physical exams, echocardiograms, electrocardiograms (EKGs), or cardiac MRI. Talk to your doctor about whether these options are right for you.
Next Steps	DISCUSS	Please share this with family members so they can talk to their doctors and learn more.
Reach Out	RESOURCES	Ambry's Cardiology Site for Families ambrygen.com/patients/cardiology National Society of Genetic Counselors nsgc.org Hypertrophic Cardiomyopathy Association 4hcm.org Sudden Arrhythmia Death Syndromes (SADS) Foundation sads.org Children's Cardiomyopathy Foundation childrenscardiomyopathy.org Genetic Information Nondiscrimination Act (GINA) ginahelp.org

Cardiomyopathy or Arrhythmia in the Family

Even though your genetic testing result was a VUS, some cardiomyopathies and arrhythmias can still run in families. All close family members of someone with an inherited cardiomyopathy or arrhythmia (like parents, brothers, sisters, children) should talk with their doctor about screening.

Please speak to 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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