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SAMPLE REPORT

Ordered By	Contact ID:1332810	Org ID:8141	Patient 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	Gender: U
			MRN #:	N/A	Collected: N/A
			Indication:	Internal Testing	Received: 11/20/2018

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

RESULTS

SCN5A Variant, Unknown Significance: p.P877R

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 testing for variants of unknown significance (VUSs) in family members may be pursued to help clarify VUS significance, but cannot be used to identify at-risk individuals at this time.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

This individual is heterozygous for the p.P877R (c.2630C>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 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) 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

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.2630C>G p.P877R

VARIANT DETAILS:

The **p.P877R** variant (also known as c.2630C>G), located in coding exon 15 of the *SCN5A* gene, results from a C to G substitution at nucleotide position 2630. The proline at codon 877 is replaced by arginine, an amino acid with dissimilar properties. This amino acid position is highly conserved in available vertebrate species. In addition, this alteration is predicted to be deleterious by *in silico* analysis. Since supporting evidence is limited at this time, the clinical significance of this alteration 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:

SCN5A (NM_198056.2) encodes the alpha subunit of the type V voltage-gated sodium channel. The *SCN5A* gene is located at 3p22.2 and contains 27 coding exons. This protein drives the initial upstroke on an electrocardiogram. Loss of function mutations in *SCN5A* are attributed to approximately 15-40% of Brugada syndrome, which is inherited in an autosomal dominant manner (Crotti et al. 2012 *J Am Coll Cardiol*). Gain of function mutations in *SCN5A* are detected in up to 10% of individuals with a clinical diagnosis of autosomal dominant long QT syndrome (Alders and Christiaans GeneReviews: Long QT Syndrome, last updated Feb 2018, available from http://www.ncbi.nlm.nih.gov/books/NBK1129/). In addition, mutations in *SCN5A* have been associated with other clinical conditions including cardiac conduction defects, sick sinus syndrome, atrial fibrillation, dilated cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy. There is significant variability in penetrance associated with *SCN5A* variants.

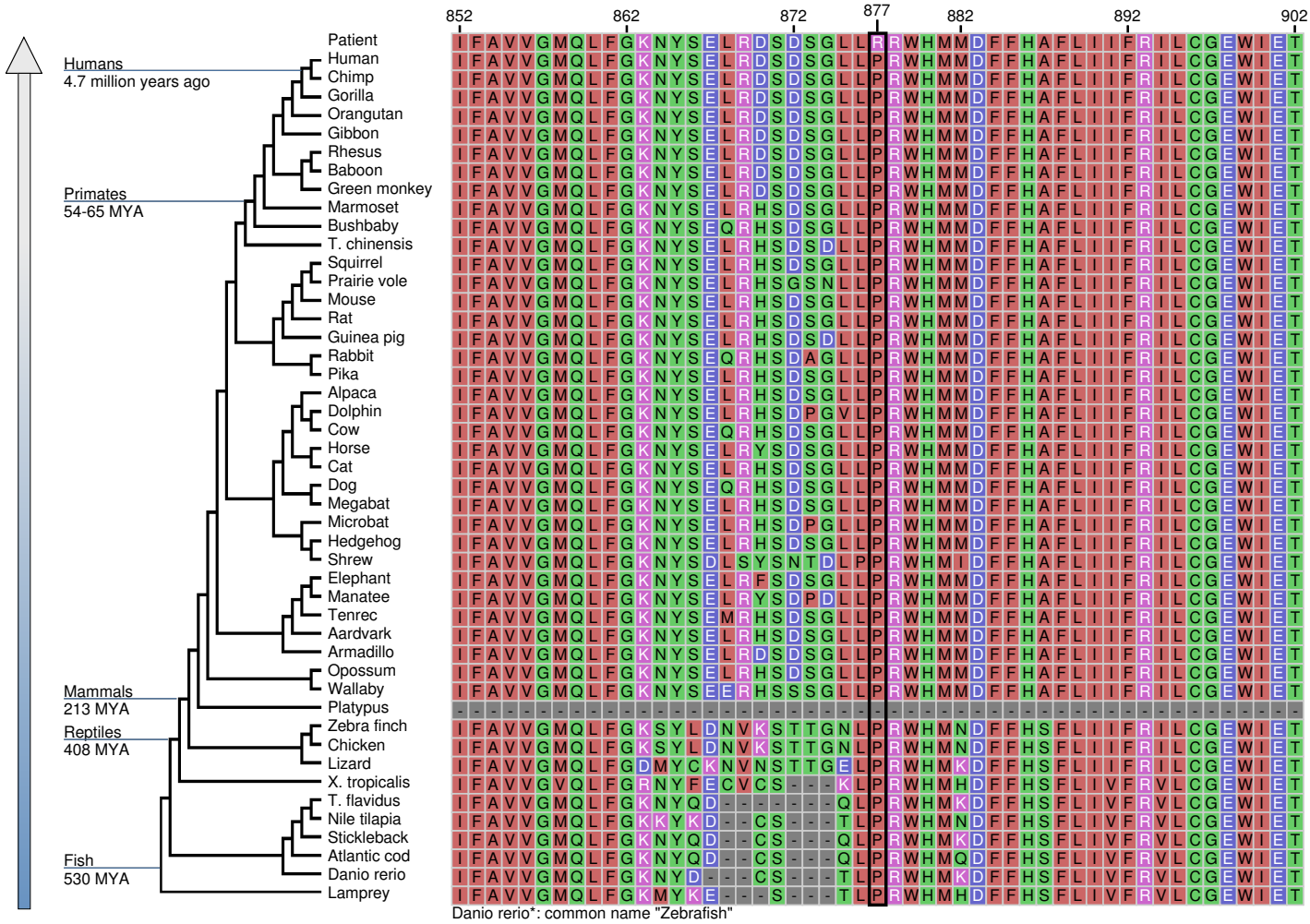
ADDITIONAL SUPPORTING INFORMATION:

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	103 (dissimilar amino acid substitution)
in silico	Deleterious

SCN5A NM_198056 c.2630C>G p.P877R

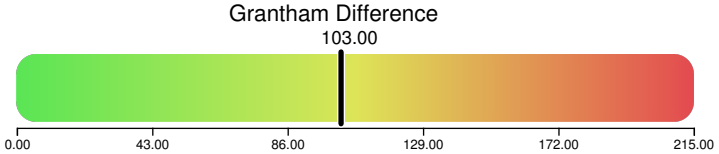
Evolutionary conservation diagram: Amino Acid Alignment

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



Amino Acid Change:

Trait	Pro (P)	Arg (R)
Amino Acid Name	Proline	Arginine
Polarity/Charge	non-polar	positively charged
pH	neutral	basic
Residue Weight	97	156
Hydrophobicity Score	-1.6	-4.5
Hydrophilicity Score	0	3
Secondary Structure Propensity	strong α breaker / strong β breaker	α indifferent / β indifferent



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Understanding Your VUS Cardiovascular Genetic Test Result

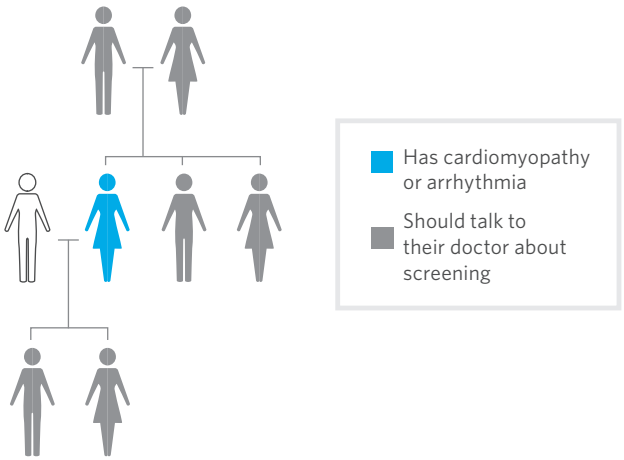
INFORMATION FOR PATIENTS WITH A **VARIANT OF UNKNOWN SIGNIFICANCE**

Result	**VUS**	Your testing shows that you have a variant of unknown significance (VUS) in a gene that causes an inherited cardiovascular disorder. A VUS is a gene change, but we do not know if it causes your cardiovascular condition or not.
Diagnosis	**NO CHANGE**	This testing does not change your cardiovascular diagnosis. If you have been diagnosed with a cardiomyopathy, arrhythmia, or another cardiovascular disorder, that remains the same.
Further Testing	**FOR FAMILY MEMBERS**	Testing your family members that have this condition may help explain this VUS. Talk with your doctor or genetic counselor about which family members may be helpful to test.
	FOR YOU	More genetic testing may be right for you. Please talk about this with your doctor or genetic counselor.
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 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 • 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 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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