Reinterpretation of Genetic Variants from a Cohort of Pediatric Arrhythmia Patients

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Madison Nicole Bernhardt

Graduate Program in Genetic Counseling

The Ohio State University

2018

Thesis Committee

Fitzgerald-Butt, Sara M.

McBride, Dr. Kim L.

Allain, Dawn C.

Abstract

Background: Genetic testing plays an important role in the diagnosis and management of inherited arrhythmia syndromes. Classification of genetic variants is a challenge and discordant interpretations occur often. In 2015, the American College of Medical Genetics and Genomics (ACMG) published guidelines to standardize interpretation of genetic variants. Although genetic testing in this field has been available for thirty years, there are currently no guidelines for variant reinterpretation.

Objective: To apply the 2015 ACMG Guidelines to pathogenic variants, likely pathogenic variants, and variants of known significance (VUS) identified in pediatric patients with suspected inherited arrhythmia syndromes, as well as determine factors associated with a higher likelihood of variant reclassification and propose a framework for determining the need for reinterpretation.

Methods: Electronic medical records were searched to identify patients with a diagnosis of an inherited arrhythmia syndrome or abnormal ECG who were seen between 2009 and 2015. Genetic testing results demonstrating a VUS or pathogenic/likely pathogenic variant were reevaluated using the 2015 ACMG Guidelines. Statistical analysis was performed using descriptive statistics and chi square tests. This information was used to develop a decision tree to assist in evaluating the need for variant reevaluation.

Results: We identified 346 patients with an inherited arrhythmia syndrome or abnormal ECG diagnosis; 118 of which had undergone genetic testing. Of the 61 unique variants, 21 (34.4%) received a new classification. 26.4% of pathogenic/likely pathogenic variants were reclassified compared to 47.8% of VUS. For VUS, clinical characteristics including prescription of beta-blocker medication (p=0.019) and having a clinical diagnosis of an inherited arrhythmia syndrome (p=0.033) were associated with variant reclassification toward pathogenic. For pathogenic/likely pathogenic variants, having an ICD (p=0.065) was trending toward association with reclassification toward benign. When analyzing all variants together, year of testing showed a trend towards association (p=0.160). Based on these results we developed a decision tree in which providers should first consider the certainty of the clinical diagnosis and then the year of the clinical report if considering a pathogenic/likely pathogenic variant.

Conclusion: As reclassification of genetic variants based on the implementation of ACMG's 2015 variant interpretation guidelines is common among this cohort and may affect patient and family management, development of a variant reassessment plan is necessary.

Dedication

This document is dedicated to my parents, brother, and the 2018 OSUGC Graduate Program Class, all of whom have endlessly supported my aspirations to become a genetic counselor.

Acknowledgments

I would like to acknowledge the efforts of my thesis committee, Sara Fitzgerald-Butt, MS, LGC, Kim McBride, MD, and Dawn Allain, MS, LGC for their invaluable mentorship. This project would not have been possible without the support and guidance of the Nationwide Children's Hospital Heart Center Faculty including Anna Kamp, MD, MPH, Vidu Garg, MD, and Jeffrey Bennett, MD, PhD, as well as the Nationwide Children's Hospital laboratory directors Shalini Reshmi, PhD, and Erik Zmuda, PhD.

Vita

2011……Upper Arlington High School

2016……..…….B.S. Biology, The Ohio State University

2018……...…M.S. Genetic Counseling, The Ohio State University

Publications

Wei L., Allain D., **Bernhardt M.**, Gillespie J., Peters S., Iwenofu O., Nelson H., Arron S., Toland A. Variants at the OCA2/HERC2 locus affect time to first cutaneous squamous cell carcinoma in solid organ transplant recipients collected using two different study designs. *Br J Dermatol*. 2017 Apr 29.

Fields of Study

Major Field: Genetic Counseling

Table of Contents

List of Tables

List of Figures

1. Background

Within the past decade, the discipline of hereditary cardiac channelopathies has expanded rapidly with the discovery of underlying predisposing genetic variants in genes encoding cardiac ion channel subunits and membrane proteins. Elucidation of genetic arrhythmogenic mechanisms has allowed for the advent of clinically available genetic testing in the setting of inherited arrhythmias (Ackerman, 2015). Indications for genetic testing and its utility in the clinical setting depend on the benefits of cascade testing and guiding management, as well as the ability to provide prognostic information. Variable expressivity, reduced penetrance, and difficulty assessing functional and clinical effects of novel mutations are common challenges across all cardiac channelopathy phenotypes (Wilde et al., 2013).

Cardiac Channelopathy Phenotypes

Long QT Syndrome

The most common of the inherited arrhythmia conditions is a genetically heterogeneous disorder of myocardial repolarization known as Long QT Syndrome (LQTS). LQTS has an estimated prevalence of one in two thousand Caucasians (Lieve and Wilde, 2015). Schwartz and Crotti (2011) developed a point scoring system for the

clinical diagnosis of LQTS, which takes into account medical and family history to provide a probability for the diagnosis (Schwartz & Crotti, 2011; Priori et al., 2013). A prolonged heart rate-corrected QT interval (QTc) of greater than or equal to 480 milliseconds (ms) in conjunction with *torsade de pointes* or other electrocardiogram (ECG) abnormalities, stress induced syncope, and/or a family history of LQTS or sudden cardiac death indicate a high probability of the condition (Schwartz & Crotti, 2011). Despite the development of clinical diagnostic criteria, securing a diagnosis of LQTS based on clinical features alone can be challenging.

Incomplete penetrance and variable expressivity are common complicating factors of reaching a diagnosis. Approximately 20-25% of individuals harboring a pathogenic mutation in an LQTS-susceptibility gene will have a normal QTc interval on electrocardiogram testing (Priori et al., 2003; Goldenberg et al., 2011). In addition, variability is common between multiple ECG evaluations for a single individual. Other non-genetic factors known to lengthen QT interval must be ruled out. These include QT-prolonging medications, electrolyte abnormalities, acute illness, hypokalemia, certain neurological conditions and structural heart problems.

There are currently fifteen known LQTS-susceptibility genes that have been implicated in the pathogenesis of the disorder. However, as many as 60-75% of patients with clinically definitive LQTS have a pathogenic mutation in one of three major LQTS-susceptibility genes: *KCNQ1, KCNH2*, and *SCN5A*. The remaining twelve minor LQTS-susceptibility genes include *ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B,*

AKAP9, SNTA1, KCNJ5, CALM1, and *CALM2*. Each of these genes account for less than one percent of LQTS cases (Alders & Christiaans, 2015).

LQTS can be divided into subtypes depending on the causative genetic variant. LQTS type 1 is caused by mutations in the *KCNQ1* gene, LQTS type 2 is caused by mutations in the *KCNH2* gene, and LQTS type 3 is caused by mutations in the *SCN5A* gene. Each subtype may have a slightly different presentation and approach to treatment and gene specific differences in risk for cardiac events have been described. For example, patients with an LQTS1 genotype are at higher risk for arrhythmic events trigged by exercise, whereas patients with an LQTS2 genotype experience events associated with sudden, loud noises. In contrast, patients with LQTS3 are at greatest risk for experiencing a cardiac event during sleep or at rest (Barsheshet et al., 2013).

Historically, pathogenic mutations known to cause LQTS have localized to pore and transmembrane domains of proteins that form sodium and potassium channels. These pathogenic variants have been shown to delay repolarization in comparison to wild type alleles (Morita et al., 2008, Kapa et al., 2009). However, loss of protein function, gain of protein function, and dominant-negative effects have all been reported in LQTS patients. The mechanism of disease appears to be gene and protein function specific (Campuzano et al., 2015). KCNQ1 and KCNH2 related LQTS are typically associated with loss of function mutations, while SCN5A related LQTS is typically associated with gain of function mutations (Alders et al., 2003).

LQTS is typically inherited in an autosomal dominant manner. The presence of two pathogenic variants in either *KCNQ1* or *KCNE1* causes Jervell and Lange-Nielsen

syndrome (Morita et al., 2008). This condition is more severe than LQTS and causes congenital profound bilateral sensorineural hearing loss and a more severe presentation of cardiac related symptoms compared to autosomal dominant LQTS. Individuals with Jervell and Lange-Nielsen syndrome typically have QTc intervals greater than 500 ms and, if untreated, a 50% chance of experiencing a cardiac event before age three (Schwartz et al., 2006).

No cure currently exists for LQTS. Instead, management is focused on the prevention of symptoms and sudden cardiac arrests and/or death. Life-style modifications including the restriction of strenuous exercise and loud, abrupt noises, as well as discontinuing use of QT-prolonging medications should be initiated promptly upon diagnosis. Beta-blocker medication is indicated for all individuals harboring a pathogenic variant in an LQTS-associated gene regardless of disease presentation (Priori et al., 2013; Vincent et al., 2009). This medication may also be considered in those patients for whom a diagnosis of LQTS is being considered. Implantable cardiac defibrillators (ICDs) are recommended for those individuals who have been resuscitated from a cardiac arrest or display beta-blocker resistance. ICDs are also recommended for individuals with continued LQTS-related syncope or have a contraindication for beta-blocker therapy such as asthma (Priori et al., 2013). Left cardiac sympathetic denervation (LCSD) is reserved for high-risk patients for whom both beta-blocker therapy and ICD therapy are refused, not effective, not tolerated or contraindicated (Priori et al. 2013).

Catecholaminergic Polymorphic Ventricular Tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare inherited arrhythmogenic disorder of aberrant intracellular calcium handling. CPVT is characterized by ventricular arrhythmias that become exacerbated by exercise and emotional stress. A diagnosis of CPVT is made in an individual with a structurally normal heart and normal ECG findings, but who develops an unexplained, bidirectional or polymorphic ventricular tachycardia during exercise or stress at an age younger than 40 (Priori et al., 2013). Individuals diagnosed with CPVT are at an increased risk for syncope and sudden cardiac death (Giudicessi &Ackerman, 2013). If untreated, as many as 30% of affected individuals will experience at least one cardiac arrest during their lifetime (Napolitano et al., 2016). Even among patients treated with beta-blocker therapy, as many as 13% of individuals with CPVT will experience a fatal or near-fatal cardiac event (Hayashi et al., 2009).

Four different genes have been implicated in the pathogenesis of CPVT, with *RYR2* being the most common accounting for 50-55% of cases. Mutations in *CASQ2, CALM1* and *TARDN* are much less common and are collectively identified in fewer than ten percent of individuals with CPVT. For a large proportion of clinically definitive CPVT cases (35-45%), no identifiable pathogenic genetic mutation is found. This suggests that more undiscovered genes that cause CPVT exist (Napolitano et al., 2004).

CALM1- and *RYR2*-related CPVT are inherited in autosomal dominant fashion, whereas *CASQ2*- and *TARDN*-related CPVT are inherited in an autosomal recessive

manner. Similarly to LQTS, CPVT displays variable expressivity and reduced penetrance, with the mean penetrance of *RYR2* pathogenic variants estimated at 83% (Napolitano et al., 2016). Therefore, 17% of individuals who harbor a pathogenic variant within *RYR2* will remain asymptomatic. A syncopal episode in childhood is the most common initial presentation of CPVT. Tragically, sudden cardiac death may be the first sign of the disorder in a previously healthy individual with no history of dizziness or syncope.

Brugada Syndrome

Brugada Syndrome (BrS) is characterized by cardiac conduction abnormalities, specifically elevation of the ST-segment on an electrocardiogram, which increases the risk for ventricular arrhythmias. These ECG findings can be transient and may be brought out only in specific situations, making diagnosis and risk assessment difficult. In the case of BrS, cardiac episodes typically occur during rest or while asleep. Common presenting features include palpitations, syncope, and sudden cardiac death. The suspected prevalence of BrS among individuals of European decent is five in ten thousand, however, one study of an adult Japanese population showed a prevalence of 66 per 10,420 individuals (Tohyou et al., 1995; Giudicessi & Ackerman, 2013; Namiki et al., 1995).

Pathogenic variants in *SCN5A* account for the majority, 15-30%, of BrS cases; however, a total of twenty-three genes have been associated with the development of the disease. Compared to LQTS and CPVT, BrS has a much lower penetrance. Among

individuals with an *SCN5A* variant known to cause BrS, approximately 20-30% will have an electrocardiogram diagnostic of BrS. Most commonly BrS is inherited in an autosomal dominant fashion with the exception of BrS due to mutations in the *KCNE5* gene. *KCNE5*-related BrS is inherited in an X-linked manner (Juang & Horie, 2016).

Clinical Cardiac Genetic Testing: Past and Present

Detection of a disease-predisposing pathogenic variant within one of the various cardiac channelopathy-associated genes has many benefits for both the proband and the proband's family members. Finding a causative pathogenic variant can help solidify a borderline diagnosis and provide the proband with a more accurate risk assessment by stratifying the disease into subtypes. If a proband has had positive genetic testing, cascade testing of the proband's at-risk relatives is possible, which allows for the identification of asymptomatic at-risk family members and initiation of therapeutic interventions in all genotype-positive family members (Guidicessi 2013). In cases of LQTS and CPVT, preventative screening, life-style modifications and initiation of prophylactic medication decrease morbidity and mortality, specifically the incidence of sudden cardiac death (Ackerman 2015). In addition, family members who test negative for the causative familial genetic variant would not need to undergo any additional clinical cardiac screening, as their risk for an adverse cardiac event would be reduced to the risk attributed to individuals within the general population (Ackerman 2011). For

these reasons, genetic testing can be helpful in the evaluation of individuals with cardiac arrhythmias.

In the past, genetic testing was limited to single gene sequencing in which the gene was chosen based on a patient's specific clinical phenotype. This made interpretation of rare variants straightforward, because the gene in which the variant was found was closely associated with the clinical phenotype of the patient (Ackerman, 2015). With this method of testing, it is also possible that assumptions of pathogenicity were made based on the fact that the mutation was identified in the gene where most suspicion was focused. More recently, next generation sequencing (NGS) has revolutionized how genetic testing is ordered. It allows for the sequencing of millions of small DNA fragments in parallel so that multiple genes or even an entire genome can be evaluated quickly, efficiently, and cost effectively (Behjati & Tarpey, 2013). Now the cost to sequence multiple genes at once via next generation sequencing is equal to or less than the cost of sequencing only one or a few specific genes via Sanger sequencing (Niroula et al., 2016).

With increasing availability and access, genetic testing is frequently being ordered to evaluate for mutations in a growing number of genes. A comprehensive arrhythmia panel today can evaluate as many as 75 genes at once. Because providers are also using genetic testing to assess individuals with borderline diagnoses, many genetic testing laboratories are also designing gene panels that encompass multiple overlapping clinical phenotypes. This is especially true in the field of cardiogenetics, because many of the associated genes have been reported in more than one cardiac phenotype. For example,

mutations in the *SCN5A* gene have been associated with both LQTS and BrS. Genetic testing is quickly becoming part of the diagnostic process to confirm or rule out a particular diagnosis (Pugh et al., 2016). Utilizing NGS technology to perform bigger and better gene panels appears to be an obvious next step as the field of clinical cardiogenetics continues to expand. However, our technology to generate genetic information has far outpaced our ability to interpret genetic alterations (Ackerman, 2015).

As more genetic information is assessed and analyzed, the probability of identifying a rare "variant of unknown significance" (VUS) increases (Giudicessi & Ackerman, 2013). VUS are genetic variants whose association with disease risk is undetermined or controversial. They tend to be rare and have limited data regarding their impact on protein structure and function. The non-informative nature of receiving a VUS result can pose challenges for both health care providers and patients. Individuals who receive a VUS test result are more likely to misunderstand their results as compared to individuals with either positive or negative results (Richter et al., 2013). In addition, patients with VUS genetic testing results are more likely to inappropriately attribute health problems to these findings. One publication studying VUS in the setting of cancer genetic counseling, found that 50% of physicians did not discuss the possibility of a VUS as a test outcome, and that 100% of physicians report that they would inappropriately refer siblings of a VUS carrier for predictive testing (Richter et al., 2013). These concepts illustrate the idea that generating more genetic information is not always better. Unclear results are a cause of confusion for both patients and providers.

Variant Classification & Interpretation

Challenges in variant interpretation have been at the forefront of recent discussion and debate among genetics professionals. In 2008, the American College of Medical Genetics and Genomics (ACMG) published an initial set of guidelines to aide in variant interpretation (Richards et al., 2008). These guidelines, however, did not address how to weigh the various forms of evidence used to come to classify a given alteration. As a result, many commercial genetic testing laboratories created their own methods for classifying genetic variants. This led to discordant interpretations of the same variant when comparing multiple genetic testing laboratories. Each laboratory relied upon unpublished, private data to determine pathogenicity of a given variant (Pepin et al., 2015, Balamana et al., 2016). This practice has created problems in the clinical setting for probands who have testing ordered through one laboratory, while family members have testing performed by a different laboratory. When variant interpretation differs between laboratories identifying the same genetic variant within a family, challenges arise with clinical utilization of the result and implementing disease management.

In an effort to combat the discordance, ACMG published an updated set of guidelines in 2015 known as the "Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology"(Richards et al., 2015). ACMG's manuscript describes the clinical significance of a given sequence variant as falling along a five-tiered gradient, ranging from pathogenic to benign. This approach to classification is more defined than the previous practice recommendations

for genetic testing laboratories. The primary goal of implementing these guidelines is two fold: 1) increase standardization in variant classification and 2) reduce the large number of variants being reported as causative of disease without sufficient evidence to support that classification (Richards et al., 2015).

Determining the significance of a given genetic variant is complex and time-consuming. Classifications are made based on evidence collected from multiple sources including *in vivo* and *in vitro* functional studies, primary literature regarding functionality of protein domains, allele frequencies within population databases, computational *in silico* predictive programs, and segregation analysis (Richards et al., 2015). Navigating these numerous resources can be challenging even for experienced genetics professionals (Amendola et al., 2016). Of particular challenge are rare or novel missense mutations, in which a single amino acid is substituted for a different amino acid. Interpretation of missense variants relies on a familiarity with protein structure and critical functional domains, as well as on *in silico* predictive programs. If the function of a gene or genetic mutations within a gene have not been well characterized or studied, the significance of novel missense variants can be extremely challenging to assess (Pepin et al., 2015). Even with guidelines in place, there is no 'one-size fits all' method that pertains to every individual variant. There is also no way to truly verify accuracy of a classification at the time the call is made (Amendola et al., 2016).

Given the enormous amount of benign variation present in the human genome, the majority of rare variants are unlikely to contribute to human disease. However, various publications have found that, in general, the pathogenicity of a given genetic variant

tends to be overestimated (Amendola et al., 2016). Several studies that have reinterpreted genetic variants among dilated and hypertrophic cardiomyopathy patients have demonstrated that variants are most likely to be reinterpreted from a "positive" result to an "inconclusive" or "negative" result based on an over representation of the variant in population databases. These studies advocate for periodic reassessment of genetic variants and believe this practice to be essential in the ongoing management of families with cardiomyopathy (Pugh et al., 2016, Das et al., 2013).

Data has been collected to assess patients of high socioeconomic background and their reactions to clinical reinterpretation of genetic variants. Those individuals who received a reinterpretation of their carrier status had low levels of perceived ambiguity and negative emotions, as well as high intentions to share the information with family members (Taber et al., 2016). No adverse patient reactions to reinterpretation of variants have been reported to date, however, more research is needed to explore responses to medically actionable changes, as well as patient reactions among individuals of lower socioeconomic background.

One research study demonstrated reclassification of genetic variants among African American adults in which variants were downgraded from pathogenic to benign. Reclassifications were made based on the exclusion of ancestry-matched controls within population databases at the time of the original testing (Manrai et al., 2016). Historically, population databases include a large majority of DNA from Caucasian individuals. The updated genetic testing results invalidated prior risk assessment for family members and required changes to medical management (Manrai et al., 2016). Although not directly

assessed in the study, these findings demonstrate how health disparities between ethnic groups may arise from limited data in available population databases for interpreting genetic variants.

Given the quickly evolving landscape of genetic testing practices, more research is needed regarding the reinterpretation of genetic data. The aim of this study is to provide information about variant reinterpretation in the setting of inherited arrhythmia patients. To our knowledge, this is the first study to address variant reinterpretation among this patient cohort.

Study Aim & Objectives

The aim of this study was to evaluate the impact of applying the 2015 ACMG Guidelines to genetic variants detected within a cohort of pediatric patients diagnosed with cardiac arrhythmias. The three primary research objectives are:

1. To determine the portion of genetic variants previously identified through cardiac arrhythmia clinical genetic testing that should be reclassified based on the updated 2015 ACMG guidelines.
2. To determine the genetic, demographic and clinical factors that affected changes in variant classification.
3. To create a decision tree to help cardiologists evaluate the need for variant reevaluation among patients with previous genetic testing.

2. Methods

This study reexamined genetic variants among a cohort of inherited arrhythmia patients by applying the 2015 ACMG Standards and Guidelines for Sequence Variant Interpretation. It was approved by the Institutional Review Board at Nationwide Children's Hospital (NCH) in Columbus, Ohio.

Because genetic testing was not a searchable field within the NCH electronic medical record, a query was performed using the NCH Heart Center database using the following criteria to identify patients who had undergone genetic testing for evaluation of an inherited arrhythmia syndrome:

1) Individuals with a clinical diagnosis of an inherited arrhythmia syndrome (ie Long QT Syndrome, CPVT, or Brugada syndrome) or an abnormal EKG
2) Individuals who completed a clinic visit with an electrophysiologist in the Heart Center at NCH between 2009 and 2015.
    a. Individuals with an inherited arrhythmia syndrome diagnosis must have had at least one appointment with an electrophysiologist.
    b. Individuals with an abnormal EKG must have had at least two appointments with a cardiologist.

Data Collection

For each patient identified as having a likely inherited arrhythmia syndrome diagnosis through the Heart Center database, electronic medical records were reviewed to determine whether or not genetic testing had been ordered. Individuals that did not undergo genetic testing were excluded from the study.

The patient's clinical genetic testing reports were examined and the following information was extracted for all pathogenic variants, likely pathogenic variants and VUS: the name of the clinical laboratory that performed the testing, the year of testing, the gene in which the variant was found, the genomic reference transcript used, the DNA and protein nomenclature of the variant, and the reported classification of the variant. It is important to note that a send out laboratory used by NCH clinicians for testing did not use conventional nomenclature for classifying variants. Instead, the lab used a three tiered system including class I variants, class II variants, and class III variants. For the purpose of this study, reported class I variants were categorized as pathogenic/likely pathogenic and class II variants were categorized as variants of unknown significance. Class III variants were categorized as benign and thus not reinterpreted for this study.

Each genetic variant was reinterpreted according to ACMG's Standards and Guidelines for Sequence Variant Interpretation (Richards et al., 2015). These guidelines propose that all genetic variants should be classified using a five-tiered system including pathogenic variants, likely pathogenic variants, variants of unknown significance, likely benign variants, and benign variants. Evidence for pathogenicity can be stratified into four categories: very strong (PVS), strong (PS), moderate (PM), and supporting (PP).

Evidence for a benign effect can be stratified into three categories: standalone (BA), strong (BS), and supporting (BP). Table 1 outlines the necessary criterions to be satisfied for each classification to be made.

Table 1: Necessary ACMG Criterions Applied to Reach Each Classification

Pathogenic	**Likely Pathogenic**	**Likely Benign**	**Benign**
1 PVS AND one of the following: ≥ 1 PS ≥ 2 PM 1 PM and 1 PP ≥ 2 PP	1 PVS AND 1 PM	1 BS AND 1 BP	1 BA
2 PS	1 PS AND one of the following: 1 PM ≥ 2 PP	≥ 2 BS	≥ 2 BS
1 PS AND one of the following: ≥ 3 PM 2 PM and ≥ 2 PP 1 PM and ≥ 4 PP	≥ 3 PM		
	2 PM AND ≥ 2 PP		
	1 PM AND ≥ 4 PP		

PVS= Pathogenic, very strong criteria
PS= Pathogenic, strong criteria
PM= Pathogenic, moderate criteria
PP= Pathogenic, supporting criteria

BA= Benign, standalone criteria
BS= Benign, strong criteria
BP= Benign, supporting criteria

For example, in order for a variant to be considered pathogenic, it must satisfy the following: one very strong criterion and any one of the following 1) one or more strong

criterions or 2) two or more moderate criterions or 3) one moderate and one supporting criterion or 4) two or more supporting criterions. A classification of pathogenic can also be given to variants that satisfy two strong criterions or one strong criterion and any one of the following: 1) three or more moderate criterions 2) two moderate and two or more supporting criterions 3) one moderate and four or more supporting criterions.

Table 2 describes each ACMG criterion in detail and the application of each criterion specific to this study.

Table 2: Application of ACMG Guidelines Specific to this Study

Criterion	**ACMG Guideline**	**Application of Criterion Specific to this Study**
PVS1	Null variant (nonsense, frameshift, +/- 1 or 2 splice site, initiation codon, single or multi-exon deletion) in a gene where loss of function is a known mechanism of disease	Applied as stated
PS1	Same amino acid change as previously established pathogenic variant regardless of nucleotide change	Applied as stated
PS2	*De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history of the disease	Applied as stated
PS3	Well established in vitro or in vivo function studies supportive of a damaging effect on the gene or gene product	One or more functional studies demonstrated a damaging effect on the gene or gene product
PS4	Prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls (RR/OR >5.0; CI does not include 1.0)	Applied as stated
PM1	Located in a mutational hotspot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation	Five or more pathogenic variants were identified five amino acid residues upstream or downstream of the variant and/or the variant was located in a

		well-established functional domain without benign variation
PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium	Variant not found in GnomAD
PM3	For recessive disorders, detected in *trans* with a pathogenic variant	Applied as stated
PM4	Protein length changes as a result of in-frame deletion/insertions in a non-repeat region or stop-loss variants	Applied as stated
PM5	Novel missense change at an amino acid residue where a different missense change determine to be pathogenic has been seen before	Applied as stated
PM6	Assumed *de novo*, but without confirmation of paternity and maternity	Applied as stated
PP1	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Variant identified in the proband was found in two or more affected family members
PP2	Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease	When missense variants were known to be a common mechanism of disease and the ExAC constraint data showed that the tolerance for missense variation in the gene had a standard deviation greater than two (Z>2)
PP3	Multiple lines of computational evidence support deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)	When the majority of the *in silico* programs (>75%) on Varsome predicted a deleterious effect for the variant
PP4	Patient's phenotype or family history is highly specific for a disease with a single gene etiology	This criterion did not apply to any of the variants in our study because they are all heterogeneous disorders
PP5	Reputable source recently reports variants as pathogenic, but the evidence is not available to the laboratory to do an independent evaluation	This criterion was not used as we wanted to rely on our own assessment of the relevant evidence

BA1	Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project or Exome Aggregation Consortium	Variant was found at an allele frequency of >5% in GnomAD
BS1	Allele frequency is greater than expected for disorder	Allele frequency in GnomAD was greater than 1.17e-06 for LQTS, 3.31e-07 for CPVT, 2.5e-06 for BrS
BS2	Seen in healthy adult for recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, full penetrance expected at an early age	This criterion was not relevant to the variants in this study as they are all expected to display reduced penetrance
BS3	Well established in vitro or in vivo functional studies show no damaging effect on protein function or splicing	One or more functional studies showing no effect on protein function or splicing
BS4	Lack of segregation in affected family members	One or more of the proband's affected family member's tested negative for the variant
BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	This criterion did not apply to any of the genes in this study
BP2	Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder OR in *cis* with a pathogenic variant in any inheritance pattern	Applied as stated
BP3	In-frame deletion/insertions in a repetitive region without a known function	Applied as stated
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	If a majority (>75%) of the *in silico* programs predicted a benign effect
BP5	Variant found in a case with an alternate molecular basis for disease	Applied as stated
BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	We chose not to use this criterion and rely on our own assessment of the relevant evidence
BP7	Synonymous (silent) variant, no predicted impact to splice consensus, no creation of new splice site AND nucleotide not highly conserved	Applied as stated

Family history information was extracted for those participants who had undergone genetic testing. The following information was recorded from the electronic medical record system, paper chart, and/or genetic testing report to help determine segregation of variants with disease phenotype: relatives who underwent genetic testing including the results of said testing, and whether those family members were affected or unaffected. The family history data collected was utilized to assess PS2, PM6, and BS4.

Data from problem lists, progress notes, ECGs, stress tests and physician letters were extracted to determine each participant's phenotype. This information was used to assess if specific clinical characteristics correlated with the original interpretation or the reinterpretation.

Next, multiple sources and online databases were consulted to compile additional supporting evidence for each variant's classification. VarSome, a human genomic variant search engine, was used to compile the following information for each genetic variant: the genomic coordinates, dbSNP RS ID, Gerp RS conservation scores, and multiple *in silico* prediction software outputs. The *in silico* program outputs reported on this website include MutationTaster, MutationAssessor, FATHMM, FATHMM-MKL, MetaSVM, MetalR, Provean, SIFT, PolyPhen, and LRT. Outputs were not reported for every program for every variant. VarSome was accessed between August 2017 and December 2017. The data collected from this database was utilized to assess PP3 and BP4.

The databases Uniprot and InterPro were consulted to determine the location of each variant and document any occurrences within an established domain or functional

site of a protein. The Online Mendelian Inheritance in Man (OMIM) was used to identify and record the following information for each gene in this study: reported phenotypes, mechanism for disease if known, and important functional domains and hotspots if known. This information was accessed between August 2017 and December 2017. The data collected from these sources was used to evaluate for PM1.

The most common type(s) of disease-causing variants for each gene within the cohort were determined using the Human Genome Mutation Database (HGMD). Each variant was also searched for within HGMD. If present, relevant literature and functional studies were recorded. Other reported pathogenic variants at the same amino acid position were also noted, as well as pathogenic variants within five residues upstream or five residues downstream of each variant. HGMD was access between August 2017 and December 2017. The data collected from this database was utilized to assess PS1, PS3, PM1, and PM5.

Next each variant was searched for in ClinVar, a freely accessible public archive of genetic variation and phenotype information. If the variant was present, we recorded the following information: all submitters and their corresponding interpretations, relevant literature and supporting evidence if provided, and other reported pathogenic missense variants at the same amino acid position. If the variant was not found in ClinVar, this was noted as well. ClinVar was accessed between August 2017 and December 2017. The data collected from this database was used to assess PS1, PS3, PS4, and PM5.

The Genome Aggregation Database Browser, GnomAD, was used to determine general population allele frequencies for all genetic variants in our study. Coverage data

was recorded for each gene within the cohort as well. This database contains both exome and genome sequencing data from a variety of large-scale sequencing projects. It is considered to be the most comprehensive and up-to-date aggregation of genomic data from healthy controls. GnomAD was accessed between August 2017 and December 2017. The data collected from this database were used to assess PM2, BA1, and BS2.

The Exome Aggregation Consortium (ExAC) browser was used to record constraint data for each gene within the cohort. This data provides a measure of a gene's intolerance to genetic variation. Genes with a reported missense variation Z score of greater than two were considered intolerant to missense variation. This method was determined by committee consensus, as this level of standard deviation is typically considered to be statistically significant. The ExAC browser was also utilized to assess for the presence of benign variation with allele frequencies greater than 0.1% for variants occurring within protein functional domains. The ExAC browser was accessed between August 2017 and December 2017. The data collected from this database were utilized to assess PM1 and PP2.

Lastly, PubMed was searched for additional relevant literature using the variant's one letter amino acid nomenclature, the three-letter amino acid nomenclature, and the DNA level nomenclature. Gene-specific literature regarding functional domain knowledge and information regarding any published hotspots was also searched. These searches occurred between August 2017 and December 2017. The data collected were used to assess PS3, PS4, PM1, and BS3.

The methods for variant classification in this study and the modifications made to the ACMG guidelines as stated above were established by committee consensus. Following evidence collection, the committee reviewed and discussed all interpretations. Any discrepancies in interpretation of the evidence or application of the guidelines were deliberated until a consensus was reached.

A letter was mailed to the cardiologist of those participants for whom a genetic variant was reclassified during our study. This letter encouraged providers to refer patients to a local cardiogenetics clinic and/or to seek reevaluation of the variant by the clinical laboratory that originally performed the testing. It was outside of the scope of this study to determine changes in clinical care (Appendix A).

Data Analysis

Descriptive statistics were used to describe variant reinterpretation rates. Chi square analyses were performed to determine factors associated with variant reclassification. Pathogenic variants, likely pathogenic variants, and class I variants were condensed into one group for the purpose of these analyses in order to evaluate "meaningful" changes in variant interpretation. Years were also condensed into three blocks: 2007 through 2011, 2012 through 2015, and post ACMG. Variant types were condensed into two groups: missense and single amino acid deletions versus frameshift, splice site, nonsense, and synonymous variants. When comparing the effects of having a diagnosis, all syndromes including LQTS, CPVT, and BRS were condensed into one

group and compared to individuals with dizziness, ventricular fibrillation, and a prolonged QT interval.

3. Results

Study Population

The initial NCH Heart Center database query identified 346 patients. Of those, 118 individuals had genetic testing reports available in their charts. 45 patients with completely negative or benign results were excluded for this study. Familial variants reported in multiple patients were condensed. This left a total of 61 unique genetic variants within a cohort of 73 pediatric arrhythmia patients (Figure 1). Twenty-three of these variants were VUS, 5 were likely pathogenic, 13 were pathogenic, and 20 were reported as class I variants (Figure 2). These variants were found in a total of twelve genes, including: *KCNQ1, KCNH2, SCN5A, RYR2, ANK2, DSP, CACA1C, CACNB2, CALM3, KCNE1, KCNE2,* and *PRKAG2* (Table 3). The vast majority of variants were present in *KCNQ1* (36%), followed by *KCNH2* (26%), and *SCN5A* (13%). Figure 3 illustrates these results.

When considering the primary proband for each unique variant, the cohort represented six clinical diagnoses including dizziness, ventricular fibrillation, prolonged QT interval, LQTS, CPTV, and BrS (Table 4). LQTS was by far the most common diagnosis, comprising 70% of the cohort (Figure 4). Original genetic testing report dates ranged from 2007 to 2017 (Table 5). 34% of variants were reported between 2007 and 2011, 36% were reported between 2012 and 2015, and 30% were reported in either 2016

or 2017 (Figure 5). While the study methodology intended to only capture patients who had received genetic testing prior to ACMG guideline implementation, there were several patients with appointment dates within the defined inclusion criteria who actually had genetic testing ordered post ACMG guideline publication. These variants were included in this study given the small number of unique variants identified.

Figure 1: NCH Heart Center Database Query Flowchart

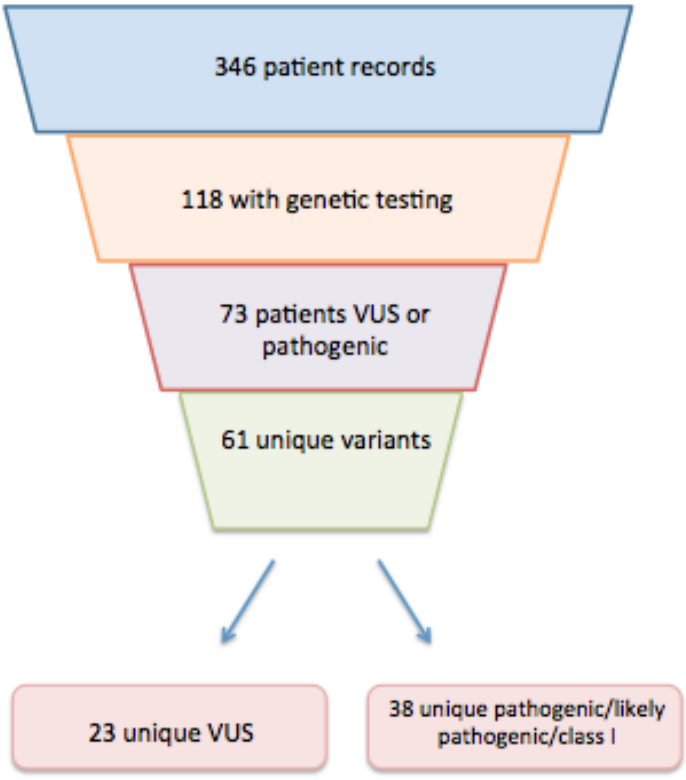


Figure 2: Reported Variant Classifications

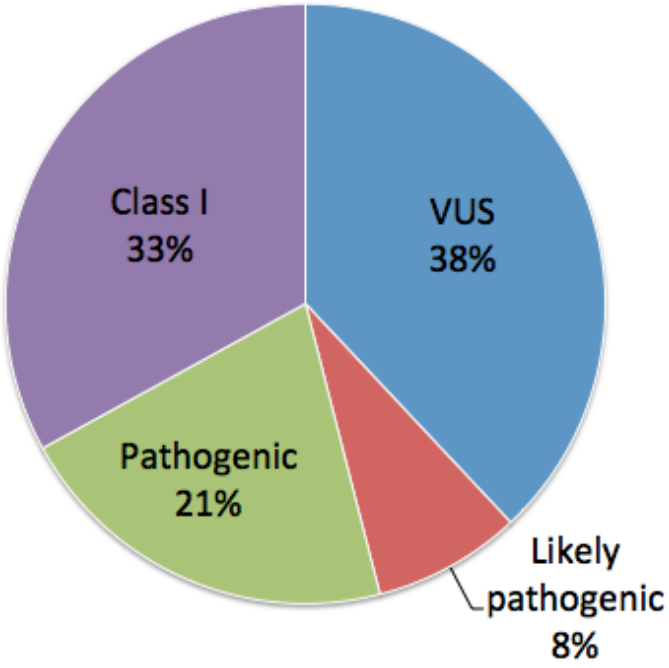


Table 3: Genes with Reported Variants

Gene	Frequency	Percentage
KCNQ1	22	36.1%
KCNH2	16	26.2%
SCN5A	8	13.1%
RYR2	6	9.8%
ANK2	2	3.3%
DSP	1	1.6%
CACA1C	1	1.6%

CACNB2	1	1.6%
CALM3	1	1.6%
KCNE1	1	1.6%
KCNE2	1	1.6%
PRKAG2	1	1.6%

Figure 3: Genes with Reported Variants

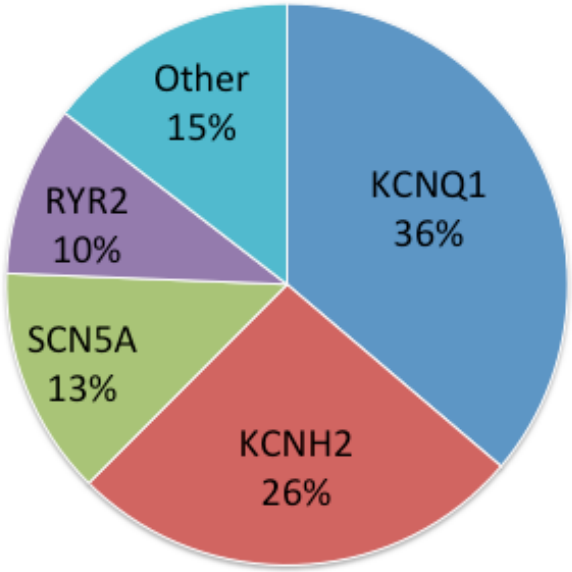


Table 4: Patient Cohort Diagnoses

Patient Diagnosis	Frequency	Percentage
Dizziness	3	4.9%
Ventricular fibrillation	2	3.3%
Prolonged QT interval	4	6.6%
LQTS	43	70.5%
CPVT	5	8.2%
BrS	4	6.6%

Figure 4: Patient Cohort Diagnoses

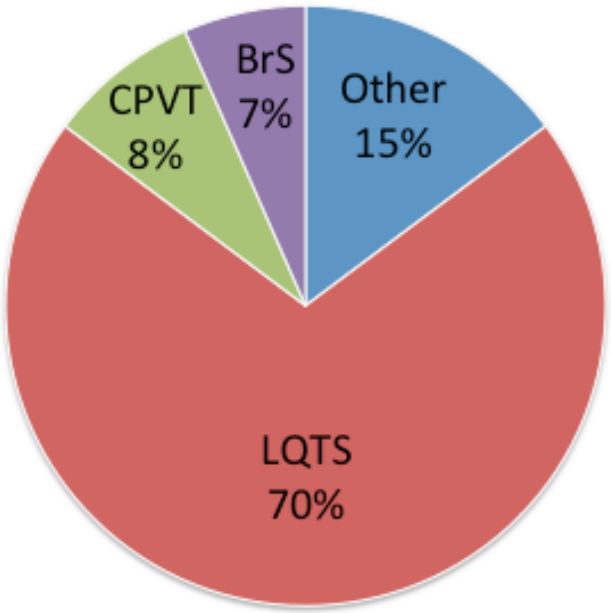
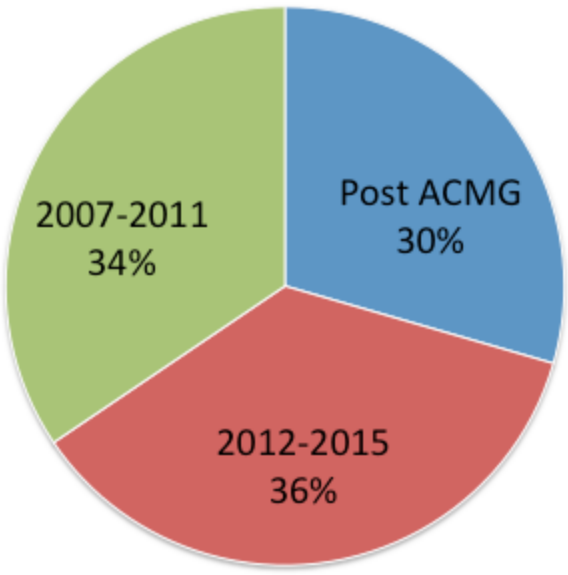


Table 5: Patient Cohort Genetic Testing Report Dates

Report Year	Frequency	Percentage
2016 and 2017	18	29.5%
2015	8	13.1%
2014	9	14.8%
2013	3	4.9%
2012	2	3.3%
2011	5	8.2%
2010	7	11.5%
2009	3	4.9%
2008	4	6.6%
2007	2	3.3%

Figure 5: Patient Cohort Genetic Testing Report Date



Frequency of Variant Reclassification

In total, 21 (34.4%) variants received a different classification as compared to the classification provided on the original report (Figure 6). Class I variants were only reclassified if they were found to be a VUS, likely benign, or benign. Of the variants reclassified, 52% were originally VUS, 10% were originally likely pathogenic, 24% were originally pathogenic, and 14% were originally class I variants (Figure 7). Of the 38 pathogenic/likely pathogenic/class I variants, 26.4% received a new classification. Of the 23 VUS variants, 11 (47.8%) received a new classification (Figure 8).

Figure 6: Percentage Reclassified Vs. Not Reclassified Variants

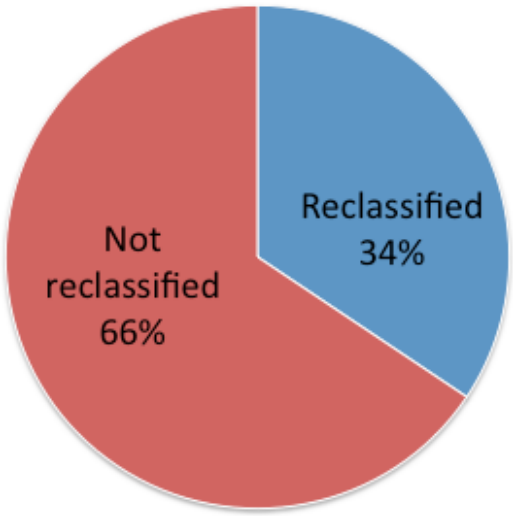


Figure 7: Original Variant Classifications of Reclassified Variants

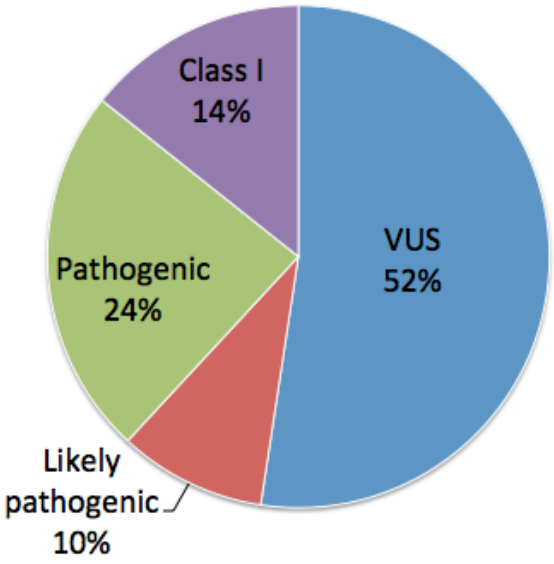
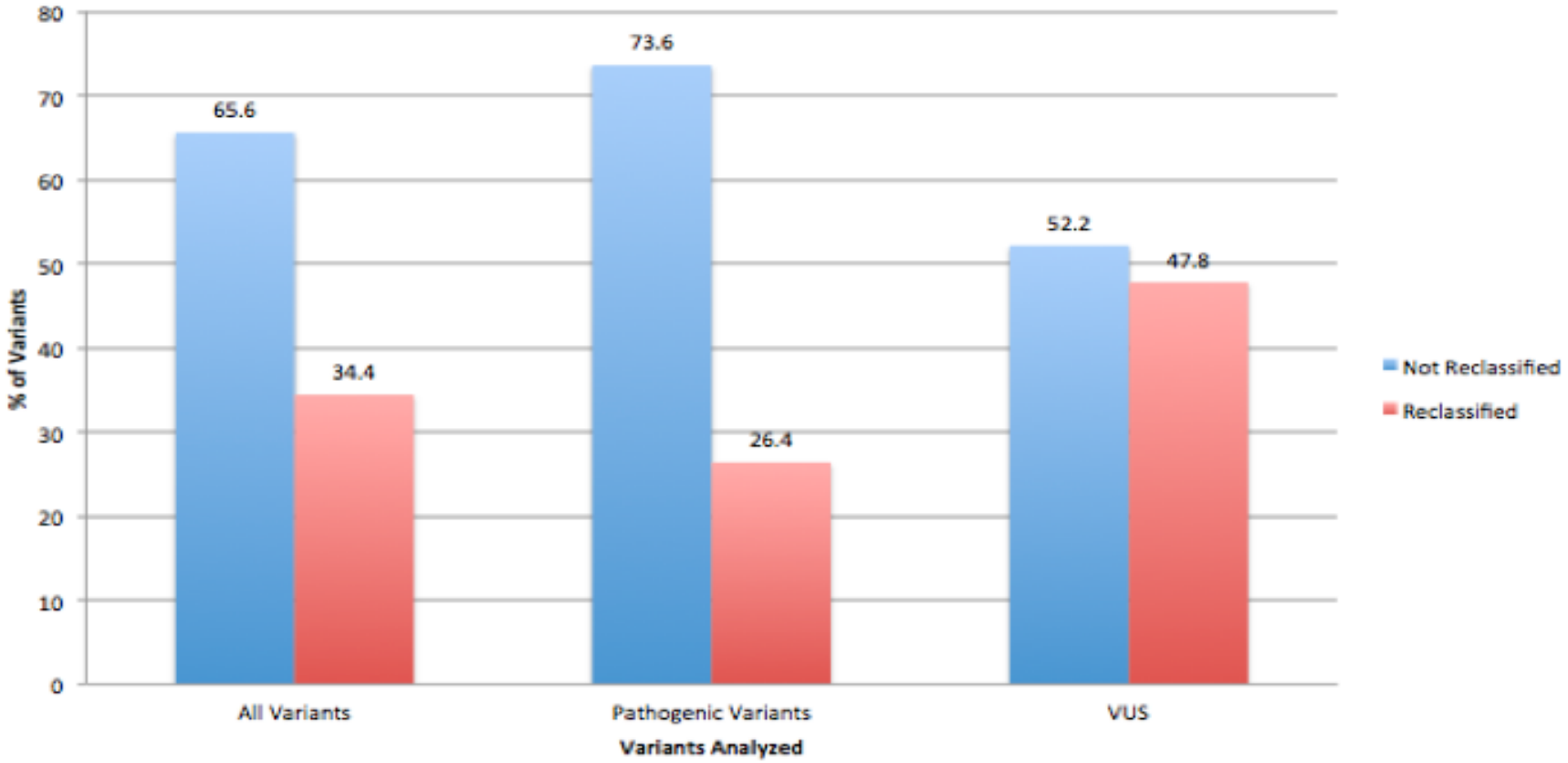


Figure 8: Percentage Reclassified Variants versus Not Reclassified Variants



The majority of variants that received a new classification (57.1%) were downgraded away from pathogenicity in their clinical significance. In addition, the majority of reclassifications (81.0%) occurred as one-step changes (a change between two adjacent classifications on the five-tiered scale). All other reclassifications (19.0%) occurred as two-step changes. 72.7% (8/11) of reclassified VUS were upgraded in their clinical significance moving toward pathogenicity, while 27.2% (3/11) were downgraded in clinical significance moving toward benign (Figure 9). Of the 38 pathogenic/likely pathogenic/class I variants, 10 (26.4%) received a new classification. The vast majority (90%) of these variants were downgraded in their clinical significance toward benign. Only one variant (10%) was reclassified from likely pathogenic to pathogenic (Figure 10, Figure 11). Table 6 below details all variants in the study and compares their reported classification to the reinterpreted classification.

Table 6: Reported Variant Classifications vs Study Variant Classifications and Clinical Significance Outcomes

Original Reported Classification	Reinterpreted Study Classification	Clinical Significance Outcome	ACMG Tiered System Outcome	# Of Variants (N=61)	%
VUS	Benign	Downgrade	Two step reclassification	1	1.6%
	Likely benign	Downgrade	One step reclassification	2	3.3%
	VUS	No change	No reclassification	12	19.7%
	Likely pathogenic	Upgrade	One step reclassification	7	11.5%
	Pathogenic	Upgrade	Two step reclassification	1	1.6%
Likely Pathogenic	VUS	Downgrade	One step reclassification	1	1.6%
	Likely pathogenic	No change	No reclassification	3	4.9%
	Pathogenic	Upgrade	One step reclassification	1	1.6%
Pathogenic	VUS	Downgrade	Two step reclassification	2	3.3%
	Likely pathogenic	Downgrade	One step reclassification	3	4.9%
	Pathogenic	No change	No reclassification	8	13.1%
Class I	VUS	Downgrade	One step reclassification	3	4.9%
	Likely pathogenic	No change	No reclassification	10	16.4%
	Pathogenic	No change	No reclassification	7	11.5%

Figure 9: Clinical Significance Outcomes for Variants of Unknown Significance

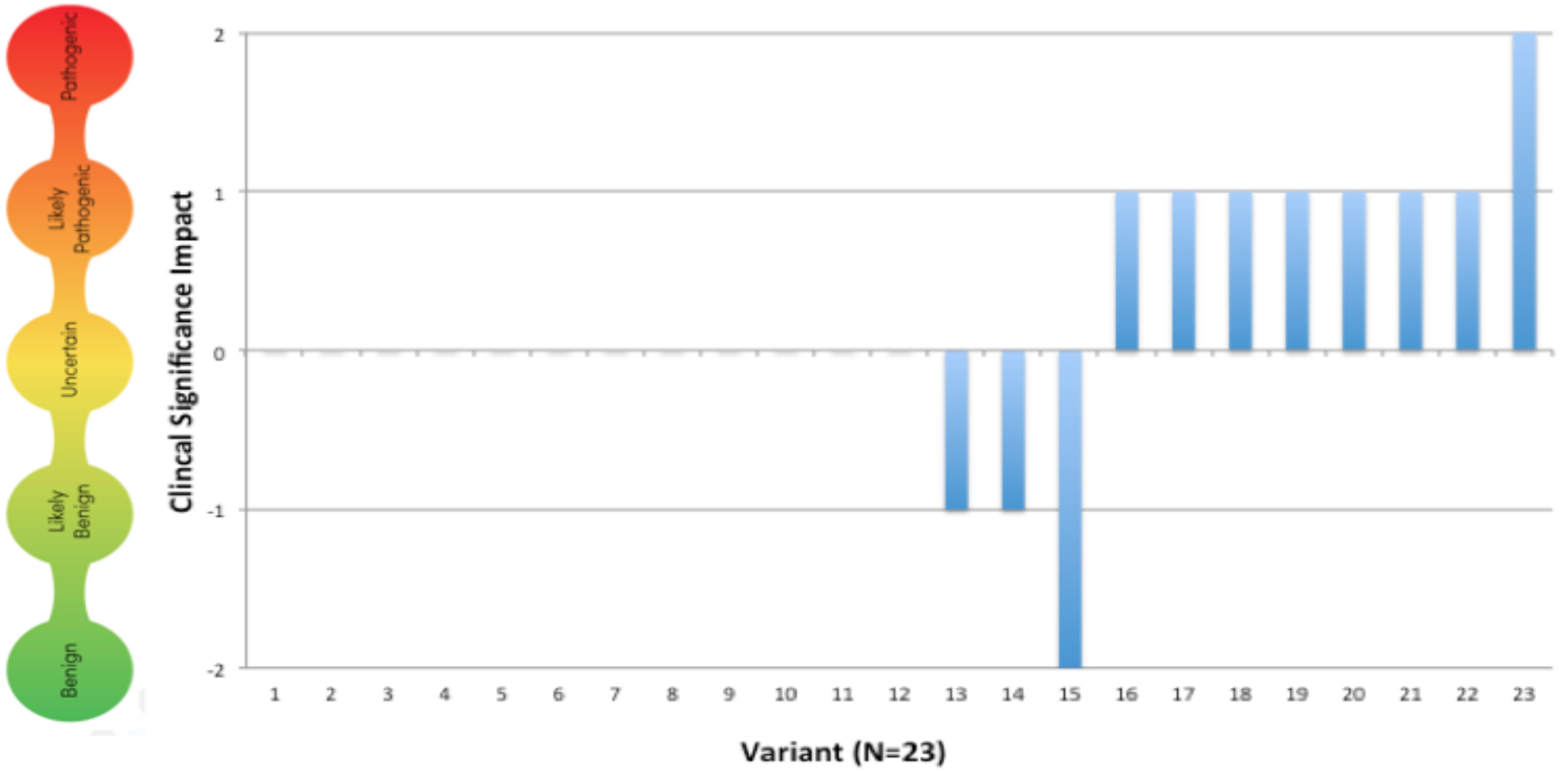


Figure 10: Clinical Significance Outcomes for Pathogenic Variants

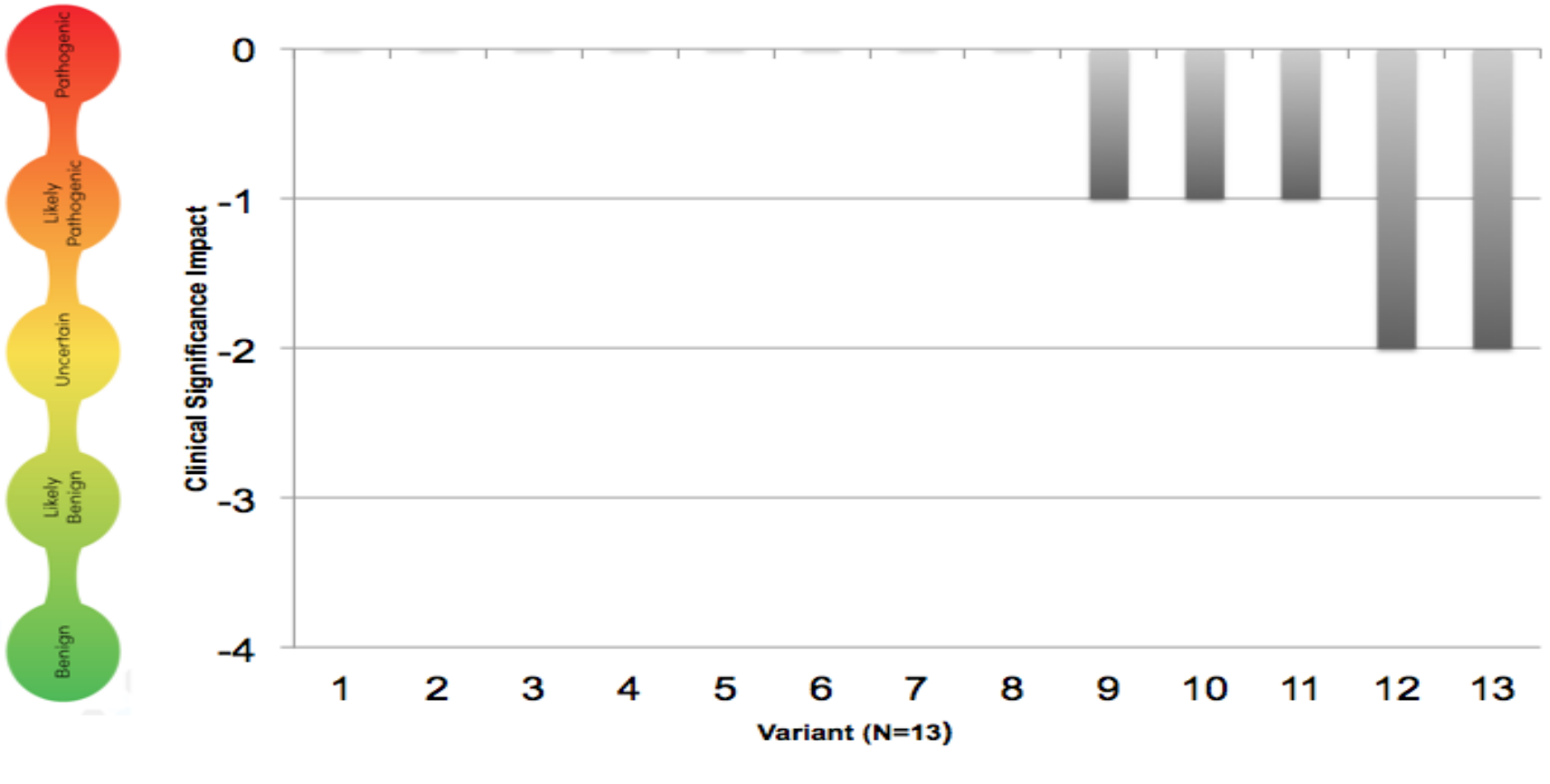
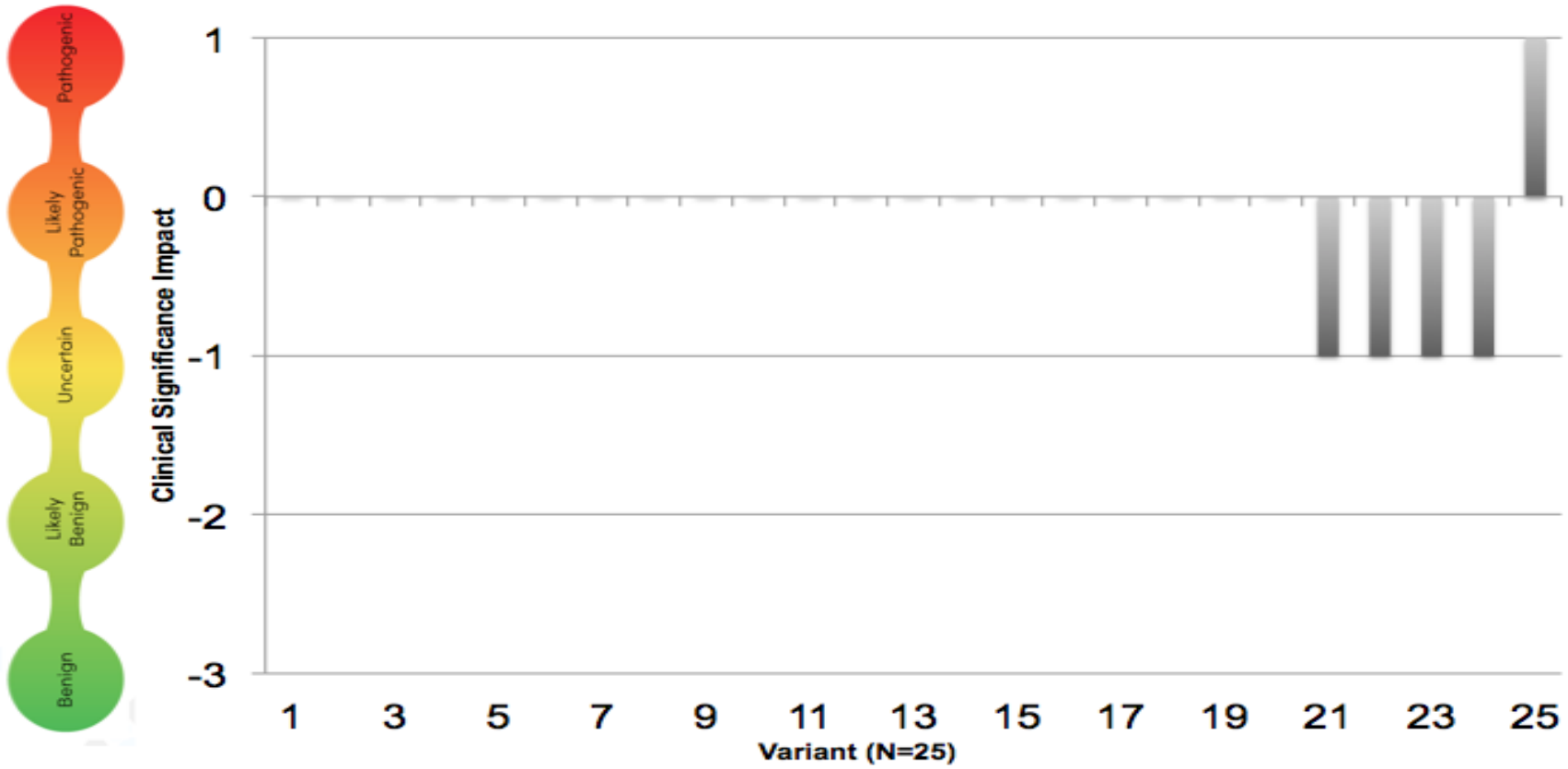


Figure 11: Clinical Significance Outcomes for Likely Pathogenic and Class I Variants



Factors Associated with Variant Reclassification

When analyzing all variant classifications together, a trend towards an association was found between reclassification and variant type. Missense variants and single amino acid deletion variants were more likely to be reclassified than nonsense, frameshift, and splice site, and synonymous variants ($p = 0.109$). A trend was also found between reclassification and years since laboratory test report date. Variants were more likely to be reclassified the longer it has been since the original report date ($p = 0.160$).

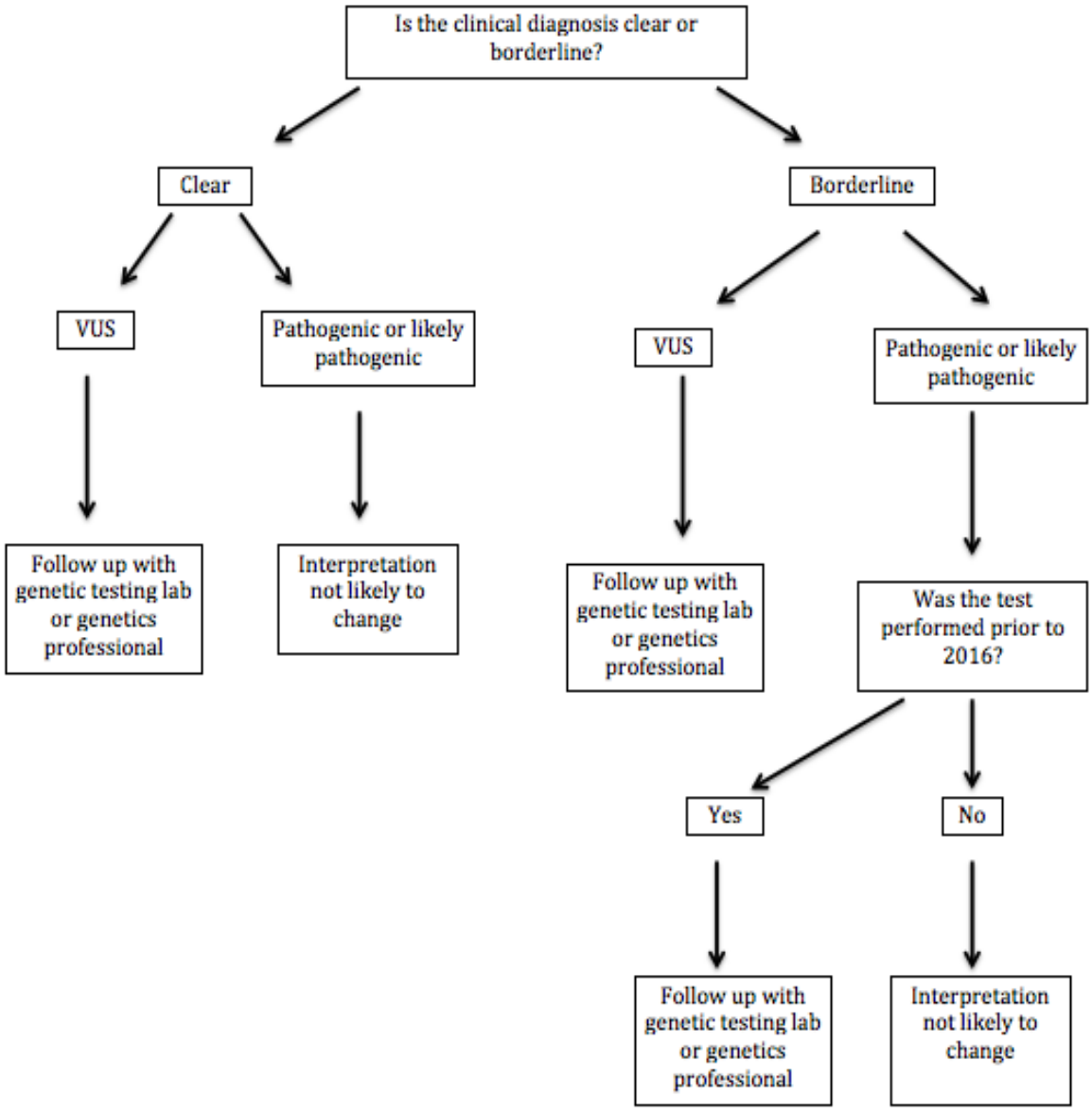
VUS and pathogenic/likely pathogenic/class I variant reclassifications were also analyzed separately. When analyzing VUS variants alone, trends toward an association were found between reclassification and medication use and having a clinical diagnosis of an inherited arrhythmia. Individuals prescribed beta-blocker medication harboring a VUS were more likely to be reclassified toward pathogenic ($p = 0.019$), as did those with a clinical diagnosis of an inherited arrhythmia syndrome ($p = 0.033$).

When analyzing the pathogenic/likely pathogenic/class I variants alone, trends toward an association were found between reclassification and variant type, implantation of an ICD, and whether or not the genetic testing was done prior to or post 2015 ACMG guideline publication. Patients without an ICD were more likely to have pathogenic/likely pathogenic variants reclassified ($p = 0.065$). Individuals with testing after ACMG guideline publication were less likely to be reclassified ($p = 0.168$). No pathogenic/likely pathogenic variants that were originally reported in either 2016 or 2017 received a new classification in this study. When only considering those pathogenic/likely pathogenic

variants reported prior to ACMG guideline publication, 10 out of 30 (33%) received a new classification.

The following decision tree was created to assist cardiologists evaluate the need for variant reevaluation in patients with previous genetic testing results (Figure 12).

Figure 12: Variant Reevaluation Decision Tree



4. Discussion

This study is the first, to our knowledge, to assess genetic variant reclassification based on the application of the 2015 ACMG Guidelines among a cohort of pediatric arrhythmia patients. There is an abundance of published literature on the challenges of interpreting genetic data (Ackerman, 2015; Allen et al., 2013; Balamana et al., 2016; Campuzano et al., 2015; Guidicessi et al., 2013; Kapa et al., 2009; Pepin et al., 2016; Quintans et al., 2014; Richter et al., 2013), but few studies have assessed the fluidity of genetic data interpretation and classification (Das et al., 2014; Manrai et al., 2016). This study demonstrates the impact of applying the 2015 ACMG Guidelines for variant interpretation and is the first to investigate factors that may be associated with variant reclassification. Clinical characteristics and treatment implementation often correlated with variant reclassification, demonstrating the need to incorporate both molecular genetic information and phenotype information together in the practice of variant evaluation.

Recommendations Regarding Variant Reevaluation

Our understanding of a genetic variant's disease impact is not static. This study demonstrates the need to revisit previous genetic testing results, as more than one third of the variants evaluated in this study received a different classification than that provided

on the original genetic testing laboratory report. It is important to note, however, that no pathogenic or likely pathogenic variants reported after ACMG guideline publication (ie reported in 2016 or 2017) received a clinically "meaningful" reclassification. Of the pathogenic/likely pathogenic variants reported prior to ACMG guideline publication, 15.8% (6/38) received a clinically "meaningful" reclassification to VUS.

The frequency of reclassification found in this study is higher than other groups have reported. Das et al. (2014), reinterpreted variants among a cohort of hypertrophic cardiomyopathy patients and found that 10% of the variant analyzed were reclassified. Das's study, however, was conducted prior to the publication of the 2015 ACMG Guidelines and only evaluated for meaningful changes (ie only had a three tiered classification system including benign, unknown, and pathogenic). The difference seen in rate of reclassification between Das's study and this study could be due to the fact that this study evaluated for small changes in variant classification.

The results of this study suggest that variant reassessment based off of the 2015 ACMG Standards and Guidelines for Interpretation of Sequence Variants may reclassify genetic variants and affect clinical management of inherited arrhythmia patients. It would be extremely beneficial to have a systematic variant reinterpretation protocol in place to help ensure that every patient and their family members are being managed based on the most up-to-date information available. While providing the most accurate result is important on the laboratory side, it is important to keep in mind that a patient's genetic testing results could have implications for their own medical management and the management of their family members. Thus, especially for conditions that include a risk

for sudden death, as these inherited arrhythmias do, regular reanalysis of variants may be necessary.

Cascade genetic testing of family members to provide risk assessment for unaffected individuals is only indicated when a likely pathogenic or pathogenic variant has been identified in the proband. As variants of unknown significance were commonly reclassified toward pathogenicity in our cohort, there may be asymptomatic family members who are actually at risk for sudden cardiac death who are not receiving prophylactic screening and therapies. These family members would be recognized as needing access to these therapies in a more timely, and perhaps life-saving, fashion with the implementation of a variant reevaluation process.

There is currently no standardized protocol for informing patients if and when their genetic variant receives a new classification. Most laboratories will only follow-up and issue a new, updated report if the variant is come across again while testing a different, unrelated patient or at the request of a patient's treating physician. With the current practice in place, individuals with a novel VUS variant may never have their variant reassessed unless prompted to do so by their physician.

Making the suggestion to implement a variant reinterpretation protocol begs the question: should variant reevaluation follow-up be the responsibility of the laboratory that performs the genetic testing or the patient's ordering provider? Many providers who order genetic testing may have only a limited background in genetics (Barry et. al, 2012). Conversely, genetic counselors have extensive training in clinical genetics and can be an asset in variant reevaluation. Variant classification, however, is not currently considered

within their scope of practice (NSGC Scope of Practice, Accessed 2017). The National Society of Genetic Counselors (NSGC) should consider creating practice guidelines to outline the role of genetic counselors in the interpretation of genetic sequence variants. While most genetic counselors do not have the molecular genetics background necessary to adequately evaluate a genetic variant's disease impact, they do have training in navigating genetic databases, relaying phenotype information to the testing laboratory and facilitating follow-up variant testing. Genetic counselors and other genetic professionals should work in collaboration with laboratory personnel to gather evidence for variant classification. Interpreting the disease impact of a genetic variant relies strongly on both clinical information and molecular knowledge.

Follow-up variant analysis of a proband's parents to determine whether a variant was inherited or occurred *de novo* can help provide evidence of pathogenicity. Variants that occur *de novo* are more likely to be disease causing than variants inherited from an unaffected parent. *De novo* variants in which maternity and paternity is confirmed provides more weight toward pathogenicity compared to *de novo* variants for which maternity and paternity is not confirmed. Confirmed *de novo* status is considered strong evidence for pathogenicity, while assumed *de novo* status is considered moderate evidence for pathogenicity based on the ACMG guidelines. Verifying maternity and paternity for *de novo* variant carriers is not currently a common clinical practice in the setting of genetic testing for inherited arrhythmia syndromes.

Although maternity and paternity testing for cases of *de novo* VUS's could help to elucidate their disease impact, one can imagine several barriers to implementing this

testing into clinical practice. While pretest counseling and informed consent would be extremely important to ensure that families were aware of the risk to discover non-paternity, several pitfalls would still exist. First, families worried about revealing non-paternity may not be willing to participate in the follow-up testing. Clinicians may also find themselves revealing and discussing cases of non-paternity with their patients, which could harm rapport between the family and the treating physician. Lastly, learning this information could also be extremely disruptive to a family dynamic and challenging for a child to cope with.

To investigate the disease impact of inherited VUS, follow-up variant analysis of affected family members can also be ordered. This practice can help to determine whether or not the variant in question is tracking with the disease phenotype within the family. Cosegregation of disease in multiple affected family members is supporting evidence for pathogenicity based on ACMG guidelines. Alternatively, lack of segregation in affected family members is strong evidence against pathogenicity based on ACMG guidelines.

Challenges with this practice worth noting, however, are the difficulties in defining who within a family are "affected," and the potential for decreased penetrance in individuals harboring a pathogenic variant. A proband's parents, siblings or other family members who are being evaluated as apart of a VUS investigation should receive appropriate cardiac evaluation, depending on the specific diagnosis, to determine whether or not they are affected. It is not sufficient to rely on the presence or absence of outward

features such as syncope. These familial investigations may help to elucidate the impact of a given VUS and influence its interpretation for other, unrelated patients.

Application of the 2015 ACMG Guidelines

The implementation of the 2015 ACMG Guidelines has only begun to scratch the surface of addressing the current challenges facing variant interpretation and reporting. For the purpose of implementing a systematic methodology in this research study, many of the criterions were given more specific definitions regarding their application. This illustrates a limitation with the current guidelines for variant interpretation. Differences in how one chooses to apply the guidelines may result in discordant interpretations of the same variant.

The 2015 ACMG Guidelines could be improved by making the criterions more specific. For example, criterion BS1 states: "allele frequency is greater than expected for disorder." How to determine an appropriate, yet conservative, expected allele frequency for a given disorder has been of recent debate (Kobayashi et al., 2017). This challenge is especially relevant when considering conditions like the inherited arrhythmia syndromes that display reduced penetrance. Pathogenic variants known to be causative of an inherited arrhythmia syndrome may be present in healthy population databases given the fact that not all individuals who harbor a pathogenic mutation show symptoms of the disease.

As an additional example, criterion PP1 states "cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease."

This guideline does not outline how many family members must be affected in order for this criterion to be applied, or how to define an "affected individual." This is an extremely important consideration for conditions that can be extremely variable in their presentations. In the case of the inherited arrhythmia syndromes, an individual may have no indication that they are affected until an electrocardiogram is performed or genetic testing reveals a pathogenic mutation. The current ACMG guidelines are not specific enough to address many of the intricacies associated with the genetics of cardiovascular disease.

There are also many criteria that are not applicable to the genes that cause the inherited arrhythmia syndromes. For example, the criterion PP4 states: "patient's phenotype or family history is highly specific for a disease with a single genetic etiology." The inherited arrhythmia syndromes are heterogeneous and, often times, the same gene can be responsible for more than one phenotype and a single phenotype can be caused by multiple genes. For example, mutations in the *SCN5A* gene are known to cause both LQTS and BrS. The 2015 ACMG Guidelines are not as effective for interpreting variants that have more complex inheritance patterns compared to variants with Mendelian inheritance. Many of the criteria do not apply to heterogeneous conditions with reduced penetrance. Some groups have proposed gene-specific guidelines for variant interpretation as a solution to the challenge of interpreting more molecularly complex conditions (Gelb et al., 2018; Kelly et al., 2018; Patel et al., 2017).

Another challenge in applying the ACMG Guidelines is navigating the many databases and online resources used to find supporting or refuting evidence for each

variant's pathogenicity. Currently, there is no single place to go to in order to find all supporting evidence. Several resources must be consulted, making this is a very time consuming process. Many of the available resources are not routinely updated and they often contain out-of-date variant interpretations. In addition, many of the public variant catalogues can be updated by anyone; no specific credentials or genetics background is necessary. This could potentially lead to the presence of unreliable information on these websites given the extremely complex nature of interpreting variants.

Finding evidence can be further complicated by the use of alternative variant nomenclature. A single variant may be described using a handful of 'names.' This makes it very easy to miss information about a particular variant if you are not using the correct nomenclature for that resource. Additionally, practices in variant nomenclature have changed in the past couple of years. Several search terms must be attempted when conducting a PubMed search to determine whether or not a variant has been reported before. These searches may not come back with a hit if the variant is only mentioned within a table or figure within an article. For rare variants that may not have an entire publication dedicated to them, this is a very important consideration. It would be extremely beneficial to have a publically available one-stop resource for interpreting genetic variants. Equal and easy access to the information used to evaluate variants would help reduce the rate of discordant interpretations between multiple reviewers and increase efficiency of the variant classification process.

Study Limitations

This study had several limitations. Our initial cohort of patients was obtained using an NCH Heart Center database query. This system was unable to filter based on the presence or absence of genetic testing within a patient's chart. It is possible that the inclusion criteria used did not identify all patients during our specified time frame that actually had genetic testing for an inherited arrhythmia syndrome. Because of this, additional unique variants may have been missed. The method used to identify participants also captured individuals who had their genetic testing ordered after the publication of the ACMG guidelines. We elected to include these variants in our analysis due to our study's small sample size. Although this study was able to demonstrate trends, many of the statistical analyses generated were not statistically significant. This could be due, in part, to a small sample size.

Another limitation to this study is that not all of the genetic testing laboratories provided the genetic transcript used on their reports. In this situation, the most common transcript for the gene was assumed. It is possible that a variant determined not to be present within a database, was actually present listed under an alternative nomenclature or a different genetic transcript.

This study also defined specific applications for several of the ACMG guideline criteria. In addition, some criteria were not used. Other reviewers may reach a different classification for a particular variant than what was reported in this study due to having different specifications for how and when to apply a particular guideline. In order to maintain consistency in the interpretation process, evaluation of variants was very

systematic throughout this research study. The same databases were consulted and the same methods for collecting evidence were used for each variant. Application of the 2015 ACMG guidelines may not be as systematic in real-life variant evaluation.

It is also important to note that we did not have access to every published journal article. It is possible that some information available to other reviewers was missed and thus not incorporated into our variant interpretations.

Directions for Future Research

This study was able to demonstrate several trends related to variant reclassification; however, the small sample size made it challenging to reach statistical significance for many comparisons. More research is needed on this topic to generate a larger sample size of unique variants. Given the large proportion of variants receiving a new classification in this study, it would be advantageous to examine patient and family member reactions to variant reinterpretation, as well as determine the specific changes to clinical care when a reclassification is made. This study showed that variant reclassification is common, but an appropriate interval for variant reevaluation has not yet been determined. In addition, this study was focused on evaluating variants from a small cohort of pediatric arrhythmia patients. Assessment regarding the impact of variant reinterpretation among other patient populations should be considered.

Study Conclusions

This study demonstrates that variant reclassification is common in the setting of genetic testing for inherited arrhythmia syndromes. Approximately one third of the variants analyzed in this study were reclassified with the application of the recommendations detailed in "Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology." Given these results, it is reasonable to consider implementing a protocol for periodic variant reevaluation in order to ensure up-to-date management of all patients and their family members. Clinicians and laboratory personnel should work together in collaboration to investigate the disease impact of genetic variants. Both phenotype information and genetic data should be incorporated together in clinical practice.

The application of the 2015 ACMG Guidelines can be improved to include more exact specifications regarding when and how to apply each criterion. Considerations should be made for those conditions that display heterogeneity, reduced penetrance and variable expressivity. Introducing gene specific guidelines may provide a solution to these challenges. In addition, the creation of a publically available one-stop resource for variant interpretation may help to reduce the rate of discordant interpretations between reviewers and increase the efficiency of the variant interpretation process. Having a variant reinterpretation protocol in place and improving upon the 2015 ACMG Guidelines would result in better care of inherited arrhythmia patients and their family members.

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Appendix A: Letter Sent To Referring Provider

Date:

Re: Patient Name
DOB:
MRN:

Dear [physician's name],

As you are aware, NAME had genetic testing for Long QT syndrome/CPVT/Brugada/etc in YEAR. The result of this test was positive/variant of unknown significance.

Recently, we undertook a study to re-evaluate previously completed cardiac genetic testing in light of the recent American College of Genetics and Genomics (ACMG) guidelines on variant interpretation.

Therefore, we are contacting you in regard to your patient, [name], to inform you that our recent research interpretation of [his/her] variant was different than the original interpretation. As this may affect medical management we wanted to inform you of this research result and also provide you with contact information for programs that could provide clinical reinterpretation of this variant.

The variant found in your patient was [variant] in the [gene] gene, originally classified as [previous classification]. Based on our research findings, a new classification of [new classification] may be more appropriate.

Several institutions in Ohio offer cardiovascular genetics clinics and are listed below.

Nationwide Children's Hospital
Cardiogenetics Clinic, Heart Center
700 Children's Dr., Columbus, OH 43205
(614) 722-2530

The Ohio State Wexner Medical Center
Richard M. Ross Heart Hospital
452 W 10th Ave, Columbus, OH 43210

(614) 293-7677

Cincinnati Children's Hospital
Heart Institute
3333 Burnet Avenue, Cincinnati, Ohio 45229-3026
(513)-636-4200

The Cleveland Clinic
Miller Family Heart and Vascular Institute
9500 Euclid Ave, Cleveland, OH 44106
(800) 659-7822

We urge you to consider this reinterpretation and to follow-up with [patient's name] as you see fit.

Best,

Anna Kamp, MD, MPH
Kim McBride, MD
Vidu Garg, MD
Sara Fitzgerald-Butt, MS, LGC
Madison Bernhardt, Master's Candidate
Jeffrey Bennett, MD, PhD

Appendix B: Supplementary Variant Interpretation Data

DNA Result	Protein Result	Gene	Transcript	Criterions Applied
c.2985G>A	p.Glu995Glu	DSP	NM_004415.3	BS1
c.1277C>T	p.Pro426Leu	KCNH2	NM_000238.3	PM1, PM2, PM5, PP1, PP2, PP3
c.1882G>A	p.Gly628Ser	KCNH2	NM_000238.3	PS3, PM1, PM2, PM5, PP2, PP3
c.3099_3109delII	p.Pro1034fs	KCNH2	NM_000238.3	PVS1, PM1
c.1778T>A	p.Ile593Lys	KCNH2	NM_000238.3	PM1, PM5, PP3, BS1
c.3017delG	p.Gly1006fs	KCNH2	NM_000238.3	PVS1, PM2, PP1
c.2398+1G>T	NA	KCNH2	NM_000238.3	PVS1, PM2, PP3
c.2145+1G>A	NA	KCNH2	NM_000238.3	PVS1, PM2, PP1, PP3
c.2759G>A	p.Arg920Gln	KCNH2	NM_000238.3	PM1, PM5, PP2, BS1
c.2467C>T	p.Arg823Trp	KCNH2	NM_000238.3	PS3, PM1, PM2, PP1, PP2,

				PP3
c.1684C>T	p.His562Tyr	KCNH2	NM_000238.3	PM1, PM2, PM5, PP2, PP3
c.1886A>T	p.Asn629Ile	KCNH2	NM_000283.3	PM1, PM2, PM5, PP2, PP3
c.1942G>T	p.Gly648Cys	KCNH2	NM_000283.3	PM1, PM2, PM5, PP2, PP3
c.565_568delGGCG	p.Gly189ProfsX11	KCNH2	NM_000283.3	PVS1, PM2
c.1781G>A	p.Arg594Gln	KCNQ1	NM_000218.2	PS3, PM1, PM5, PP2, PP3, BS1
c.499_501delTCC	p.Phe167del	KCNQ1	NM_000218.2	PS1, PM2
c.935C>T	p.Thr312Ile	KCNQ1	NM_000218.2	PM1, PM2, PP2, PP3
c.107dupT	p.Ser37fs	KCNQ1	NM_000218.2	PVS1, PM2, PP1
c.1394-1G>T	NA	KNQ1	NM_000218.2	PVS1, PM2, PP3
c.401T>C	p.Leu134Pro	KCNQ1	NM_000218.2	PM2, PP2, PP3
c.488delT	p.Leu163fs	KCNQ1	NM_000218.2	PVS1, PM2
c.502G>A	p.Gly168Arg	KCNQ1	NM_000218.2	PS1, PS3, PM1, PM2, PP2, PP3
c.1031C>A	p.Ala344Glu	KCNQ1	NM_000218.2	PM1, PM2, PM5, PP2, PP3
c.108insT	p.Phe36fs+247X	KCNQ1	NM_000218.2	PVS1, PM2

c.1031C>T	p.Ala344Val	KCNQ1	NM_000218.2	PS3, PM1, PM2, PM5, PP2, PP3
c.458C>T	p.Thr153Met	KCNQ1	NM_000218.2	PP2, PP3, BS1
c.1032G>A	p.Ala344Ala	KCNQ1	NM_000218.2	PM5, PM1, PS1
c.1178A>T	p.Lys393Met	KCNQ1	NM_000218.2	PS3, PM2, PM5, PM1, PP3, PP2
c.781G>C	p.Glu261Gln	KCNQ1	NM_000218.2	PM1, PM2, PM5, PP2, PP3
c.797T>C	p.Leu266Pro	KCNQ1	NM_000218.2	PS3, PM1, PM2, PP2, PP3
c.973G>A	p.Gly325Arg	KCNQ1	NM_000218.2	PS1, PS3, PM1, PM2, PM5, PP2, PP3
c.1394-1G>T	NA	KCNQ1	NM_000218.2	PVS1, PM2
	Del-exon3	RYR2	NM_001035.2	PVS1, PM2
c. 1603C>T	p.Arg535Ter	SCN5A	NM_001160161.1	PVS1, PM1
c.1338+2T>A	NA	SCN5A	NM_001160161.1	PVS1, PM2, PP3
c.4519_4527del	p.Gln1507_Pro1509del	SCN5A	NM_001160161.1	PVS1, PM2
	Del-exon4-6	SCN5A	NM_001160161.1	PVS1, PM2
c.11823G>T	p.Lys3941Asn	ANK2	NM_001148.4	PM2, PP3, BS4

c.8395G>A	p.Asp2799Asn	ANK2	NM_001148.4	BS1, BP4
c.911T>C	p.Ile304Thr	CACNA1C	NM_000119.6	PP2, BS1, BP4
c.1347G>T	p.Arg449Ser	CACNB2	NM_201590	PM2
c.286G>C	p.Asp96His	CALM3	NM_005184.2	PM1, PM2, PM5, PP2, PP3
c.23C>T	p.Ala8Val	KCNE1	NM_000219.5	PS3, BS1
c.229C>T	p.Arg77Trp	KCNE2	NM_172201.1	BS1, BS3
c.1895G>A	p.Ser620Asn	KCNH2	NM_000283.3	PM1, PM2, PP1, PP2, PP4
c.2312A>G	p.His771Arg	KCNH2	NM_000238.3	PM1, PM2, PP1, PP2, PP3
c.1652T>C	p.Phe551Ser	KCNH2	NM_000238	PM2, PP2, PP3
c.1097G>T	p.Arg366Leu	KCNQ1	NM_000218.2	PM2, PM5, PP1, PP3
c.1135T>C	p.Trp379Arg	KCNQ1	NM_000218.2	PM2, PM6, PP2, PP3
c.1576A>G	p.Lys526Glu	KCNQ1	NM_000218.2	PS3, PP2, PP3, BS1
c.1655G>A	p.Ser548Ser	PRKAG2	NM_016203.3	PM5, BS1, BP5
c.14093T>C	p.Leu4698Pro	RYR2	NM_001035.2	PM2, PP2, PP3

c.14288A>G	p.Asn4763Ser	RYR2	NM_001035.2	PM1, PM2, PM6, PP2, PP3, PP5
c.1259G>A	p.Arg420Gln	RYR2	NM_001035.2	PS3, PM2, PM5, PM6, PP2, PP3
c.6430C>T	p.Arg2144Cys	RYR2	NM_001035.2	PP2, PP3, BS1
c.11318T>C	p.Val3773Ala	RYR2	NM_001035.2	PM2, PP2, PP3, BP5
c.3540G>A	p.Ala1180Ala	SCN5A	NM_198056.2	BS1, BP6
c.694G>A	p.Val232Ile	SCN5A	NM_000335.4	PP2, PP3, BS1
c.5038G>A	p.Ala1680Thr	SCN5A	NM_198056.2	PP2, PP3, BS1
c.529A>G	p.Met1766Val	SCN5A	NM_198056.2	PM1, PM2, PM5, PP2, PP3