HeartRhythm

The Official Journal of the Heart Rhythm Society, The Cardiac Electrophysiology Society, and The Pediatric & Congenital Electrophysiology Society

Heart Rhythm Society SM

The frequency of gene variant reclassification and its impact on clinical management in the inherited arrhythmia clinic

William J. Young, PhD,[1,2] Soe Maung, MBBS,[1] Selda Ahmet, MBBS,[1] Claire Kirkby, MSc,[1] Charlotte Ives, PGCert,[1] Richard J. Schilling, MD,[1] Martin Lowe, MD,[1] Pier D. Lambiase, PhD, FHRS[1,3]

ABSTRACT

BACKGROUND Genetic testing in the inherited arrhythmia clinic informs risk stratification, clinical management, and family screening. Periodic review of variant classification is recommended as supporting evidence accrues over time. However, there is limited reporting of real-world data on the frequency and impact of variant reclassification.

OBJECTIVE The purpose of this study was to determine the burden of variant reclassification in our inherited arrhythmia clinic and the impact on clinical management.

METHODS Genetic testing reports for patients referred to our clinic from 2004–2020 were reviewed. Reported variants were reinvestigated using ClinVar, VarSome, and a literature review. Classification was updated using the American College of Medical Genetics and Genomics (ACMG) criteria and tested for association with arrhythmic events and modification of medical management.

RESULTS We identified 517 patients (median age 37 years) who underwent gene panel testing. A variant of uncertain significance (VUS) was reported for 94 patients (18.2%) and more commonly identified when using large gene panels ($P < .001$). A total of 28 of 87 unique VUSs (32.2%) were reclassified to pathogenic/likely pathogenic (n = 11) or benign/likely benign (n = 17). Of 138 originally reported pathogenic variants, 7 (5.1%) lacked support using ACMG criteria. Variant reclassification was not associated with arrhythmic events; however, it did impact genotype-specific counseling and future therapeutic options.

CONCLUSION In our large real-world patient cohort, we identify a clinically important proportion of both pathogenic variants and VUSs with evidence for reclassification. These findings highlight the need for informed pretest counseling, a regular structured review of variants reported in genetic testing, and the potential benefits to patients for supporting genotype-guided therapy.

KEYWORDS Inherited arrhythmia; Channelopathy; Genetic testing; Variant of uncertain significance; Reclassification

(Heart Rhythm 2024;■:1–8)

Introduction

Genetic testing in specialist inherited arrhythmia clinics has diagnostic, prognostic, and therapeutic implications, particularly for long QT syndrome (LQTS).[1] It also informs cascade screening and identification of at-risk family members. Next-generation sequencing and broad multigene panels have increased the potential to detect disease-causing (pathogenic) variants.[2] However, 20%–40% of variants reported are variants of uncertain significance (VUSs), which are not actionable and can create uncertainty for patients and clinicans.[2–5]

A variant's classification may change over time as additional evidence is accrued from functional evaluation, co-segregation with clinical phenotypes, and reporting of allele frequencies from large population datasets. The American College of Medical Genetics and Genomics (ACMG) guidelines recommend periodic review of VUSs and emphasize the role of the patient's leading physician for this

From the [1]Barts Heart Centre, St Bartholomew's Hospital, London, United Kingdom, [2]Centre for Clinical Pharmacology and Precision Medicine, Queen Mary University of London, London, United Kingdom, and [3]Institute of Cardiovascular Science, University College London, London, United Kingdom.

https://doi.org/10.1016/j.hrthm.2024.01.008

responsibility.[6] However, the frequency of variant reclassification and the impact on clinical management in the inherited arrhythmia clinic are unclear because of limited reporting of real-world data.

The aims of this study were to determine the burden of pathogenic or VUS reclassifications in a large patient cohort that underwent genetic testing in our inherited arrhythmia clinic and evaluate the impact on clinical management.

Methods

Genetic testing reports were retrieved for patients referred to our inherited arrhythmia clinic at St. Bartholomew's Hospital and the Heart Hospital (London, United Kingdom) from 2004 to 2020. For each patient, the indication for genetic testing, variants reported, and original classification were recorded. Family files were reviewed to identify support for variant co-segregation with the clinical phenotype. Arrhythmia events were defined as syncope suspected to be secondary to arrhythmia; documented ventricular arrhythmia; or bradyarrhythmia (if diagnosed with progressive cardiac conduction disease).

A search was performed for each variant using ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/)[7] and VarSome (https://varsome.com/),[8] which are publicly available databases that aggregate evidence for the assessment of pathogenicity. This includes variant annotation and *in silico* predictions for deleteriousness using >20 different tools and combined meta-scores. Allele frequencies from the Genome Aggregation Database were extracted.[9] An allele frequency threshold <0.0001 (0.01%) was used to report rare variants with potential to cause disease in isolation based on previous LQTS studies.[10,11] A PubMed literature review was independently performed for each variant to identify supporting functional evidence and previously reported associations with inherited cardiac conditions. Using all available data, variant classification was updated using the ACMG standardized criteria.[6]

Statistical analyses were undertaken using R Version 4.2.1.[12] Comparisons between groups were made using either the Student *t* test or χ^2 test. Plots were created using the R package "ggplot2" Version 3.3.6.[13]

This project was registered and approved by the Barts Health National Health Service trust clinical effectiveness unit (ID 12910). The research reported in this paper adhered to the Helsinki Declaration guidelines as revised in 2013.

Abbreviations

ACMG: American College of Medical Genetics and Genomics

CRDS: calcium release deficiency syndrome

CPVT: catecholaminergic polymorphic ventricular tachycardia

LOF: loss of function

LQTS: long QT syndrome

VF: ventricular fibrillation

VUS: variant of uncertain significance

Results

Cohort summary

We identified 700 consecutive patients who underwent genetic testing. A total of 424 patients (60.5%) were probands; the remaining 276 patients underwent genetic testing for family screening. Of those who underwent family screening, 183 (66.3%) had predictive testing for a specific variant identified in the proband that was originally classified as either pathogenic or a VUS. We have not included these patients in the remaining summary statistics because they did not undergo testing with a full gene panel and will not identify any additional pathogenic variants or VUSs. However, their clinical phenotyping and genetic testing data were used to identify evidence for variant co-segregation with disease. Of the remaining 93 individuals undergoing family screening, the proband did not undergo follow-up in our center and was not one of the 424 probands in this dataset.

Indications for genetic testing

Excluding patients tested for a specific variant only, 517 patients remained from 440 different families. Median age was 37 years [interquartile range 27–48], and there was a higher proportion of females (54%). The most common indication for genetic testing was a clinical diagnosis of LQTS (331/517 [64.0%]) (Figure 1). Other indications included Brugada syndrome (76 [14.7%]) and a suspected channelopathy diagnosis after documented ventricular fibrillation (VF) arrest (59 [11.4%]).

In total, 352 patients (68.1%) underwent testing using a LQTS panel containing 2–28 genes depending on the year of referral and testing provider. For 52 (10.0%) and 35 (6.8%) patients, Brugada (1–17 genes) and general arrhythmia (100–256 genes) panels were used, respectively. Large general arrhythmia panels were predominantly used from 2010 (30/35 patients). The remaining patients underwent testing using catecholaminergic polymorphic ventricular tachycardia (CPVT) (14), short QT syndrome (3), and conduction disease (12) panels, or multiple panels combining channelopathy and cardiomyopathy genes (49). An overview of variant reporting per year is shown in Figure 2. Over time, the number of VUSs reported has increased.

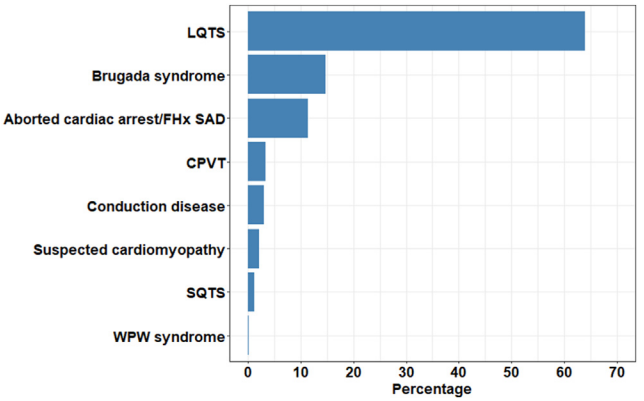

Figure 1
Genetic testing indications summary. x-axis: Percentage of patients in the full cohort (total = 517). y-axis: Indication for genetic testing from clinical data when referred. CPVT = catecholaminergic polymorphic ventricular tachycardia; FHx = family history; LQTS = long QT syndrome; SAD = sudden arrhythmic death; SQTS = short QT syndrome; WPW = Wolff-Parkinson-White.

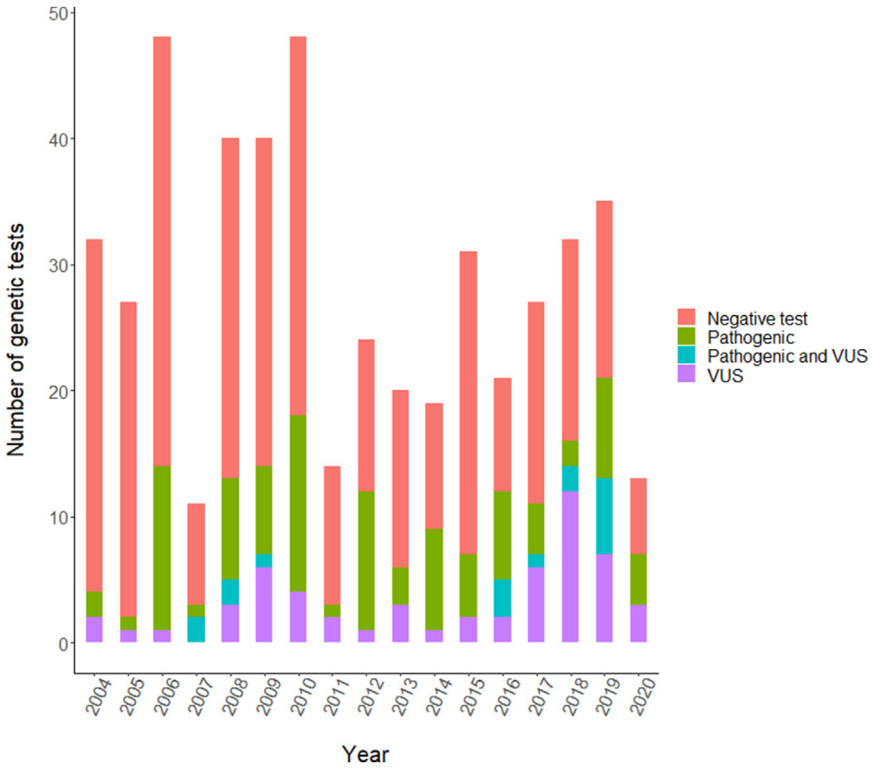

Figure 2
Genetic testing findings by reporting year. x-axis: Year genetic testing performed. y-axis: Number of genetic tests performed. Findings from each report are color coded: negative *(red)*, variant of uncertain significance (VUS) *(purple)*, pathogenic *(green)*, or pathogenic and a VUS *(blue)*.

VUS

A total of 94 patients (18.2%) from 78 families had a VUS reported, comprising 87 unique VUSs (Supplemental Table 1). Of these patients, 11 (from 9 families) had 2 VUSs and 20 (18 families) had an additional variant classified either pathogenic or likely pathogenic. Genes with the highest number of VUSs reported were *KCNH2* (14/89 [15.7%]), *SCN5A* (14 [15.7%]), *KCNQ1* (12 [13.5%]), and *RYR2* (9 [10.1%]). Figure 3 shows the number of genetic tests reporting a VUS vs the number of genes included in the panel. A VUS was more likely with increasing gene panel size (difference in mean number of genes tested; 55 vs 17; t = 3.7; P <.001). This was driven by the broad arrhythmia panels containing >190 genes. In these cases, a VUS was more often reported in a known cardiomyopathy gene or one in which the relationship with monogenic ion channel disease is less well characterized (*AKAP9* [1], *KCNJ5* [1], *KCNQ1* [1], *MYBPC3* [2], *PKP2* [1], *RYR2* [3], *SCN3B* [1], *TBX5* [1], *TMEM43* [1], *TPM1* [1], *TRPM4* [1], *TTN* [1], *VCL* [1]).

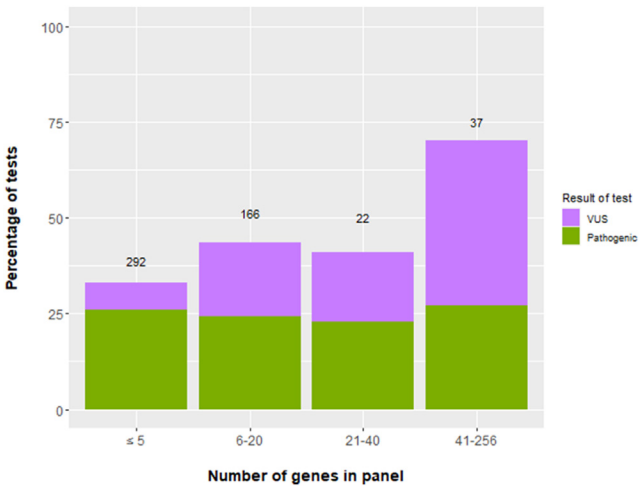

Figure 3
Percentage of tests reporting a pathogenic variant or variant of uncertain significance (VUS) grouped by number of genes in the panel. x-axis: Number of genes in the panels tested. The total number of tests performed for each group is provided above each bar. y axis: Percentage of tests in which a variant was identified as either pathogenic *(green)* or uncertain significance *(purple)*.

Of the 87 unique VUSs, 11 (12.6%) fulfilled ACMG criteria to support reclassification to pathogenic or likely pathogenic (Table 1). The original reporting year ranged from 2004 to 2019. Two were reported after release of the ACMG criteria (since 2015). All variants were rare with an allele frequency <0.005%, and 6 were not reported in the Genome Aggregation Database despite good gene coverage. The majority were missense (9); the remaining 2 were an in-frame insertion and a nonsense variant. Experimental evidence supporting a pathogenic classification was available for 6 reclassified variants. For an additional 2 variants, functional studies were available for variants at either the same or nearby residue. Of the 11 reclassified VUSs, the results of segregation analyses were available for 7 families, and all co-segregated with the clinical phenotype in multiple relatives.

Table 1 VUSs with support for reclassification to pathogenic or likely pathogenic

Year of report	Indication for test	Gene	HGVS protein	AF (%, gnomAD)	ACMG criteria	Functional evidence	Segregation in our cohort	Conclusion
2006	LQTS	*KCNH2*	Ser660Leu	0.0006	PS2, PM2, PP1, PP3, PP4, PP5	No. Segregation across multiple families.	Y	LP
2011	LQTS	*KCNH2*	Ala78Thr	Not found	PS3, PM1, PM2, PM5, PP5	Impairs protein stability with reduction in ion current (PMID:27761169)	No information	P
2015	LQTS	*KCNQ1*	Arg518Pro	0.0046	PS4 (moderate classification), PM1, PM5, PP3	No. Alternative variant is pathogenic.	No information	LP
2008	Idiopathic VF	*KCNQ1*	Ala150Thr	0.0019	PS3, PP2, PP1	Reduction in K^+ channel current (PMID 31899541, 29532034)	Y	LP
2014	LQTS	*KCNQ1*	Gly179Ala	Not found	PM1, PM5, PP3, PP5, PP1	No. Variants at the same residue are pathogenic with functional support (PMID: 29532034, 31785541).	Y	LP
2009	LQTS	*KCNQ1*	Asp242Asn	Not found	PS3, PS4, PM1, PM2, PM5, PP1, PP3, PP5	Reduced current compared to wild type (PMID: 25705178, 28739325, 29167462)	Y	P
2004	LQTS	*KCNQ1*	T247dup	Not found	PS3, PM1, PM2, PM4	Severe channel dysfunction in the basal state (https://doi.org/10.1093/europace/euy015.258)	Y	P
2009	LQTS	*KCNQ1*	Phe279Ile	Not found	PM1, PM2, PM5, PP1, PP3	No. Alternative variant is classified as likely pathogenic.	Y	LP
2009	Idiopathic VF	*RYR2*	Asp4646Tyr	Not found	PM5, PM2, PM1, PP3, PP1	No. Missense variant at same residue is pathogenic. Causes cardiac electrophysiological remodeling (PMID: 33536282).	Y	LP
2009	Brugada syndrome	*SCN5A*	Arg1638Ter	0.0008	PVS1, PS3, PS4, PM2, PP5	Reduction in Na^+ current (PMID: 27784737)	No information	P
2019	LQTS	*SCN5A*	Met1792Lys	0.0006	PP3, PM2, PM1, PP5	No. Variants at nearby residues are associated with arrhythmia.	No information	LP

ACMG = American College of Medical Genetics and Genomics; AF = allele frequency; gnomAD = Genome Aggregation Database; HGVS = Human Genome Variation Society; LP = likely pathogenic; LQTS = long QT syndrome; P = pathogenic; PMID = PubMed ID; VF = ventricular fibrillation; VUS = variant of uncertain significance.

Reclassification has potentially significant implications. For example, within 1 family, 3 individuals suffered a cardiac arrest during exercise or intense emotional stress. The missense variant (*RYR2*, p.Asp4646Tyr) co-segregated with affected family members. It is located within a highly conserved region containing a cluster of pathogenic variants including p.Asp4646Ala, a loss-of-function (LOF) variant that suppresses spontaneous calcium waves in mouse models unlike CPVT-linked gain-of-function mutations.[14] The variant was described along with other *RYR2* LOF variants in individuals who do not exhibit the characteristic crescendo ventricular ectopy observed in CPVT at elevated heart rates (eg, during exercise) and likely represents a different condition recently described as calcium release deficiency syndrome (CRDS).[14] All affected individuals were already taking a beta-blocker; however, reclassification now increases therapeutic options (eg, flecainide).

In total, 17 VUSs had support for reclassification to benign or likely benign (Table 2). The allele frequency of these variants ranged from 0.001%–35.7%. Of these, 9 variants are reported as "conflicting interpretation" in ClinVar; however, their allele frequencies are above the threshold to cause disease in isolation. Five patients (all probands) with a VUS reclassified to benign or likely benign also had a pathogenic variant identified during testing. Of the remaining 12 patients, 4 had a diagnosis of idiopathic VF, and 7 met clinical diagnostic

Table 2 VUSs with support for reclassification to benign or likely benign

Year of report	Indication for test	Gene	HGVS coding	HGVS protein	AF (%, gnomAD)	ACMG criteria	Conclusion
2018	SQT clinical diagnosis, documented VT	*CACNA1A*	c.3053G>A	Arg1018Gln	Failed QC	BS1, BP4	LB
2017	Suspected cardiomyopathy	*DSC2*	c.304G>A	Glu102Lys	0.075	BS1, BP4	LB
2008	LQTS diagnosis, suspected coexisting cardiomyopathy, pathogenic *DSC2* carrier	*DSG2*	c.1550C>T	Ala517Val	0.166	BS1, BS2, BP4	B
2010	Idiopathic VF	*DSP*	c.2815G>A	Gly939Ser	1.113	BS1, BS2, BP4	B
2011	Idiopathic VF	*DSP*	c.5498A>T	Glu1833Val	0.87	BS1, BS2	B
2009	LQTS, previous VF arrest	*KCNE1*	c.112A>G	Ser38Gly	35.7	BA1, BS1, BP4, BP6	B
2009	LQTS clinical diagnosis	*KCNE2*	c.22A>G	Thr8Ala	0.382	BS1, BS2, BP4, BP6	B
2007	LQTS and HCM clinical diagnoses, pathogenic *MYBPC3* variant	*KCNH2*	c.1039C>T	Pro347Ser	0.0637	BS1, BP4	LB
2009	LQTS, previous VF arrest	*KCNH2*	c.2690A>C	Lys897Thr	20.3	BA1, BS1, BP4, BP6	B
2009	Idiopathic VF	*KCNH2*	c.2729C>T	Pro910Leu	0.0204	BS2, BP4	LB
2010	LQTS clinical diagnosis	*KCNH2*	c.2941A>G	Ser981Gly	0.0237	BS1, BS2,	B
2008	LQTS clinical diagnosis	*KCNH2*	c.442C>T	Arg148Trp	0.0644	BS1, BP4	LB
2008	Family history of SCD, pathogenic *SCN5A* variant	*PKP2*	c.1420G>A	Ala474Thr	0.0651	BS1, BS2, BP4	B
2018	ARVC clinical diagnosis	*PKP2*	c.1627G>A	Val543Ile	0.241	BS1, BS2, BP4	B
2008	LQTS clinical diagnosis, pathogenic *SCN5A* variant	*SCN5A*	c.1381T>G	Leu461Val	0.325	BS1, BS2, BP4, BP6	B
2007	LQTS clinical diagnosis, pathogenic *KCNQ1* variant	*SCN5A*	c.1673A>G	His558Arg	22	BA1 BS1, BP4	B
2013	Idiopathic VF	*SCN5A*	c.3508+11G>A	NA	0.0013	BS2, BP4	LB

ARVC = arrhythmogenic right ventricular cardiomyopathy; B = benign; HCM = hypertrophic cardiomyopathy; LB = likely benign; NA = not applicable; QC = quality control; SCD = sudden cardiac death; SQT = short QT; VT = ventricular tachycardia; other abbreviations as in Table 1.

criteria for either LQTS (5), short QT syndrome, or arrhythmogenic cardiomyopathy. Of these 7 patients, 3 had a history of ventricular arrhythmia.

Detailed information for each VUS and clinical characteristics of the proband is provided in Supplemental Table 2. In total, 76 patients with a VUS reported had follow-up data available (median 7 years). Forty-three patients had documented arrhythmia or an event highly suspected to be due to arrhythmia (sudden death 3, aborted cardiac arrest 19, sustained ventricular tachycardia 9, nonsustained ventricular tachycardia 5, syncope 3, high-degree atrioventricular block or sinus node disease 4). Reclassification to either pathogenic/likely pathogenic or benign/likely benign was not associated with arrhythmia (χ^2 test; $P = .758$ and $P = .242$, respectively), indicating variant reclassification does not correlate with severity of the clinical phenotype. Reclassification direction may be more likely predicted by the clinical phenotype in question and the certainty of diagnosis. For each patient, reclassification of a VUS did not lead to an immediate change in medical therapy as medical management had been guided by the clinical phenotype.

Pathogenic variants

A total of 138 variants were classified as likely pathogenic or pathogenic at original reporting (Supplemental Table 1). The majority were in either *KCNQ1* (57/138 [41.3%]), *KCNH2* (28 [20.3%]), *SCN5A* (24 [17.2%]), and *RYR2* (10 [7.2%]). There was no association between pathogenic variant reporting and the number of genes included in the panel ($P = .49$) (Figure 3). Using ACMG criteria, 7 of these 138 variants lacked support for a pathogenic or likely pathogenic classification, with 3 reclassified to benign/likely benign and 4 to VUS (Table 3). These variants were originally reported between 2008 and 2014, before publication of the ACMG guidelines. This has had important implications for patients. For example, the *SCN5A* missense variant p.Thr1303Met initially was classified as pathogenic; however, this is now downgraded to VUS (Table 3). It has been reported in healthy individuals and has an allele frequency greater than would be expected to cause disease alone (0.2%). The original classification created diagnostic uncertainty for a patient carrying the variant whose son was a victim of sudden cardiac death with evidence for arrhythmogenic cardiomyopathy on postmortem examination. The patient did not meet criteria for a clinical diagnosis of LQTS, and screening had identified clinical features of arrhythmogenic cardiomyopathy in other family members. The previous label of "possible LQTS type 3" has since been revised; however, it initially led to inappropriate therapy (beta-blockers) based on genotype alone and created significant apprehension for the patient and family.

Table 3 Previously reported pathogenic variants with support for reclassification

Year of report	Indication for testing	Gene	HGVS protein	AF (%, gnomAD)	ClinVar	VarSome	Evidence	ACMG Criteria	Conclusion
2008	Suspected cardiomyopathy, diagnosis unclear	*DSC2*	Glu102Lys	0.075	Conflicting interpretation of pathogenicity	Likely benign	AF higher than expected. *In silico* prediction: Benign verdict.	BS1, BP4	Likely benign
2008	Suspected cardiomyopathy, diagnosis unclear	*DSG2*	Val920Gly	0.43	Benign/likely benign	Benign	AF higher than expected. Reported in healthy individuals. *In silico* prediction: Benign verdict.	BS1, BS2 BP4, BP6	Benign
2010	Clinical diagnosis of LQTS	*KCNH2*	Gln81His	NF	VUS	VUS	Absent in controls. In a mutation hotspot associated with LQTS.	PM2, PM1, PP3	VUS
2010	Clinical diagnosis of LQTS	*KCNH2*	Pro952Arg	NF	VUS	VUS	Absent in controls. *In silico* prediction tools inconclusive.	PM2	VUS
2014	VF arrest, recurrent VT not polymorphic/ exercise induced	*RYR2*	Glu4659Gly	0.00066	VUS	VUS	Variant not reported previously. *In silico* tools suggest pathogenic.	PM2, PP3	VUS
2008	Suspected diagnosis of LQTS, family history of sudden cardiac death	*SCN5A*	Leu461Val	0.325	Benign/likely benign	Benign	AF higher than expected. Reported in healthy individuals. *In silico* prediction tools: Benign verdict.	BS1, BS2 BP4, BP6	Benign
2013	Clinical diagnosis of LQTS	*SCN5A*	Thr1303Met	0.0217	Conflicting interpretation of pathogenicity	VUS	AF higher than expected. Conflicting functional evidence.	PP3, BS1	VUS

Abbreviations as in Tables 1 and 2.

For 2 other families, previously "genotype negative" individuals were rescreened and alternative diagnoses were reconsidered. Two patients with a reclassified pathogenic variant had an implantable cardioverter-defibrillator inserted; however, the indication was driven by a history of VF arrest and documented ventricular tachycardia rather than the genotype.

Discussion

In this study of 517 patients who underwent genetic testing in our inherited arrhythmia clinic, we identify a clinically relevant proportion of VUSs with support for reclassification (32.2%). Reclassification was not associated with occurrence of arrhythmia or an immediate change in medical therapy; however, there were implications for genotype-guided counseling and future clinical management. A VUS finding was more likely when broad gene panels were used, without the benefit of an increase in yield of pathogenic variants. We also report a clinically relevant proportion of variants that do not meet ACMG criteria for their original "pathogenic" classification.

There has been limited reporting of variant reclassification from inherited arrhythmic clinics. A study of 49 suspected inherited channelopathy cases reported a higher burden of VUSs (69.4%), and 20% had support for reclassification after 5 years.[15] All patients included in the study underwent testing with a broad 78-gene sudden cardiac death panel, which may account for the higher VUS burden compared with our own. A study of 116 pediatric patients with inherited arrhythmia syndromes reported 52% of VUSs were reclassified after implementation of the 2015 ACMG criteria; however, the impact of reclassification on clinical management was not reported.[3] The authors found a VUS was more likely to be identified if the clinical diagnosis was unclear; however, they did not evaluate the impact of gene panel size. They also reported the mean time to reclassification for a VUS was 7 years, which may be impacted by the availability of data from familial phenotyping and cascade screening.

Multiple factors may influence an institution's practice for review of variants and awareness of a reclassified status. We received formal notification of reclassification from a genetic

testing provider for only 4 variants. Although the ACMG advises the lead physician and local multidisciplinary team are responsible for the review of variant classifications, there is a lack of consensus on this recommendation in the health care community.[16] Economic barriers may prevent re-evaluation of variants in many health care systems globally as they may lead to additional costs for patients depending on insurance requirements, genetic test performed, and frequency of review.[6,16] Given the potential for a variant to change classification and the relevant implications, these considerations need to be explained to patients when counseling for genetic testing.

In addition to economic barriers, the focus on pathogenic variants (as they are actionable) may prevent recognition of reclassified VUSs. To correctly identify reclassified variants, centers should ensure regular maintenance of patient genetic databases with linkage to family data, including documentation of VUSs. This requires accurate phenotype data from cascade screening, which may inform assessment of classification.[1] It is a labor-intensive task, so it requires genetic data software developments to enable automated collation and highlighting of variant reclassification in individual/family cases with integration into electronic health records. Confirmation of reclassification should be obtained within a specialized inherited cardiac conditions multidisciplinary team including cardiologists, geneticists, and specialist nursing staff. However, because of the rapid evolution of cardiovascular genetics over the last decade, local and national educational programs are necessary to address deficits in the knowledge and deliver updates in best practice for variant catagorization.[17] Improvements of the guidelines are needed to aid clinicians in the cardiovascular genetics clinic. Currently, there is no established standard or consensus on the recommended frequency to review variants. In a recent study evaluating genetic panels across multiple medical specialties including cardiology, mean time between original testing and reclassification was 22.4 months for variants reclassified to pathogenic or likely pathogenic.[18] Therefore, review of VUSs ideally should occur at least biennially with an automated approach directly linked to a contemporary database, highlighting variant reclassification as "alerts" in the patient electronic heath care record that are also communicated to the responsible physician.

In our study, a VUS was more likely identified when large gene panels were used, consistent with a previous study evaluating arrhythmia gene panels.[2] This observation was without a corresponding increase in pathogenic variants detection. Therefore, our study supports the latest consensus statement for genetic testing of cardiac diseases in which there is increased emphasis on testing genes with the strongest support in the first instance.[1] However, this is not possible in all cases, and for patients with idiopathic VF, wider gene panel testing is necessary.[1]

We did not identify any association between a change in VUS classification and occurrence of arrhythmic events, confirming decision-making on a variant's pathogenicity should not be influenced by clinical phenotype severity. For patients with a VUS reclassified as benign or likely benign, 11 of 17 (64.7%) had documented arrhythmic events. Therefore, although a benign reclassification may indicate a lower probability of monogenic disease, our study indicates that it translates to a more benign form of disease. It is possible that the causal variant or gene has yet to be identified. In addition, the current ACMG criteria are limited by an assumption that the clinical phenotype is caused by a single variant; however, it is possible that variants classified as VUS (or benign) are disease modifiers or cause disease in combination (compound VUSs).[19]

Although reclassification of a VUS to pathogenic did not influence medical management (patients were established on therapy targeting the clinical phenotype), it has implications for genotype-guided risk stratification and future therapies,[20] for example, use of mexiletine for LQTS type 3 or future access to preimplantation genetic diagnosis, as was the case for 1 family.[21,22] It also impacts family screening because noncarriers can be reassured and discharged from regular follow-up, which is not possible for those with a VUS. In addition, reclassification for 1 family in our study (*RYR2*, p.Asp4646Tyr) may have identified support for a diagnosis of CRDS, characterized by an *RYR2* LOF mutation and a normal exercise treadmill test.[14] In the absence of demonstrating a long-burst, long-pause spontaneous ventricular ectopy pattern before initiation of VF, genetic testing is the only method of screening families for CRDS.[23]

We also identified in our cohort a clinically significant proportion (5%) of variants that no longer have support for a pathogenic classification. All variants were reported before the release of large-scale population-level allele frequency data and the introduction of standardized criteria for variant classification. As is the case for some previously reported VUSs (eg, *SCN5A* p.His588Arg), early variant reporting was susceptible to inconsistencies across laboratories and was affected by case ascertainment bias due to small cohort sizes and unmatched control populations.[24] Our findings illustrate the importance of regularly reviewing pathogenic variants and VUSs as guidelines for classification change over time and additional functional evidence is reported. Overclassification has significant potential to cause harm through incorrect diagnoses, medical therapy not targeted at the true underlying disease, and lowering of thresholds for implantable cardioverter-defibrillator insertion. In our study, it resulted in diagnostic uncertainty, inappropriate medical therapy, and repeated clinical phenotyping of family members.

Conclusion

We have identified a clinically important proportion of patients for whom a variant (VUS or pathogenic) had support for reclassification. These findings affect final diagnoses, genotype-guided therapy, and family screening. Our study highlights the need for a regular, structured review of variants and the need to include this information when counseling patients for genetic testing.

Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrthm.2024.01.008.

Funding Sources: Dr Young recognizes the National Institute for Health and Care Research (NIHR) Integrated Academic Training programme, which supports his Academic Clinical Lectureship post. Dr Lambiase is supported by the Stephen Lyness Memorial Fund and University College Hospitals Biomedicine NIHR and Barts Biomedical Research Centre.

Disclosures: The authors have no conflicts to disclose.

Authorship: All authors attest they meet the current ICMJE criteria for authorship.

Address reprint requests and correspondence: Dr Pier Lambiase, Institute of Cardiovascular Science, University College London, Gower Street, London, United Kingdom WC1E 6BT. E-mail address: p.lambiase@ucl.ac.uk

References

1. Wilde AAM, Semsarian C, Márquez MF, et al. European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) expert consensus statement on the state of genetic testing for cardiac diseases. Heart Rhythm 2022;19:e1–e60.
2. van Lint FHM, Mook ORF, Alders M, Bikker H, Lekanne Dit Deprez RH, Christiaans I. Large next-generation sequencing gene panels in genetic heart disease: yield of pathogenic variants and variants of unknown significance. Neth Heart J 2019;27:304–309.
3. Bennett JS, Bernhardt M, McBride KL, et al. Reclassification of variants of uncertain significance in children with inherited arrhythmia syndromes is predicted by clinical factors. Pediatr Cardiol 2019;40:1679–1687.
4. Federici G, Soddu S. Variants of uncertain significance in the era of high-throughput genome sequencing: a lesson from breast and ovary cancers. J Exp Clin Cancer Res 2020;39:46.
5. Makhnoon S, Shirts BH, Bowen DJ. Patients' perspectives of variants of uncertain significance and strategies for uncertainty management. J Genet Couns 2019;28:313–325.
6. Richards S, Aziz N, Bale S, et al. 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. Genet Med 2015;17:405–424.
7. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018;46:D1062–D1067.
8. Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: the human genomic variant search engine. Bioinformatics 2019;35:1978–1980.
9. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020;581:434–443.
10. Kaltman JR, Evans F, Fu YP. Re-evaluating pathogenicity of variants associated with the long QT syndrome. J Cardiovasc Electrophysiol 2018;29:98–104.
11. Walsh R, Thomson KL, Ware JS, et al. Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. Genet Med 2017;19:192–203.
12. R core team. R: a language and environment for statistical computing Vienna, Austria: R Foundation for Statistical Computing; 2022.
13. Wickham H. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag; 2016.
14. Sun B, Yao J, Ni M, et al. Cardiac ryanodine receptor calcium release deficiency syndrome. Sci Transl Med 2021;13:eaba7287.
15. Sarquella-Brugada G, Fernandez-Falgueras A, Cesar S, et al. Clinical impact of rare variants associated with inherited channelopathies: a 5-year update. Hum Genet 2022;141:1579–1589.
16. Berger SM, Appelbaum PS, Siegel K, et al. Challenges of variant reinterpretation: opinions of stakeholders and need for guidelines. Genet Med 2022;24:1878–1887.
17. Lopez Santibanez Jacome L, Dellefave-Castillo LM, Wicklund CA, et al. Practitioners' confidence and desires for education in cardiovascular and sudden cardiac death genetics. J Am Heart Assoc 2022;11:e023763.
18. Chen E, Facio FM, Aradhya KW, et al. Rates and classification of variants of uncertain significance in hereditary disease genetic testing. JAMA Netw Open 2023;6:e2339571.
19. Giudicessi JR, Roden DM, Wilde AAM, Ackerman MJ. Classification and reporting of potentially proarrhythmic common genetic variation in long QT syndrome genetic testing. Circulation 2018;137:619–630.
20. Giudicessi JR, Ackerman MJ. Genotype- and phenotype-guided management of congenital long QT syndrome. Curr Probl Cardiol 2013;38:417–455.
21. Mazzanti A, Maragna R, Faragli A, et al. Gene-specific therapy with mexiletine reduces arrhythmic events in patients with long QT syndrome type 3. J Am Coll Cardiol 2016;67:1053–1058.
22. Yeates L, McDonald K, Burns C, Semsarian C, Carter S, Ingles J. Decision-making and experiences of preimplantation genetic diagnosis in inherited heart diseases: a qualitative study. Eur J Hum Genet 2022;30:187–193.
23. Roston TM, Wei J, Guo W, et al. Clinical and functional characterization of ryanodine receptor 2 variants implicated in calcium-release deficiency syndrome. JAMA Cardiol 2022;7:84–92.
24. Cassa CA, Tong MY, Jordan DM. Large numbers of genetic variants considered to be pathogenic are common in asymptomatic individuals. Hum Mutat 2013;34:1216–1220.