Original Article

Genetic Testing in the Evaluation of Unexplained Cardiac Arrest

From the CASPER (Cardiac Arrest Survivors With Preserved Ejection Fraction Registry)

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Background—Unexplained cardiac arrest may be because of an inherited arrhythmia syndrome. The role of genetic testing in cardiac arrest survivors without a definite clinical phenotype is unclear.

Methods and Results—The CASPER (Cardiac Arrest Survivors with Preserved Ejection Fraction Registry) is a large registry of cardiac arrest survivors where initial assessment reveals normal coronary arteries, left ventricular function, and resting ECG. Of 375 cardiac arrest survivors in CASPER from 2006 to 2015, 174 underwent genetic testing. Patients were classified as phenotype-positive (n=72) or phenotype-negative (n=102). Genetic testing was performed at treating physicians' discretion in line with contemporary guidelines and availability. All genetic variants identified from original laboratory reports were reassessed by the investigators in line with modern criteria. Pathogenic variants were identified in 29 (17%) patients (60% channelopathy-associated and 40% cardiomyopathy-associated genes) and 70 variants of unknown significance were identified in 32 (18%) patients. Prior syncope (odds ratio, 4.0; 95% confidence interval, 1.6–9.7) and a family history of sudden death (odds ratio, 3.2; 95% confidence interval, 1.1–9.4) were independently associated with the presence of a pathogenic variant. In phenotype-negative patients, broad multiphenotype genetic testing led to higher yields (21% versus 8%; P=0.04) but was associated with more variants of unknown significance (55% versus 5%; P<0.01).

Conclusions—Genetic testing identifies a pathogenic variant in a significant proportion of unexplained cardiac arrest survivors. Prior syncope and family history of sudden death are predictors of a positive genetic test. Both arrhythmia and cardiomyopathy genes are implicated. Broad, multiphenotype testing revealed the highest frequency of pathogenic variants in phenotype-negative patients.

Clinical Trial Registration—https://www.clinicaltrials.gov. Unique Identifier: NCT00292032

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The majority of sudden cardiac arrest occurs in older individuals and is because of underlying coronary artery disease or structural heart disease.[1] When initial evaluation with ECG, coronary angiography, and echocardiography is normal, a wider differential diagnosis exists. Comprehensive clinical evaluation of initially unexplained cardiac arrest survivors will diagnose an inherited arrhythmia syndrome phenotype in ≤56%.[2] When a phenotype is identified, genetic testing to identify the causative pathogenic variant is recommended.[3] Genetic testing may produce 3 types of results: (1) a positive test where a definitive pathogenic genetic variant has been identified consistent with the clinical phenotype; (2) a negative test where no rare or disease-causing variants are identified; (3) identification of one or more variants of unknown significance (VUS). VUS are rare genetic changes where there is insufficient evidence to classify them as either pathogenic or benign. Interpretation of VUS requires significant experience and expertise in the assessment of pathogenicity, a process that is frequently difficult and time-consuming.[4]

See Editorial by Semsarian and Wilde
See Clinical Perspective

The current expert consensus on genetic testing in channelopathies and cardiomyopathies,[3] published in 2011, does

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not recommend genetic testing of a cardiac arrest survivor when the cardiac arrest remains unexplained, despite comprehensive clinical testing. The document highlights the high costs involved and difficulties interpreting VUS as the reasons for this recommendation.

Next-generation sequencing has reduced the costs of genetic testing, and broad panels with large numbers of genes are now widely available. A recent report on postmortem genetic testing in unexplained sudden cardiac death using broad-panel genetic testing reported pathogenic variants in 27% of victims.[5] However, little literature exists on the use of such broad panels in unexplained cardiac arrest survivors, where the yield may be expected to be higher than in sudden unexplained death. Therefore, the clinical utility of broad panel genetic testing in cardiac arrest survivors remains unclear.

We analyzed a large registry of unexplained cardiac arrest survivors in whom genetic testing had been undertaken to identify the yield of pathogenic variants and look for predictors of a positive finding. In particular, we aimed to compare phenotype-guided and phenotype-negative testing in terms of identifying pathogenic variants and VUS. We hypothesized that genetic testing in phenotype-negative cases would have a lower but yet significant yield when compared with those with a clinical phenotype.

Methods

Patient Enrollment

Details of the CASPER (Cardiac Arrest Survivors with Preserved Ejection fraction Registry) have previously been reported.[2] Briefly, CASPER is a national registry that examines phenotype–genotype correlation and assesses test performance in familial sudden death and unexplained cardiac arrest. Cardiac arrest survivors were eligible for enrollment if they had experienced an unexplained cardiac arrest with documented ventricular tachycardia or ventricular fibrillation requiring direct current cardioversion or defibrillation, and subsequent testing demonstrated normal left ventricular function (left ventricular ejection fraction ≥50%) and normal coronary arteries (no coronary stenosis >50%). Patients with known causes for cardiac arrest, including an ECG diagnostic of long QT syndrome (LQTS; persistent resting QTc >460 ms for males and 480 ms for females[6]) or Brugada syndrome,[7] hypertrophic cardiomyopathy, marked hypokalemia, drug overdose, or commotio-cordis, were excluded. Furthermore, patients with recognized forms of idiopathic ventricular tachycardia were also excluded. In addition, enrollment in this study required undertaking of genetic testing. Cases where there was a missing or incomplete genetic report were excluded.

The protocol was approved by the Health Sciences Research Ethics Board of the University of British Columbia and at each enrolling center, and all patients included in this study have given informed consent (Registry of Unexplained Cardiac Arrest).

Clinical Testing

Patients received standard testing to determine the cause of the cardiac arrest, including a resting 12-lead ECG, echocardiography, and assessment of coronary anatomy by selective angiography, or computed tomography. Patients who met the enrollment criteria underwent further testing, including high-lead ECG,[8] signal-averaged ECG, exercise stress testing, ambulatory monitoring, cardiac magnetic resonance imaging, and procainamide and epinephrine provocation tests. Investigators had discretion to perform testing as guided by the clinical presentation and results of previous testing. Exercise stress tests were performed using a Bruce or modified Bruce protocol. ECGs were recorded before, during, and ≤6 minutes after exercise. Procainamide and epinephrine infusions were performed using standard techniques.[9,10] right ventricular (RV) angiography and RV biopsy was performed in selected cases where arrhythmogenic RV cardiomyopathy was suspected. In the majority of cases, family cardiac evaluation did not inform the original assessment of phenotype in the proband. This may have been able to guide genetics testing in some cases. Furthermore, because of the limited availability of data on family members, we were not able to use cosegregation of variants to inform the diagnosis of the proband.

Genetic Testing

Genetic testing was performed at the discretion of the investigating physician in keeping with contemporary guidelines and availability. Testing before 2007 was restricted to limited research testing. Thereafter, clinical testing was performed through local hospital or commercial laboratories using either direct Sanger sequencing or, more recently, next-generation sequencing techniques (with variants confirmed using Sanger sequencing). Genetic tests undertaken were grouped for analysis as single gene; single phenotype; limited multiphenotype where <20 genes in total were tested for ≥1 clinical phenotype; and broad multiphenotype, where large panels of arrhythmia or cardiomyopathy genes were tested. Pathogenic variants and VUS were initially identified from laboratory reports

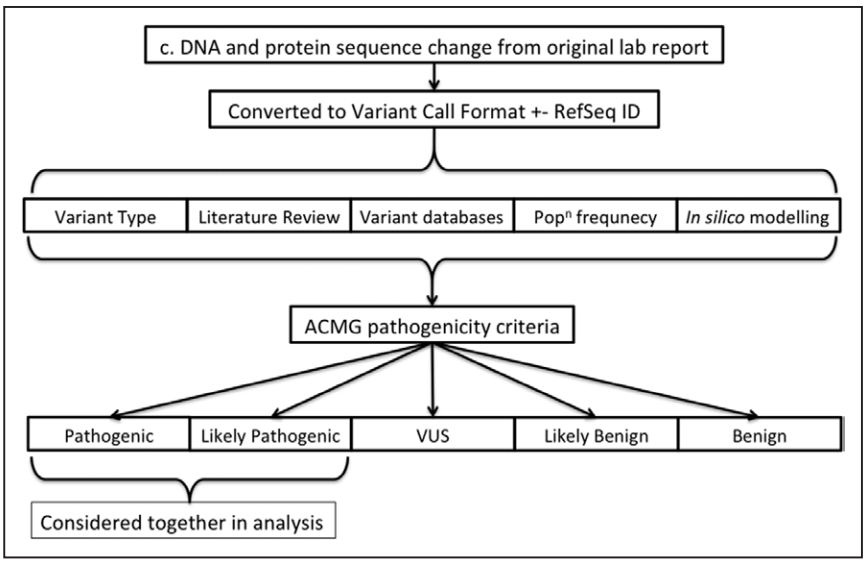

Figure 1. Variant reclassification process. Variants classified as either pathogenic or likely pathogenic were considered together for analyses of genetic testing yields. ACMG indicates American College of Genetics and Genomics; and VUS, variants of unknown significance.

issued at the time of testing. The clinical significance of all identified variants were reassessed by the investigators in line with recent guidance from the American College of Medical Genetics and Genomics (Figure 1). Features of each variant were analyzed and assigned a level of evidence for pathogenicity as very strong; strong; moderate; or supporting (see Data Supplement). The aggregate score was then used to classify variants as pathogenic; likely pathogenic; VUS; likely benign; or benign. Factors considered included variant type and location within the gene; functional studies; cosegregation within families; previous publication in peer-reviewed literature; documentation in ClinVar,[11] the public access archive of phenotype–genotype relationships or in disease/gene-specific databases where available[12,13]; general population variant frequencies, identified through the Exome Aggregation Consortium,[14] a public access database of genetic variation in 60 706 unrelated individuals; and in silico pathogenicity prediction, performed using the Complementary Annotation Dependent Depletion algorithm.[15] Public access databases were interrogated in September 2016.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 (Institutional Software, SPSS, Armonk, NY). Continuous variables were compared by using the 2-tailed Student's *t* test or analysis of variance when comparing ≥3 groups. Categorical variables were compared using the χ^2 test. *P* values <0.05 were considered significant. Continuous variables are expressed as mean±SD. A logistic regression model was used to identify independent associations with a positive genetic test. Variables were inspected for any significant interactions. Available clinical variables were initially incorporated with backwards elimination used to identify the best fit. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the article as written.

Results

Clinical Characteristics

Of 375 cardiac arrest survivors in CASPER, genetic testing was undertaken in 175 (mean age 39±15 years, 56% male). One case was excluded because the genetic report was incomplete and could not be verified, giving a cohort of 174. Patients who underwent genetic testing were younger than those who did not and were less likely to have a clinical phenotype (see Data Supplement). A pathogenic variant (1 homozygous) was identified in 29 (17%) individuals. One or more VUS were identified in 32 (18%) patients. There were 70 VUS identified in total (2.2 VUS/patient; range 1–9). Prior syncope (odds ratio, 4.0; 95% confidence interval, 1.6–9.7) and a family history of sudden death (odds ratio, 3.2; 95% confidence interval, 1.1–9.4) were independent predictors of a pathogenic variant. Identification of a clinical phenotype was associated with a higher yield on univariate analysis (*P*=0.01) but not when corrected for prior syncope and family history of sudden death (odds ratio, 2.3; 95% confidence interval, 0.9–5.5). Clinical characteristics are shown in Table 1.

There were 27 unique pathogenic variants identified in 15 genes (see Table 2). Three unrelated individuals had the same variant in *CACNA1C* (c.2570C>T, p.Pro857Leu). Pathogenic variants in genes associated with LQTS and arrhythmogenic RV cardiomyopathy were most commonly identified. Pathogenic variants in genes associated with cardiomyopathy were identified in 11 (38%) individuals.

Table 1. Clinical Characteristics Stratified by the Presence of a Pathogenic Variant

	All	Pathogenic +	Pathogenic –	Univariate *P*	Multivariate OR*
n	174	29 (17)	145 (83)	…	…
Age, y, mean±SD	39.1±15.2	36.0±17.3	39.7±14.8	0.23	…
Male (%)	98 (56)	15 (52)	83 (57)	0.58	…
Prior syncope (%)	39 (22)	14 (48)	25 (17)	<0.01	4.0 (1.6–9.7)
Fhx SD (%)†	22 (13)	7 (25)	15 (11)	0.04	3.2 (1.1–9.4)
Clinical phenotype (%)	72 (41)	18 (62)	54 (37)	0.01	2.3 (0.9–5.5)

Fhx SD indicates family history of sudden death; and OR, odds ratio.

*Prior syncope, Fhx SD, and clinical phenotype included in multivariate regression.

†Family history of sudden death was unavailable in 5 (3%) patients.

Of these 11, five (45%) had no apparent clinical phenotype. The mean age of these patients was 31 years (range 14–44) and was similar to phenotype-negative cases with pathogenic variants in arrhythmia genes (*P*=0.75). One patient with a *LMNA* variant developed severe left ventricular systolic impairment 4 years after the cardiac arrest. One patient with a *DSP* variant had minor diagnostic criteria for arrhythmogenic RV cardiomyopathy. Imaging was normal in the remaining 3 (echocardiogram 2 and cardiac magnetic resonance imaging 1). Follow-up imaging 2 years postcardiac arrest remained normal in 1 patient, with a pathogenic nonsense variant in PLN (c.116T>G, p.Leu39Ter).

There were 70 unique VUS identified in 30 genes. A full list of all VUS is provided in the Data Supplement.

Role of Clinical Phenotype

A clinical phenotype was identified in 72 (41%) individuals. Pathogenic variants were identified in 18 (25%) of phenotype-positive patients, most commonly in those with an arrhythmogenic RV cardiomyopathy phenotype. The frequencies of pathogenic variants in the most common phenotypes are shown in Table 3. VUS were identified in 13 (18%) of phenotype-positive individuals.

A pathogenic variant was identified in 11 (11%) phenotype-negative patients, with a higher yield in broad multiphenotype testing than other types of testing (21% versus 8%; *P*=0.04). The frequency of VUS identification also significantly increased with broad multiphenotype panels (55% versus 5%; *P*<0.01). The yields of different genetic testing strategies in phenotype-negative patients are shown in Figure 2.

Variant Reclassification and Inter-Laboratory Comparison

The original laboratory reports identified 141 variants in 66 patients (29 pathogenic and 112 VUS). Fifty (35%) variants were reclassified after independent assessment

Table 2. Summary of Identified Pathogenic Variants

Case No.	Age	Sex	Ethnicity	Clinical Phenotype	Gene	Sequence Change	Variant Type	Protein Change	Pathogenicity	ACMG Criteria Satisfied
1	22	Female	South Asian	LQTS	KCNH2	c.3020G>A	Missense	p.Arg1007His	Likely pathogenic	PM+4PP
7	15	Female	White	CPVT	RYR2	c.11934G>A	Missense	p.Met3978Ile	Likely pathogenic	2 PM+4PP
8	32	Female	White	Idiopathic	LMNA	c.673C>T	Nonsense	p.Arg225Ter	Pathogenic	PVS+PS+PM+2PP
14	10	Female	Middle Eastern	ARVC	DSC2	c.712_714delGAT	In-frame deletion	p.Asp238del	Likely pathogenic	2 PM+2PP
19	57	Male	White	ARVC	SCN5A	c.2893C>T	Missense	p.Arg965Cys	Pathogenic	PS+PM+4PP
22	33	Female	White	LQTS	RYR2	c.12587C>T	Missense	p.Thr4196Ile	Likely pathogenic	2 PM+2PP
53	27	Male	White	ARVC	PKP2	c.368G>A	Nonsense	p.Trp123Ter	Pathogenic	PVS+PS+3PP
58	16	Male	White	Idiopathic	KCNQ1	c.727C>T	Missense	p.Arg243Cys	Pathogenic	PS+3 PM+3PP
60	53	Male	White	ARVC	DSG2	c.523+1G>A	Splice site	…	Pathogenic	PVS+PM+2PP
65	51	Male	White	Idiopathic	SCN5A	c.1943 C>T	Missense	p.Pro648Leu	Likely pathogenic	PS+PM+PP
69	24	Female	White	LQTS	CACNA1C	c.2570C>T	Missense	p.Pro857Leu	Pathogenic	2 PM+3PP
93	71	Male	White	BrS	SCN5A	c.1066G>A	Missense	p.Asp356Asn	Pathogenic	PS+2 PM+4PP
100	42	Male	White	BrS	SCN5A	c.1064T>G	Missense	p.Phe355Cys	Likely pathogenic	2 PM+3PP
104	43	Male	White	ARVC	DSC2	c.1521G>A	Splice site	…	Pathogenic	PVS+PM+2PP
112	59	Male	White	HCM	MYBPC3	c.2373dupG	Frameshift	p.Trp792ValfsTer41	Pathogenic	PVS+PS+3PP
116	35	Male	White	Idiopathic	SCN5A	c.2550_2551dupGT	Frameshift	p.Phe851CysfsTer19	Pathogenic	PVS+PS+PM+2PP
117	56	Male	White	ARVC	DSG2	c.941C>A	Nonsense	p.Ser314Ter	Pathogenic	PVS+PM+3PP
118	28	Male	White	Idiopathic	DSP	c.4541T>C	Missense	p.Leu1514Pro	Likely pathogenic	2 PM+2PP
132	15	Male	White	Idiopathic	SCN5A	c.1099C>T	Missense	p.Arg367Cys	Pathogenic	PS+2 PM+3PP
135	19	Female	South Asian	CPVT	RYR2	c.7159G>A	Missense	p.Ala2387Thr	Pathogenic	PS+2 PM+4PP
139	45	Female	White	Idiopathic	KCNE1	c.200G>T	Missense	p.Arg67Leu	Likely pathogenic	2 PM+2PP
141	69	Male	White	LQTS	KCNE2	c.29C>T	Missense	p.Thr10Met	Pathogenic	PVS+PS+2PP
152	14	Female	White	Idiopathic	PLN	c.116T>G	Nonsense	p.Leu39Ter	Pathogenic	PVS+PS+2PP
154	37	Female	White	LQTS	CACNA1C	c.2579G>A	Missense	p.Arg860Gln	Pathogenic	PS+2 PM+3PP
156	49	Female	Aboriginal	LQTS	CACNA1C	c.2570C>T	Missense	p.Pro857Leu	Pathogenic	PS+2 PM+3PP
160	25	Female	White	Idiopathic	SCN5A	c.5872C>T	Nonsense	p.Arg1958Ter	Pathogenic	PVS+PM+2PP
170	16	Female	White	Idiopathic	CACNA1C	c.2570C>T	Missense	p.Pro857Leu	Pathogenic	2 PM+2PP
172	36	Female	White	Idiopathic	TTN	c.78820del	Deletion	p.Asp26274MetfsTer66	Pathogenic	PVS+PM+PP
175	44	Male	South Asian	Idiopathic	DSC2	c.2200C>T	Nonsense	p.Gln734Ter	Pathogenic	PVS+PM+PP

ACMG indicates American College of Genetics and Genomics; ARVC, arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; ERS, early repolarization syndrome; HCM, hypertrophic cardiomyopthy; LQTS, Long QT syndrome; PM, moderate evidence of pathogenicity; PP, supporting evidence of pathogenicity; PS, strong evidence of pathogenicity; PVS, very strong evidence of pathogenicity; and SCA, sudden cardiac arrest.

(Figure 3) Pathogenic variants were reclassified as VUS in 4 cases. One *KCNE1* (c.253G>A p.Asp85Asn) variant was reported as a modulator of QT interval but not associated with LQTS per se. A truncating variant in AKAP9 (c.7438C>T, p.Gln2480Ter) was reclassified despite low population frequency because loss of function in AKAP9 has not been proven to be a mechanism of disease. A novel DSP (c.7784C>T, p.Thr3595Ile) variant was reclassified because of a lack of supporting evidence of pathogenicity. Similarly, an in-frame deletion in SCN5A (c.4140_4142delCAA, p.1380delA) was reclassified. This variant had previously been reported as a VUS in ClinVar.

Table 3. Yield of Pathogenic Variants by Clinical Phenotype

Phenotype	n	Pathogenic Variant, %
LQTS	25	5 (20)
BrS	7	2 (29)
CPVT	8	2 (25)
ARVC	10	6 (60)
Phenotype negative	102	13 (13)

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; and LQTS, Long QT syndrome.

VUS were reclassified as pathogenic in 3 cases and as likely pathogenic in 1 case. Two of these cases concerned the *CACNA1C* variant described earlier. A missense variant in *SCN5A* (c.1099C>T, p.Arg367Cys) was also revised to pathogenic. This variant was previously reported as pathogenic multiple times, resides in a functionally important region, is rare in control populations, and is predicted as deleterious by in silico modeling (Complementary Annotation Dependent Depletion score 35). A missense variant in RYR2 (c.12587C>T, p.Thr4196Ile) was revised to be likely pathogenic. Although not reported previously, it resides in an area of the gene where multiple other pathogenic missense variants have been reported. It is also absent from Exome Aggregation Consortium and has a high Complementary Annotation Dependent Depletion score (30). In addition, the patient experienced cardiac arrest during exercise.

VUS were reclassified as benign polymorphisms in 42 instances, largely based on high general population frequencies. The majority of VUS were identified in phenotype-negative individuals who underwent broad multiphenotype genetic testing. These panels were performed by 3 commercial laboratories in 88% of cases. The number of genes included in the next-generation sequencing panels was different for each laboratory, although the yield of pathogenic variants was similar. The number of VUS/patient and VUS/genes tested/patient, as originally reported, also differed significantly (See Table 4).

Cascade Family Screening

Assessment of first-degree relatives of survivors with either genetic or clinical evaluation was performed in 11 of the 29 cases where a pathogenic/likely pathogenic variant was identified. Focused genetic testing was performed in 21 relatives from 9 families, with variants identified in 12 (57%). Details of the cascade screening are included in the Data Supplement.

Discussion

The Yield of Genetic Testing

Genetic testing in this large heterogeneous cohort of unexplained cardiac arrest survivors revealed a pathogenic variant in 17% of cases. This illustrates the principle that genetic cardiac disease may first present with life-threatening arrhythmia even in the absence of an identifiable clinical phenotype. Therefore, genetic testing in unexplained cardiac arrest survivors may be considered. Future studies are required to fully assess the clinical utility, effect on patient management, and cost-effectiveness of such a strategy.

A family history of sudden death and a history of prior syncope were independently associated with a higher yield of pathogenic variants. This is understandable because a genetically mediated substrate is more likely to present with recurrent arrhythmic episodes and affect multiple family members. The presence of these features may be used to direct genetic testing to those groups where the yield is highest, especially in healthcare systems where resources may be limited.

Identification of a clinical phenotype may have also been expected to lead to a higher yield of pathogenic variants because it allows for directed genetic testing, while those with no identifiable clinical phenotype may have been expected to have negative testing; familial cases of idiopathic ventricular fibrillation are rare and, other than the Dutch founder mutation in *DPP6*,[16,17] genetic etiologies have not readily been identified.

However, while the presence of a clinical phenotype was associated with a higher yield on univariate analysis, it was of borderline significance when corrected for other significant

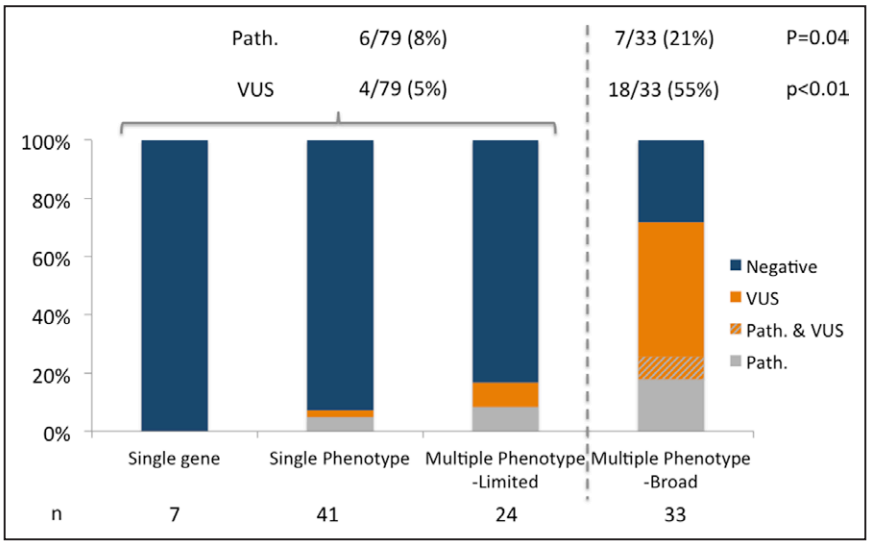

Figure 2. Yields of genetic testing by testing type in phenotype-negative cardiac arrest survivors. Broad multiple phenotype testing identifies more pathogenic variants in cardiac arrest survivors with no clinical phenotype but is also associated with a higher rate of identification of VUS. Path. indicates pathogenic/likely pathogenic; and VUS, variant of unknown significance.

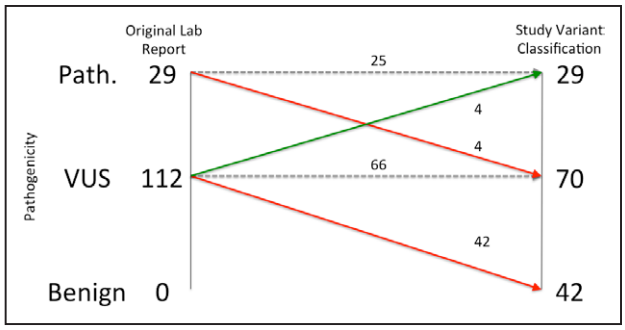

Figure 3. Variant reclassification results after application of independent assessment algorithm based on 2015 ACMG criteria. Variants were reclassified in 53 (38%) instances. ACMG indicates American College of Genetics and Genomics; Path., pathogenic/likely pathogenic; and VUS, variant of unknown significance.

clinical features. This may be explained by the limitations of phenotypic testing and the difficulties of making an accurate diagnosis based on mild phenotypic features. Only 20% of those with a definite or probable clinical diagnosis of LQTS had a pathogenic variant identified compared with 75% expected in usual practice.[3] Because all these patients had normal resting QTc measurements, as per the study inclusion criteria, diagnosis was largely based on provocative testing with exercise ECG or epinephrine infusion. It has previously been shown that epinephrine infusion in particular is a sensitive but not specific test for LQTS.[10] The borderline effect of clinical phenotype on yields of pathogenic variants may, therefore, be explained by physician bias toward sensitive rather than specific investigations in the setting of an unexplained cardiac arrest leading to overdiagnosis on clinical grounds.

The genetic testing strategy used also had an influence on the yield of both pathogenic variants and VUS. Focusing on phenotype-negative patients, where a commonly accepted strategy is not available, yields of pathogenic variants were higher when broad multiphenotype panels were used compared with other strategies. The use of broad panels (including

Table 4. Comparison of Laboratories Performing Broad-Panel Testing in Phenotype-Negative Patients

	Laboratory 1	Laboratory 2	Laboratory 3	*P* Value
Patients tested, n	16	5	8	…
Genes tested/patient (mean±SD)	55.7±24.6	43.2±35.2	91.4±55.4	0.05
Patients with pathogenic variant, n	1	1	2	0.41
Patients with VUS, n	13	3	7	0.47
Sum total VUS, n	23	5	53	<0.01
VUS/patient (mean±SD)	1.4±1.4	1.0±1.2	6.6±4.6	<0.01
VUS/gene/patient (mean±SD)	0.024±0.016	0.032±0.051	0.083±0.067	0.01

Figures are based upon original laboratory variant interpretation. VUS indicates variants of unknown significance.

≤150 genes associated with arrhythmia syndromes or cardiomyopathies) identified pathogenic variants in 21% of phenotype-negative cases. Extrapolation of this yield to the 72 phenotype-negative cases where alternative testing strategies were used would have led to the identification of a pathogenic variant in an additional 9 cases. Given the limitations of phenotypic testing discussed earlier, it may be possible that the yield in the phenotype-positive group would also have been increased if such a hypothesis-free strategy was undertaken. Interestingly, recent molecular autopsy studies using similarly large gene panels in the investigation of sudden arrhythmic death syndrome, which can be thought of as analogous to unexplained cardiac arrest, have reported comparable yields: Nunn et al[18] reported a yield of 29% using a 135 gene panel in 59 sudden arrhythmic death syndrome victims, and more recently, Bagnall et al[5] reported a 26% yield testing 46 genes in 113 unexplained sudden cardiac deaths.

Broad multiphenotype panels also identified ≥1 VUS in 55% of cases with ≤7 VUS in a single patient. The VUS is currently the biggest challenge in clinical cardiogenetics, and freeing patients and physicians from genetic purgatory[19] requires significant time, resources, and expertise. We, therefore, conclude that broad-panel testing should be undertaken only in dedicated clinics with the appropriate infrastructure and expertise to not only interpret and act on pathogenic variants, but also to critically appraise the nearly inevitable VUS when testing is performed. Such reappraisal of variants must also be serially repeated because their significance may change over time: 35% of variants originally reported were subsequently reclassified when reassessed. Time since the original report for reclassified variants was 3.4±2.2 years and was similar for variants whose classification did not change (P=0.45). Furthermore, similar to recent studies,[20] we observed significant variation between laboratories regarding variant ascertainment, perhaps reflecting contrasting philosophies with respect to the balance between sensitivity and specificity of findings, meaning an uninformed clinician is not able to always simply rely on the interpretation of the reporting laboratory. It is hoped that with increasing sizes of control populations, greater understanding of the functional implications of identified variants, and evolving guidelines for variant classification,[21] less heterogeneity will be seen between laboratories and that the proportion of VUS will decrease in the future. VUS were frequently identified in minor arrhythmia genes where substantial evidence connecting them to sudden death is limited or absent. Subsequently, the signal/noise ratio for these genes is low. It may be that omitting such genes from future sudden death panels may also reduce numbers of VUS without significantly reducing rates of pathogenic variant identification.

Genes Implicated in Unexplained Cardiac Arrest

The majority of genes in which pathogenic variants were identified have been well described in association with LQTS, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia. Several minor *LQTS* genes (representing LQTS4-12) were also implicated. Of note, a single variant in *CACNA1C* (c.2570C>T, p.Pro857Leu) was identified in 3 unrelated individuals. Two had a clinical phenotype of LQTS, while the third had minor findings insufficient for a

clinical diagnosis. This variant was first reported in 2013 in association with autosomal dominant LQTS[22] with a history of sudden death in the affected family. CACNA1C encodes the α-subunit of the L-type calcium channel (Cav 1.2).[23] The variant localizes to a key proline, P, glutamic acid, E, serine, S, and threonine, T domain in which several other pathogenic variants have been identified. proline, P, glutamic acid, E, serine, S, and threonine, T domains act to signal protein degradation.[24] Functional studies of other pathogenic variants within the same domain (eg, Pro857Arg) have shown an increased $I_{Ca,L}$ current density because of increased channel stability at the cell membrane. Further investigation is required to ascertain whether this particular variant is associated with an increased risk of sudden death.

Cardiomyopathy genes were also implicated in phenotype-negative patients. This is again in keeping with the recent molecular autopsy study by Bagnall et al,[5] in which a significant proportion of pathogenic variants identified in cases with structurally normal hearts at postmortem were in cardiomyopathy-associated genes. The notion that pathogenic variants in cardiomyopathy genes may result in sudden death without manifest structural change both prompts a reassessment of the accepted mechanisms of arrhythmia in such cases and supports a humble approach to investigation of sudden death, where the limitations of phenotyping are recognized and genetic testing is undertaken with an open mind, as discussed earlier. No clinical features were identified as predicting pathogenic variants in cardiomyopathy genes in phenotype-negative cases, although the small numbers prevent detailed analysis.

Because of the retrospective nature of this study, it is possible that referral bias may have led to an overestimate of the incidence of pathogenic variants in unexplained cardiac arrest. Large-scale prospective investigation with systematic broad-panel genetic testing is necessary to identify the true incidence, which may then inform whether such a strategy should be recommended for all unexplained cardiac arrest survivors. As stated earlier, the apparent high rate of VUS should certainly limit current testing to experienced centers, which may be focused on those with prior syncope or a family history of sudden death.

Study Limitations

Although the cohort is large, genetic testing was not systematic and was limited by available resources and contemporary technologies in some cases. Data regarding segregation of variants within families of cardiac arrest survivors is currently unavailable; at present, there are insufficient first-degree relatives enrolled in the registry to perform any meaningful analysis. This will be developed in the future. Because of the retrospective nature of the study, referral bias cannot be excluded.

Conclusion

Genetic testing identifies a pathogenic variant in a significant proportion of unexplained cardiac arrest even in the absence of an identifiable clinical phenotype. Prior syncope and a family history of sudden death are predictors of a higher yield of pathogenic variants. Broad multiphenotype panels have the highest yield in phenotype-negative cases but frequently identify VUS. Genetic testing may, therefore, be considered in unexplained cardiac arrest, but should be limited to specialist clinics with genetic interpretation expertise. Implicated genes include those associated with cardiomyopathy even in the absence of a clinical phenotype.

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Disclosures

None.

Appendix

From the Heart Rhythm Services, Division of Cardiology (G.M., Z.W.M.L., A.D.K.) and Department of Medical Genetics (L.A., R.L.), University of British Columbia, Vancouver, Canada; Cardiovascular Genetics Center, Department of Medicine, Montreal Heart Institute, Université de Montréal, Quebec, Canada (R.T., M.T.); Section of Cardiac Electrophysiology, Division of Cardiology, Department of Medicine, Western University, London, ON, Canada (J.D.R., G.J.K.); Libin Cardiovascular Institute, University of Calgary, AB, Canada (B.G.); Department of Cardiology, Queen's University, Kingston, ON, Canada (C.S.S.); Quebec Heart and Lung Institute, Canada (J.C., C. Steinberg); QEII Health Sciences Center, Halifax, NS, Canada (M.G.); University of Ottawa Heart Institute, ON, Canada (D.H.B); Division of Cardiology, St Michael's Hospital, University of Toronto, ON, Canada (P.A.); Childrens Heart Centre, BC Children's Hospital, Vancouver, Canada (S.S.); University Health Network, Toronto, ON, Canada (V.S.C.); WHRA Cardiac Sciences Program, St Boniface Hospital, Winnipeg, Manitoba (C. Seifer); and Population Health Research Institute, Hamilton, ON, Canada (J.S.H.).

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CLINICAL PERSPECTIVE

Unexplained cardiac arrests may be because of inherited arrhythmia syndromes. Identification of the underlying genetic variant responsible for the cardiac arrest can help refine clinical diagnoses, allowing tailored treatment and facilitate cascade screening of other at-risk family members. This retrospective study of a large cohort of cardiac arrest survivors, where structural and ischemic heart disease had been excluded, analyses the role of genetic testing in diagnosing the cause of an apparently unexplained cardiac arrest. The study shows that 18% of cardiac arrest survivors are identified with a pathogenic variant in a causative gene. Clinical predictors of a positive genetic test include the presence of prior syncope and of a family history of sudden death. Identification of a clinical phenotype after in-depth clinical evaluation only led to a borderline improvement in the yield of a positive test. Genetic variants of uncertain significance were also identified frequently, particular in phenotype-negative cases where large gene panels were used. Genetic variants were identified in both arrhythmia syndrome and cardiomyopathy genes, mirroring findings from recent molecular autopsy studies. This highlights that arrhythmic risk may occur before structural manifestations of cardiomyopathy and questions the accepted mechanisms of arrhythmia induction in these conditions. This study shows that genetic testing may be considered as an adjunct to clinical evaluation for survivors of an unexplained cardiac arrest. However, the frequent identification of variants of unknown significance means that genetic testing should only be performed by those centers with the appropriate experience, expertise, and infrastructure to independently evaluate such findings.

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Supplemental Materials

Table S1. Comparison with probands who did not have genetic testing. *Fhx SD= Family history of sudden death*

	No Genetic Testing	Genetic Testing	p
n	201	174	
Age at cardiac arrest	42.6±13.9	39.1±15.2	0.02
Male (%)	130 (65)	98 (56)	0.08
Prior syncope	33 (17)	39 (22)	0.12
Fhx SD	32 (16)	22 (13)	0.35
Clinical phenotype	35 (18)	72 (41)	<0.01
- ARVC	0 (0)	10 (6)	<0.01
- BrS	4 (2)	7 (4)	0.24
- CPVT	1 (1)	8 (5)	0.01
- LQTS	8 (4)	25 (14)	<0.01

Figure S1. Age distribution of cohort.

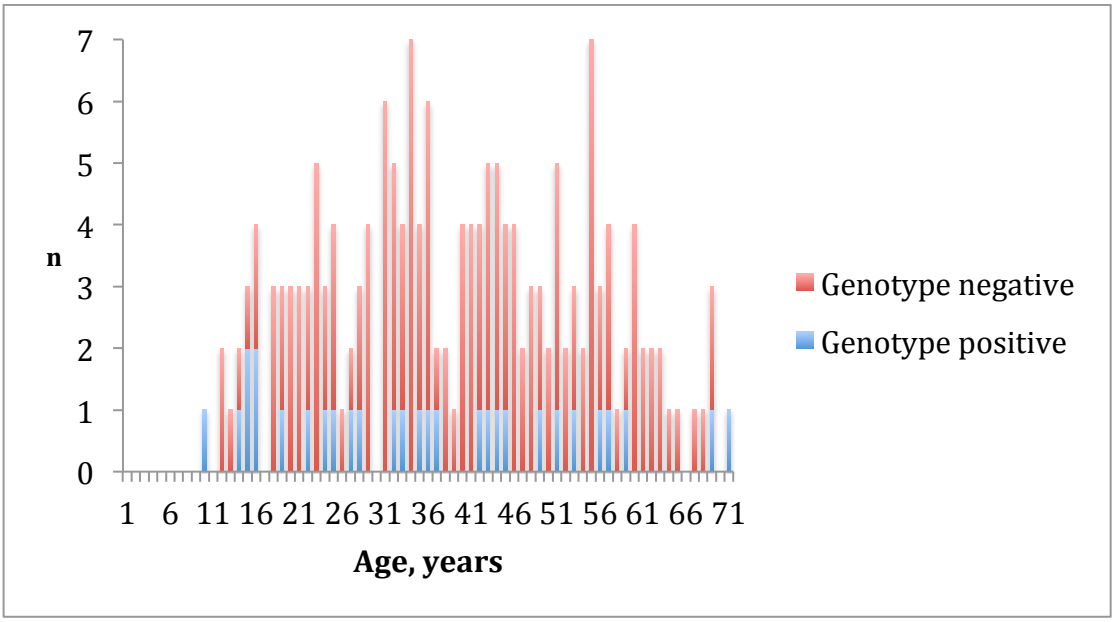

Table S2. Criteria used to ascertain evidence of pathogenicity. Criteria are based upon 2015 ACMG guidelines for variant classification. Italicized text states specific criteria used in this study where appropriate. Evidence was aggregated as per 2015 ACMG guidelines to give final variant classification of pathogenic; likely pathogenic; VUS; likely benign; or benign. *ARVC=Arrhythmogenic Right Ventricular Cardiomyopathy, BrS=Brugada Syndrome, CPVT=Catecholaminergic Polymorphic Ventricular Tachycardia, LQTS=Long QT Syndrome, HCM=Hypertrophic Cardiomyopathy; MAF=Minor Allele Frequency.*

Evidence of pathogenic variant				**Evidence of benign variant**		
Very Strong (PVS)	**Strong (PS)**	**Medium (PM)**	**Supporting (PP)**	**Stand alone (BA)**	**Strong (BS)**	**Supporting (BP)**
Null Variant	Same amino acid change as established pathogenic variant *(i.e. ≥2 unequivocal reports in ClinVar)*	Located in known mutational hot spot or functional domain	Co-segregation in family	Allele Frequency ≥5%	Allele frequency greater than expected for disorder *(i.e. >1:1000)*	Missense in gene where truncating variant usual cause
	Well established in vitro or in vivo functional studies demonstrating pathogenicity	Absent from control populations *(MAF<1:50,000 in ExAC)*	Missense variant in gene commonly affected by missense (i.e. in major arrhythmia or CM gene)		Well established in vitro or in vivo functional studies demonstrating no damaging effect on protein function	In frame ins/del in a repetitive region with no known function
		Protein length change (from in frame ins/del)	In silico tests predict pathogenicity *(i.e. CADD ≥20)*		Lack of segregation in affected family members	In silico tools predict no affect on protein function *(i.e. CADD<20)*
		Same amino acid affected in established pathogenic variant	Phenotype suggestive of monogenic disease *(i.e. phenotype positive for LQTS/CPVT/BrS/HCM/ARVC)*			Found in a case with an alternative molecular cause for disease *(i.e. in patient where another pathogenic variant detected)*
			Published article stating as pathogenic			Synonymous variant with no effect on splicing and not highly conserved

Table S3. Summary of Variants of Uncertain Significance. *ARVC=Arrhythmogenic Right Ventricular Cardiomyopathy, BrS=Brugada Syndrome, CPVT=Catecholaminergic Polymorphic Ventricular Tachycardia, Dup=duplication, ERS=Early Repolarization Syndrome, LQTS=Long QT Syndrome*

Study ID	Age	Sex	Ethnicity	Phenotype	Gene	Sequence Change	Variant type	Protein Change
13	42	Female	Caucasian	Idiopathic	DSP	c.7784C>T	mis-sense	Thr2595Ile
35	52	Female	Caucasian	LQTS	KCNJ2	c.1199C>T	mis-sense	Thr400Met
40	69	Female	Caucasian	Idiopathic	DSP	c.3820 G>C	mis-sense	Ala1274Thr
40	69	Female	Caucasian	Idiopathic	DSP	c.5753 A>C	mis-sense	Glu1918Ala
40	69	Female	Caucasian	Idiopathic	CACNA1C	c.959 C>T	mis-sense	Thr320Met
64	16	Male	Unknown	Idiopathic	TPM1	c.24G>C	mis-sense	Glu8Asp
71	40	Male	S Asian	Idiopathic	DSC2	c.1787C>T	mis-sense	Ala596Val
77	23	Female	S Asian	Idiopathic	SCN1B	c.560G>A	mis-sense	Arg187His
77	23	Female	S Asian	Idiopathic	SNTA1	c.1210G>A	mis-sense	Ala404Thr
79	67	Male	Caucasian	Idiopathic	CACNB2	c.1122+3A>T	splice site	-
79	67	Male	Caucasian	Idiopathic	CACNB2	c.1558G>A	mis-sense	Gly520Ser
81	13	Male	Caucasian	Idiopathic	MYH6	c.2612G>A	mis-sense	Arg871His
83	34	Male	S Asian	Idiopathic	AKAP9	c.8345C>T	mis-sense	Thr2782Ile
83	34	Male	S Asian	Idiopathic	DSC2	c. 2197G>A	mis-sense	Ala733Thr
86	0	Female	Caucasian	CPVT	ANK2	c.6206G>A	mis-sense	Arg2069His
86	0	Female	Caucasian	CPVT	AKAP9	c.11378C>G	mis-sense	Ser3793Cys
113	69	Female	Caucasian	BrS	SCN5A	c.4140_4142delCAA	in frame deletion	1380delA
116	35	Male	Caucasian	SCIVF	DSG2	c.601G>A	mis-sense	Val201Ile

121	12	Male	Caucasian	Idiopathic	RYR2	c.10024G>C	mis-sense	Ala3342Pro
123	32	Female	Caucasian	Idiopathic	DSC2	c.2687_2688insAG	frameshift	A897KfsX3
125	33	Female	Caucasian	ARVC	DSP	c.5178C>A	mis-sense	Asn1726Lys
130	21	Male	Caucasian	Idiopathic	AKAP9	c.6037G>A	mis-sense	Glu2013Lys
130	21	Male	Caucasian	Idiopathic	KCNA5	c.1790G>A	mis-sense	Arg597Gln
130	21	Male	Caucasian	Idiopathic	DSG2	c.3040G>A	mis-sense	Val1014Ile
131	14	Male	Caucasian	LQTS	RYR2	c.2131G>A	mis-sense	Glu711Lys
131	14	Male	Caucasian	LQTS	TTN	c.30247A>G	mis-sense	Met100831Val
144	46	Male	Caucasian	Idiopathic	NEXN	c.613G>A	mis-sense	Glu205Lys
144	46	Male	Caucasian	Idiopathic	TTN	c.74470G>A	mis-sense	Val24824Ile
144	46	Male	Caucasian	Idiopathic	TTN	c.50521G>A	mis-sense	Glu1684Lys
144	46	Male	Caucasian	Idiopathic	TTN	c.10880C>T	mis-sense	Pro3627Leu
144	46	Male	Caucasian	Idiopathic	DSP	c.4372C>G	mis-sense	Arg1458Gly
144	46	Male	Caucasian	Idiopathic	KCNJ8	c.763G>A	mis-sense	Glu255Lys
144	46	Male	Caucasian	Idiopathic	MYH11	c.5800A>T	mis-sense	Thr1934Ser
145	23	Female	Caucasian	Idiopathic	TTN	c.74816T>A	mis-sense	Leu24939Gln
145	23	Female	Caucasian	Idiopathic	TTN	c.63097G>T	mis-sense	Val21033Phe
145	23	Female	Caucasian	Idiopathic	TTN	c.36394A>G	mis-sense	Ile12132Val
145	23	Female	Caucasian	Idiopathic	TTN	c.8329G>T	mis-sense	Val2777Phe
145	23	Female	Caucasian	Idiopathic	KCNE3	c.248G>A	mis-sense	Arg83His
146	20	Male	Caucasian	ARVC	DSG2	c.44T>A	mis-sense	Leu15Gln
148	34	Female	S Asian	LQTS	SNTA1	c.1118G>A	mis-sense	Arg373His
151	34	Male	Caucasian	Idiopathic	ANK2	c.343G>A	mis-sense	Val115Ile
152	14	Female	Caucasian	Idiopathic	SCN5A	c.52C>T	mis-sense	Arg18Trp
153	28	Male	Caucasian	Idiopathic	TRPM4	c.678C>G	mis-sense	Asp226Glu
154	37	Female	Caucasian	LQTS	KCNE1	c.314C>T	mis-sense	Ser105Leu

155	45	Female	Caucasian	Idiopathic	DSG2	c.437G>T	mis-sense	Arg146Leu
156	49	Female	Inuit	LQTS	ANK2	c.478A>T	mis-sense	Thr160Ser
158	15	Male	S Asian	Idiopathic	AKAP9	c.11276C>T	mis-sense	Pro3759Leu
158	15	Male	S Asian	Idiopathic	CACNB2	c.1429T>G	mis-sense	Ser477Ala
159	43	Female	Caucasian	Idiopathic	ANK2	c.5509G>A	mis-sense	Ala1837Thr
162	18	Male	S Asian	Idiopathic	RYR2	c. 9352G>A	mis-sense	Gly3118Arg
162	18	Male	S Asian	Idiopathic	SCN5A	c.1840C>T	mis-sense	Pro614Ser
162	18	Male	S Asian	Idiopathic	MYBPC3	c.3118T>G	mis-sense	Ser1040Ala
162	18	Male	S Asian	Idiopathic	MYBPC3	c.503T>C	mis-sense	Val168Ala
163	55	Male	Caucasian	Idiopathic	AKAP9	c.4825_4286delADinsCA	mis-sense	Arg1609Gln
163	55	Male	Caucasian	Idiopathic	PKP2	c.964G>T	mis-sense	Gly322Cys
165	32	Female	S Asian	ERS	AKAP9	c.7438C>T	non-sense	Gln2480X
165	32	Female	S Asian	ERS	DSP	c.7916G>A	mis-sense	Arg2639Gln
166	34	Female	Caucasian	LQTS	KCNE1	c.253G>A	mis-sense	Asp85Asn
170	16	Female	Caucasian	Idiopathic	PLEC	c.677G>A	mis-sense	Arg226Gln
170	16	Female	Caucasian	Idiopathic	ABCC9	c.4570_4572delinsAAAT	frameshift	Leu1524Lysfs*5
171	20	Female	Caucasian	LQTS	VCL	c.565G>A	mis-sense	Val189Met
171	20	Female	Caucasian	LQTS	MYH6	c.1048G>A	mis-sense	Val350Ile
173	53	Male	Caucasian	Idiopathic	RYR2	c.6022+5G>A	splice site	-
173	53	Male	Caucasian	Idiopathic	CACNB2	c.169-8C>T	splice site	-
174	48	Female	Caucasian	Idiopathic	RYR2	c.7375G>A	mis-sense	Gly2459Arg
175	44	Male	S Asian	Idiopathic	SYNE1	c.22765C>T	mis-sense	Arg7589Trp
175	44	Male	S Asian	Idiopathic	SYNE1	c.15200C>T	mis-sense	Ala5067Val
175	44	Male	S Asian	Idiopathic	AKAP9	c.2609G>A	mis-sense	Cys870Tyr
175	44	Male	S Asian	Idiopathic	JUP	c.2138C>T	mis-sense	Pro713Leu
175	44	Male	S Asian	Idiopathic	LDLR	c.1241T>G	mis-sense	Leu414Arg

Table S4. Findings from cascade screening of first-degree relatives

Proband 7; CPVT phenotype, RYR2, c.11934G>A	
	Three first-degree relatives identified as carriers of the variant.
	CPVT phenotype and prior syncope in all 3
	Treated with BBblocker (n=3) Ca blocker (n=20, flecainide (n=1) and ICD (n=1)
	One clinically unaffected relative has not had genetic testing
Proband 8; LMNA c.673C>T, phenotype neg. (developed DCM during follow-up)	
	3 unaffected relatives with negative genetic testing
Proband 13; phenotype negative DSP c.7784C>T	
	1 family member carries variant. No evidence of ARVC on imaging
Proband 18; phenotype negative, RYR2 c.76G>A	
	7 relatives assessed (genetic testing in 5, positive in 2 (40%)
	1 of 2 gene positive relatives diagnosed with ARVC
	1 of remaining relatives (gene negative) under surveillance following "borderline right ventricular abnormalities on MRI"
Proband 69; LQTS phenotype, CACNA1C c.2570C>T	
	2 relatives clinically unaffected but no genetic testing
Proband 104; ARVC phenotype DSC2 c.1521G>A	
	2 relatives with genetic testing, 1 positive
	no evidence of ARVC phenotype in either
Proband 112; HCM phenotype, MYBPC3 c.2373dupG	
	3 relatives with genetic testing, 2 positive
	Both gene positive affected, ICD in 1
Proband 117; ARVC phenotype, DSG2 c,941C>A	
	Single clinically unaffected relative, no genetic testing
Proband 154; LQTS phenotype; CACNA1C c.2579G>A	
	1 relative, gene positive but clinically unaffected
Proband 156; LQTS phenotype; CACNA1C c.2570C>T	
	1 relative, gene positive, borderline phenotype, treated with beta-blocker
Proband 160; phenotype negative, SCN5A c.1C>T	
	1 relative, gene positive, LQTS phenotype, treatment unknown