








# Evaluation of gene validity for CPVT and short QT syndrome in sudden arrhythmic death

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## Aims

Catecholaminergic polymorphic ventricular tachycardia (CPVT) and short QT syndrome (SQTS) are inherited arrhythmogenic disorders that can cause sudden death. Numerous genes have been reported to cause these conditions, but evidence supporting these gene–disease relationships varies considerably. To ensure appropriate utilization of genetic information for CPVT and SQTS patients, we applied an evidence-based reappraisal of previously reported genes.

## Methods and results

Three teams independently curated all published evidence for 11 CPVT and 9 SQTS implicated genes using the ClinGen gene curation framework. The results were reviewed by a Channelopathy Expert Panel who provided the final classifications. Seven genes had definitive to moderate evidence for disease causation in CPVT, with either autosomal dominant (*RYR2*, *CALM1*, *CALM2*, *CALM3*) or autosomal recessive (*CASQ2*, *TRDN*, *TECL1*) inheritance. Three of the four disputed genes for CPVT (*KCNJ2*, *PKP2*, *SCN5A*) were deemed by the Expert Panel to be reported for phenotypes that were not representative of CPVT, while reported variants in a fourth gene (*ANK2*) were too common in the population to be disease-causing. For SQTS, only one gene (*KCNH2*) was classified as definitive, with three others (*KCNQ1*, *KCNJ2*, *SLC4A3*) having strong to moderate evidence. The majority of genetic

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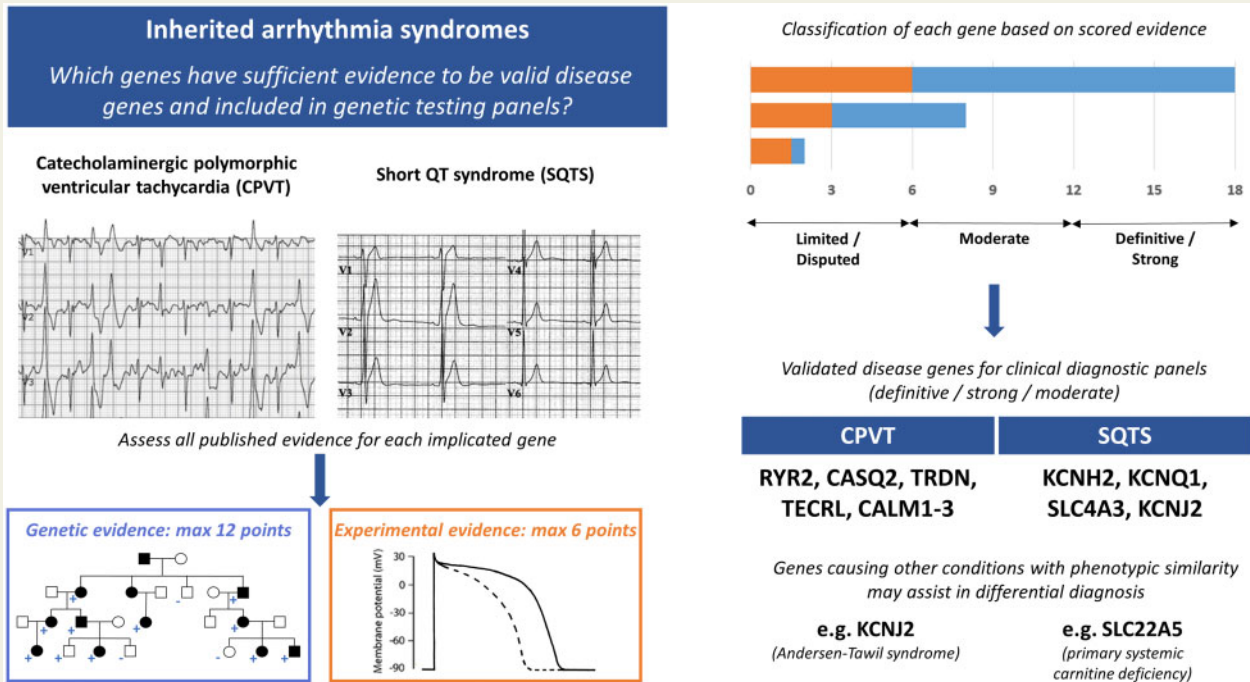
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evidence for SQTS genes was derived from very few variants (five in *KCNJ2*, two in *KCNH2*, one in *KCNQ1/SLC4A3*).

## Graphical Abstract



## Keywords

Catecholaminergic polymorphic ventricular tachycardia • Short QT syndrome • Genetic testing • Mendelian genetics

## Translational perspective

This study comprehensively reappraises the evidence for genes implicated in the inherited arrhythmias catecholaminergic polymorphic ventricular tachycardia and short QT syndrome using the ClinGen curation framework. The genes identified as having substantial evidence for disease causation should be included in clinical genetic testing panels for these diseases while genes with insufficient evidence, or those causing other arrhythmia phenotypes, should be sequenced and interpreted more cautiously. These findings will enable a more evidence-based approach to diagnostic genetic testing and help to reduce the number of uncertain or false positive findings obtained from the inclusion of wrongly implicated genes.

## Introduction

In contemporary practice, genetic testing is routinely used in the evaluation of inherited cardiac syndromes, enabling the implementation of precision medicine for patients and genetic cascade screening to identify at-risk family members. This has been made possible by the identification of disease genes over the past three decades, initially through linkage analysis in large family pedigrees and more recently by unbiased whole exome sequencing and through candidate gene

studies (where plausible disease genes are selected for sequencing in patient cohorts). While the latter approach has been successful in identifying some disease-causing genes in situations where large families are unavailable, its limitations in establishing valid gene–disease relationships have been exposed in recent years. We now recognize that many genetic variants previously described as pathogenic are too common in populations to be disease causing and that rare but benign variants are collectively common in many genes. These developments have prompted the re-evaluation of many gene–disease

relationships and the recognition that more rigorous approaches are needed to define Mendelian disease genes.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) and short QT syndrome (SQTS) are rare inherited arrhythmogenic disorders that can lead to sudden cardiac death (SCD) at a young age. It is therefore imperative to diagnose patients early to implement primary prevention measures such as appropriate medical therapy or implantable cardioverter–defibrillator insertion. Genetic testing is now widely applied for CPVT and SQTS, but the interpretation of results is hampered by challenges in accurate phenotyping of these conditions, despite the availability of diagnostic criteria.<sup>1,2</sup>

Catecholaminergic polymorphic ventricular tachycardia often presents in children or young adults with syncope or SCD triggered by  $\beta$ -adrenergic stimulation related to exercise or emotional stress. Patients have normal electrocardiograms (ECGs) at rest with usually no evidence of structural heart disease on cardiac imaging. Diagnosis is made through the use of exercise testing (or Holter monitor in children) to detect exercise-induced arrhythmias, most commonly bi-directional and polymorphic ventricular tachycardia (VT).<sup>2</sup> These observations upon exercise but not at rest potentially enable clinicians to distinguish CPVT from other arrhythmogenic disorders that can induce syncope and/or cardiac arrest in patients with structurally normal hearts.

Short QT syndrome is characterized by abbreviation of the cardiomyocyte action potential duration, which manifests as a short QT interval on ECG. SQTS patients have a propensity to develop atrial fibrillation at a young age and are at risk of SCD.<sup>3</sup> Due to the near absence of large families with SQTS, genetic research using linkage analysis is impossible and our knowledge of SQTS genetics is almost solely dependent on data derived from single patients or small kindreds.

Genes reported in CPVT and SQTS are often incorporated into clinical genetic testing panels irrespective of the strength of evidence provided for these assertions. We have previously reassessed the genetic aetiology of the arrhythmogenic disorders Brugada syndrome (BrS)<sup>4</sup> and long QT syndrome (LQTS)<sup>5</sup> using a standardized, evidence-based framework for examining reported gene–disease relationships.<sup>6</sup> Here, we report the reappraisal of all previously published genes reported to cause CPVT and SQTS, to enable more accurate interpretation of genetic data and appropriate clinical management in patients and families with these potentially life-threatening conditions.

## Methods

### Gene curation framework

The evidence for gene–disease relationships was evaluated as previously described for BrS and LQTS.<sup>4,5</sup> Briefly, three teams of biocurators independently curated each gene, applying the ClinGen Gene Curation Framework version 7 of the standard operating procedure.<sup>6</sup> This framework uses a semi-quantitative scoring system for genetic (maximum 12 points) and experimental (maximum 6 points) evidence from published studies only, which categorizes each gene–disease relationship into a clinical validity classification level—*Definitive* (total of 12–18 points and replicated over time in the literature), *Strong* (12–18 points), *Moderate* (7–11 points), and *Limited* (1–6 points). A gene curation expert panel

(GCEP), comprising 10 individuals with collectively dozens of years of experience in clinical care and/or research in the field of CPVT, SQTS, and clinical genetics, was tasked with performing final evaluation and classification on a gene-by-gene basis. The panel had the option of modifying the findings of the curation template scores (upgrade, no change, downgrade) based on the available evidence. See [Supplementary material online](#) for full details of Methods.

## Results

### Summary of findings

Eleven genes were evaluated as reported causes of CPVT: eight with autosomal dominant (AD) inheritance, two with autosomal recessive (AR) inheritance, and one gene, *CASQ2*, was analysed separately for both ([Table 1](#)). The literature search identified nine genes that have been previously reported as causing SQTS ([Table 2](#)). Detailed curation summaries and classification matrices for each gene are available in the [Supplementary material online](#).

### Definitive genes specific for catecholaminergic polymorphic ventricular tachycardia

*RYR2* (AD inheritance) and *CASQ2* (AR inheritance) were the first genes reported to cause CPVT (in 2001<sup>7</sup> and 2002,<sup>8</sup> respectively). Both genes were classified as *Definitive* after reaching the maximum amount of genetic and experimental points with curation of only a small subset of available published evidence ([Figure 1](#)). Gain-of-function missense variants in *RYR2* are detected in at least 50% of CPVT cases with a definitive diagnosis,<sup>9</sup> with the gene–disease relationship supported by multiple reports of familial segregation in large pedigrees, numerous examples of *de novo* inheritance in sporadic cases<sup>7</sup> and an abundance of functional characterization of variants detected in patients. Biallelic loss-of-function variants in *CASQ2* have been described in numerous CPVT cases, with the relationship further supported by a plethora of experimental evidence including knockout mice that recapitulate the disease phenotype.

As some recent reports have suggested that heterozygous loss-of-function variants in *CASQ2* may also be disease-causing,<sup>10,11</sup> evidence for AD inheritance for *CASQ2* was evaluated separately. Most of this evidence came from a recent multi-centre study on *CASQ2*-related CPVT that described 12 CPVT probands with heterozygous *CASQ2* variants and a CPVT phenotype in 8/37 (22%) of heterozygous relatives of biallelic probands.<sup>10</sup> After discussion, the GCEP came to the consensus that these data should be cautiously interpreted and constituted *Moderate* evidence for causing AD CPVT ([Figure 1](#)). The reasons included the absence of adjudicated phenotyping of cases and uncertainty about the pathogenicity of some variants, i.e. population frequencies incompatible with AD CPVT, loss-of-function variants with uncertain effects (presumed nonsense-mediated decay-escaping C-terminal truncating variants and a splice region variant of unproven effect), and missense variants with limited functional characterization. More research is required to define the penetrance of *CASQ2* loss-of-function variants in the heterozygous state.

**Table 1** Classification of evidence for genes reported as causing catecholaminergic polymorphic ventricular tachycardia

Gene	Protein	HGNC ID	Chromosomal location	Inheritance	Presence on GTR panels, n = 12 (%)	Scoring classification	Final expert classification	Other arrhythmia conditions with valid gene-disease relationship
<i>RYR2</i>	Ryanodine receptor 2	10484	1q43	AD	100%	Definitive	Definitive	—
<i>CASQ2</i>	Calsequestrin-2	1513	1p13.1	AR	100%	Definitive	Definitive	—
<i>TRDN</i>	Triadin	12261	6q22.31	AR	92%	Definitive	Definitive	LQTS
<i>TECRL</i>	Trans-2,3-enoyl-CoA reductase like	27365	4q13.1	AR	25%	Definitive	Definitive	—
<i>CALM1</i>	Calmodulin-1	1442	14q32.11	AD	92%	Moderate	Moderate <sup>a</sup>	LQTS
<i>CALM2</i>	Calmodulin-2	1445	2p21	AD	58%	Moderate	Moderate <sup>a</sup>	LQTS
<i>CALM3</i>	Calmodulin-3	1449	19q13.32	AD	67%	Limited	Moderate <sup>a</sup>	LQTS
<i>KCNJ2</i>	Potassium voltage-gated channel sub-family J member 2	6263	17q24.3	AD	92%	Limited	Disputed	Andersen-Tawil syndrome, SQTS
<i>SCN5A</i>	Sodium voltage-gated channel alpha sub-unit 5	10593	3p22.2	AD	25%	Limited	Disputed	LQTS, BrS
<i>PKP2</i>	Plakophilin-2	9024	12p11.21	AD	0%	Limited	Disputed	ARVC
<i>ANK2</i>	Ankyrin-2	493	4q25-q26	AD	75%	Limited	Disputed	—

Genes implicated in CPVT that were reappraised in this study.

AD, autosomal dominant; AR, autosomal recessive; ARVC, arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; GTR, Genetic Testing Registry; LQTS, long QT syndrome; SQTS, short QT syndrome.

<sup>a</sup>See Discussion regarding limitations of the gene curation template for the CALM gene family.

## Genes with both catecholaminergic polymorphic ventricular tachycardia and long QT syndrome phenotypes

While *RYR2* and *CASQ2* are associated with a distinct CPVT phenotype, a number of recently described arrhythmia genes have been shown to present with phenotypes that can include CPVT or LQTS (or features of both conditions). *TRDN* (encoding triadin) and *TECRL* (encoding trans-2,3-enoyl-CoA reductase like) were recently reported in AR CPVT and classified as *Definitive* genes based on multiple reports of patients with biallelic loss-of-function variants and supporting experimental data including *TRDN* knockout mice (Figure 1). Most of these published cases were early onset and presented in childhood with syncope or cardiac arrest. *TRDN* was previously classified with *Strong* evidence for causing an atypical and early onset form of LQTS.<sup>5</sup> Several of the *TECRL* CPVT patients also displayed a longer than normal QT interval (though rarely >500 ms), but this gene was not assessed in the LQTS gene curation study.

Three calmodulin genes (*CALM1*, *CALM2*, *CALM3*) are located on different chromosomes but encode identical 149 amino acid proteins. All three were previously classified as having *Definitive* evidence for LQTS with atypical features such as presentation in infancy or early childhood and with functional heart block and severe QT prolongation.<sup>5</sup> The recently published International Calmodulinopathy Registry described 74 cases with calmodulin variants, with a LQTS

phenotype more prevalent than CPVT (49% vs. 28%).<sup>12</sup> Based on curation of the available genetic and experimental evidence for CPVT, the genes scored as *Moderate*, *Moderate*, and *Limited* for *CALM1*, *CALM2*, and *CALM3*, respectively (Figure 1). After discussion, the GCEP decided unanimously to upgrade *CALM3* to *Moderate* on the curation matrix. However, the GCEP believed the restrictions of the general scoring framework yielded overly conservative classifications for the calmodulin genes and that all three genes can cause CPVT (see Discussion for details).

## Genes disputed as a cause for catecholaminergic polymorphic ventricular tachycardia

Four genes (*ANK2*, *KCNJ2*, *PKP2*, and *SCN5A*) scored with *Limited* evidence as single gene causes for CPVT and were subsequently classified as *Disputed* by the GCEP (Figure 1). Variants in *ANK2* were detected in patients with a reported CPVT-like phenotype in three families, with mutant ankyrin-B unable to rescue the arrhythmia phenotype of ankyrin-B heterozygous null mice upon transfection, in contrast to wild-type protein.<sup>13</sup> However, each of these variants are now recognized as too common in the population to be dominant causes of CPVT, with maximum gnomAD population prevalence in the range of 1/250 individuals. Therefore, this gene-disease

**Table 2** Classification of evidence for genes reported as causing short QT syndrome

Gene	Protein	HGNC ID	Chromosomal location	Inheritance	Presence on GTR panels, n = 19 (%)	Scoring classification	Final expert classification	Other arrhythmia conditions with valid gene–disease relationship
<i>CACNA1C</i>	Calcium voltage-gated channel subunit alpha1 C	1390	12p13.33	AD	89%	Limited	Disputed	Timothy syndrome
<i>CACNA2D1</i>	Calcium voltage-gated channel auxiliary subunit alpha2 delta 1	1399	7q21.11	AD	63%	Limited	Disputed	—
<i>CACNB2</i>	Calcium voltage-gated channel auxiliary subunit beta 2	1402	10p12.3	AD	89%	Limited	Disputed	—
<i>KCNH2</i>	Potassium voltage-gated channel subfamily H member 2	6251	7q36.1	AD	100%	Definitive	Definitive	LQTS
<i>KCNJ2</i>	Potassium voltage-gated channel subfamily J member 2	6263	17q24.3	AD	95%	Moderate	Moderate	Andersen–Tawil syndrome
<i>KCNQ1</i>	Potassium voltage-gated channel subfamily Q member 1	6294	11p15.5-p15.4	AD	100%	Strong	Strong	LQTS
<i>SLC22A5</i>	Solute carrier family 22 member 5	10969	5q31.1	AR	11%	Limited	Disputed (SQTS-mimic) <sup>a</sup>	—
<i>SLC4A3</i>	Solute carrier family 4 member 3	11028	7q36.1	AD	0%	Moderate	Moderate–Strong <sup>b</sup>	—
<i>SCN5A</i>	Sodium voltage-gated channel alpha subunit 5	10593	3p22.2	AD	0%	Limited	Disputed	BrS, LQTS

Genes implicated in SQTS that were reappraised in this study.

AD, autosomal dominant; AR, autosomal recessive; BrS, Brugada syndrome; GCEP, gene curation expert panel; GTR, Genetic Testing Registry; LQTS, long QT syndrome; SQTS, short QT syndrome

<sup>a</sup>This gene does not cause SQTS but primary systemic carnitine deficiency which may mimic SQTS.

<sup>b</sup>The GCEP was divided between moderate and strong classifications (see text for details).

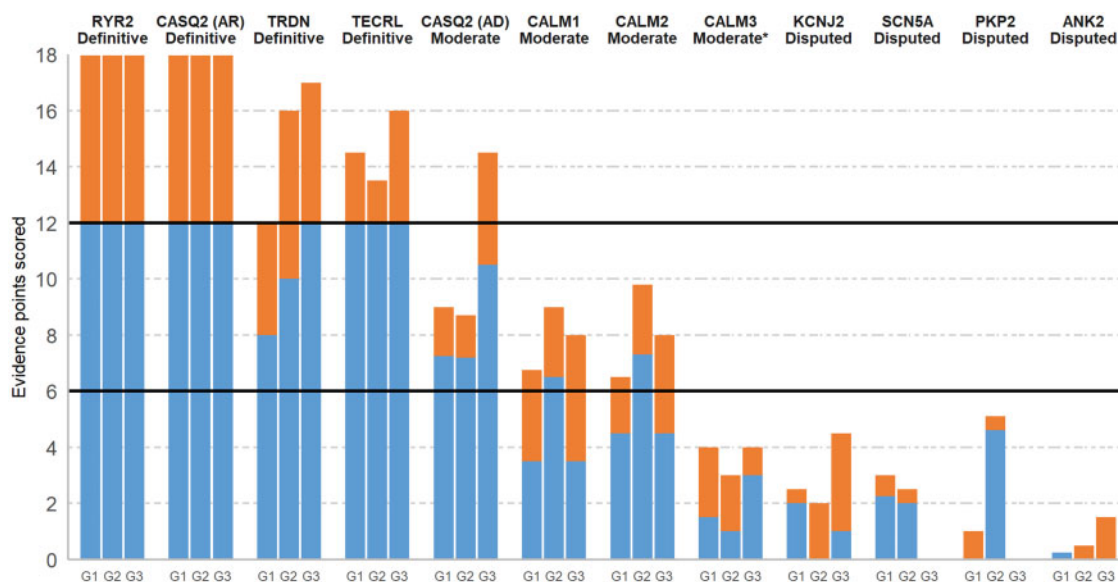
relationship was classified as *Disputed* (as it has previously been for BrS<sup>4</sup> and LQTS<sup>5</sup>).

Variants in the other three genes (*KCNJ2*, *PKP2*, and *SCN5A*) were described in patients that had some phenotypic features that were clinically interpreted to represent CPVT. Although the variants reported in these genes were highly likely to be responsible for the arrhythmogenic phenotypes of these cases (based on evidence from functional assays, the enrichment of truncating variants in cases and segregation in a large family pedigree for *KCNJ2*, *PKP2*, and *SCN5A*, respectively), the GCEP deemed that despite the ventricular arrhythmia burden described, these cases did not have CPVT but rather represented cardiac-restricted expression of Andersen–Tawil syndrome (ATS) (*KCNJ2*),<sup>14–16</sup> the concealed cardiomyopathy stage of arrhythmogenic cardiomyopathy (*PKP2*),<sup>17</sup> and a phenotype of multifocal ectopic Purkinje-related premature contractions (MEPPC) (*SCN5A*).<sup>18</sup> The three genes were therefore downgraded to *Disputed* for CPVT (see Discussion).

## Definitive short QT syndrome-causing gene

*KCNH2* was the only gene classified as *Definitive* for disease causality in SQTS, with variants identified in 18 SQTS probands, including seven unrelated probands of both Caucasian and Asian ethnicity with p.Thr618Ile and six with p.Asn588Lys. This genetic evidence, coupled with supporting experimental evidence, was deemed sufficient by the GCEP for classification of *KCNH2* as definitively causing SQTS (Figure 2). It should be noted that the curation scores for *KCNH2* were lower than those of other *Definitive* genes in previous gene–disease curation efforts. According to the gene curation framework utilized here, the maximum genetic evidence score cannot be reached based on identification of non-*de novo* missense variants alone. Additional evidence from *de novo* or loss-of-function variants, segregation, or case–control data would be required for a maximal score, which are not available for *KCNH2* due to the disease mechanism (gain-of-function) and the paucity of cases. Accordingly, the GCEP recognized this





**Figure 1** Disease causality classification and level of evidence scores for genes implicated in catecholaminergic polymorphic ventricular tachycardia. For each curated gene, the genetic (up to a maximum of 12 points, blue) and experimental (up to a maximum of 6 points, orange) evidence scores of the three blinded curating teams (G1, G2, G3) are detailed, along with the consensus classifications obtained. \*Although *CALM3* scored with *Limited* evidence, the gene curation expert panel unanimously decided to upgrade this to *Moderate*.

limitation in the framework and did not view the relatively lower curation scores as dissuasion towards *Definitive* classification.

### Genes with moderate-strong level of evidence for short QT syndrome

Variants in *KCNQ1* were identified in nine SQTs probands, eight of which were the p.Val141Met variant. The presentation of all cases with p.Val141Met was similar: severe bradycardia was identified *in utero* or at birth, with atrial fibrillation also diagnosed in six cases and no reports of cardiac arrest or SCD.<sup>19</sup> It was reported to be *de novo* in three cases (although paternity was not proven in all). The other variant, p.Val307Leu, was described in a single case with a cardiac arrest at the age of 70,<sup>20</sup> much older than other reported SQTs cases. Three other variants (p.Phe279Ile,<sup>21</sup> p.Arg259His,<sup>22</sup> and p.Ala287Thr<sup>23</sup>) were reported in suspected cases of SQTs; however, due to incomplete or inconclusive phenotype data, the GCEP unanimously agreed not to score these variants. The final score for *KCNQ1* and the reproducibility of supporting evidence over time was sufficient for a *Definitive* classification; however, the fact that almost all genetic evidence was derived from a single variant led the GCEP to limit the classification of *KCNQ1* to *Strong* for SQTs.

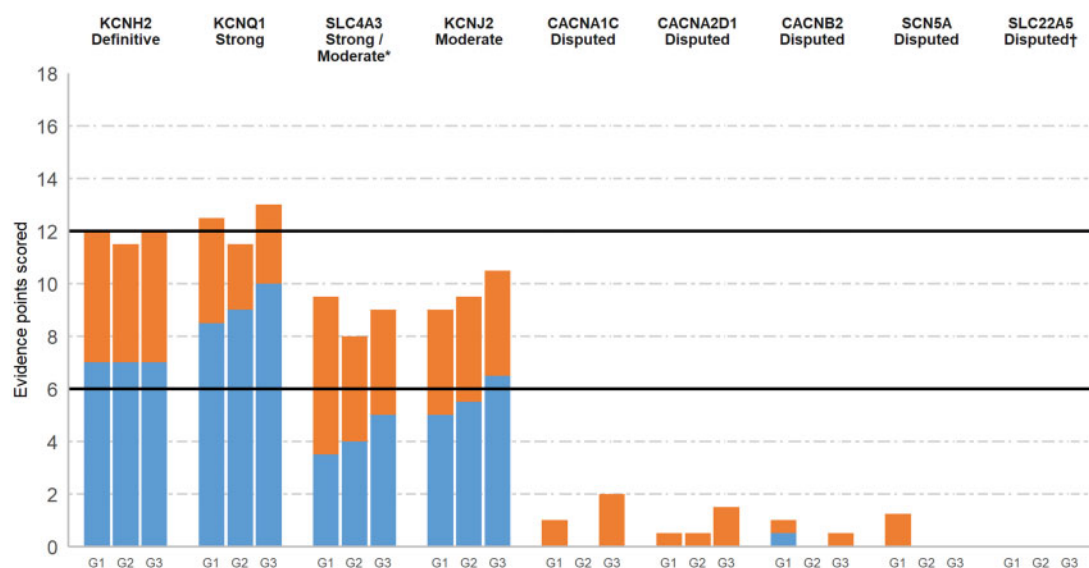
Genetic evidence supporting *SLC4A3* as an SQTs-causing gene is derived from one recent publication in which exome sequencing in two families, including one large pedigree, detected the same p.Arg370His rare variant.<sup>24</sup> Experimental evidence from *in vitro* and zebrafish models suggests that reduced membrane localization of the mutated protein leads to intracellular alkalinization and shortening of the action potential duration. While the unbiased gene discovery approach and segregation evidence strongly supports causality of this

variant, the lack of other publications supporting this gene–disease relationship led to a score in the *Moderate* range using the gene curation template. The GCEP discussed upgrading the final classification but was divided on this issue with four panellists voting for *Strong* and five for *Moderate*.

Genetic variants in *KCNJ2* have been identified in six SQTs patients from five families, including at least two probands with a *de novo* variant (paternity not confirmed). Experimental evidence demonstrated that these variants lead to gain-of-function of the late repolarizing, *KCNJ2*-encoded  $I_{K1}$  current in the heart and abbreviation of the action potential duration.<sup>25</sup> These data were considered sufficient for classifying the gene–disease relationship of *KCNJ2* as *Moderate*.

### Genes disputed as a cause for short QT syndrome

Four genes encoding calcium (*CACNA1C*, *CACNA2D1*, *CACNB2*) or sodium (*SCN5A*) channels were classified as *Disputed* genes for SQTs (Figure 2). The main limitation of published data associating these genes with SQTs was the absence of a typical SQTs phenotype in isolation, with a Brugada ECG pattern in addition to a commonly recognized relatively abbreviated QT interval seen in BrS cases observed in three reported probands with a *CACNA1C* variant, one proband with a *CACNB2* variant and one proband with a *SCN5A* variant. The GCEP concluded that these were BrS cases and therefore not scored, though it should be noted that previous gene curation of the calcium channel genes for BrS by this GCEP also resulted in a *Disputed* classification.<sup>4</sup> Similarly, another case with a *CACNA1C* variant was found to have hypertrophic cardiomyopathy without clinical findings typical of SQTs. Two other reported variants in SQTs cases



**Figure 2** Disease causality classification and level of evidence scores for genes implicated in short QT syndrome. Validity scores according to the ClinGen curation framework and final classifications of the gene curation expert panel. Genetic (up to a maximum of 12 points, blue) and experimental (up to a maximum of 6 points, orange) evidence scores of each of the three blinded curating teams (G1, G2, G3) are detailed. Complete list of references used for scoring of these genes is available in the supplements. \*The gene curation expert panel was divided between *Moderate* and *Strong* classifications. †This gene is a short QT syndrome-mimic. See text for further details.

are observed too frequently in the Ashkenazi Jewish population to be monogenic causes of SQTs (prevalence of  $<1/10\,000$ )—*CACNA2D1*:p.Ser755Thr with an Ashkenazi prevalence of 1/50 and *CACNA1C*:p.Arg1977Gln (1/800) (see gene summaries in [Supplementary material online](#) for details of these reports).

Variants in *SLC22A5* cause AR primary systemic carnitine deficiency (PSCD), a syndrome characterized by hypoketotic hypoglycaemia, hyperammonemia, liver dysfunction, hypotonia, and cardiomyopathy.<sup>26</sup> Nevertheless, homozygote or compound heterozygote variants have been identified in unexplained SCD or resuscitated cardiac arrest cases without overt extra-cardiac manifestations.<sup>27,28</sup> Furthermore, a short QT interval has been demonstrated in a carnitine-deficient mouse model<sup>27</sup> as well as in patients with PSCD.<sup>27,28</sup> Importantly, however, the QT interval in these patients returns to normal with carnitine supplementation treatment,<sup>27,28</sup> leading the GCEP to conclude that PSCD is a metabolic and reversible SQTs-mimic and that the relationship between *SLC22A5* and true SQTs is *Disputed* (Figure 2).

### Gene panel coverage

Twelve CPVT-specific and 19 SQTs-specific clinical genetic testing panels were listed in the NCBI Genetic Testing Registry<sup>29</sup> (Figure 3, full details in [Supplementary material online](#)). *RYR2* and *CASQ2* were included in every CPVT testing panel, with the other *Definitive*/*Moderate* genes present in 25–92% and the *Disputed* genes present in 0–92%. *KCNQ1* was present in 3/12 panels despite having no reported relationship with CPVT. Of the validated SQTs genes, *KCNH2* and *KCNQ1* were included in all SQTs testing panels, *KCNJ2* in 95% but *SLC4A3* was present in none and the SQTs-mimic gene

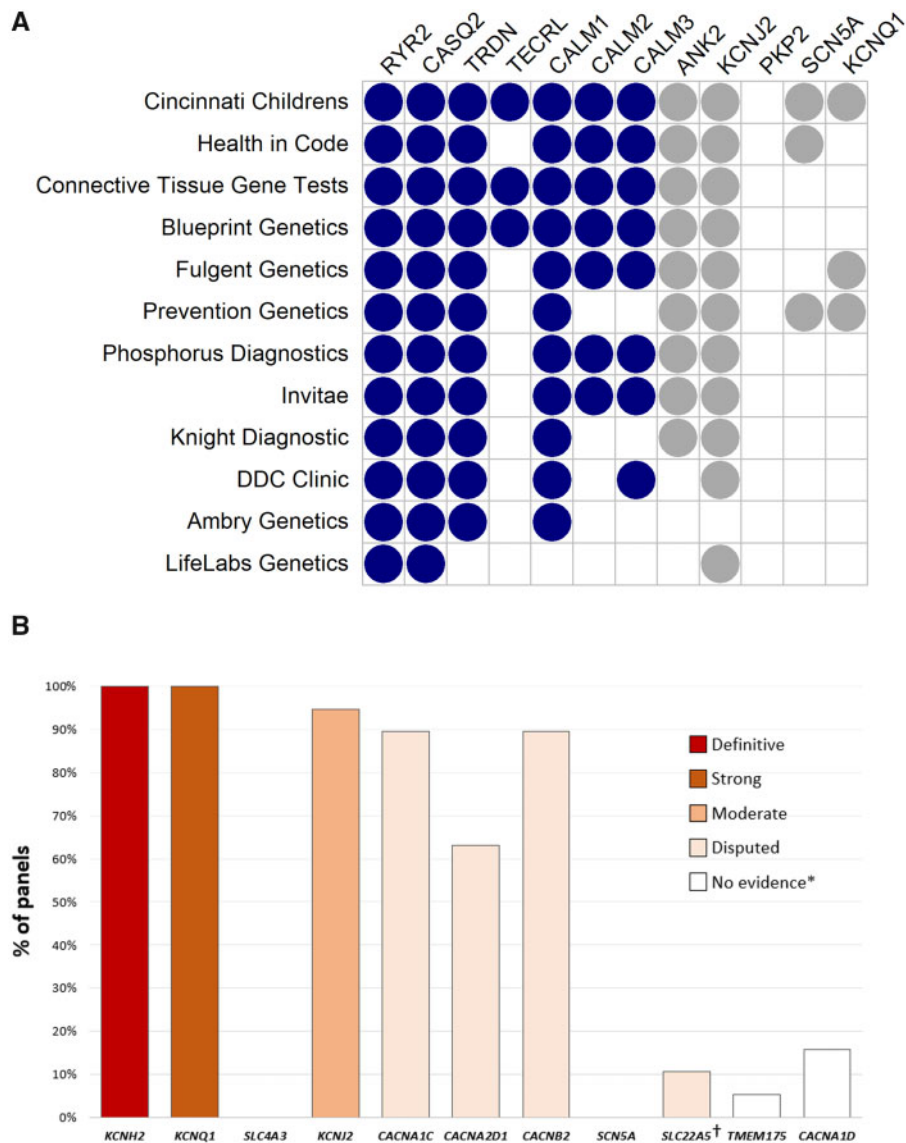
*SLC22A5* was included in only 11%. The *Disputed* calcium channel genes were included in most panels (63–89%), with *CACNA1D* present in 15% despite no reports in the medical literature suggesting a relationship with SQTs.

## Discussion

An essential pre-requisite of clinical genetic testing in patients with conditions like CPVT and SQTs, as recommended by the American College of Medical Genetics and Genomics guidelines,<sup>30</sup> is to restrict analysis to genes with a proven role for the disease in order to avoid misinterpretation of rare variants found in genes with little evidence for disease causality. In this study, we utilized a previously published evidence-based framework<sup>6</sup> to reappraise the genes implicated in CPVT and SQTs by evaluating the genetic, experimental, and phenotypic data in published studies. We identified seven CPVT and four SQTs genes that currently have sufficient evidence to be regarded as valid Mendelian disease genes for these disorders.

### Genes disputed as causes of catecholaminergic polymorphic ventricular tachycardia and short QT syndrome based on genetic evidence

*ANK2* reported for CPVT and *CACNA1C* and *CACNA2D1* reported for SQTs represent the few genes disputed based on genetic evidence, with the reported variants now being recognized as far too common in human populations to cause these rare diseases. The reasons why CPVT and SQTs have not been encumbered



**Figure 3** Composition of catecholaminergic polymorphic ventricular tachycardia and short QT syndrome genetic testing panels. Data derived from the NCBI Genetic Testing Registry (as of December 2020) excluding single gene or broad arrhythmia/cardiac panels. (A) Validated catecholaminergic polymorphic ventricular tachycardia genes are shown in dark blue, disputed and non-catecholaminergic polymorphic ventricular tachycardia genes are shown in grey. Although *PKP2* is not currently on any panels, it was included in this reappraisal process based on a recent report where truncating variants were identified in patients previously diagnosed with catecholaminergic polymorphic ventricular tachycardia. (B) Percentage of genetic panels including genes previously reported as causing short QT syndrome. *SLC4A3* (moderate evidence) was not present in any of the panels we investigated. \*These are candidate genes with no evidence for gene–disease relationship and were not curated by the Biocurator teams. <sup>†</sup>This gene is a short QT syndrome-mimic and should be considered in short QT syndrome-specific genetic panels. See text for further details.

with many erroneous genetic associations (in contrast to other inherited cardiac conditions<sup>4,5</sup>) may include the relative rarity of the conditions (~1/10 000 for CPVT and likely rarer still for SQTS), which precludes the assembly of cohorts for candidate gene studies that led to many disputed associations in other diseases. Catecholaminergic polymorphic ventricular tachycardia is also frequently observed as an early onset disease due to *de novo*

variants or AR inheritance—pathogenic variants in such cases are easier to unambiguously identify (through trio analysis of probands and their parents) compared to later-onset diseases and AD inheritance. In contrast, the majority of *Disputed* classifications for both CPVT and SQTS related to disputing the phenotypes of the reported cases and the adjudication of the GCEP that these were unlikely to be CPVT/SQTS.



## Genes disputed as causes of catecholaminergic polymorphic ventricular tachycardia and short QT syndrome based on phenotypic miscues

For CPVT, three genes (*KCNJ2*, *PKP2*, *SCN5A*) were classified as *Disputed* despite the likely pathogenicity of the reported variants, as the phenotypes in these reports were concluded by the GCEP to represent distinct conditions other than CPVT. Variants in *KCNJ2* are causative of ATS, a condition characterized by dysmorphic features, periodic paralysis, and prominent U waves on ECG. For cases with a cardiac-restricted phenotype, distinguishing ATS from CPVT can be challenging as arrhythmia burden may increase during exercise and resemble CPVT. However, the observed high ventricular arrhythmia burden in the resting state, occasionally including the presence of bidirectional VT,<sup>15,31</sup> and/or the presence of abnormal U waves or prolonged QUc on ECG,<sup>14,16,31</sup> suggests that each of the cases reappraised in this study are likely to represent ATS lacking extra-cardiac features rather than the reported CPVT diagnosis.

Truncating variants in *PKP2* were detected in 5/18 patients who had previously been diagnosed with CPVT.<sup>17</sup> One of these patients was subsequently diagnosed with arrhythmogenic right ventricular cardiomyopathy (ARVC) and eventual right ventricular structural changes were observed in two others. The GCEP therefore deemed that these were highly likely to be ARVC cases in the concealed cardiomyopathy stage of the disease upon initial presentation, a conclusion indeed shared by the authors of the original study,<sup>17</sup> and that *PKP2* cannot be considered a causal gene for CPVT. The *SCN5A* variant p.Ile141Val was shown to segregate with an arrhythmogenic phenotype in a large Finnish pedigree (LOD score 3.56) after identification by linkage analysis and exome sequencing.<sup>18</sup> The phenotype in this family displayed some similarities with CPVT, with individuals developing premature ventricular contractions during exercise testing (but also abundantly at rest in some), which progressed to non-sustained polymorphic VT in the majority of cases. However, the presence of features not typical of CPVT, in particular the abundant ventricular ectopy at rest in some patients, suggests that this is a distinct MEPPC arrhythmogenic phenotype that shares similarities with other MEPPC-associated gain-of-function *SCN5A* variants. Furthermore, the observed *in vitro* electrophysiological abnormalities of p.Ile141Val were comparable to those of the well-recognized MEPPC variant p.Arg222Gln.

The above examples highlight the importance of accurate phenotyping and diagnosis in patients with suspected arrhythmogenic disorders like CPVT, for both research studies seeking to identify novel gene–disease relationships and applying and interpreting routine CPVT genetic testing in the clinic. The detection of ventricular arrhythmias upon exercise testing, but not at rest, is the essential diagnostic feature for CPVT cases but other clinical characteristics atypical for CPVT should also be considered during evaluation (Figure 4).

Four of the genes implicated in SQTS were also classified as *Disputed* based on the patient phenotype in the published studies. For three of these genes (*CACNA1C*, *CACNB2*, and *SCN5A*), most of the reported cases had BrS with a relatively short QT interval and were not regarded by the GCEP as an SQTS phenotype. *SLC22A5*, which

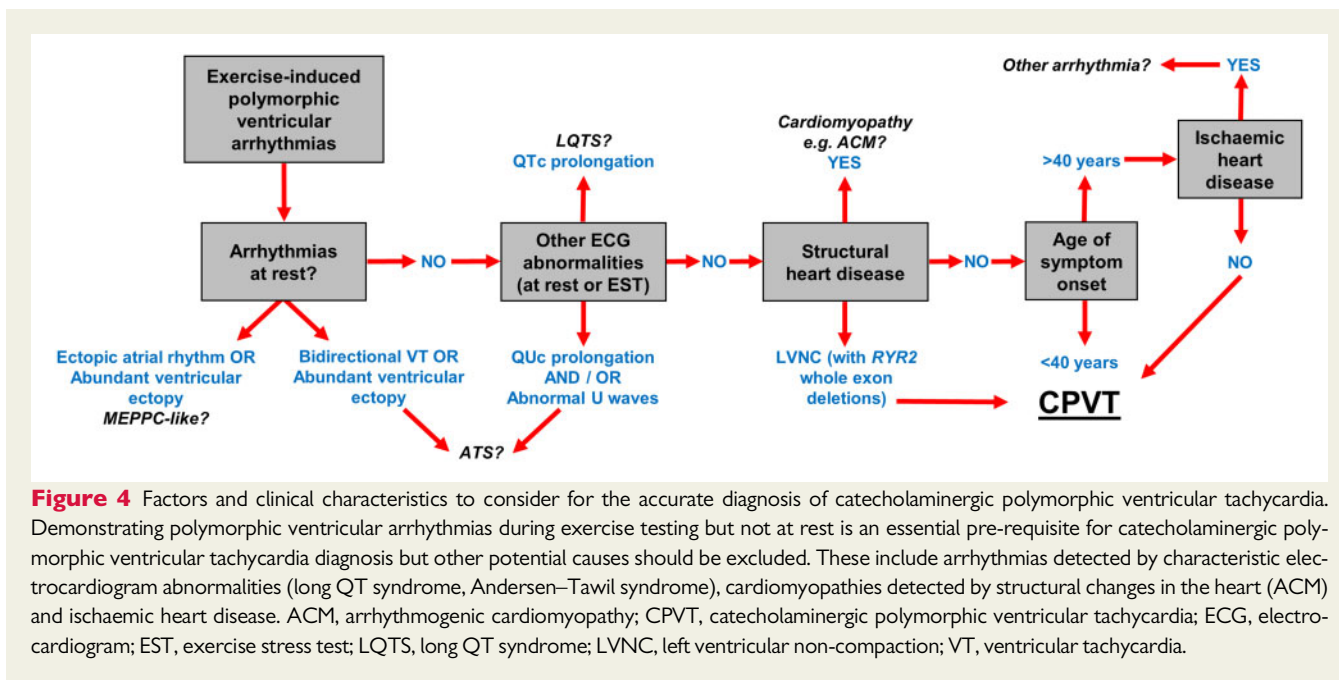
causes the AR disorder PSCD, is a unique case of an SQTS-mimic as biallelic *SLC22A5* variants may rarely present with an isolated cardiac phenotype that resembles SQTS. While the molecular mechanism of this observation is not clearly understood, it should be considered in the genetic investigation of SQTS cases despite being *Disputed* as a cause of SQTS, given that the phenotype can be readily treated with orally supplemented carnitine.

## Validated short QT syndrome genes

Four causal genes for SQTS were identified by this reappraisal: *KCNH2* with *Definitive* evidence and three other genes (*KCNQ1*, *KCNJ2*, and *SLC4A3*) with substantial evidence leading to classifications ranging from *Moderate* to *Strong* (Table 2 and Figure 2). Given the rarity of SQTS, it is not surprising that a paucity of cases precluded these genes from being classified as definitive. *KCNQ1* is the second most common gene reported in SQTS after *KCNH2*. Interestingly, almost all cases were found to harbour the same genetic variant (p.Val141Met) with a similar clinical presentation. While the pathogenicity of this variant is irrefutable, there is no convincing evidence of pathogenicity for other *KCNQ1* variants with SQTS. Unlike the other established SQTS genes, *SLC4A3* does not encode a cardiac potassium channel but rather a chloride–bicarbonate exchanger. Loss-of-function of this exchanger is thought to lead to intracellular alkalinisation, which shortens the cardiac action potential duration. This gene's recent association with SQTS by a single study may explain its absence from SQTS commercial genetic testing panels (Figure 3B). Nevertheless, the evidence supporting this gene's relationship with SQTS was seen as robust by the GCEP, with future reports of *SLC4A3* variants in SQTS cases likely to strengthen the level of evidence.

## Validated definitive catecholaminergic polymorphic ventricular tachycardia genes: RYR2, TRDN, CASQ2, and TECRL

*RYR2* (AD inheritance) and *CASQ2* (AR inheritance) were first reported as causes of CPVT nearly 20 years ago, with repeated descriptions of both familial and *de novo* cases robustly establishing their pathogenic role in CPVT. Experimental data in both *in vitro* systems and animal models have established the net effect of these genetic defects in promoting spontaneous cytosolic fluxes of calcium release from the sarcoplasmic reticulum (SR) during adrenergic stimulation in cardiac diastole, leading to delayed afterdepolarizations (DADs) and triggered arrhythmias. *TRDN* (encoding triadin) and *TECRL* (encoding trans-2,3-enoyl-CoA reductase like) were more recently reported with AR CPVT and classified as *Definitive* genes based on multiple reports of patients with biallelic loss-of-function variants and supporting experimental data including *TRDN* knockout mice and iPSC-derived cardiomyocytes harbouring *TECRL* variants. These genes encode proteins regulating *RYR2*-mediated SR calcium release and their loss-of-function provokes the classical CPVT cellular physiology of inappropriate calcium release and DADs. *TRDN*-related disease may also present with a phenotype of LQTS on resting ECGs, in addition to exercise-provoked polymorphic VT seen in CPVT, and thus, may present a mixed clinical picture in some cases. Similarly,



some cases related to *TECRL* variants have been described with prolonged QT, although not to the degree as seen with *TRDN*.

## The *CALM* gene family and the limitations of the ClinGen gene curation template

The family of *CALM* genes represents a rare phenomenon in human genetics. These genes, *CALM1*, *CALM2*, and *CALM3*, encode the identical protein, all are functionally active and highly expressed in the myocardium but are localized to distinct chromosomal regions within the human genome. The encoded protein calmodulin plays a critical role in intracellular calcium regulation, directly binding to the L-type calcium channel, *RYR2*, and the delayed rectifier potassium channel (*I<sub>ks</sub>*) in its role in modulating calcium levels and action potential duration. Variants in any one of these genes may cause a phenotype of LQTS, and numerous reports have described a phenotype of CPVT in isolation, or a hybrid phenotype of LQTS and CPVT features (prolonged QT at rest, and exercise-induced polymorphic ventricular arrhythmia). Most interestingly, 93% of these *CALM* variants are *de novo* and frequently the identical *de novo* variant is present in more than one of the *CALM* genes. When curated for the phenotype of isolated CPVT using the standard ClinGen curation template, gene validity scores of moderate, moderate and limited were obtained for the three *CALM* genes, respectively. However, the GCEP considered significant limitations of the curation template in the context of the rare scenario of the *CALM* gene family encoding identical proteins, and the common observations of repeated, paralogous *de novo* variants in these genes. In particular, the application of usual scoring for *de novo* variants in the template is designed for single gene–single protein scenarios and does not account for or recognize the added strength of evidence when identical *de novo* variants occur in any of three genes that encode the same protein sequence. Of the 18

isolated CPVT phenotypes described for the *CALM* genes, 14 cases from two families are due to the *CALM1* p.Asn54Ile variant. Fewer cases are reported due to *CALM2* or *CALM3* variants. However, the GCEP considered the observation of an identical *de novo* variant at amino acid residue Asp132 seen on six occasions in patients with *CALM*-related LQTS or CPVT (or hybrid) phenotypes. The p.Asp132Glu amino acid change, described in *CALM2* and *CALM3* patients with clinical features consistent with CPVT, was considered as indisputable evidence for disease causation for CPVT from either *CALM2* or *CALM3* in addition to *CALM1*. Variants at Asp132 are absent from gnomAD for all 3 *CALM* genes, and the probability of this identical *de novo* variant occurring in each of these genes presenting with *CALM*-related LQTS or an isolated CPVT phenotype as reported for p.Asp132Glu in *CALM3* would be extraordinarily low. In addition, all three *CALM* genes have demonstrated the classical CPVT physiology in functional experiments when mutant *CALMs* are expressed, including the *CALM3* p.Ala103Val variant reported in CPVT, which demonstrated provocation of DADs when expressed in mice myocytes. As such, the GCEP concluded that all three *CALM* genes have unequivocal evidence for causation of isolated CPVT, in addition to LQTS and hybrid phenotypes.

## Clinical implications

The classification of genes reported herein should be considered when designing clinical genetic testing panels for CPVT and SQTS and interpreting variants identified in such patients. Based on these findings, we recommend that CPVT panels should include, but be restricted to, all genes with *Definitive* to *Moderate* evidence (i.e. *RYR2*, *CASQ2*, *TRDN*, *TECRL*, *CALM1*, *CALM2*, *CALM3*) and be employed for patients diagnosed with CPVT by standard exercise stress testing (or Holter monitoring in younger patients). For cases where this is unavailable or where atypical features are observed, and where no

causative variants have been identified with the standard seven gene panel, an expanded panel could be applied to identify potentially pathogenic variants in non-CPVT genes that cause conditions that may be misinterpreted as CPVT. Results from such expanded panels should be cautiously analysed, however, and interpreted in the context of the phenotype of the patient being tested, e.g. if a *KCNJ2* variant is detected, does the patient have cardiac or non-cardiac features that would suggest a diagnosis of ATS?

For SQTS, although the three genes encoding cardiac calcium channels (*CACNA1C*, *CACNB2*, *CACNA2D1*) are included in the majority of commercial SQTS panels (Figure 3B), rare variants in these genes will almost certainly be interpreted as variants of unknown significance (VUS) (a not uncommon occurrence given that rare missense variants in these genes will be found in ~8% of the general population). Their inclusion in SQTS-specific panels would not increase the yield of the panel but would add to turnaround time and, more importantly, may lead to anxiety and uncertainty if a VUS is reported. As the relationship of *CACNB2* and *CACNA2D1* with BrS has also been previously disputed, the inclusion of these genes in wider arrhythmia or pan-cardiac genetic panels should also be reconsidered. It is noteworthy that the genetic evidence supporting the relationship of *KCNH2*, *KCNQ1*, and *SLC4A3* with SQTS is based on very few variants (*KCNH2*:p.Thr618Ile, *KCNH2*:p.Asn588Lys, *KCNQ1*:p.Val141Met, and *SLC4A3*:p.Arg370His) identified in multiple patients. Therefore, other rare variants in these genes should be cautiously interpreted, particularly for the potassium channel genes as gain-of-function variants in *KCNH2*, *KCNQ1*, and *KCNJ2* that cause SQTS are expected to be very rare.

## Lessons learned in gene discovery research

Most of the *Disputed* genes described here and in previous reappraisals were identified using candidate gene approaches. Although this remains a valuable application to gene discovery where large family pedigrees are unavailable, significant limitations exist in the absence of appropriately controlled study designs. Rare variants are known to be collectively relatively common for many genes and therefore will often be incidentally detected. While demonstrating an excess of rare variants in case vs. control cohorts supports disease causation, this has been rarely performed in published candidate gene studies and moreover requires very large case cohorts for less prevalent disease genes. Where human genetic evidence is limited, *in vitro* functional assays are often used to support the pathogenicity of rare variants. However, rare control variants are almost never functionally assessed and therefore the threshold of functional abnormality necessary to cause disease is often unknown, particularly for human physiological parameters that span a range of normal values, such as the QTc interval (360–450 ms). Rare variants may display functional differences *in vitro* but not reach a threshold of difference needed to induce disease, as has been observed for variants whose pathogenicity was previously supported by functional assays but are now recognized as being too common to cause rare disease.<sup>4</sup> Due to these limitations, the candidate gene approach needs to consider innovative designs and appropriate control experiments to elevate the evidence of genes proposed to cause rare disease.

## Conclusions

This reappraisal of gene–disease relationships for CPVT and SQTS provides an evidence-based analysis of which genes can currently be considered as valid disease genes and should be included in clinical genetic testing panels. Our findings suggest that a systematic, evidence-based approach should be applied to assess the validity of any reported or novel gene–disease relationships before use in patient care and that both genetic and phenotype data should be carefully assessed when investigating novel genetic causes of these conditions.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

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## Data availability

The data underlying this article are available in the article and in its online supplementary material, as well as on the ClinGen website (<https://www.clinicalgenome.org/>).

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