SUDDEN CARDIAC DEATH AND CHANNELOPATHIES

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SUDDEN CARDIAC DEATH AND CHANNELOPATHIES

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EDITORIAL
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Editorial: Sudden Cardiac Death and Channelopathies

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Keywords: sudden cardiac death (SCD), channelopathies, arrhythmias, genetics, risk stratification

Editorial on the Research Topic

Sudden Cardiac Death and Channelopathies

Sudden cardiac death has serious consequences for the patient's relatives and for society. Each year in Europe and the USA, around 350,000 people, or around 0.1% of the general population, have an out-of-hospital cardiac arrest, of which only a small percentage survive with no sequelae.

The etiology of sudden death has been extensively studied at a population level. Ischemic heart disease occupies a prominent position and is responsible for up to 70% of these deaths; other structural heart diseases make up 10%, and primary arrhythmias cause a further 10%.

In young patients (less than 35 years of age), in whom the incidence of sudden death is 100 times lower than in the general population, arrhythmic etiology, mainly from channelopathies, in the absence of structural heart disease is much more common and is the predominant cause of sudden death in patients aged between 14 and 25 years. In older patients channelopathies such as Brugada syndrome are more likely to occur in the fourth decade of life.

The channelopathies responsible for sudden cardiac death are: Long QT syndrome, Short QT syndrome, Brugada syndrome, Catecholaminergic Polymorphic Ventricular Tachycardia syndrome, and Early repolarization Syndrome.

Early identification and risk stratification is of major importance in patients with a channelopathy who remain asymptomatic, for several reasons. First, sudden death may be the first manifestation of the disease, with no previous warning symptoms. In addition, it should be considered that when we diagnose a patient with a hereditary disease, we also diagnose their family. Identification of an individual with this condition must be accompanied by meticulous familial screening. It is true that in these diseases the risk of sudden death decreases with age, but even a diagnosis in an elderly patient is relevant, as it allows identification of the disease in relatives and their appropriate work-up.

The scope of the Research Topic is to collect state-of-the art papers on what we know so far for all types of Long QT syndromes, Brugada Syndrome, Early repolarization Syndrome, Catecholaminergic Polymorphic Ventricular Tachycardia, and Short QT syndrome. Furthermore, our aim is to provide further insight on the current research areas, and which should be the area's future perspectives.

Ezeani explored one of the numerous mechanisms with which the arrhythmias present. Identifying the sources that control Ca^{2+} is novel in understanding arrhythmogenesis. The TRP channels mediate Ca^{2+} flux and voltage changes across membranes. Regulation of Ca^{2+}-handling by the TRP channels indicate that they can potentially boost Ca^{2+} cycling disorders. The plasma membrane sensory and metabotropic TRPM4 subgroup is a drug candidate for Brugada syndrome and familial heart blockers.

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Novelli et al. dealt with *Pleiotropic Phenotypes Associated with PKP2 Variants.* Their review on plakophilin-2 (PKP2) coded by the gene Pkp2, whose pathogenic role has recently been recognized in different inherited cardiac arrhythmias syndromes, ranging from Arrhythmogenic Cardiomyopathy (ACM or ARVC), Brugada Syndrome (BrS), idiopathic ventricular fibrillation, hypertrophic cardiomyopathy (HCM), and dilated cardiomyopathy (DCM). The expansion and increased availability of genetic testing has challenged the concept of "one gene-one disease" and has shown that different phenotypes can be caused by variants on one same gene. The interpretation of these findings in light of human variation data is complex and casts some warning on the clinical application of this information. The evidence of the pleiotropy of a gene suggested by genetic variants and their functional effect *in vitro* has the important value of discovering different protein functions and suggest arrhythmia mechanisms.

Badone et al. contributed to the field by reviewing the Functional Effects of Calmoduline Mutations and Their Relationship with Clinical Phenotypes. Based on the information reviewed above, mechanism-guided therapeutic approaches to calmodulinopathies should ideally address the interaction of mutant CaM with its targets. Particularly in the case of LQTS-type mutations, this approach is justified by the role of the high target affinity of mutant CaMs in causing negative dominance of the mutation. Tools for this purpose are not available yet, but possibilities exist and are currently explored.

Kotta et al. present in a detailed CaM's sequence, structure and function, the genetic spectrum of CaM mutations and the associated phenotypes, as well as available therapies. They also provide an overview of the underlying disease mechanisms of calmodulinopathy (reviewed in detail in the accompanying article by Badone et al.) and present the thus far used *in vitro* methods for deciphering these disease mechanisms.

Casado Arroyo et al. provided an overview for the current evidence supporting different theories explaining Early Repolarization Syndrome. Along with future developments in the field directed toward individualized treatment, strategies are also examined. Bourier et al. add significantly to the field of ER syndrome, suggesting a risk stratification approach and therapeutic management.

Baltogiannis et al. presented Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) in this collection, as arrhythmogenesis, therapeutic Management, and Future Perspectives are still challenging. Dutsi et al. provided, in detail, the therapeutic impact of Cardiac Sympathetic Denervation in Cardiac Channelopathies. clinical data from well-conducted multicenter registries largely confirmed the preclinical findings, showing that LCSD is an effective treatment for drug-refractory ventricular arrhythmias in both LQTS and CPVT, and that LCSD is now recommended in recent guidelines. Not surprisingly, considering the mechanism of action, the efficacy and potential indication of LCSD in channelopathies goes far beyond secondary prevention, potentially including many still asymptomatic patients with high-risk features for SCD, despite optimized medical therapy.

Campuzano et al. offered a mini review in Recent Advances in Short QT Syndrome. Nearly 20 years ago, SQTS was reported as a familial arrhythmogenic entity. Nowadays, a low number of families have been reported but with a high lethality. This lack reported families, impedes the establishment of a conclusive risk stratification scale, particularly in asymptomatic cases carrying a genetic alteration. New development of hiPSC-CMs from patients may allow unraveling pathophysiological mechanism, helping to understand or treat the disease. Patients suffering of SQTS are at high risk of syncope and SCD. Implantation of an ICD remains the most effective preventive measure after aborted SCD and malignant ventricular arrhythmia, although pharmacological therapies may be used in certain cases, especially in children. Currently, regardless of advances being made in genetics, almost 70–80% of families remain without a genetic cause identified after a comprehensive analysis. Genotype-phenotype analysis are necessary to improve current guidelines in early identification as well as prevention in families suffering from SQTS. On the other hand, Wilders and Verkerk dealt with Long QT Syndrome and Sinus Bradycardia. Sinus bradycardia has been reported in relation to a large number of LQTS mutations. The occurrence of both QT prolongation and sinus bradycardia on a family basis is almost completely limited to LQT3 and Ankyrin-B syndrome ("LQT4"). However, the mechanisms of the associated ventricular arrhythmias and sudden death are largely different. Cardiac events, including nocturnal sudden death, are provoked by the bradycardia and associated excessive QT prolongation in case of LQT3, whereas disturbed calcium homeostasis leads to dysfunction of the SAN cells in case of the Ankyrin-B syndrome, with sudden death occurring after physical exertion and emotional stress.

Moreau and Chahine have added significant value on the topic by introducing a New Cardiac Channelopathy Associated with $Na_v1.5$ Gating Pores. The potential cardiac cellular effects of gating pores and their blockers are also presented here. The increasing knowledge regarding gating pores and their pathologic implication, potentially highlights novel biophysical defects and consequently novel channelopathies. In the current dynamic toward more precise and personalized medicine, this growing knowledge could in the future orientate clinicians in their day to day practice in the management of cardiac channelopathies. This could also help to develop specifically targeted novel medication to accurately and precisely block gating pores, finally benefitting patients.

Verkerk et al. analyzed all possible disease modifiers of Inherited SCN5A Channelopathy. Genetic modifiers, (common) co-morbidities, environmental influences, and life style factors including diet and exercise may modify disease expressivity and severity, and as such significantly modulate the risk for arrhythmia occurrence and survival in *SCN5A* channelopathy. Importantly, the impact of modulatory factors may differ between distinct mutations but may also vary with age and gender. Hence, the clinical management of patients with *SCN5A* mutations should include careful and continuous assessment of co-existing diseases and other modulatory factors, in addition to rigorous treatment of relevant co-morbidities. Identification of disease modifiers will be an essential step in further research

related to *SCN5A* channelopathies and may help to design better risk stratification algorithms and to improve development of novel diagnostic and therapeutic strategies. Tse et al. provided electrocardiographic evidence that higher levels of dispersion in conduction and repolarization are found in type 1 than non-type 1 BrS patients. This may potentially explain the higher incidence of ventricular arrhythmias in the former group. Indices reflecting cumulative conduction and repolarization abnormalities may provide additional value for risk stratification.

Finally, Cheniti et al. dealt with Mapping and Ablation of Idiopathic Ventricular Fibrillation. Insights in pathophysiology of idiopathic VF are presented. Idiopathic VF is diagnosed in around one third of survivors of unexplained SCD aged under 35 years. Genetic testing allows identification of a likely causative mutation in around one quarter of unexplained sudden deaths in children and young adults. Ablation of the PVCs that trigger VF in this setting is associated with high rates of acute success and long-term freedom from VF recurrence. Importantly, almost two thirds of patients have subtle structural abnormalities identified by high density electrogram mapping which are missed by current imaging tools. This localized substrate, which co-locates with regions of VF drivers, provides an explanation for so called unexplained SCD and represents a novel potential target for ablation.

AUTHOR CONTRIBUTIONS

GB has written the editorial. GC, JS, GD, and PB have edited the editorial. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Long QT Syndrome and Sinus Bradycardia–A Mini Review

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Congenital long-QT syndrome (LQTS) is an inherited cardiac disorder characterized by the prolongation of ventricular repolarization, susceptibility to Torsades de Pointes (TdP), and a risk for sudden death. Various types of congenital LQTS exist, all due to specific defects in ion channel-related genes. Interestingly, almost all of the ion channels affected by the various types of LQTS gene mutations are also expressed in the human sinoatrial node (SAN). It is therefore not surprising that LQTS is frequently associated with a change in basal heart rate (HR). However, current data on how the LQTS-associated ion channel defects result in impaired human SAN pacemaker activity are limited. In this mini-review, we provide an overview of known LQTS mutations with effects on HR and the underlying changes in expression and kinetics of ion channels. Sinus bradycardia has been reported in relation to a large number of LQTS mutations. However, the occurrence of both QT prolongation and sinus bradycardia on a family basis is almost completely limited to LQTS types 3 and 4 (LQT3 and Ankyrin-B syndrome, respectively). Furthermore, a clear causative role of this sinus bradycardia in cardiac events seems reserved to mutations underlying LQT3.

Keywords: mutations, sinus bradycardia, human, long-QT syndrome, heart rate, sinoatrial node, ion channel, computer simulation

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INTRODUCTION

Congenital long-QT syndrome (LQTS) is an inherited cardiac disorder characterized by the prolongation of ventricular repolarization, susceptibility to Torsades de Pointes (TdP), and a risk for sudden death (1). Various types of congenital LQTS exist, but the most common forms of LQTS, accounting for ≈90% of genotype-positive LQTS cases (2), are LQT1, LQT2, and LQT3, caused by mutations in the genes encoding the pore-forming α-subunits of the ion channels carrying the slow delayed rectifier K^+ current (I_{Ks}), rapid delayed rectifier K^+ current (I_{Kr}), and fast Na^+ current (I_{Na}), respectively [for reviews, see (3, 4) and, more recent, (5, 6)]. The incidence and occurrence of phenotype is modulated by a large number of conditional factors (4), including heart rate (HR) (7). For example, LQT1 patients are found to be at greatest risk for cardiac events during conditions of elevated HR, while slower HR provokes cardiac events in LQT2 and LQT3 patients (7). Modulation of HR by exercise may also be a diagnostic criterion in LQTS (8), and treatment/prevention of cardiac events in LQTS is frequently accomplished by HR control (7, 9).

Interestingly, almost all of the ion channels affected by the various types of LQTS gene mutations are also expressed in the human sinoatrial node (SAN) (10). It is therefore not surprising that LQTS is frequently associated with a change in basal HR due to impaired SAN pacemaker activity (11). For example, bradycardia is frequently observed in LQT1 mutation carriers, especially in the fetal-neonatal period (12, 13). It has even been concluded that sinus bradycardia in the cardiotocogram

may indicate LQTS in the fetus (14) and that fetal bradycardia is an important predictor of LQTS (15). Also, basal HR was found to be significantly slower in patients with LQT1 compared with non-carriers (16). Maximum HR during exercise may also be reduced in LQTS [see (8, 11), and primary references cited therein]. Thus, LQTS may have a direct impact on HR (17), but this is not a consistent finding (18, 19). One may argue that this is because effects on HR differ between types of LQTS and between specific mutations. However, even within a single mutation different effects on HR are described. For example, the A341V mutation in *KCNQ1* may result in sinus bradycardia (13), but may also occur in absence of baseline HR changes compared to non-carriers (19).

Because LQTS-related rhythm disorders can be triggered by slow or high HR and sinus pauses (4, 11), detailed knowledge of the relation between LQTS and SAN function is required. In this mini-review, we provide an overview of known LQTS mutations with effects on HR and the underlying changes in expression and kinetics of mutant channels.

LQTS GENE MUTATIONS AND CHANGES IN BASAL HEART RATE

In **Tables S1–S8**, which are part of our **Supplementary Material**, we provide a detailed overview of the various autosomal dominant LQTS mutations known to date that are associated with sinus bradycardia, together with data on the mutation-induced changes in expression and kinetics of the respective ion channels. Below, we provide a brief overview of the various types of congenital LQTS and the extent to which each type is associated with sinus bradycardia. This overview is accompanied by **Table 1**, which summarizes the data of **Tables S1–S8**.

TABLE 1 | Mutations observed in patients with both sinus bradycardia and LQTS[a].

LQTS type	Gene	Protein	Patient groups	Mutations
LQT1	*KCNQ1*	K_V7.1	Single patient	c.387-5 T>A, R174H, L175fsX, G179S, G325R, S338F, F339S, F339del, A344V, K422fsX, T587M, A590T
			Multiple single patients	R231C, A341V, D611Y
LQT2	*KCNH2*	K_V11.1	Single patient	R534C, A561V
			Small family	K638del
LQT3	*SCN5A*	Na_V1.5	Small family	QKP1507–1509del
			Large family	1795insD
			Multiple families	KPQ1505–1507del (ΔKPQ), E1784K
LQT4	*ANK2*	Ankyrin-B	Single patient	I1855R
			Multiple single patients	R1788W
			Multiple families	E1425G
LQT5	*KCNE1*	KCNE1 (minK)	Single patient	A8V, D85N, R98W
			Multiple single patients	D85N
			Small family	D85N
LQT6	*KCNE2*	KCNE2 (MirP1)	Multiple single patients	M54T
LQT7	*KCNJ2*	Kir2.1	–	–
LQT8	*CACNA1C*	Ca_V1.2	Single patient	A582D, P857R, R858H
LQT9	*CAV3*	Caveolin-3	Multiple single patients	T78M
LQT10	*SCN4B*	$Na_V\beta$4	Single patient	L179F
LQT11	*AKAP9*	Yotiao	–	–
LQT12	*SNTA1*	α1-syntrophin	–	–
LQT13	*KCNJ5*	Kir3.4 (GIRK4)	–	–
LQT14	*CALM1*	Calmodulin	Single patient	E105A
			Multiple single patients	F142L
LQT15	*CALM2*	Calmodulin	Single patient	D96V, N98I, D132H
LQT16	*CALM3*	Calmodulin	Single patient	D96H, F142L

[a] *Further details are provided in* ***Tables S1–S8****, which are part of our* ***Supplementary Material****.*

LQT1

LQT1 is due to loss-of-function mutations in *KCNQ1*, the gene encoding the pore-forming α-subunit of the I_{Ks} channel (K_V7.1). A decrease in I_{Ks} will result in a prolongation of the ventricular action potential (AP) and a prolongation of the QT interval on the ECG (20). Of note, four K_V7.1 α-subunits assemble in a tetramer to create the pore of an I_{Ks} channel. Therefore, a mutation in *KCNQ1* may affect a large majority of the I_{Ks} channels as wild-type and mutant subunits co-assemble in heterotetramers.

Many *KCNQ1* mutations exist and some are associated with sinus bradycardia (**Table 1**). These bradycardia-associated mutations result in "loss-of-function" by a reduced level of channel expression, expression of non-functional channels, activation at more positive membrane potentials, faster deactivation kinetics, and/or inhibited cAMP-dependent stimulation. For example, the A341V mutation strongly suppresses the increase in I_{Ks} in response to cAMP (21), which may also explain the more pronounced phenotype during exercise. Sinus bradycardia in LQT1 patients seems limited to isolated, often neonate cases (Table S1).

It is somewhat difficult to envision how a loss of repolarizing I_{Ks} *per se* would lead to a profound increase in the cycle length of SAN cells, thus generating sinus bradycardia. Such loss would lengthen AP duration (APD), but at the same time shorten the considerably longer (22) diastolic phase by increasing the rate of diastolic depolarization. An increase in repolarizing I_{Ks}, on the other hand, as observed in short QT syndrome type 2 (SQT2), will inhibit diastolic depolarization and substantially increase cycle length, despite an accompanying decrease in APD, as observed in simulations by Fabbri et al. (23) using their recently developed comprehensive computer model of a single human SAN pacemaker cell. Clinically, sinus bradycardia is indeed relatively common in SQT2 patients [see, e.g., (24)].

LQT2

LQT2 is due to loss-of-function mutations in *KCNH2*, the gene encoding the pore-forming α-subunit of the I_{Kr} channel ($K_V11.1$). Observation of sinus bradycardia in LQT2 patients seems rare (25, 26) and limited to a few isolated cases and a small family (**Table 1**). Bradycardia does occur in the fetal-neonatal period, but is due to 2:1 atrioventricular block rather than sinus bradycardia (12). Such cases are not included in **Table 1**. In contrast, Horigome et al. (13) reported that the incidence of sinus bradycardia was comparable between groups of young (<1 year, mostly fetal-neonatal) LQT1, LQT2, and LQT3 patients. However, whether the LQTS observation is due to the bradycardia or the bradycardia results from the mutations (4, 11) is less clear. Bradycardia-associated mutations in *KCNH2*, so far characterized, result in a decrease in current density, non-functional channels, a shift in voltage of half-activation, and faster deactivation and inactivation rates (Table S2).

The above consideration regarding the potential association between sinus bradycardia and the increase in repolarizing I_{Ks} in case of SQT2 similarly holds for the increase in repolarizing I_{Kr} in case of short QT syndrome type 1 (SQT1). Sinus bradycardia is indeed observed in SQT1 patients, although being less common than in SQT2 patients (27).

LQT3

LQT3 is due to gain-of-function mutations in *SCN5A*, the gene encoding the pore-forming α-subunit of the I_{Na} channel ($Na_V1.5$). Unlike LQT1 and LQT2, the occurrence of sinus bradycardia is not limited to isolated cases. Several families, including the large Dutch family with the 1795insD founder mutation (28), show both QT prolongation and sinus bradycardia (**Table 1**). A common feature is the increased late current, also named persistent or sustained current, underlying the QT prolongation. Another common feature is the decrease in "window current" due to a positive shift in the steady-state activation curve and/or a negative shift in the steady-state inactivation curve (Table S3).

Figures 1A–D illustrate the effects of the 1795insD mutation, based on data from recent computer simulations (29), as set out in the **Supplementary Material**. The observed increase in cycle length (**Figure 1A**) is largely due to a decrease in net inward current (I_{net}) during diastole (**Figure 1B**), which in turn is due to a striking change in the time course of I_{Na} (**Figure 1C**). Where I_{Ks} and I_{Kr} channels are tetramers, the pore of the I_{Na} channel is formed by a single $Na_V1.5$ protein. As a consequence, "mutant I_{Na}" (**Figure 1D**, dotted red trace) is partly flowing through pure wild-type channels (solid green trace) and partly through pure mutant channels (solid orange trace). There is hardly any current flowing through these mutant channels during diastole due to the decrease in window current. There is, on the other hand, some late current flowing during the AP, albeit with a negligible effect on APD, in contrast to ventricular myocytes, in which the current density of I_{Na} is much larger. These effects are more pronounced during vagal activity (**Figures 1E–H**). The slight decrease in diastolic depolarization rate and increase in cycle length as a result of the inhibition of I_{Na} (**Figures 1A,E**) are in line with experimental observations on isolated rabbit SAN cells (30).

The increase in late current ("gain-of-function") is a prerequisite for QT prolongation, but not for sinus bradycardia. A sole decrease in window current ("loss-of-function"), as for example observed in case of the R376C and D1275N mutations in *SCN5A* (31, 32), is sufficient to cause sinus bradycardia.

LQT4

Ankyrin-B syndrome, originally named LQT4, is due to heterozygous loss-of-function mutations in *ANK2*, encoding the widely distributed ankyrin-B adaptor protein. Loss of ankyrin-B results in Ca^{2+} homeostasis dysfunction by reduced Na^+-Ca^{2+} exchange current (I_{NCX}), L-type Ca^{2+} current ($I_{Ca,L}$), Na^+-K^+-ATPase, and IP3 receptor expression (Table S4). Mutations in *ANK2* associated with both QTc prolongation and sinus bradycardia are observed in both large families and single patients (**Table 1**).

LQT5

LQT5 is due to loss-of-function mutations in *KCNE1*. The encoded protein, named KCNE1 or minK, is a β-subunit that may affect both I_{Ks} and I_{Kr} function. Reports of bradycardia in LQT5 patients are scarce (**Table 1**). The observations made to date show reduced I_{Ks} or I_{Kr} density or a shift of I_{Ks} activation to more positive potentials (Table S5). Interestingly, the A8V mutation affects I_{Ks} but not I_{Kr}, whereas the R98W mutation affects I_{Kr} but not I_{Ks}.

LQT6

LQT6 is due to loss-of-function mutations in *KCNE2*. The encoded protein, named KCNE2 or MirP1, is a β-subunit that may affect various ion currents. Mutations in *KCNE2* may result in an accelerated inactivation time course of I_{Kr} (33, 34), but also in an increase of $I_{Ca,L}$ (35), and a reduction of the hyperpolarization-activated current (I_f) (36), the latter important for pacemaker activity in human SAN cells (22). Despite its multiple ion current modulations, *KCNE2* mutations associated with sinus bradycardia are limited to M54T and V65M. In case of the M54T mutation, both I_{Kr} and I_f are inhibited (Table S6). It is conceivable that the V65M mutation also acts through I_f, given the well-established effect of KCNE2 on I_f (37).

LQT7

Andersen-Tawil syndrome ("LQT7") is a multisystem disorder due to loss-of-function mutations in *KCNJ2,* the gene encoding the Kir2.1 protein, which assembles in tetramers to build the channels that carry the inward rectifier K^+ current (I_{K1}) (38). Given the low expression of Kir2.1 in human SAN (10), it is not surprising that HR seems not affected in Andersen-Tawil syndrome patients (39).

LQT8

Timothy syndrome (TS) is a severe multisystem disorder due to gain-of-function mutations in *CACNA1C*, encoding the pore-forming α-subunit of the $I_{Ca,L}$ channel ($Ca_V1.2$), and results in bradycardia in almost all patients known, but caused by 2:1 atrioventricular block rather than sinus bradycardia (see footnote

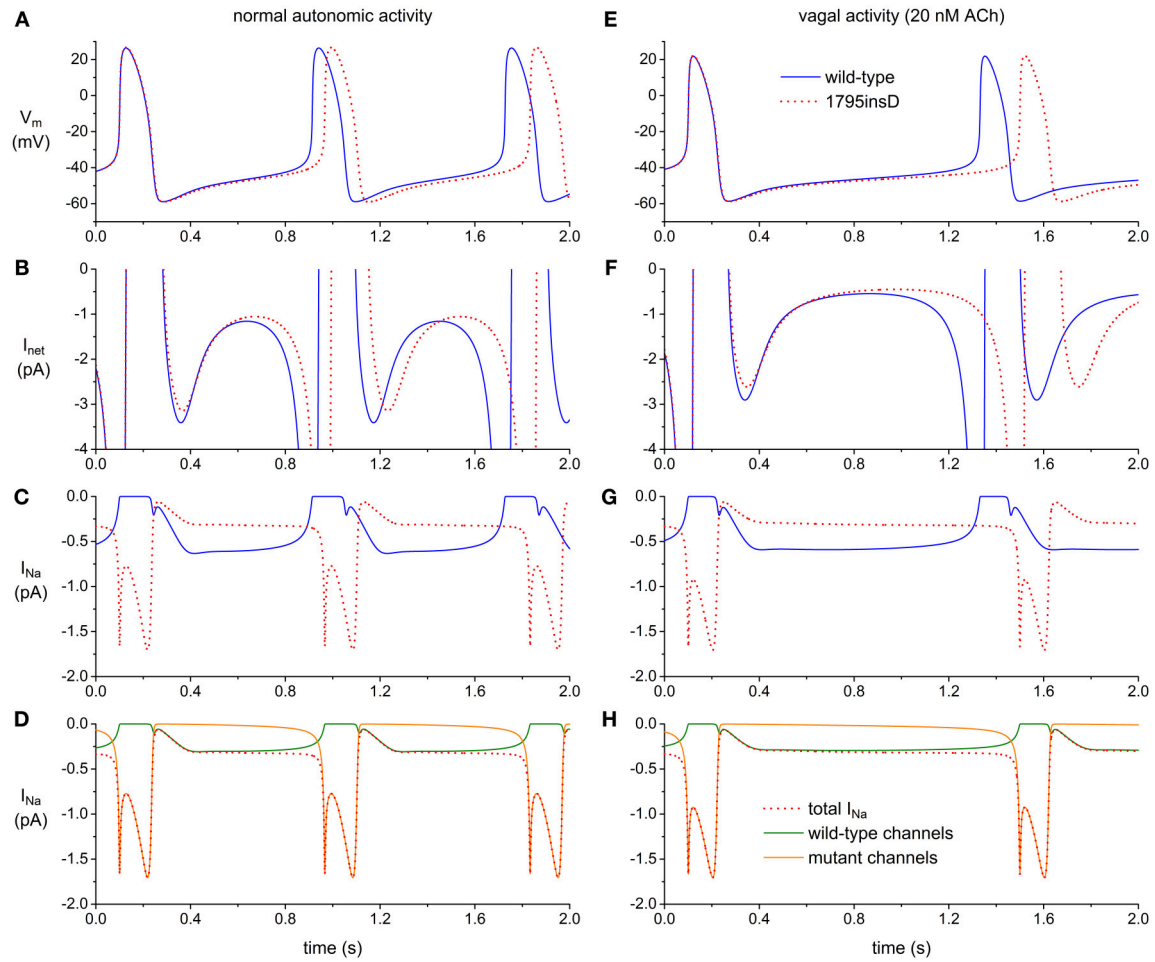

FIGURE 1 | Effect of the 1795insD mutation in *SCN5A* on the electrical activity of the Fabbri–Severi model cell of a human SAN pacemaker cell **(A–D)** under control conditions (normal autonomic activity) and **(E–H)** during vagal activity [based on data from recent computer simulations (29)]. **(A,E)** Membrane potential (V_m) of wild-type and mutant cell (solid blue and dotted red trace, respectively). **(B,F)** Associated net membrane current (I_{net}). (**C,G**) Associated fast Na^+ current (I_{Na}). **(D,H)** Contribution of wild-type and mutant channels (solid green and orange trace, respectively) to I_{Na} of the mutant cell (dotted red trace).

to Table S7). Although TS is also known as LQT8, because of the extreme QT prolongation in TS patients (40, 41), we restricted the LQT8 data in Table S7 to non-TS patients. In isolated cases, these show sinus bradycardia (**Table 1**). Mutant $I_{Ca,L}$ shows an increase in density or slowing of inactivation (Table S7).

LQT9

LQT9 is due to mutations in *CAV3*, encoding caveolin-3, an important structural component of caveolae membrane in muscle cells (42). *CAV3* mutations in heart have been shown to increase the late I_{Na}, thus causing QT prolongation as in LQT3 (43, 44). More recently, it has been shown that mutations in *CAV3* may affect several other membrane currents (see footnote to Table S8). Sinus bradycardia has been observed in two patients carrying the T78M mutation (**Table 1**).

LQT10

LQT10 is due to gain-of-function mutations in *SCN4B*, encoding the $Na_V\beta4$ β-subunit of the I_{Na} channel. A case report exists for an *SCN4B*-L179F mutation with impact on SAN function (**Table 1**). In a 21-month-old girl, profound QT prolongation and bradycardia (<60 bpm) were observed (45). The *SCN4B*-L179F mutation increases late I_{Na} (Table S8) and may thus have effects comparable to LQT3 mutations.

LQT11–LQT16 and Beyond

To the best of our knowledge, no sinus bradycardia has been reported in relation to the rare LQTS types LQT11–LQT13 (see footnote to Table S8). Genetic variation in *KCNJ3* and *KCNJ5*, encoding the pore-forming Kir3.1 and Kir3.4 ion channel subunits of the acetylcholine-sensitive K^+ current ($I_{K,ACh}$), and which the latter may underlie LQT13, seems not involved in pathogenesis of SAN dysfunction (46). However, it is suggested that identification of susceptibility genes for SAN dysfunction requires the construction of a large database of patients and controls whose phenotype should be identified with standard criteria to ensure adequate power for cause-effect studies (47).

Thus, the incidence of some LQTS types may be too low to determine clear associations with bradycardia.

Several reports exist of mutations in the *CALM1–CALM3* genes, each encoding the ubiquitous Ca^{2+} sensing protein calmodulin, in relation to LQTS and sinus bradycardia (**Table 1** and Table S8). Calmodulin regulates multiple Ca^{2+}-related processes in the cardiomyocyte (48), including, e.g., gating of the I_{Ks} channel (49). Mutations in *CALM1* and *CALM2* may impair Ca^{2+}-dependent inactivation of $I_{Ca,L}$ (50, 51), functionally comparable to the slowed inactivation of $I_{Ca,L}$ in case of LQT8 (Table S7).

In **Table 1** and Table S8, we, like others (52, 53), used LQT14–LQT16 in relation to mutations in *CALM1–CALM3*. We are, however, well aware that the naming LQT16 has been used in other review articles (54, 55) in relation to mutations in *SCN1B* (56) and in *TRDN* (57). Altmann et al. identified autosomal recessive homozygous or compound heterozygous mutations in *TRDN*, encoding triadin, associated with LQTS, and themselves proposed that "triadin knockout syndrome" or "*TRDN*-mediated autosomal-recessive LQTS" should be used rather than "LQT17," because of the atypical phenotype that was observed (57).

DISCUSSION AND CONCLUSION

SAN action potentials are generated from a delicate balance of several inwardly and outwardly directed ionic currents, and "Ca^{2+} clock" mechanisms [for reviews, see (58–60)]. While LQTS gene mutations may affect HR by changes in SAN action potential repolarization, it is highly likely that they also affect the intrinsic SAN cycle length by changes in the diastolic, phase 4, depolarization rate, as illustrated by the computer simulations of **Figure 1**.

It is important to realize that a mutation in a single LQTS-related gene may affect several ion currents. This does not only hold for mutations in a Ca^{2+} sensing protein like calmodulin, but also for mutations in the α-subunit of a specific ion channel. The LQT1-related T587M mutation in *KCNQ1* for example does not only reduce I_{Ks}, but also fails to increase membrane localization of the *KCNH2*-encoded $K_V11.1$ protein, as opposed to wild-type *KCNQ1*, thus also reducing I_{Kr} (61). Furthermore, we have to keep in mind that the LQTS-induced changes in rhythm may in turn induce changes in expression of specific ion channels, as demonstrated in studies by Tsuji et al. (62), Yeh et al. (63), and D'Souza et al. (64).

LQT2 and LQT3, but not LQT1, patients have a more pronounced risk for arrhythmias at slower HR (7). LQT2 and LQT3 gene mutations may therefore increase the risk for cardiac events via a direct effect on HR, as indeed clinically was found in a large family with LQT3 (65, 66). In LQT1 patients, on the other hand, cardiac events tend to occur during exercise (7). These differences between LQTS types 1–3 underscore the differences in underlying mechanisms and the potential role of sinus bradycardia in cardiac events.

As shown in **Tables S1–S8** and summarized in **Table 1**, sinus bradycardia has been reported in relation to a large number of LQTS mutations. However, observations are limited to one or a few single patients for most of these mutations (**Table 1**). The occurrence of both QT prolongation and sinus bradycardia on a family basis is almost completely limited to LQT3 and Ankyrin-B syndrome ("LQT4"). However, the mechanisms of the associated ventricular arrhythmias and sudden death are largely different. Cardiac events, including nocturnal sudden death, are provoked by the bradycardia and associated excessive QT prolongation in case of LQT3 (65, 66), whereas disturbed calcium homeostasis leads to dysfunction of the SAN cells in case of the Ankyrin-B syndrome, with sudden death occurring after physical exertion and emotional stress (67–70).

We conclude that, although sinus bradycardia has been reported in relation to a large number of LQTS mutations, a causative role of this sinus bradycardia in cardiac events is limited to mutations underlying LQTS type 3.

AUTHOR CONTRIBUTIONS

RW and AV: experimental design, data acquisition, analysis and interpretation of data, drafting manuscript, editing manuscript, and approval.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2018.00106/full#supplementary-material

REFERENCES

1. Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, MacCluer J, et al. The long QT syndrome: prospective longitudinal study of 328 families. *Circulation* (1991) 84:1136–44. doi: 10.1161/01.CIR.84.3.1136
2. Obeyesekere MN, Antzelevitch C, Krahn AD. Management of ventricular arrhythmias in suspected channelopathies. *Circ Arrhythm Electrophysiol.* (2015) 8:221–31. doi: 10.1161/CIRCEP.114.002321
3. January CT, Gong Q, Zhou Z. Long QT syndrome: cellular basis and arrhythmia mechanism in LQT2. *J Cardiovasc Electrophysiol.* (2000) 11:1413–8. doi: 10.1046/j.1540-8167.2000.01413.x
4. Wehrens XHT, Vos MA, Doevendans PA, Wellens HJJ. Novel insights in the congenital long QT syndrome. *Ann Intern Med.* (2002) 137:981–92. doi: 10.7326/0003-4819-137-12-200212170-00012
5. Bohnen MS, Peng G, Robey SH, Terrenoire C, Iyer V, Sampson KJ, et al. Molecular pathophysiology of congenital long QT syndrome. *Physiol Rev.* (2017) 97:89–134. doi: 10.1152/physrev.00008.2016
6. Giudicessi JR, Wilde AAM, Ackerman MJ. The genetic architecture of long QT syndrome: a critical reappraisal. *Trends Cardiovasc Med.* (2018) doi: 10.1016/j.tcm.2018.03.003. [Epub ahead of print].
7. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation* (2001) 103:89–95. doi: 10.1161/01.CIR.103.1.89
8. Swan H, Viitasalo M, Piippo K, Laitinen P, Kontula K, Toivonen L. Sinus node function and ventricular repolarization during exercise stress test in long QT syndrome patients with KvLQT1 and HERG potassium channel defects. *J Am Coll Cardiol.* (1999) 34:823–9. doi: 10.1016/S0735-1097(99)00255-7

9. Kass RS, Moss AJ. Long QT syndrome: novel insights into the mechanisms of cardiac arrhythmias. *J Clin Invest.* (2003) 112:810–5. doi: 10.1172/JCI19844
10. Chandler NJ, Greener ID, Tellez JO, Inada S, Musa H, Molenaar P, et al. Molecular architecture of the human sinus node: insights into the function of the cardiac pacemaker. *Circulation* (2009) 119:1562–75. doi: 10.1161/CIRCULATIONAHA.108.804369
11. Schwartz PJ. Idiopathic long QT syndrome: progress and questions. *Am Heart J.* (1985) 109:399–411. doi: 10.1016/0002-8703(85)90626-X
12. Lupoglazoff J-M, Denjoy I, Villain E, Fressart V, Simon F, Bozio A, et al. Long QT syndrome in neonates: conduction disorders associated with *HERG* mutations and sinus bradycardia with *KCNQ1* mutations. *J Am Coll Cardiol.* (2004) 43:826–30. doi: 10.1016/j.jacc.2003.09.049
13. Horigome H, Nagashima M, Sumitomo N, Yoshinaga M, Ushinohama H, Iwamoto M, et al. Clinical characteristics and genetic background of congenital long-QT syndrome diagnosed in fetal, neonatal, and infantile life: a nationwide questionnaire survey in Japan. *Circ Arrhythm Electrophysiol.* (2010) 3:10–7. doi: 10.1161/CIRCEP.109.882159
14. Beinder E, Grancay T, Menéndez T, Singer H, Hofbeck M. Fetal sinus bradycardia and the long QT syndrome. *Am J Obstet Gynecol.* (2001) 185:743–7. doi: 10.1067/mob.2001.117973
15. Mitchell JL, Cuneo BF, Etheridge SP, Horigome H, Weng H-Y, Benson DW. Fetal heart rate predictors of long QT syndrome. *Circulation* (2012) 126:2688–95. doi: 10.1161/CIRCULATIONAHA.112.114132
16. Henrion U, Zumhagen S, Steinke K, Strutz-Seebohm N, Stallmeyer B, Lang F, et al. Overlapping cardiac phenotype associated with a familial mutation in the voltage sensor of the KCNQ1 channel. *Cell Physiol Biochem.* (2012) 29:809–18. doi: 10.1159/000178470
17. Lane CM, Bos JM, Rohatgi RK, Ackerman MJ. Beyond the length and look of repolarization: defining the non-QTc electrocardiographic profiles of patients with congenital long QT syndrome. *Heart Rhythm* (2018) doi: 10.1016/j.hrthm.2018.04.033. [Epub ahead of print].
18. Takenaka K, Ai T, Shimizu W, Kobori A, Ninomiya T, Otani H, et al. Exercise stress test amplifies genotype-phenotype correlation in the LQT1 and LQT2 forms of the long-QT syndrome. *Circulation* (2003) 107:838–44. doi: 10.1161/01.CIR.0000048142.85076.A2
19. Brink PA, Crotti L, Corfield V, Goosen A, Durrheim G, Hedley P, et al. Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population. *Circulation* (2005) 112:2602–10. doi: 10.1161/CIRCULATIONAHA.105.572453
20. Tristani-Firouzi M, Chen J, Mitcheson JS, Sanguinetti MC. Molecular biology of K^+ channels and their role in cardiac arrhythmias. *Am J Med.* (2001) 110:50–9. doi: 10.1016/S0002-9343(00)00623-9
21. Heijman J, Spätjens RLHMG, Seyen SRM, Lentink V, Kuijpers HJH, Boulet IR, et al. Dominant-negative control of cAMP-dependent I_{Ks} upregulation in human long-QT syndrome type 1. *Circ Res.* (2012) 110:211–9. doi: 10.1161/CIRCRESAHA.111.249482
22. Verkerk AO, Wilders R, Van Borren MMGJ, Peters RJG, Broekhuis E, Lam K, et al. Pacemaker current (I_f) in the human sinoatrial node. *Eur Heart J.* (2007) 28:2472–8. doi: 10.1093/eurheartj/ehm339
23. Fabbri A, Fantini M, Wilders R, Severi S. Computational analysis of the human sinus node action potential: model development and effects of mutations. *J Physiol.* (2017) 595:2365–96. doi: 10.1113/JP273259
24. Villafañe J, Fischbach P, Gebauer R. Short QT syndrome manifesting with neonatal atrial fibrillation and bradycardia. *Cardiology* (2014) 128:236–40. doi: 10.1159/000360758
25. Couderc J-P, McNitt S, Xia J, Zareba W, Moss AJ. Repolarization morphology in adult LQT2 carriers with borderline prolonged QTc interval. *Heart Rhythm* (2006) 3:1460–6. doi: 10.1016/j.hrthm.2006.08.009
26. Beery TA, Shooner KA, Benson DW. Neonatal long QT syndrome due to a de novo dominant negative *hERG* mutation. *Am J Crit Care* (2007) 16:412–5.
27. Harrell DT, Ashihara T, Ishikawa T, Tominaga I, Mazzanti A, Takahashi K, et al. Genotype-dependent differences in age of manifestation and arrhythmia complications in short QT syndrome. *Int J Cardiol.* (2015) 190:393–402. doi: 10.1016/j.ijcard.2015.04.090
28. Postema PG, Van den Berg MP, Van Tintelen JP, Van den Heuvel F, Grundeken M, Hofman N, et al. Founder mutations in the Netherlands: *SCN5a* 1795insD, the first described arrhythmia overlap syndrome and one of the largest and best characterised families worldwide. *Neth Heart J.* (2009) 17:422–8. doi: 10.1007/BF03086296
29. Wilders R. Sinus bradycardia in carriers of the *SCN5A*-1795insD mutation: unraveling the mechanism through computer simulations. *Int J Mol Sci.* (2018) 19:634. doi: 10.3390/ijms19020634
30. Baruscotti M, DiFrancesco D, Robinson RB. A TTX-sensitive inward sodium current contributes to spontaneous activity in newborn rabbit sino-atrial node cells. *J Physiol.* (1996) 492:21–30. doi: 10.1113/jphysiol.1996.sp021285
31. Detta N, Frisso G, Limongelli G, Marzullo M, Calabrò R, Salvatore F. Genetic analysis in a family affected by sick sinus syndrome may reduce the sudden death risk in a young aspiring competitive athlete. *Int J Cardiol.* (2014) 170:e63–5. doi: 10.1016/j.ijcard.2013.11.013
32. Moreau A, Janin A, Millat G, Chevalier P. Cardiac voltage-gated sodium channel mutations associated with left atrial dysfunction and stroke in children. *Europace* (2018) doi: 10.1093/europace/euy041. [Epub ahead of print].
33. Isbrandt D, Friederich P, Solth A, Haverkamp W, Ebneth A, Borggrefe M, et al. Identification and functional characterization of a novel *KCNE2* (MiRP1) mutation that alters HERG channel kinetics. *J Mol Med.* (2002) 80:524–32. doi: 10.1007/s00109-002-0364-0
34. Lu Y, Mahaut-Smith MP, Huang CL-H, Vandenberg JI. Mutant MiRP1 subunits modulate HERG K^+ channel gating: a mechanism for pro-arrhythmia in long QT syndrome type 6. *J Physiol.* (2003) 551:253–62. doi: 10.1111/j.1469-7793.2003.00253.x
35. Liu W, Deng J, Wang G, Zhang C, Luo X, Yan D, et al. KCNE2 modulates cardiac L-type Ca^{2+} channel. *J Mol Cell Cardiol.* (2014) 72:208–18. doi: 10.1016/j.yjmcc.2014.03.013
36. Nawathe PA, Kryukova Y, Oren RV, Milanesi R, Clancy CE, Lu JT, et al. An LQTS6 MiRP1 mutation suppresses pacemaker current and is associated with sinus bradycardia. *J Cardiovasc Electrophysiol.* (2013) 24:1021–7. doi: 10.1111/jce.12163
37. Decher N, Bundis F, Vajna R, Steinmeyer K. KCNE2 modulates current amplitudes and activation kinetics of HCN4: influence of KCNE family members on HCN4 currents. *Pflügers Arch.* (2003) 446:633–40. doi: 10.1007/s00424-003-1127-7
38. Nguyen H-L, Pieper GH, Wilders R. Andersen-Tawil syndrome: clinical and molecular aspects. *Int J Cardiol.* (2013) 170:1–16. doi: 10.1016/j.ijcard.2013.10.010
39. Zhang L, Benson DW, Tristani-Firouzi M, Ptacek LJ, Tawil R, Schwartz PJ. Electrocardiographic features in Andersen-Tawil syndrome patients with *KCNJ2* mutations: characteristic T-U-wave patterns predict the *KCNJ2* genotype. *Circulation* (2005) 111:2720–6. doi: 10.1161/CIRCULATIONAHA.104.472498
40. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, et al. $Ca_V1.2$ calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* (2004) 119, 19–31. doi: 10.1016/j.cell.2004.09.011
41. Splawski I, Timothy KW, Decher N, Kumar P, Sachse FB, Beggs AH, et al. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. *Proc Natl Acad Sci USA.* (2005) 102:8089–96. doi: 10.1073/pnas.0502506102
42. Gazzerro E, Sotgia F, Bruno C, Lisanti MP, Minetti C. Caveolinopathies: from the biology of caveolin-3 to human diseases. *Eur J Hum Genet.* (2010) 18:137–45. doi: 10.1038/ejhg.2009.103
43. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, et al. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. *Circulation* (2006) 114:2104–12. doi: 10.1161/CIRCULATIONAHA.106.635268
44. Cronk LB, Ye B, Kaku T, Tester DJ, Vatta M, Makielski JC, et al. Novel mechanism for sudden infant death syndrome: persistent late sodium current secondary to mutations in caveolin-3. *Heart Rhythm* (2007) 4:161–6. doi: 10.1016/j.hrthm.2006.11.030
45. Medeiros-Domingo A, Kaku T, Tester DJ, Iturralde-Torres P, Itty A, Ye B, et al. *SCN4B*-encoded sodium channel β4 subunit in congenital long-QT syndrome. *Circulation* (2007) 116:134–42. doi: 10.1161/CIRCULATIONAHA.106.659086

46. Holmegard HN, Theilade J, Benn M, Duno M, Haunso S, Svendsen JH. Genetic variation in the inwardly rectifying K^+ channel subunits *KCNJ3* (*GIRK1*) and *KCNJ5* (*GIRK4*) in patients with sinus node dysfunction. *Cardiology* (2010) 115:176–81. doi: 10.1159/000279319
47. Lian J, Yan G-X, Kowey PR. Born to be bradycardic? *Cardiology* (2010) 115:229–31. doi: 10.1159/000297573
48. Sorensen AB, Søndergaard MT, Overgaard MT. Calmodulin in a heartbeat. *FEBS J.* (2013) 280:5511–32. doi: 10.1111/febs.12337
49. Shamgar L, Ma L, Schmitt N, Haitin Y, Peretz A, Wiener R, et al. Calmodulin is essential for cardiac I_{KS} channel gating and assembly: impaired function in long-QT mutations. *Circ Res.* (2006) 98:1055–63. doi: 10.1161/01.RES.0000218979.40770.69
50. Limpitikul WB, Dick IE, Joshi-Mukherjee R, Overgaard MT, George AL Jr, Yue DT. Calmodulin mutations associated with long QT syndrome prevent inactivation of cardiac L-type Ca^{2+} currents and promote proarrhythmic behavior in ventricular myocytes. *J Mol Cell Cardiol.* (2014) 74:115–24. doi: 10.1016/j.yjmcc.2014.04.022
51. Pipilas DC, Johnson CN, Webster G, Schlaepfer J, Fellmann F, Sekarski N, et al. Novel calmodulin mutations associated with congenital long QT syndrome affect calcium current in human cardiomyocytes. *Heart Rhythm* (2016) 13:2012–9. doi: 10.1016/j.hrthm.2016.06.038
52. Giudicessi JR, Ackerman MJ. Calcium revisited: new insights into the molecular basis of long-QT syndrome. *Circ Arrhythm Electrophysiol.* (2016) 9:e002480. doi: 10.1161/CIRCEP.116.002480
53. Mascia G, Arbelo E, Solimene F, Giaccardi M, Brugada R, Brugada J. The long-QT syndrome and exercise practice: the never-ending debate. *J Cardiovasc Electrophysiol.* (2018) 29:489–96. doi: 10.1111/jce.13410
54. Campuzano O, Allegue C, Partemi S, Iglesias A, Oliva A, Brugada R. Negative autopsy and sudden cardiac death. *Int J Legal Med.* (2014) 128:599–606. doi: 10.1007/s00414-014-0966-4
55. Gray B, Behr ER. New insights into the genetic basis of inherited arrhythmia syndromes. *Circ Cardiovasc Genet.* (2016) 9:569–77. doi: 10.1161/CIRCGENETICS.116.001571
56. Riuró H, Campuzano O, Arbelo E, Iglesias A, Batlle M, Pérez-Villa F, et al. A missense mutation in the sodium channel β1b subunit reveals *SCN1B* as a susceptibility gene underlying long QT syndrome. *Heart Rhythm* (2014) 11:1202–9. doi: 10.1016/j.hrthm.2014.03.044
57. Altmann HM, Tester DJ, Will ML, Middha S, Evans JM, Eckloff BW, et al. Homozygous/compound heterozygous triadin mutations associated with autosomal-recessive long-QT syndrome and pediatric sudden cardiac arrest: elucidation of the triadin knockout syndrome. *Circulation* (2015) 131:2051–60. doi: 10.1161/CIRCULATIONAHA.115.015397
58. Verkerk AO, Van Ginneken ACG, Wilders R. Pacemaker activity of the human sinoatrial node: role of the hyperpolarization-activated current, I_f. *Int J Cardiol.* (2009) 132:318–36. doi: 10.1016/j.ijcard.2008.12.196
59. Lakatta EG, DiFrancesco D. What keeps us ticking: a funny current, a calcium clock, or both? *J Mol Cell Cardiol.* (2009) 47:157–70. doi: 10.1016/j.yjmcc.2009.03.022
60. Monfredi O, Maltsev VA, Lakatta EG. Modern concepts concerning the origin of the heartbeat. *Physiology* (2013) 28:74–92. doi: 10.1152/physiol.00054.2012
61. Biliczki P, Girmatsion Z, Brandes RP, Harenkamp S, Pitard B, Charpentier F, et al. Trafficking-deficient long QT syndrome mutation KCNQ1-T587M confers severe clinical phenotype by impairment of KCNH2 membrane localization: evidence for clinically significant I_{Kr}-I_{Ks} α-subunit interaction. *Heart Rhythm* (2009) 6:1792–801. doi: 10.1016/j.hrthm.2009.08.009
62. Tsuji Y, Zicha S, Qi X-Y, Kodama I, Nattel S. Potassium channel subunit remodeling in rabbits exposed to long-term bradycardia or tachycardia: discrete arrhythmogenic consequences related to differential delayed-rectifier changes. *Circulation* (2006) 113:345–55. doi: 10.1161/CIRCULATIONAHA.105.552968
63. Yeh Y-H, Burstein B, Qi XY, Sakabe M, Chartier D, Comtois P, et al. Funny current downregulation and sinus node dysfunction associated with atrial tachyarrhythmia: a molecular basis for tachycardia-bradycardia syndrome. *Circulation* (2009) 119:1576–85. doi: 10.1161/CIRCULATIONAHA.108.789677
64. D'Souza A, Bucchi A, Johnsen AB, Logantha SJRJ, Monfredi O, Yanni J, et al. Exercise training reduces resting heart rate via downregulation of the funny channel HCN4. *Nat Commun.* (2014) 5:3775. doi: 10.1038/ncomms4775
65. Van den Berg MP, Wilde AAM, Viersma JW, Brouwer J, Haaksma J, Van der Hout AH, et al. (2001). Possible bradycardic mode of death and successful pacemaker treatment in a large family with features of long QT syndrome type 3 and Brugada syndrome. *J Cardiovasc Electrophysiol.* 12, 630–6. doi: 10.1046/j.1540-8167.2001.00630.x
66. Beaufort-Krol GCM, Van den Berg MP, Wilde AAM, Van Tintelen JP, Viersma JW, Bezzina CR, et al. Developmental aspects of long QT syndrome type 3 and Brugada syndrome on the basis of a single *SCN5A* mutation in childhood. *J Am Coll Cardiol.* (2005) 46:331–7. doi: 10.1016/j.jacc.2005.03.066
67. Schott J-J, Charpentier F, Peltier S, Foley P, Drouin E, Bouhour JB, et al. Mapping of a gene for long QT syndrome to chromosome 4q25-27. *Am J Hum Genet.* (1995) 57:1114–22.
68. Mohler PJ, Schott J-J, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* (2003) 421:634–9. doi: 10.1038/nature01335
69. Mohler PJ, Splawski I, Napolitano C, Bottelli G, Sharpe L, Timothy K, et al. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. *Proc Natl Acad Sci USA.* (2004) 101:9137–42. doi: 10.1073/pnas.0402546101
70. Le Scouarnec S, Bhasin N, Vieyres C, Hund TJ, Cunha SR, Koval O, et al. Dysfunction in ankyrin-B-dependent ion channel and transporter targeting causes human sinus node disease. *Proc Natl Acad Sci USA.* (2008) 105:15617–22. doi: 10.1073/pnas.0805500105

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MINI REVIEW
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Mapping and Ablation of Idiopathic Ventricular Fibrillation

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Idiopathic ventricular fibrillation (IVF) is the main cause of unexplained sudden cardiac death, particularly in young patients under the age of 35. IVF is a diagnosis of exclusion in patients who have survived a VF episode without any identifiable structural or metabolic causes despite extensive diagnostic testing. Genetic testing allows identification of a likely causative mutation in up to 27% of unexplained sudden deaths in children and young adults. In the majority of cases, VF is triggered by PVCs that originate from the Purkinje network. Ablation of VF triggers in this setting is associated with high rates of acute success and long-term freedom from VF recurrence. Recent studies demonstrate that a significant subset of IVF defined by negative comprehensive investigations, demonstrate in fact subclinical structural alterations. These localized myocardial alterations are identified by high density electrogram mapping, are of small size and are mainly located in the epicardium. As reentrant VF drivers are often colocated with regions of abnormal electrograms, this localized substrate can be shown to be mechanistically linked with VF. Such areas may represent an important target for ablation.

Keywords: idiopathic ventricular fibrillation, mapping, ablation, Purkinje, localized substrate

INTRODUCTION

Idiopathic ventricular fibrillation (IVF) is a rare cause of sudden cardiac death (SCD). It is reported in 6.8% of all patients who survive an out-of-hospital cardiac arrest and is more frequent in young adults (1). Indeed up to 35% of cases of sudden death remain unexplained in patients between 18 and 35 years old (2). Current guidelines define IVF as a diagnosis of exclusion in patients who have survived a VF episode without any identifiable structural or metabolic cause (3). An implantable cardioverter defibrillator (ICD) is usually recommended for primary and secondary prevention of SCD in this population (3, 4). However, around one third of patients with IVF, experience VF recurrence in the 5 years following diagnosis (5). VF ablation is recommended to prevent VF recurrence and reduce the number of ICD shocks (3, 4).

We aim to review the mechanisms underlying IVF and the different ablation strategies in this setting.

VF PATHOPHYSIOLOGY

VF Initiation

VF is initiated by premature ventricular complexes (PVCs) or by the transition from a ventricular tachycardia (VT). In patients with IVF, PVCs that trigger the arrhythmia originate from the Purkinje system in up to 93% of the cases (6, 7). More rarely, they originate from the ventricular myocardium including the right ventricular outflow tract (RVOT) (7–11) or the papillary muscle (12, 13). These PVCs may result from abnormal automaticity, triggered activities, or more rarely from reentry, either phase 2 reentry (14) or reentry using the Purkinje system (15). Purkinje cells have distinctive anatomical and electrophysiological properties (16). Abnormal automaticity in the Purkinje fibers likely results from a deficient calcium regulation by the sarcoplasmic reticulum (16, 17). Triggered activities such as early afterdepolarizations (EADs) or delayed afterdepolarizations (DADs) are commonly recorded in the Purkinje cells (18–20) and can result from Ca^{2+} overload (17). These arrhythmogenic mechanisms become more prevalent in the presence of electrolyte imbalance, exposure to drugs, and in the presence of myocardial ischemia (21).

VF Maintenance

Mechanisms that maintain VF are as yet, incompletely elucidated. Animal studies suggest reentrant activities and multiple wavelets as main mechanisms maintaining early VF (22) and Purkinje system as principal mechanism that maintains long duration VF (23). Structural heterogeneities are critical for the occurrence of reentries by decreasing the conduction velocities and thereby anchoring reentries (24–26). Complex myocardial fiber arrangement at the papillary muscle insertions and at the Purkinje tissue can maintain fibrillatory activities in the absence of additional pathology (27).In a mammalian 3-dimensional model, Berenfeld et al. (28) simulated the evolution of reentrant activity at the Purkinje-muscle junction and demonstrated that Purkinje activity is essential to the reentry at its initial stage and led to intra-myocardial reentries that sustained the arrhythmia. Subsequently, Pak et al. (29) demonstrated the contribution of both Purkinje activities and the ventricular myocardium (left postero-septum and papillary muscles) in maintaining VF. Newton et al.(30) and Tabereaux et al. (31) demonstrated that Purkinje fibers are highly active during the VF, mainly 1 min after the initiation. This activation was associated with an endocardial to epicardial gradient (32) and is explained by the resistance of the Purkinje cells to prolonged ischemia. Additional evidence supporting the role of Purkinje fibers in the initiation and maintenance of VF comes from canine heart studies in which chemical ablation of the Purkinje fibers using Lugol's solution significantly increased the VF induction thresholds (33) and was associated to early VF termination (34).

Genetics of IVF

Several familial cases of IVF have been reported, suggesting that a subset of IVF is hereditary and has a genetic transmission. This has been demonstrated by Alders et al. (35) who performed a genome wide haplotype sharing analysis to identify the responsible gene for IVF in 3 distantly related families from the Netherlands. The authors identified a mutation located on the chromosome7q36 harboring a part of the dipeptidyl peptidase-like protein-6 (DPP6) gene that encodes for a component of the transient outward current (36). The correlation between DPP6 mutation and IVF was confirmed in a larger population of 26 families including 601 family members from the Netherlands (37). The mutation increased levels of the DPP6 mRNA 20 fold compared to controls. Xiao et al. (38) demonstrated that DPP6 overexpression selectively increases the I_{TO} current in the Purkinje fibers leading to abnormal depolarization which may explain a part of the pathogenesis of VF in this group. Other genes have been linked to IVF including CALM1 (39), RYR2 (40), IRX3 (41).

Whole exome sequencing represents the latest approach to genetic testing in patients with IVF, allowing diagnosis of a wide range of sudden death-susceptibility genes (42, 43). Of note however, genetic screening frequently reveals rare variants and variants of uncertain significance that require further classification (20, 44–49).

MAPPING AND ABLATION OF VF TRIGGERS

Clinical Experience

So far, mapping and ablation of the premature ventricular contractions (PVCs) triggering VF remains the gold standard for IVF ablation. Multiple cases of successful ablation of triggering PVCs have been reported and are represented in **Table 1**. Ashida et al. (50) first reported successful ablation of right ventricular outflow tract (RVOT) triggers in a patient with recurrent VF episodes. PVC morphology was reproduced by pace-mapping at the septal RVOT. Ablation at this site abolished the arrhythmia and the episodes of syncope. Later, Kusano et al. (51) and Takatsuki et al. (52) also reported successful ablation of ectopics triggering VF arising from the RVOT; this was associated with freedom from VF recurrence. Additional sites of PVCs triggering VF have been reported at the infero-lateral RV (53, 54), Purkinje system (55–57, 59–63), moderator band (67), and papillary muscles (12, 13).

So far, two large studies of IVF ablation have been published. The first study included 27 patients with recurrent episodes VF (7). A Purkinje origin was demonstrated in 23/27 (93%) patients. This was located in the left ventricular septum in 10 patients, in the anterior right ventricle in 9 patients, and in both ventricles in 4 patients. The second study was a multicenter study that included 38 patients (11). The PVC origin was in the Purkinje system in 33/38 (87%) patients. They arose from the left Purkinje fibers in 14 patients, from the right Purkinje fibers in 16 patients and from both chambers in 3 patients. A myocardial origin was identified in 5 patients, the majority being from the RVOT (4/5).

In other studies, Noda et al. (10) explored 101 patients with normal structural hearts who presented with PVCs arising from the RVOT. Among this group, 16 patients presented with spontaneous episodes of VF (5 cases) and syncope (11 cases).

TABLE 1 | Case reports of successful ablation of PVCs triggering VF.

References	Patient history	Mapping and ablation	Outcome
Ashida et al. (50)	18 y.o female Syncope	Septal RVOT	No VF recurrence after 3 years
Kusano et al. (51)	65 y.o female Syncope	RVOT	No VF recurrence after 18 months
Takatsuki et al. (52)	62 y.o male	Postero-septal RVOT	No VF recurrence after 20 months
Saliba et al. (53)	41 y.o female	PVC coupling interval = 240 ms Duration 140 ms Inferolateral border of the right ventricle Late sharp potential recorded in sinus rhythm and preceding the PVCs	No VF recurrence after 6 months
Betts et al. (54)	27 y.o male Coupling interval 260–300 ms	Free wall of the RVOT Sharp potential 80 ms before PVC onset	No VF recurrence after 11 months of follow-up
Pasquie et al. (55)	3 patients, mean age 62 y.o VF during fever episodes	Coupling intervals = 240 and 320 ms Purkinje potential preceding the PVC (anterior RV)	No VF recurrence after 9, 18 and 22 months
Kohsaka et al. (56)	21 y.o female Electrical storm	Purkinje from the right bundle preceding the PVC initiation VF by 72 ms	No VF recurrence after 12 months
Naik et al. (57)	24 y.o male Syncope	Coupling interval = 280–320 ms 2 PVC morphologies = RVOT + RV apex Few PVCs recorded during the procedure Ablation based on pacemapping and targeting Purkinje potential in the RV apex	VF recurrence after 9 months due to PVCs of similar morphology Redo ablation was associated with VF Freedom after 1 year-follow-up
Cho et al. (58)	17 y.o male Aborted sudden cardiac death due to IVF	Coupling interval = 360 ms Ablation at the anterolateral wall of the RVOT based on the earliest activation site and pacemapping	Acute success with no VF/PVT recurrence during the 2 weeks after the procedure
Szumowski et al. (59)	25 y.o female syncope 150 ICD therapies in 9 years	PVCs originating from the Purkinje network	No VF recurrence after 2 years
Saba et al.(60)	10 y.o male syncope Atrial fibrillation 30 ICD shocks in 2 months	4 PVC morphologies: 2 short coupled (268+/110 ms) with a large QRS (161 ± 7 ms) 2 longer coupled PVCs (422 ± 25 ms) and narrower QRS (118 ± 9 ms) Mapping performed using a basket catheter	No VF recurrence after 21 months using quinidine
Santoro et al.(13)	5 patients, mean age 39 ± 12 years Multiple ICD shocks and electrical storm	PVCs arising from the left ventricular posteromedial papillary muscle in 4 cases and from the right ventricular postero lateral papillary muscle in 1 case.	No VF recurrence after 58 ± 11 months
Nagase et al.(61)	29 y.o female Multiple ICD shocks for VF episodes	PVC with different morphologies Ablation targeting earliest anterior and posterior Purkinje potentials	Recurrence of 3 VF episodes after 96 months No VF recurrence after administration of atenolol and disopyramide
Kleissner et al.(62)	Male Electrical storm	2 PVCs initiating VF The first arising from the right Purkinje preceding the PVC by 28 ms. The second arising from the RVOT.	–
Rosu et al., (63)	39 y.o male Multiple syncopes	PVCs arising from the right Purkinje preceding the PVC by 15 ms.	2 early recurrences of VF episodes initiated by PVC s from the RV with different morphologies. No VF recurrence after 3 years
Chan and Sy (64)	2 patients = 40 and 24 y.o females syncope and cardiac arrest respectively	PVCs arising from the posterior fascicle in the first case and from the RVOT in the second case	No VF recurrence after 17 and 42 months respectively
Ho et al. (65)	44 y.o male Electrical storm	PVCs arising from the moderator band mapped using the Pentaray catheter Purkinje potentials preceding the PVC by 103 ms Ablation targeted Purkinje potentials at the moderator band	No VF recurrence after ablation
Martin et al. (66)	32 y.o male Syncope	PVC arising from the posterior fascicle Purkinje potentials preceding the PVC by 34 ms Ablation based on pacemapping and the site of earliest activation	Recurrence of 1 VF episode after 2 year follow-up

ICD, implantable cardioverter defibrillator; PVC, premature ventricular contraction; PVT, polymorphic ventricular tachycardia; RV, right ventricle; RVOT, right ventricular outflow tract; VF, ventricular fibrillation.

Ablation targeting the PVCs from the RVOT eliminated episodes of syncope and VF in all patients during a follow-up period of 54 ± 39 months. Santoro et al. (13) explored 5 patients with IVF using intracardiac echocardiography (ICE). They identified a PVC origin in the left ventricular posteromedial papillary muscle in 4 cases and in the right ventricular posterolateral papillary muscle in 1 case. Sadek et al. (67) mapped PVCs triggering VF in a group of 36 patients with VF using ICE. They identified the PVC origin at the moderator band in 7 patients.

Mapping of VF Triggers

The procedure is best scheduled during or as soon as possible after an electrical storm, a period during which the culprit PVCs tend to be frequent. Thereafter, PVCs become less frequent which reduces the likelihood of success. The PVC morphology on the 12 lead ECG is of particular interest as it guides mapping techniques and allows focus on the area of interest (**Figure 1**). When originating from the left Purkinje fibers, the PVCs are usually narrow (<120 ms) with a right-bundle-branch block

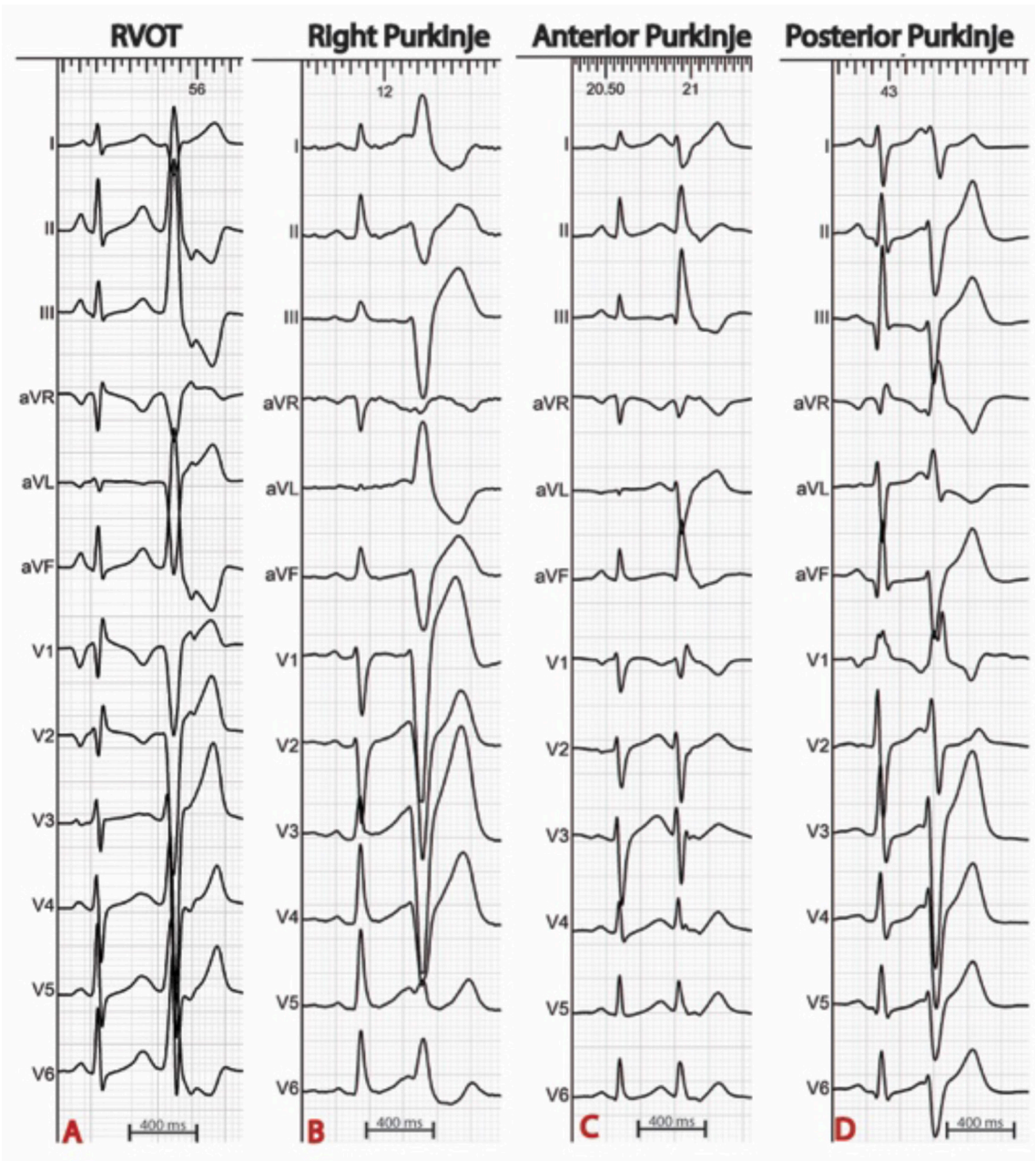

FIGURE 1 | Examples of ECGs in patients with IVF. The origin of premature ventricular complexes triggering VF may be the right ventricular outflow tract **(A)**, the right Purkinje system **(B)**, or the left anterior **(C)** or posterior **(D)** Purkinje system.

morphology (6, 7, 11). They demonstrate right or left axis deviation when originating from the anterior or the posterior Purkinje fibers respectively. Discrete morphology changes are frequently observed in the left Purkinje PVCs. When originating from the right Purkinje arborization, the PVCs are usually wide and have a left-bundle-branch block morphology (6, 7, 11). More rarely, PVCs can originate from a non-conductive tissue source, particularly from the RVOT. In this case, the PVCs have an inferior axis. The coupling interval is classically variable and VF is usually triggered by short coupled PVCs. Discrete PVC morphology changes are frequently observed before VF initiation, potentially due to different exit sites.

As described by our group (6, 7, 16), the PVC origin may be mapped endocardially and is located at the earliest electrogram site relative to the onset of the PVC on the 12 lead ECG. The right ventricle is accessed by a venous femoral approach using a long sheath. The left ventricle is accessed either by a transeptal approach or a by a retrograde approach. The transeptal approach is effective in reaching most of the left ventricular myocardium and may provide more stability to map and ablate the anterior Purkinje and the antero-lateral papillary muscle. The retrograde approach is more effective in accessing the left basal septum and the left ventricular outflow tract. The transeptal approach is preferred in patients with aortic atherosclerosis and in the presence of aortic valve stenosis. A decapolar catheter is helpful in mapping the His-Purkinje arborization in both chambers. A lasso catheter can be used to map the RVOT (68). A multispline catheter is also useful for mapping over a wide area of ventricular endocardium with high spatial sampling and resolution.

In sinus rhythm, in the absence of intraventricular conduction abnormalities, distal Purkinje potentials are usually sharp

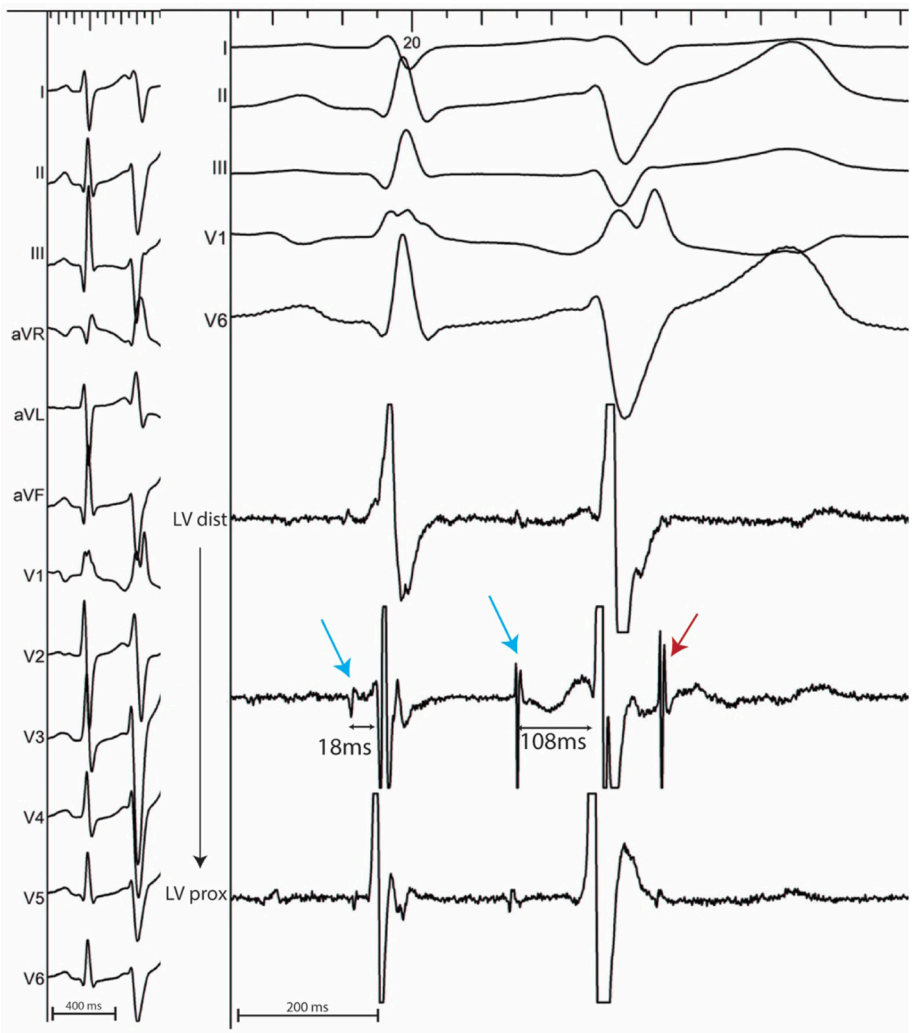

FIGURE 2 | 12 lead ECG with associated endocardial electrograms of a PVC arising from the posterior Purkinje network. Purkinje fascicular potentials precede QRS onset by 18 ms during sinus rhythm. During the PVC, Purkinje potentials precede QRS onset by 108 ms (blue arrows). Notice the presence of a concealed Purkinje potential (red arrow).

(≤10 ms) and precede the QRS complex by ≤15 ms. Longer intervals indicate a fascicular origin. During a PVC, Purkinje potentials precede the local EGM by variable intervals that are usually greater than 15 ms (**Figure 2**). Purkinje activation can be blocked and concealed and can be activated retrogradely (**Figure 3**). Purkinje potentials become concealed within the local EGMs in the presence of intraventricular conduction abnormalities. Therefore, special care should be made during mapping to avoid inadvertent bumping of the left or right bundles. The absence of Purkinje potential at the earliest ventricular activation site during sinus rhythm indicates a myocardial origin. Whenever needed, PVCs can be induced by pacing maneuvers (atrial or ventricular) and/or more rarely by intravenous infusion of Isoproterenol (1–2 mcg/kg/min) or Ajmaline (1 mg/kg).

In the absence of spontaneous or inducible PVCs, pace-mapping may give an indication of the area of interest. However, pace-mapping cannot reliably reproduce the morphology of the Purkinje triggered PVCs due to simultaneous capture of the surrounding myocardium. Pace mapping is performed with the lowest pacing output (twice the diastolic threshold, range 2–15 mA) with a pulse width of 2 ms in order to capture the local ventricular myocardium. Different systems allow analysis of the degree of similarity between the recorded PVC and the original one and express it as a percentage. This comparison may also be performed for mechanically induced PVCs.

Electrocardiographic imaging (ECGi) represents an additional tool that may accurately identify the origin of the PVCs triggering VF (69–71). It is of particular interest in patients with rare PVCs (**Figure 4**).

IS THERE A SUBSTRATE IN PATIENTS WITH IVF?

In order to sustain, VF requires a substrate, either anatomical or electrical. However, current diagnostic tools are limited and may miss subtle structural abnormalities. In addition, the lack of mapping resolution during VF in humans, as well as the unknown effects of acute or dynamic phenomena, may explain the lack of data in this group.

In a recent study (20), we evaluated 24 patients who survived IVF. All patients benefited from non-invasive mapping to characterize the drivers maintaining VF during the initial 5 s of VF. In addition, all patients benefited from high density endocardial and epicardial biventricular mapping. A decapolar catheter was used to map the endocardium of the right and left ventricles, while 20-pole catheters with 2 mm inter-electrode spacing (Pentaray, BiosenseWebster, CA; Lasso, BiosenseWebster, CA) were used for biventricular epicardial mapping.

A total of 19 VF episodes were analyzed. VF occurred spontaneously in 3 patients and was induced by electrical stimulation in 16, whereas it was not inducible in 5 patients. A mean of 28 ± 3 VF cycles were recorded during the initial 5 s and the mean VF cycle length was 183 ± 23 ms. A mean of 2.8 ± 0.7 activities (including focal and reentrant activities) were recorded per cycle, being reentrant in 87% and focal in 13%. A ventricle was considered as dominant when hosting more than 50% of the activities during the initial 5 s. This was the case in 9 patients while the rest demonstrated biventricular distribution of the fibrillatory activities. High density mapping during sinus rhythm identified abnormal electrograms [>70 ms

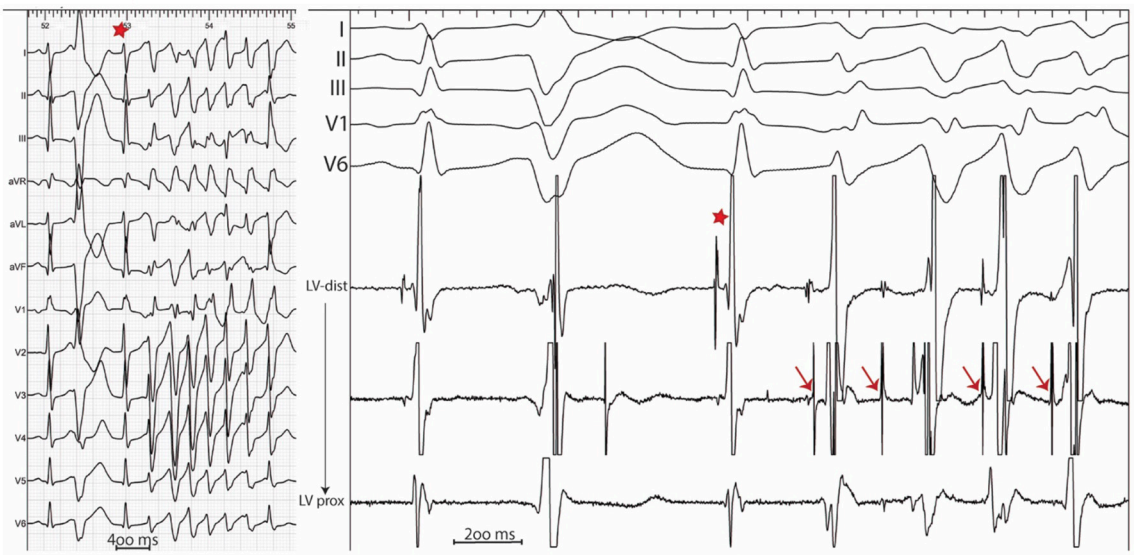

FIGURE 3 | 12 lead ECG **(Left)** with associated endocardial tracings **(Right)** showing spontaneous polymorphic PVCs from a patient with idiopathic VF. A wide PVC likely originating from the right ventricle is followed by a concealed retrograde Purkinje potential (red star). Purkinje potentials during sinus rhythm are shown by blue arrows. PVCs originating from the Purkinje fibers are preceded by Purkinje potentials with a different coupling interval (red arrows). Notice the modifications in PVC morphology which result from the complex arborization of the left Purkinje system.

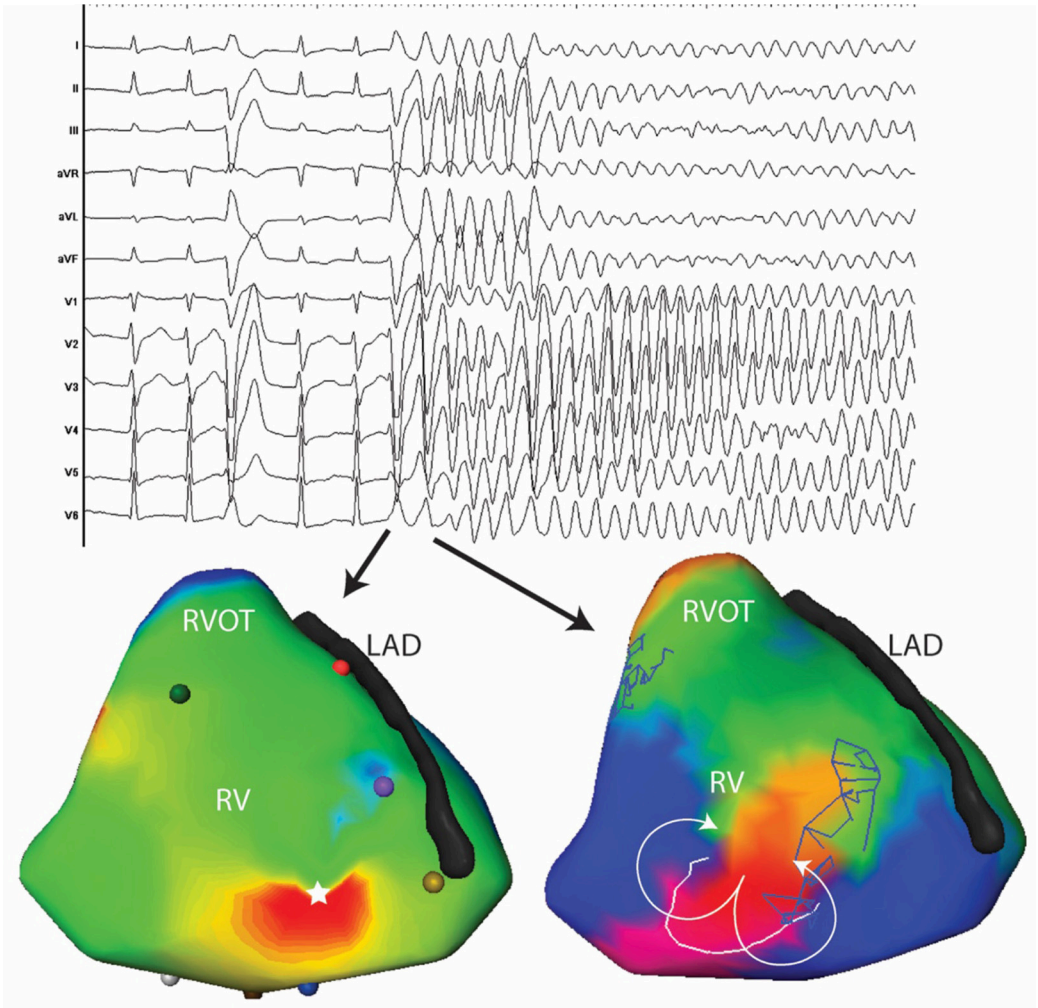

FIGURE 4 | Twelve lead ECG and activation maps of the first and second beats of spontaneously initiated VF in a 30-year-old man. The PVC initiating VF has a similar morphology as the previous PVC with subtle changes in the precordial leads (V2-V3). The PVC initiating VF is located at the antero-apical RV (white star). The subsequent beat is a figure of eight at the same site as the first PVC.

duration and more than 3 spikes (72–74)] in 15/24 patients (62.5%) (**Figure 5**). They were arranged in a confluent (rather than a distributed) pattern and covered a limited surface area (13 ± 6 cm^2), representing 5 ± 3% of the total ventricular surface area. The abnormal electrograms were located in the right ventricle in 11, the left ventricle in one and both in three, and were predominantly epicardial. The localized substrate colocated with the driver regions in 76% of cases ($p < 0.001$). The 9 patients without structural alterations had a high incidence of Purkinje triggers (7/9).

ABLATION STRATEGIES AND PROCEDURAL OUTCOME

The site of earliest ventricular activation during spontaneous PVCs is the target of choice. In patients without clinical PVCs, ablation can target the local Purkinje potentials or the site of best matched morphology by pace-mapping. Ablation may be performed using an irrigated 3.5 mm tip catheter. Power is delivered according to catheter location. PVCs originating from the RVOT or from the Purkinje network are ablated using 30 watts. The power can be increased to 50 watts on the septum when the PVC origin is intramural. Manual titration of the irrigation flow is performed to achieve the required power. In all cases, ablation is extended approximately 1–2 cm^2 around the target site. During ablation, it is common to have exacerbation of the arrhythmia (multiple PVCs leading to polymorphic VT and more rarely to VF) before the eradication of premature beats. The occurrence of QRS widening during ablation indicates potential catheter displacement toward the more proximal conduction system and ablation should be stopped (7). Knecht et al. (11) have reported the occurrence of transient left bundle branch block in one patient and nonspecific intraventricular

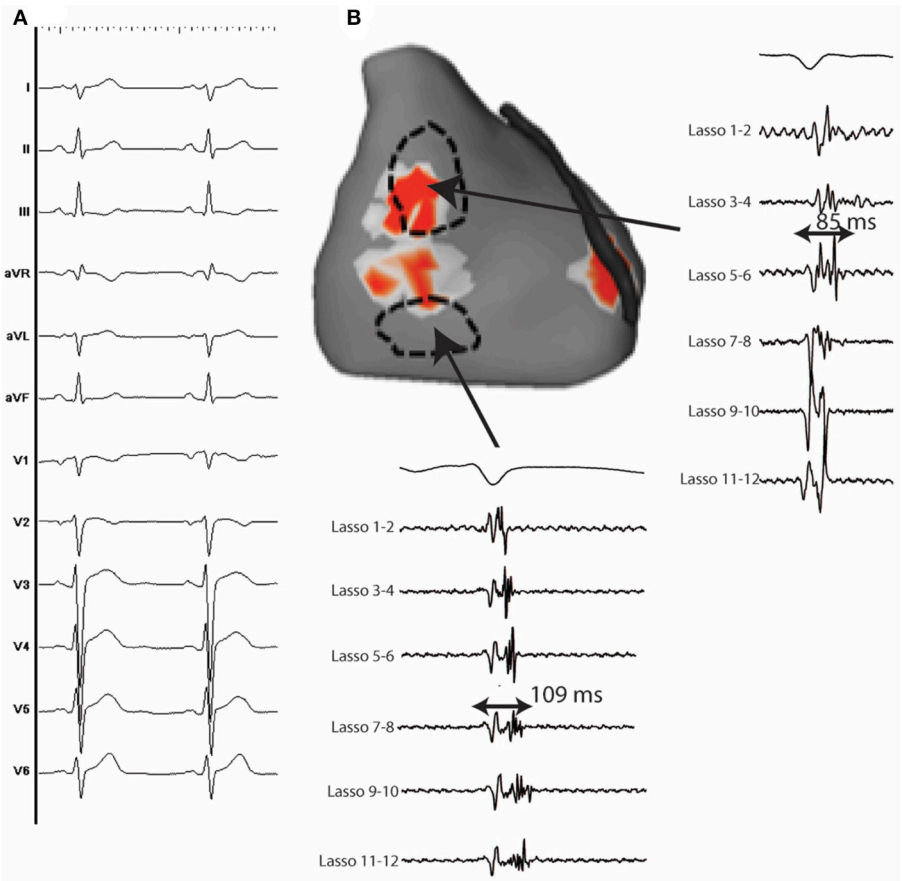

FIGURE 5 | (A) 12 lead ECG of a 37 y.o man with IVF. **(B)** Anterior view of the heart showing an area of reentrant activity located at the anterior and lateral epicardial RV. Fragmented epicardial electrograms with long duration during sinus rhythm are identified close to the driver sites.

conduction defects in 6 of 38 patients. The procedural endpoint is complete elimination of the culprit PVC and of the local Purkinje potentials. Acute procedural success rate is high. In their initial report, Haissaguerre et al. (7) achieved complete elimination of all the clinical PVCs that were recorded in 24 of 27 patients. Ablation was guided by pace-mapping in the remaining 3 patients. Two patients had early recurrence of PVCs with different morphologies that were successfully eliminated during a second procedure. Knecht et al. reported successful elimination of the culprit PVCs in all patients who presented with spontaneous PVCs.

The localized substrate identified during mapping represents a novel additional target for ablation. In a recent study, we targeted the abnormal substrate in 12 patients with IVF with recurrent episodes. Ablation was associated with VF free outcome with 14-months follow-up (20).

A summary of the diagnostic, mapping and ablation approaches in patients with IVF is provided in **Figure 6**.

PROCEDURAL OUTCOMES

Clinical and Holter monitoring is performed in all patients for at least 3 days after the procedure. ICDs are systematically implanted before discharge if not already *in situ*. Antiarrhythmic drugs are continued for at least 3 months after the procedure. After discharge, patients are followed-up at 1, 3, 6, and 12 months, then every 6 months to 1 year thereafter. Follow-up includes clinical examination, 12-lead ECG, exercise test and ICD interrogation.

Following current guidelines (3, 4), we systematically perform familial screening, including resting ECG, exercise testing and echocardiography in first degree relatives. In selected cases, Holter and signal-averaged ECGs, MRI and provocation testing (including with Class Ic antiarrhythmic drugs and epinephrine) are performed.

To date, the greatest experience with mapping and ablation of IVF was reported in a multicenter trial of 38 patients (11). After a mean follow-up of 63 months, 31 of 38 (82%) patients were free from VF recurrence. VF recurrence occurred in the remaining 7 (18%) patients after a median of 4 months with multiple episodes in 5 of them. The presence of bundle branch block before ablation was the only parameter associated with worse outcome and with VF recurrence (11). There was no difference in outcome between patients with Purkinje triggers and those with muscular triggers.

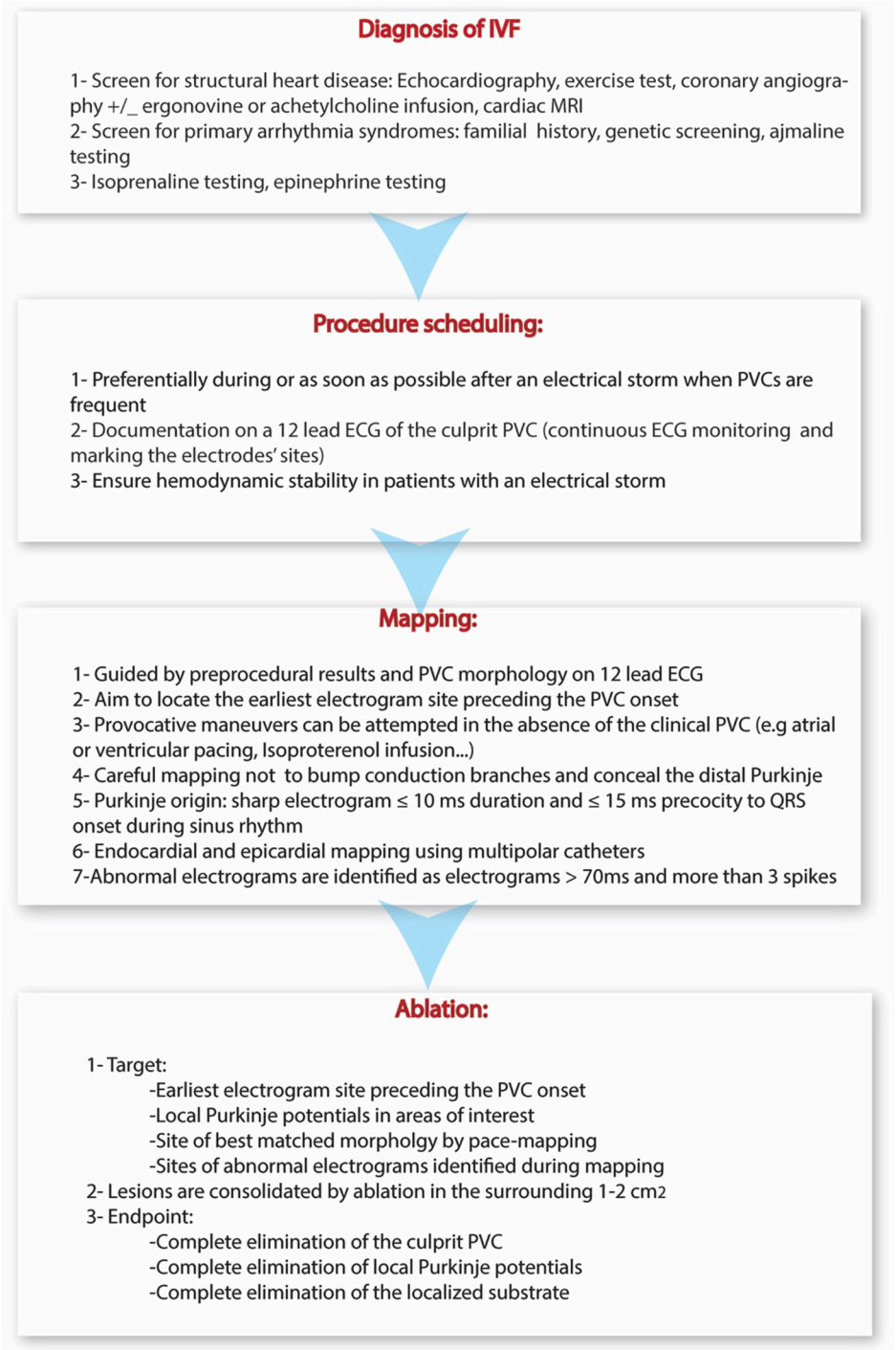

FIGURE 6 | Summary of the current diagnostic, mapping and ablation approaches in patients with IVF.

CONCLUSIONS

Idiopathic VF is diagnosed in around one third of survivors of unexplained SCD aged under 35 years. Genetic testing allows identification of a likely causative mutation in around one quarter of unexplained sudden deaths in children and young adults. Ablation of the PVCs that trigger VF in this setting is associated with high rates of acute success and long-term freedom from VF recurrence. Importantly, almost two thirds of patients have subtle structural abnormalities identified by high density electrogram

mapping and missed by current imaging tools. This localized substrate, which colocates with regions of VF drivers, provides an explanation for so called unexplained SCD and represents a novel potential target for ablation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Waldmann V, Bougouin W, Karam N, Dumas F, Sharifzadehgan A, Gandjbakhch E, et al. Characteristics and clinical assessment of unexplained sudden cardiac arrest in the real-world setting: focus on idiopathic ventricular fibrillation. *Eur Heart J*. (2018) 39:1981−7. doi: 10.1093/eurheartj/ehy098
2. Eckart RE, Scoville SL, Campbell CL, Shry EA, Stajduhar KC, Potter RN, et al. Sudden death in young adults: a 25-year review of autopsies in military recruits. *Ann Intern Med*. (2004) 141:829–34. doi: 10.7326/0003-4819-141-11-200412070-00005
3. Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC). Endorsed by: association for European Paediatric and Congenital Cardiology (AEPC). *Eur Heart J*. (2015) 36:2793–867. doi: 10.1093/eurheartj/ehv316
4. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm* (2013) 10:1932–63. doi: 10.1016/j.hrthm.2013.05.014
5. Ozaydin M, Moazzami K, Kalantarian S, Lee H, Mansour M, Ruskin JN. Long-term outcome of patients with idiopathic ventricular fibrillation: a meta-analysis. *J Cardiovasc Electrophysiol*. (2015) 26:1095–104. doi: 10.1111/jce.12737
6. Haissaguerre M, Shah DC, Jais P, Shoda M, Kautzner J, Arentz T, et al. Role of Purkinje conducting system in triggering of idiopathic ventricular fibrillation. *Lancet* (2002) 359:677–8. doi: 10.1016/S0140-6736(02)07807-8
7. Haissaguerre M, Shoda M, Jais P, Nogami A, Shah DC, Kautzner J, et al. Mapping and ablation of idiopathic ventricular fibrillation. *Circulation* (2002) 106:962–7. doi: 10.1161/01.CIR.0000027564.55739.B1
8. Shimizu W. Arrhythmias originating from the right ventricular outflow tract: how to distinguish "malignant" from "benign"? *Heart Rhythm* (2009) 6:1507–11. doi: 10.1016/j.hrthm.2009.06.017
9. Viskin S, Rosso R, Rogowski O, Belhassen B. The "short-coupled" variant of right ventricular outflow ventricular tachycardia: a not-so-benign form of benign ventricular tachycardia? *J Cardiovasc Electrophysiol*. (2005) 16:912–6. doi: 10.1111/j.1540-8167.2005.50040.x
10. Noda T, Shimizu W, Taguchi A, Aiba T, Satomi K, Suyama K, et al. Malignant entity of idiopathic ventricular fibrillation and polymorphic ventricular tachycardia initiated by premature extrasystoles originating from the right ventricular outflow tract. *J Am Coll Cardiol*. (2005) 46:1288–94. doi: 10.1016/j.jacc.2005.05.077
11. Knecht S, Sacher F, Wright M, Hocini M, Nogami A, Arentz T, et al. Long-term follow-up of idiopathic ventricular fibrillation ablation: a multicenter study. *J Am Coll Cardiol*. (2009) 54:522–8. doi: 10.1016/j.jacc.2009.03.065
12. Van Herendael H, Zado ES, Haqqani H, Tschabrunn CM, Callans DJ, Frankel DS, et al. Catheter ablation of ventricular fibrillation: importance of left ventricular outflow tract and papillary muscle triggers. *Heart Rhythm* (2014) 11:566–73. doi: 10.1016/j.hrthm.2013.12.030
13. Santoro F, Di Biase L, Hranitzky P, Sanchez JE, Santangeli P, Perini AP, et al. Ventricular fibrillation triggered by PVCs from papillary muscles: clinical features and ablation. *J Cardiovasc Electrophysiol*. (2014) 25:1158–64. doi: 10.1111/jce.12478
14. Cantalapiedra IR, Penaranda A, Echebarria B, Bragard J. Phase-2 reentry in cardiac tissue: role of the slow calcium pulse. *Phys Rev E Stat Nonlin Soft Matter Phys*. (2010) 82(1 Pt 1):011907. doi: 10.1103/PhysRevE.82.011907
15. Antzelevitch C, Burashnikov A. Overview of basic mechanisms of cardiac arrhythmia. *Card Electrophysiol Clin*. (2011) 3:23–45. doi: 10.1016/j.ccep.2010.10.012
16. Haissaguerre M, Vigmond E, Stuyvers B, Hocini M, Bernus O. Ventricular arrhythmias and the His-Purkinje system. *Nat Rev Cardiol*. (2016) 13:155–66. doi: 10.1038/nrcardio.2015.193
17. Ter Keurs HE, Boyden PA. Calcium and arrhythmogenesis. *Physiol. Rev*. (2007) 87:457–506. doi: 10.1152/physrev.00011.2006
18. Song Z, Ko CY, Nivala M, Weiss JN, Qu Z. Calcium-voltage coupling in the genesis of early and delayed afterdepolarizations in cardiac myocytes. *Biophys J*. (2015) 108:1908–21. doi: 10.1016/j.bpj.2015.03.011
19. Hirose M, Stuyvers BD, Dun W, ter Keurs HE, Boyden PA. Function of Ca(2+) release channels in Purkinje cells that survive in the infarcted canine heart: a mechanism for triggered Purkinje ectopy. *Circ Arrhythmia Electrophysiol*. (2008) 1:387–95. doi: 10.1161/CIRCEP.107.758110
20. Haissaguerre M, Hocini M, Cheniti G, Duchateau J, Sacher F, Puyo S, et al. Localized structural alterations underlying a subset of unexplained sudden cardiac death. *Circ Arrhythmia Electrophysiol*. (2018) 11:e006120. doi: 10.1161/CIRCEP.117.006120
21. Friedman PL, Stewart JR, Wit AL. Spontaneous and induced cardiac arrhythmias in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. *Circ Res*. (1973) 33:612–26. doi: 10.1161/01.RES.33.5.612
22. Nash MP, Mourad A, Clayton RH, Sutton PM, Bradley CP, Hayward M, et al. Evidence for multiple mechanisms in human ventricular fibrillation. *Circulation* (2006) 114:536–42. doi: 10.1161/CIRCULATIONAHA.105.602870
23. Jackson N, Massé S, Zamiri N, Azam MA, Lai PFH, Kusha M, et al. Mechanisms of long-duration ventricular fibrillation in human hearts and experimental validation in canine purkinje fibers. *JACC Clin Electrophysiol*. (2015) 1:187–97. doi: 10.1016/j.jacep.2015.04.003
24. Jeyaratnam J, Umapathy K, Masse S, Nair K, Farid T, Krishnan S, et al. Relating spatial heterogeneities to rotor formation in studying human ventricular fibrillation. *Conf Proc IEEE Eng Med Biol Soc*. (2011) 2011:231–4. doi: 10.1109/IEMBS.2011.6090043
25. Nair K, Umapathy K, Farid T, Masse S, Mueller E, Sivanandan RV, et al. Intramural activation during early human ventricular fibrillation. *Circ Arrhythmia Electrophysiol*. (2011) 4:692–703. doi: 10.1161/CIRCEP.110.961037
26. Cheniti G, Hocini M, Martin R, Sacher F, Dubois R, Haissaguerre M, et al. Is VF an ablatable rhythm? *Curr Treat Options Cardiovasc Med*. (2017) 19:14. doi: 10.1007/s11936-017-0511-0
27. Kim YH, Xie F, Yashima M, Wu TJ, Valderrabano M, Lee MH, et al. Role of papillary muscle in the generation and maintenance of reentry during ventricular tachycardia and fibrillation in isolated swine right ventricle. *Circulation* (1999) 100:1450–9. doi: 10.1161/01.CIR.100.13.1450
28. Berenfeld O, Jalife J. Purkinje-muscle reentry as a mechanism of polymorphic ventricular arrhythmias in a 3-dimensional model of the ventricles. Circ Res. (1998)82(10):1063-77. doi: 10.1161/01.RES.82.10.1063
29. Pak HN, Kim GI, Lim HE, Fang YH, Choi JI, Kim JS, et al. Both Purkinje cells and left ventricular posteroseptal reentry contribute to the maintenance of ventricular fibrillation in open-chest dogs and swine: effects of catheter ablation and the ventricular cut-and-sew operation. *Circ J*. (2008) 72:1185–92. doi: 10.1253/circj.72.1185
30. Newton JC, Smith WM, Ideker RE. Estimated global transmural distribution of activation rate and conduction block during porcine and canine ventricular fibrillation. *Circ Res*. (2004) 94:836–42. doi: 10.1161/01.RES.0000120860.01645.17
31. Tabereaux PB, Walcott GP, Rogers JM, Kim J, Dosdall DJ, Robertson PG, et al. Activation patterns of Purkinje fibers during long-duration ventricular

fibrillation in an isolated canine heart model. *Circulation* (2007) 116:1113–9. doi: 10.1161/CIRCULATIONAHA.107.699264
32. Worley SJ, Swain JL, Colavita PG, Smith WM, Ideker RE. Development of an endocardial-epicardial gradient of activation rate during electrically induced, sustained ventricular fibrillation in dogs. *Am J Cardiol*. (1985) 55:813–20. doi: 10.1016/0002-9149(85)90162-6
33. Damiano RJ, Jr, Smith PK, Tripp HF, Jr, Asano T, Small KW, Lowe JE, et al. The effect of chemical ablation of the endocardium on ventricular fibrillation threshold. *Circulation* (1986) 74:645–52. doi: 10.1161/01.CIR.74.3.645
34. Dosdall DJ, Tabereaux PB, Kim JJ, Walcott GP, Rogers JM, Killingsworth CR, et al. Chemical ablation of the Purkinje system causes early termination and activation rate slowing of long-duration ventricular fibrillation in dogs. *Am J Physiol Heart Circ Physiol*. (2008) 295:H883–9. doi: 10.1152/ajpheart.00466.2008
35. Alders M, Koopmann TT, Christiaans I, Postema PG, Beekman L, Tanck MWT, et al. Haplotype-sharing analysis implicates chromosome 7q36 harboring DPP6 in Familial Idiopathic ventricular fibrillation. *Am J Hum Genet*. (2009) 84:468–76. doi: 10.1016/j.ajhg.2009.02.009
36. Radicke S, Cotella D, Graf EM, Ravens U, Wettwer E. Expression and function of dipeptidyl-aminopeptidase-like protein 6 as a putative beta-subunit of human cardiac transient outward current encoded by Kv4.3. *J Physiol*. (2005) 565(Pt 3):751–6. doi: 10.1113/jphysiol.2005.087312
37. Ten Sande JN, Postema PG, Boekholdt SM, Tan HL, van der Heijden JF, de Groot NM, et al. Detailed characterization of familial idiopathic ventricular fibrillation linked to the DPP6 locus. *Heart Rhythm* (2016) 13:905–12. doi: 10.1016/j.hrthm.2015.12.006
38. Xiao L, Koopmann TT, Ordog B, Postema PG, Verkerk AO, Iyer V, et al. Unique cardiac Purkinje fiber transient outward current beta-subunit composition: a potential molecular link to idiopathic ventricular fibrillation. *Circ Res*. (2013) 112:1310–22. doi: 10.1161/CIRCRESAHA.112.300227
39. Marsman RF, Barc J, Beekman L, Alders M, Dooijes D, van den Wijngaard A, et al. A mutation in CALM1 encoding calmodulin in familial idiopathic ventricular fibrillation in childhood and adolescence. *J Am Coll Cardiol*. (2014) 63:259–66. doi: 10.1016/j.jacc.2013.07.091
40. Cheung JW, Meli AC, Xie W, Mittal S, Reiken S, Wronska A, et al. Short-coupled polymorphic ventricular tachycardia at rest linked to a novel ryanodine receptor (RyR2) mutation: Leaky RyR2 channels under non-stress conditions. *Int J Cardiol*. (2015) 180:228–36. doi: 10.1016/j.ijcard.2014.11.119
41. Koizumi A, Sasano T, Kimura W, Miyamoto Y, Aiba T, Ishikawa T, et al. Genetic defects in a His-Purkinje system transcription factor, IRX3, cause lethal cardiac arrhythmias. *Eur Heart J*. (2016) 37:1469–75. doi: 10.1093/eurheartj/ehv449
42. Bagnall RD, Das KJ, Duflou J, Semsarian C. Exome analysis-based molecular autopsy in cases of sudden unexplained death in the young. *Heart Rhythm* (2014) 11:655–62. doi: 10.1016/j.hrthm.2014.01.017
43. Bagnall RD, Ingles J, Yeates L, Berkovic SF, Semsarian C. Exome sequencing-based molecular autopsy of formalin-fixed paraffin-embedded tissue after sudden death. *Genet Med*. (2017) 19:1127–33. doi: 10.1038/gim.2017.15
44. Shanks GW, Tester DJ, Ackerman JP, Simpson MA, Behr ER, White SM, et al. Importance of variant interpretation in whole-exome molecular autopsy: population-based case series. *Circulation* (2018) 137:2705–15. doi: 10.1161/CIRCULATIONAHA.117.031053
45. Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, et al. A Prospective study of sudden cardiac death among children and young adults. *N Engl J Med*. (2016) 374:2441–52. doi: 10.1056/NEJMoa1510687
46. Suktitipat B, Sathirareuangchai S, Roothumnong E, Thongnoppakhun W, Wangkiratikant P, Vorasan N, et al. Molecular investigation by whole exome sequencing revealed a high proportion of pathogenic variants among Thai victims of sudden unexpected death syndrome. *PLoS ONE* (2017) 12:e0180056. doi: 10.1371/journal.pone.0180056
47. Narula N, Tester DJ, Paulmichl A, Maleszewski JJ, Ackerman MJ. Post-mortem Whole exome sequencing with gene-specific analysis for autopsy-negative sudden unexplained death in the young: a case series. *Pediatr Cardiol*. (2015) 36:768–78. doi: 10.1007/s00246-014-1082-4
48. Anderson JH, Tester DJ, Will ML, Ackerman MJ. Whole-exome molecular autopsy after exertion-related sudden unexplained death in the young. *Circ Cardiovasc Genet*. (2016) 9:259–65. doi: 10.1161/CIRCGENETICS.115.001370
49. Lahrouchi N, Raju H, Lodder EM, Papatheodorou E, Ware JS, Papadakis M, et al. Utility of post-mortem genetic testing in cases of sudden arrhythmic death syndrome. J Am Coll *Cardiol*. (2017) 69:2134–45. doi: 10.1016/j.jacc.2017.02.046
50. Ashida K, Kaji Y, Sasaki Y. Abolition of Torsade de Pointes after radiofrequency catheter ablation at right ventricular outflow tract. *Int J Cardiol*. (1997) 59:171–5. doi: 10.1016/S0167-5273(97)02938-0
51. Kusano KF, Yamamoto M, Emori T, Morita H, Ohe T. Successful catheter ablation in a patient with polymorphic ventricular tachycardia. *J Cardiovasc Electrophysiol*. (2000) 11:682–5. doi: 10.1111/j.1540-8167.2000.tb00031.x
52. Takatsuki S, Mitamura H, Ogawa S. Catheter ablation of a monofocal premature ventricular complex triggering idiopathic ventricular fibrillation. *Heart* (2001) 86:e3. doi: 10.1136/heart.86.1.e3
53. Saliba W, Abul Karim A, Tchou P, Natale A. Ventricular fibrillation: ablation of a trigger? *J Cardiovasc Electrophysiol*. (2002) 13:1296–9. doi: 10.1046/j.1540-8167.2002.01296.x
54. Betts TR, Yue A, Roberts PR, Morgan JM. Radiofrequency ablation of idiopathic ventricular fibrillation guided by noncontact mapping. *J Cardiovasc Electrophysiol*. (2004) 15:957–9. doi: 10.1046/j.1540-8167.2004.03655.x
55. Pasquie JL, Sanders P, Hocini M, Hsu LF, Scavee C, Jais P, et al. Fever as a precipitant of idiopathic ventricular fibrillation in patients with normal hearts. *J Cardiovasc Electrophysiol*. (2004) 15:1271–6. doi: 10.1046/j.1540-8167.2004.04388.x
56. Kohsaka S, Razavi M, Massumi A. Idiopathic ventricular fibrillation successfully terminated by radiofrequency ablation of the distal Purkinje fibers. *Pacing Clin Electrophysiol*. (2007) 30:701–4. doi: 10.1111/j.1540-8159.2007.00731.x
57. Naik N, Juneja R, Sharma G, Yadav R, Anandraja S. Malignant idiopathic ventricular fibrillation "cured" by radiofrequency ablation. *J Interv Card Electrophysiol*. (2008) 23:143–8. doi: 10.1007/s10840-008-9284-4
58. Cho YR, Park JS. Radiofrequency catheter ablation for unifocal premature ventricular complexes triggering recurrent ventricular fibrillations in a young man without structural heart disease. *Korean Circ J*. (2012) 42:575–9. doi: 10.4070/kcj.2012.42.8.575
59. Szumowski L, Walczak F, Przybylski A, Maryniak A, Szufladowicz E, Derejko P, et al. [Ablation of a catecholaminergic polymorphic VT and VF originating from Purkinje fibers–a case report]. *Kardiol Pol*. (2007) 65:319–26.
60. Saba MM, Salim M, Hood RE, Dickfeld TM, Shorofsky SR. Idiopathic ventricular fibrillation in a 10-year-old boy: technical aspects of radiofrequency ablation and utility of antiarrhythmic therapy. *Pacing Clin Electrophysiol*. (2011) 34:e85–9. doi: 10.1111/j.1540-8159.2010.02796.x
61. Nagase S, Banba K, Nishii N, Morita H, Fukushima Kusano K, Ohe T, et al. Multiform premature ventricular contractions and polymorphic ventricular tachycardia caused by Purkinje activity with slow conduction in idiopathic ventricular fibrillation. *Intern Med*. (2014) 53:725–8. doi: 10.2169/internalmedicine.53.1147
62. Kleissner M, Peichl P, Kautzner J. Idiopathic ventricular fibrillation triggered by two distinct foci. *Europace* (2014)16:1459. doi: 10.1093/europace/euu034
63. Rosu R, Muresan L, Cismaru G, Puiu M, Andronache M, Gusetu G, et al. Catheter ablation of idiopathic ventricular fibrillation using the CARTO 3 mapping system. *Egypt Heart J*. (2015) 67:349–52. doi: 10.1016/j.ehj.2015.03.001
64. Chan KH, Sy RW. Catheter ablation of recurrent ventricular fibrillation: a literature review and case examples. *Heart Lung Circ*. (2016) 25:784–90. doi: 10.1016/j.hlc.2016.02.008
65. Ho RT, Frisch DR, Greenspon AJ. Idiopathic ventricular fibrillation ablation facilitated by PENTARAY mapping of the moderator band. *JACC Clin Electrophysiol*. (2017) 3:313–4. doi: 10.1016/j.jacep.2016.08.006
66. Martin CA, Nunn L, Lambiase PD. Syncope in a young man: role of Purkinje fibres in idiopathic ventricular fibrillation. *Indian Pacing Electrophysiol J*. (2017) 17:113–5. doi: 10.1016/j.ipej.2017.05.006
67. Sadek MM, Benhayon D, Sureddi R, Chik W, Santangeli P, Supple GE, et al. Idiopathic ventricular arrhythmias originating from the moderator band: electrocardiographic characteristics and treatment by catheter ablation. *Heart Rhythm* (2015) 12:67–75. doi: 10.1016/j.hrthm.2014.08.029
68. Saleem MA, Burkett S, Passman R, Dibs S, Engelstein ED, Kadish AH, et al. New simplified technique for 3D mapping and ablation of right ventricular

outflow tract tachycardia. *Pacing Clin Electrophysiol*. (2005) 28:397–403. doi: 10.1111/j.1540-8159.2005.09547.x

69. Intini A, Goldstein RN, Jia P, Ramanathan C, Ryu K, Giannattasio B, et al. Electrocardiographic imaging (ECGI), a novel diagnostic modality used for mapping of focal left ventricular tachycardia in a young athlete. *Heart Rhythm* (2005) 2:1250–2. doi: 10.1016/j.hrthm.2005.08.019
70. Erkapic D, Greiss H, Pajitnev D, Zaltsberg S, Deubner N, Berkowitsch A, et al. Clinical impact of a novel three-dimensional electrocardiographic imaging for non-invasive mapping of ventricular arrhythmias-a prospective randomized trial. *Europace* (2015) 17:591–7. doi: 10.1093/europace/euu282
71. Jamil-Copley S, Bokan R, Kojodjojo P, Qureshi N, Koa-Wing M, Hayat S, et al. Noninvasive electrocardiographic mapping to guide ablation of outflow tract ventricular arrhythmias. *Heart Rhythm* (2014) 11:587–94. doi: 10.1016/j.hrthm.2014.01.013
72. Anter E, Tschabrunn CM, Josephson ME. High-resolution mapping of scar-related atrial arrhythmias using smaller electrodes with closer interelectrode spacing. *Circ Arrhythmia Electrophysiol*. (2015) 8:537–45. doi: 10.1161/CIRCEP.114.002737
73. Hsia HH, Callans DJ, Marchlinski FE. Characterization of endocardial electrophysiological substrate in patients with nonischemic cardiomyopathy and monomorphic ventricular tachycardia. *Circulation* (2003) 108:704–10. doi: 10.1161/01.CIR.0000083725.72693.EA
74. Cano O, Hutchinson M, Lin D, Garcia F, Zado E, Bala R, et al. Electroanatomic substrate and ablation outcome for suspected epicardial ventricular tachycardia in left ventricular nonischemic cardiomyopathy. *J Am Coll Cardiol*. (2009) 54:799–808. doi: 10.1016/j.jacc.2009.05.032

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REVIEW
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Disease Modifiers of Inherited *SCN5A* Channelopathy

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To date, a large number of mutations in *SCN5A*, the gene encoding the pore-forming α-subunit of the primary cardiac Na^+ channel ($Na_V1.5$), have been found in patients presenting with a wide range of ECG abnormalities and cardiac syndromes. Although these mutations all affect the same $Na_V1.5$ channel, the associated cardiac syndromes each display distinct phenotypical and biophysical characteristics. Variable disease expressivity has also been reported, where one particular mutation in *SCN5A* may lead to either one particular symptom, a range of various clinical signs, or no symptoms at all, even within one single family. Additionally, disease severity may vary considerably between patients carrying the same mutation. The exact reasons are unknown, but evidence is increasing that various cardiac and non-cardiac conditions can influence the expressivity and severity of inherited *SCN5A* channelopathies. In this review, we provide a summary of identified disease entities caused by *SCN5A* mutations, and give an overview of co-morbidities and other (non)-genetic factors which may modify *SCN5A* channelopathies. A comprehensive knowledge of these modulatory factors is not only essential for a complete understanding of the diverse clinical phenotypes associated with *SCN5A* mutations, but also for successful development of effective risk stratification and (alternative) treatment paradigms.

Keywords: $Na_V1.5$, LQT3, Brugada syndrome, conduction, co-morbidities

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INTRODUCTION

To date, an increasing number of mutations in *SCN5A*, the gene encoding the pore-forming α-subunit of the primary cardiac Na^+ channel ($Na_V1.5$), is found in patients with a wide range of electrocardiogram (ECG) abnormalities and cardiac syndromes (1–3). Although they are all due to mutations in the same ion channel, these syndromes show a myriad of phenotypes (4). While this may be partly explained by mutation-specific biophysical changes in the current generated by $Na_V1.5$ channels (here named Na^+ current, I_{Na}), it has now become clear that a single mutation in *SCN5A* may also result in a large number of disease phenotypes within one and the same family [for review, see (2)]. Also, disease severity often varies significantly among affected individuals, with some *SCN5A* mutation-positive patients suffering from life-threatening arrhythmias at young age while others do not display any clinical signs (i.e., reduced and incomplete penetrance).

At this moment, clinical management of *SCN5A* mutation-positive patients is hindered by this reduced penetrance as well as by the considerable variation in disease severity and risk of sudden cardiac death (SCD) observed in affected individuals. Cardiac and non-cardiac modulatory factors and co-morbidities are supposed to modify disease severity and expressivity, however, till now they are largely unexplored. A major reason for the lack of detailed information on such

disease modifiers in *SCN5A*-mutation related disorders is the large genetic heterogeneity between individual patients. In addition, different mutations result in different biophysical alterations and thus give rise to further variability between individuals. In this review, we provide an updated summary of presently identified cardiac disease entities secondary to *SCN5A* mutations, and give an overview of a broad spectrum of concomitant disorders and conditions which may modify disease severity and expressivity of *SCN5A* channelopathies.

CARDIAC DISORDERS ASSOCIATED WITH *SCN5A* MUTATIONS

$Na_V1.5$ channels are widely distributed in the mammalian heart, but the number of channels (5–7) and their electrophysiological function (6, 8–10) may differ between various parts of the heart. Consequently, *SCN5A* mutations can lead to multiple cardiac disease phenotypes, and even considerable overlap may exist, named "overlap syndrome," between these cardiac clinical entities (2). Aside from the heart, $Na_V1.5$ channels are also expressed in other tissues throughout the body, and *SCN5A* mutations therefore are also associated with extracardiac phenotypes, including gastrointestinal dysfunction (11) and epilepsy (12). Below, we first provide a brief overview of the $Na_V1.5$ channel and I_{Na} properties and subsequently introduce briefly the various *SCN5A*-related cardiac disorders in relation to the associated biophysical $Na_V1.5$ channel defects.

$Na_V1.5$ Structure and Function

As reviewed in detail elsewhere (13), the $Na_V1.5$ protein is formed by four homologous domains (D1–DIV) each composed of six transmembrane spanning helices (S1–S6) (**Figure 1**). $Na_V1.5$-based channels are voltage dependent and open upon depolarization, resulting in a rapid activation of I_{Na}. In working myocytes, this I_{Na} is large and generates the fast action potential (AP) depolarization (6, 14). Typically, $Na_V1.5$ channels also close rapidly due to inactivation. This fast inactivation, together with the reduction in driving force of Na^+ ions occurring during the AP upstroke, results in a rapid decrease of I_{Na} (**Figure 1B**). Although most $Na_V1.5$ channels show fast inactivation, some channels may inactivate slower and/or incompletely. Consequently, a small persistent or late I_{Na} current is generated (**Figure 1C**), which may affect AP repolarization (15). Moreover, a small overlap exists between the voltage dependence of activation and inactivation. Therefore, $Na_V1.5$ channels can activate but are not inactivated completely, resulting in a small I_{Na} at this range of membrane potentials, named the "window current" (**Figure 1D**). Such a window current also contributes to the AP repolarization phase. In addition, late and/or window I_{Na} may also affect pacemaker activity of sinoatrial nodal (SAN) cells (8, 10) and excitability (16). Upon return to hyperpolarized potentials, i.e., during or following the AP repolarization, $Na_V1.5$ channels can quickly recover from inactivation (14). The speed of recovery from inactivation regulates $Na_V1.5$ channel availability for subsequent APs, and is therefore responsible for the refractory period (17).

SCN5A-Related Disorders

Brugada syndrome (BrS) is characterized on the ECG by ST-segment elevation in the right-precordial leads V1 to V3. BrS is associated with ventricular arrhythmias and SCD, which occur particularly during rest and sleep in apparently healthy and young (age <40 years) individuals (18). The characteristic ST-segment elevation of the ECG is often variably present, and can be unmasked by I_{Na} blockade or exercise [see (18)]. *SCN5A* mutations linked to BrS are so called "loss-of-function" mutations, which typically result in a decreased I_{Na} (1). This reduction in I_{Na} may be due to decreased trafficking and membrane channel expression and/or altered gating properties of the channel resulting in disruption of voltage dependency of (in)activation, accelerated speed of inactivation, or slowed recovery from inactivation.

Long QT syndrome (LQTS) is characterized by a QT-interval prolongation on the ECG accompanied by an enhanced risk for SCD as a result of ventricular tachyarrhythmias. LQTS type 3 (LQT3), the subtype caused by *SCN5A* mutations, is associated with bradycardia and arrhythmias and/or SCD occurring mostly at slow heart rates such as during rest or sleep (19). *SCN5A* mutations underlying LQT3 are typically "gain-of-function" mutations inducing various biophysical alterations (such as slower I_{Na} inactivation, larger late I_{Na}, larger window I_{Na}, and/or increased I_{Na} density (1), all leading to an enhanced I_{Na} function during the AP repolarization phase and consequent AP prolongation.

Atrial fibrillation (AF), a rapid and irregular beating of the atria, is mostly found in elderly patients with structural alterations in the heart. Evidence is increasing that AF in young patients with structurally normal hearts may also be hereditary. In familial forms of AF, both *SCN5A* loss-of-function and gain-of-function mutations have been identified (20). The gain-of-function can be due to various gating changes including negative shifts in voltage dependence of activation, positive shifts in voltage dependence of inactivation, slower current inactivation, and faster recovery from inactivation [see (16), and primary references cited therein]. Loss-of-function can be the consequence of reduced I_{Na} density (21) or of a negative shift in voltage dependence of inactivation (22).

Sick sinus syndrome (SSS) is described as the "intrinsic inadequacy of the SAN to perform its pacemaking function due to a disorder of automaticity and/or inability to transmit its impulse to the rest of the atrium" [see (23)]. A number of *SCN5A* mutations have been associated with inherited SSS, and interestingly these can be both loss-of-function and gain-of-function mutations. Consequently, the occurrence of SSS has a considerable overlap with BrS (24) and LQT3 (25, 26). Loss-of-function, i.e., a reduction of I_{Na} availability, decreases the speed of the diastolic depolarization phase of SAN cells and thereby pacemaker activity (25). The overlap of SSS and gain-of-function mutations associated with LQT3 is more complex. Although an increase in late I_{Na} results in faster pacemaker activity (25), the concurrent changes in I_{Na} density and the shifts in voltage dependency of activation and inactivation counteract the enhanced late I_{Na}, resulting in a slower pacemaker activity (25).

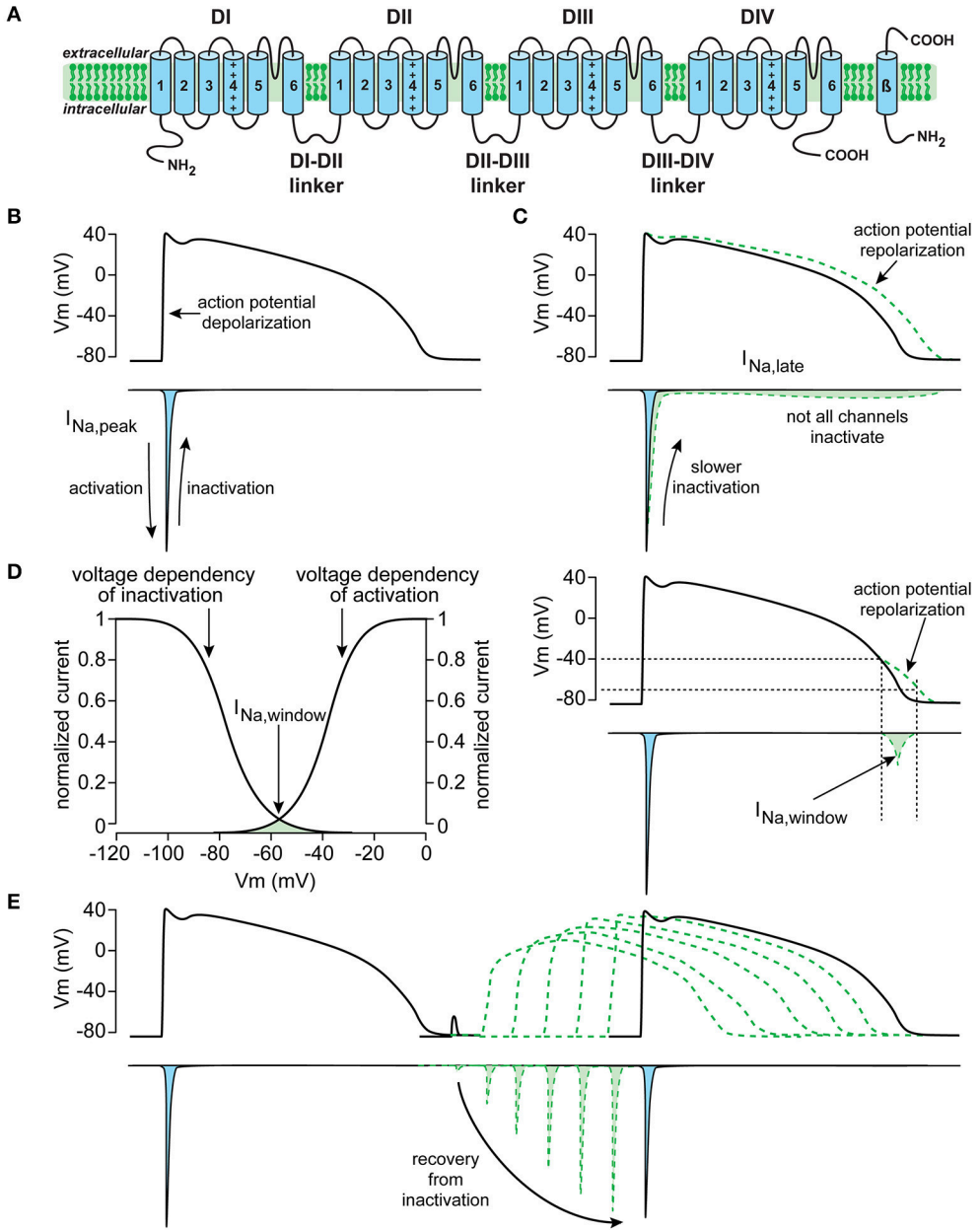

FIGURE 1 | Schematic drawings of the cardiac sodium channel $Na_V1.5$ encoded by the *SCN5A* gene **(A)**, and important biophysiological properties and function of the current generated by *SCN5A* (here named I_{Na}), including peak I_{Na} **(B)**, late I_{Na} **(C)**, window I_{Na} **(D)**, and recovery from inactivation **(E)**.

Progressive cardiac conduction defect (PCCD) is characterized by progressive conduction slowing through the His-Purkinje system. PCCD is associated with PQ- and QRS-interval prolongation, complete atrio-ventricular (AV) and right and/or left bundle branch block, syncope and SCD. PCCD is often observed in BrS patients, and similar to BrS, is due to loss-of-function mutations (18).

Multifocal ectopic Purkinje-related premature contraction (MEPPC) is characterized by frequent premature ventricular contractions originating from the Purkinje system, especially at rest (16). The *SCN5A* mutations underlying MEPPC are typically gain-of-function mutations due to an increased window I_{Na}, faster recovery from inactivation and/or increased channel availability of $Na_V1.5$ (see (16), and primary references cited therein).

Sudden infant death syndrome (SIDS) is characterized by the sudden unexplained death of a seemingly healthy infant younger than 1 year. SIDS is a disease with multiple pathophysiological mechanisms (27), and cardiac ion channel gene mutations appear to be involved in approximately 20% of the cases of SIDS, from which more than half of the mutations are related to I_{Na} [for review, see (28)]. These may include mutations in *SCN5A*, but

also in the I_{Na}-modulatory β-subunits (*SCN3B* and *SCN4B*) and other "regulatory genes" (*CAV3*, *SNTA1*, and *GPD1-L*), which could result in either I_{Na} loss-of function or gain-of-function mutations [see (28, 29), and primary references cited therein].

Dilated cardiomyopathy (DCM) is a structural heart disease characterized by dilated chambers, pump failure, and arrhythmia. DCM is a multifactorial disorder with several proposed pathophysiological mechanisms (30), including *SCN5A* mutations (31). Both loss-of-function and gain-of-function are associated with DCM, but the pathophysiological mechanisms of DCM secondary to *SCN5A* mutations are not exactly known (2, 32). As reviewed by Wilde and co-workers, DCM may be: (i) secondary to *SCN5A* mutation induced arrhythmias and/or bradycardia; (ii) due to increased late I_{Na} and consequent changes in intracellular Na^+ and Ca^{2+}; or (iii) secondary to the non-electrical role of $Na_V1.5$ as a potential anchoring protein for structural and cytoskeletal proteins (33).

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy characterized by fibrofatty replacement of the right ventricle, ventricular arrhythmias, and SCD (34). Up to 60–70% of the ARVC index cases carry a causal desmosomal [such as plakophilin-2 (*PKP2*) or desmoglein-2 (*DSG2*)] gene mutation, but various non-desmosomal genes may also be involved (35, 36), including *SCN5A* (37). Although the percentage of pathogenic *SCN5A* mutations in ARVC is very low, *PKP2* knockdown and overexpression of *Dsg2* mutations both result in a decrease in I_{Na} (38, 39), and such a decrease in I_{Na} is proposed to be a critical factor in arrhythmogenesis in ARVC (40).

VARIABLE EXPRESSIVITY IN *SCN5A* CHANNELOPATHY

Patients harboring *SCN5A* mutations demonstrate a significant variability in disease expression (41). Obviously, such variability in *SCN5A*-releated diseases can be due to different severities of the I_{Na} biophysical defect, with truncating *SCN5A* loss-of-function mutations resulting in more pronounced conduction slowing than missense *SCN5A* mutations (42). The range of biophysical alterations induced by a particular genetic defect in *SCN5A* (1) may also determine the capability of that mutation to cause cardiac rhythm disorders. Importantly, the variability in *SCN5A*-releated disease severity and expressivity is also present in family members carrying the same mutation, as exemplified in a large Dutch family with the *SCN5A*-1795insD "overlap syndrome" mutation (43). Some mutation carriers in this family display predominantly loss-of-function phenotypes with BrS and/or conduction disease, while other family members carrying this mutation show mainly a gain-of-function phenotype resulting in QT-prolongation (44). In addition, and apart from family members with a clear phenotype, other family members carrying the same *SCN5A*-1795insD mutation appear unaffected (43). Thus, independent of the mutation-specific effects, individual-specific factors also appear to contribute importantly to the regulation of disease expressivity and severity in *SCN5A* channelopathy. Moreover, the variability in $Na_V1.5$ disease expression and severity is not only related to I_{Na} defects, but likely also closely related to other cardiac ion channels which contribute to the cardiac AP. Apart from I_{Na}, the AP morphology is the consequence of a fine balance between the inwardly directed L-type Ca^{2+} current ($I_{Ca,L}$; Ca_V), and various outwardly directed K^+ currents (K_V) including the transient outward K^+ current (I_{to}), the inward rectifier K^+ current (I_{K1}) and the slow and rapid delayed rectifier K^+ currents (I_{Ks} and I_{Kr}, respectively) (**Figure 2A**). Changes in these Ca_V and various K_V currents may affect the expressivity of *SCN5A* channelopathies. For example, a decrease in $I_{Ca,L}$ and/or increase I_{to} (**Figure 2B**, in red) may increase phase-1 repolarization and lower the AP plateau phase which may promote ST-segment elevation and BrS (45), while an increase in $I_{Ca,L}$ and/or decrease of K_V currents (**Figure 2C**, in red) will result in longer APs thus promoting LQT3 (46).

Below, we provide an overview of various known genetic and non-genetic disease modifiers of inherited cardiac *SCN5A* channelopathies, which are summarized in **Figure 3**.

Genetic Modifiers of *SCN5A* Channelopathy

Genetic background and modifiers are considered important determinants of disease expressivity and/or severity in *SCN5A* channelopathies, especially among patients carrying the same mutation (47–49). This has been clearly demonstrated in experimental studies where the impact on genetic variability on disease severity was evaluated in two distinct strains (129P2 and FVBN/J background) of mice carrying the *Scn5a*-1798insD/+ mutation, the equivalent to *SCN5A*-1795insD in humans. A more severe phenotype was present in 129P2 mice as compared to FVBN/J mice (50, 51). In addition, subsequently identified potential modifiers of conduction disease severity were found. Comparison of cardiac gene expression between the 129P2 mice and FVBN/J mice demonstrated that *Scn4b* (encoding a ß-subunit of sodium channels) is an important modifier of conduction disease severity (52). Furthermore, by performing a system genetics approach on F2 progeny arising from these two mouse strains, we showed that *Tnni3k* (encoding troponin 1 interacting kinase) is another modulator of AV conduction (53). These genetic studies clearly underline the relevance of genetic background and genetic modifiers in sodium channelopathy.

Single nucleotide polymorphisms, frequently observed in the general population, may further determine disease expressivity and/or severity. For example, H558R is the most commonly found *SCN5A* polymorphism (with a 9–36% prevalence), and its distribution varies between different ethnic populations (54). Co-existence of this polymorphism and *SCN5A* mutations may affect the functional consequences of the latter, including plasma-membrane targeting of $Na_V1.5$, I_{Na} density and/or I_{Na} gating properties (55–60). Moreover, a combination of specific polymorphisms [haplotype (HapB)] within the *SCN5A* promoter region may affect conduction in BrS patients (61). HapB is frequently present in Asians, and may therefore partly explain the high prevalence of BrS in individual with an Asian background. In addition, polymorphisms in non-*SCN5A* genes may also

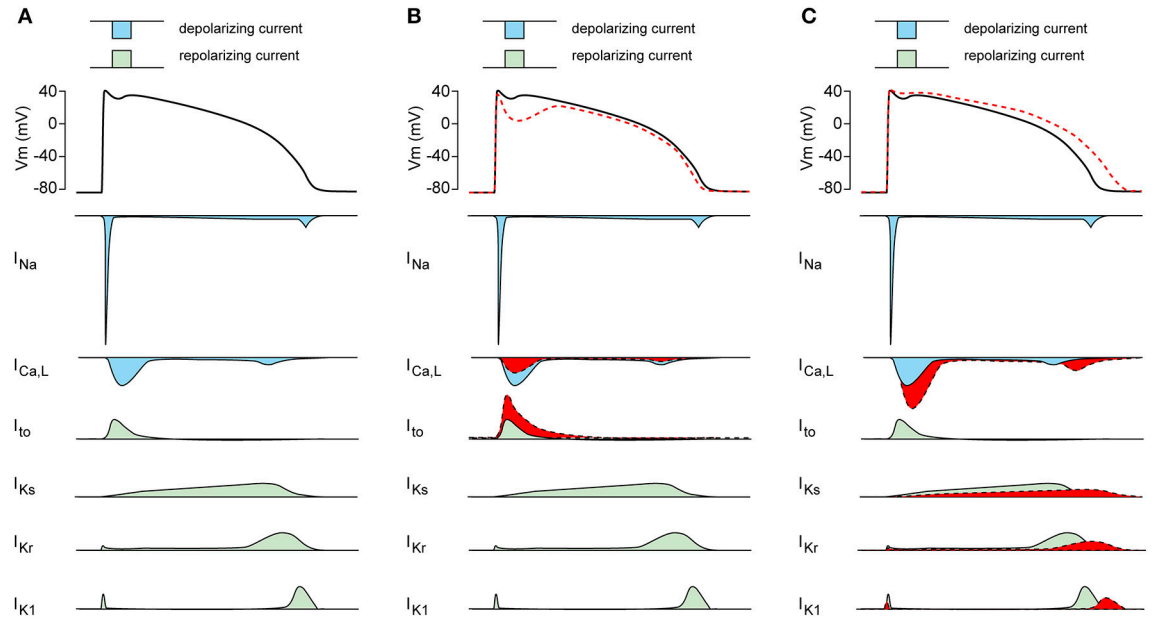

FIGURE 2 | (A) Schematic drawing of the cardiac action potential (AP) and its underlying membrane currents. I_{Na}, Na^+ current; $I_{Ca,L}$, L-type Ca^{2+} current; I_{to}, transient outward K^+ current; I_{Ks}, slow component of the delayed rectifier K^+ current; I_{Kr}, rapid component of the delayed rectifier K^+ current; I_{K1}, inward rectifier K^+ current. **(B)** Schematic drawing of AP plateau suppressing ion channels changes (in red). **(C)** Schematic drawing of AP prolonging ion channels changes (in red).

contribute to disease expressivity in sodium channelopathy. For example, Groenewegen et al. (62) demonstrated that phenotype severity of *SCN5A*-D1275N mutation carriers was importantly modulated by 2 closely linked polymorphisms forming a haplotype within the promotor region of the *GJA5*, the gene underlying the atrial-specific connexin-40 gap junction protein. *SCN5A*-D1275N mutation carriers homozygous for the *GJA5* promoter polymorphisms exhibited atrial standstill, while carriers without or with only a heterozygous *GJA5* promoter polymorphism displayed only a mild PR-interval prolongation (62).

Additionally, genetic variation due to the presence and relative expression of two important *SCN5A* alternatively spliced variants, i.e., *SCN5A*-Q1077del and *SCN5A*-Q1077 (63), may further modulate sodium channelopathy severity. The BrS phenotype severity associated with the *SCN5A*-G1406R mutation was enhanced in combination with the Q1077 variant (64). Q1077del has furthermore been shown to modulate I_{Na} density, gating properties, and recovery from inactivation of *SCN5A* mutations associated with DCM (65).

Non-genetic Modifiers of *SCN5A* Channelopathy

Gender

Gender is a clear modifier of disease severity in *SCN5A* channelopathy, exemplified by the preponderance of BrS in males (66), and LQT3 in females especially in the 30–40 year age range (67). In addition, within one family with the G1406R loss-of-function mutation, females were found to have mostly cardiac conduction defects whereas males showed predominantly a BrS phenotype (47). Gender, and particularly sex hormones, has a significant impact on ion channels responsible for repolarization, and is associated with a larger $I_{Ca,L}$ and smaller I_{to} and consequently higher QTc values in females [see (68), and primary references cited therein]. This lower repolarization reserve intrinsic to female hearts is thought to augment the detrimental impact of a mutation-induced late I_{Na}. Barajas-Martinez and colleagues reported a higher I_{Na} magnitude in male epi- and endocardial myocytes compared to female (69). In addition, they found in females a larger ventricular transmural dispersion of I_{Na} density. They suggested that in the setting of decreased I_{Na}, epicardial myocytes display more easily all-or-none repolarization leading to BrS in males (with a smaller $I_{Ca,L}$ and larger I_{to}), while females with a smaller I_{Na} are more sensitive to loss of conduction velocity.

Age

Age is another determinant of severity and expressivity of *SCN5A* channelopathies (70–72). For example, carriers of the *SCN5A*-1795insD mutation show QT-interval prolongation and conduction disorders from birth, while features of BrS mostly develop later in life (72). While peak I_{Na} density and I_{Na} availability (i.e., AP upstroke velocity) does not appear to change with age (73, 74), aging may result in an acceleration of I_{Na} inactivation and an enhanced use-dependent decrease in I_{Na} (73). In addition, aging myocytes also show AP prolongation secondary to both an increase in late I_{Na} and a reduction in K_V currents (74). These ion channel changes, together with a prolonged AP (74) (hence, a shorter time for recovery from inactivation) may promote BrS, conduction delay, and LQT3.

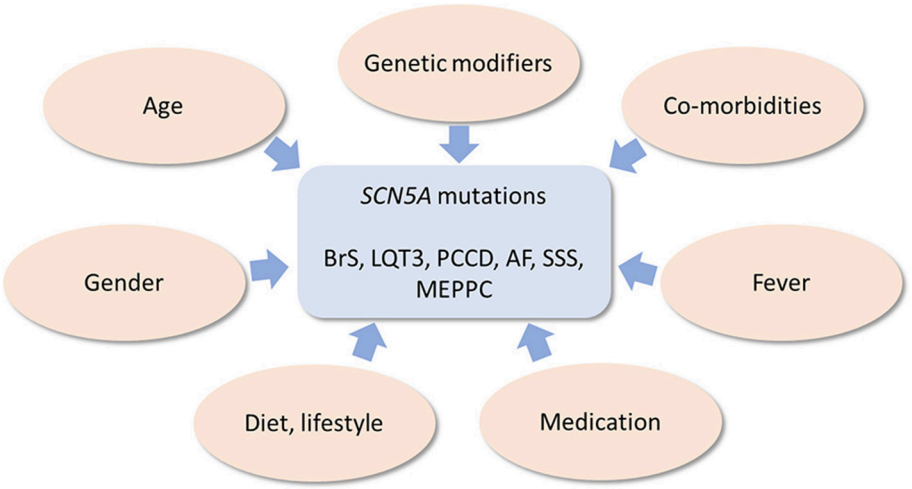

FIGURE 3 | Schematic drawing of genetic and non-genetic disease modifiers of inherited cardiac *SCN5A* channelopathies.

Furthermore, fibrosis due to aging is thought to play another major role in modulating conduction and repolarization disorder severity (75–77).

Medication

It is well known that many clinically used antiarrhythmic, psychotropic, and anesthetics drugs may induce type-1 ECG and/or arrhythmias in BrS patients (78, 79). These drugs with potential adverse effects for BrS patients (for overview, see the website www.brugadadrugs.org) are known to block I_{Na} and/or Ca_V currents significantly, thereby increasing the susceptibility for BrS. In addition, many clinically used drugs are also known to result in QT-interval prolongation (71, 80). For an overview of QT-interval prolonging drugs, see the website www.QTdrugs.org. These drugs prolong the QT-interval due to blockade of I_{Kr} or I_{Ks}, rather than an increase of late I_{Na}, and increase the arrhythmia risk in patients with inherited LQTS, including LQT3 (81).

Lifestyle

Evidence is increasing that lifestyle can have a significant impact on *SCN5A* channelopathies by either a direct modulation of I_{Na} properties or indirectly via impacting on K_V and Ca_V channels, making the heart more sensitive to (the consequences of) *SCN5A* mutations.

Alcohol

Alcohol consumption has been associated with BrS (82). Alcohol intoxication may have pro-arrhythmic actions through I_{Na} channel inhibition, thereby mimicking the actions of I_{Na} blocking drugs (83, 84). Furthermore, ethanol shortens the AP through multiple effects on Ca_V and K_V channels [see (84), and primary references cited therein]; hence, alcohol could theoretically reduce QT-interval prolongation and arrhythmias in the setting of LQT3. On the other hand, episodic excessive alcohol intake is associated with an increase in QT duration dispersion due to cardiac autonomic imbalance (85), which may in fact promote repolarization abnormalities.

Recreational drug use

Recreational drug use is another well-known factor in BrS, especially cocaine (79). Cocaine has multiple indirect and direct effects on the electrical activity of the heart as demonstrated by increases in PR-, QRS-, and QT-intervals due to inhibition of Ca_V, K_V and Na_V currents (86). The decrease in I_{Na} appears to be caused by slower recovery from inactivation in combination with a shift in voltage dependency of inactivation (86). The cocaine-induced QT-prolongation is importantly due to a blockade of I_{Kr}, and predisposes to the occurrence of EADs and TdP (86).

Tobacco

Tobacco use has many detrimental effects on general health. In addition, nicotine and carbon monoxide (CO), a major component of smoke, also cause changes in cardiac development as well as ion channel remodeling (87, 88). For example, a low plasma concentration of nicotine increased peak I_{Na} and late I_{Na}, with shifts in both inactivation and activation kinetics resulting also in a larger I_{Na} window current (88). In addition, sublethal CO exposure is frequently associated with cardiac arrhythmias, and it has been demonstrated that its effects may be due to $Na_V1.5$ channel modulation, causing an increase in late I_{Na}, but a decrease of peak I_{Na} (89).

Exercise

Exercise, especially swimming, may trigger most types of LQTS (90), but paradoxically appears to lower arrhythmia risk in LQT3 patients (91). On the other hand, exercise may aggravate the ECG defects observed in BrS patients (92). These acute effects of exercise on BrS and LQT3 may be explained by vagal activity and rapid heart rates, resulting in less recovery from inactivation in combination of a lower driving force of Na^+ ions due to intracellular Na^+ accumulation (91–93). Regular low intensity exercise and endurance training can also lead to structural and

electrical remodeling of the heart [for review, see (92)]. A well-known effect of exercise training is a reduction of resting heart rate, partially via a decrease of the hyperpolarization-activated current, I_f (94). Theoretically, such a lower resting heart rate may itself increase the susceptibility to both BrS and LQT3. On the other hand, exercise training does not affect the expression of *SCN5A* mRNA (95), but reduces I_{to} in epicardial myocytes thereby reducing the transmural gradient of I_{to} significantly (96). This could potentially suppress BrS, but may increase LQT3 due to AP prolongation (96).

Diet and dietary supplements

Diet may have both beneficial and detrimental effects on *SCN5A*-related diseases, but underlying mechanisms appear complex. For example, acute application of polyunsaturated fatty acids, in particular those of the n-3 class (PUFAs), inhibits I_{Na} (97) and therefore may facilitate BrS. Yagi et al. (98), however, suggested that n-3 PUFAs may prevent ventricular fibrillation in BrS, likely due to additional blockade of various other cardiac ion channels (68), including I_{to} (99). High cholesterol and fat intake may constitute additional diet-related modulatory factors. Both are associated with a slower recovery from I_{Na} inactivation, but with a more negative voltage dependence of I_{Na} activation, which may lower the threshold for excitation of $Na_V1.5$ channels (100). To date, the clinical impact of high cholesterol and fat intake on LQT3 and BrS patients are as yet unknown. Interestingly, consuming a large meal, resulting in vagal stimulation, may trigger sudden cardiac arrest in BrS (101, 102). In addition, glucose load (alone and in combination with insulin infusion), as well as Thai high glycemic index (HGI) meals are known to affect ST-segment elevation in BrS patients (see (103), and primary references cited therein). The mechanism behind this effect may be related to glucose-induced insulin secretion. In myocytes, insulin results in activation of the Na/K pump (104), and consequently, in an increased outwardly directed current during the AP thereby theoretically promoting repolarization. On the other hand, insulin in myocytes enhanced the depolarizing $I_{Ca,L}$ (105), while it inhibits I_{Kr} (106) and I_{Ks} (107), thereby prolonging the QTc in humans (108) which may favor LQTS. More studies are required to elucidate the exact role of glucose/insulin on BrS and LQT3, and to explain the so-called diabetic death-in-bed syndrome as mentioned by Skinner et al. (109). Furthermore, high salt and glucose intake can result in hypertension and diabetes, respectively. Both diseases have significant impact on ion channel function, and hence likely also modulate disease expressivity and severity in the setting of *SCN5A* mutations (see also below).

These days, dietary supplements, natural drugs, and/or traditional Chinese medicines are increasingly used (110). Some ingredients in these preparations shorten the cardiac AP due to I_{Na} and $I_{Ca,L}$ inhibition [for review, see (110)], thus caution for BrS patients seems appropriate. Other compounds, such as Wenxin Granule [for review, see (111)], may however have a therapeutic effect on BrS. Although Wenxin Granule was shown to reduce I_{Na}, it also suppressed the electrocardiographic and arrhythmic manifestations of BrS due to inhibition of I_{to} (112). It has also been shown to reduce late I_{Na} (113, 114), and therefore may also have an impact in LQT3 patients. Resveratrol, a polyphenol compound that is primarily derived from grapes, also inhibits late I_{Na} as well as $I_{Ca,L}$ (110); hence, LQT3 patients may have some benefit from such natural and readily available supplements. Another example of a traditional Chinese medicine is dimethyl lithospermate B (dmLSB), an extract of Chinese herbal Danshen. dmLSB slows I_{Na} inactivation, thereby potentially eliminating the arrhythmogenic substrate responsible for BrS (115). Other ingredients of natural drugs and/or traditional Chinese medicines are known to prolong the AP due to K_V blockade which may consequently predispose to arrhythmias in LQT3 patients [for review, see (110)]. Finally, apart from direct action om membrane currents, diet and dietary supplements may lead to electrolyte changes, which may have an indirect impact on ion channel function and thereby modify disease expression. For example, higher K^+ levels may shorten the QT-interval in LQT3 patients while hypokalemia is a well-known trigger of QT-interval prolongation and arrhythmias in patients with LQTS (116). Thus, diet and dietary supplements may impact on various *SCN5A*- related conditions, but randomized clinical trials are required to assess their potential beneficial and/or detrimental effects in *SCN5A* channelopathy patients.

Environmental conditions

Environmental conditions should also be considered as potential disease modifiers in *SCN5A* channelopathies. Particulate air pollution, for example, has been associated with increased QTc duration (117), and thus may theoretically increase disease severity in LQT3. In addition, sudden noises are well-known to trigger *SCN5A*-related arrhythmias (1), but evidence is increasing that more chronic, environmental noise pollution also increased incidence of arrhythmias, especially AF (118). The exact mechanism is yet unknown, but noise is a non-specific stressor that activates the autonomous nervous system and endocrine signaling with multiple effects on human health [for review, see (119)].

Fever

Some *SCN5A* mutations may induce BrS-associated symptoms especially during fever episodes, with may be due to changes in I_{Na} channel gating properties in response to increasing temperature (120, 121). We and others have shown that specific *SCN5A* mutations promote slow inactivation of I_{Na} at higher temperatures (i.e., enhanced slow inactivation), thereby causing reduced peak I_{Na} availability (122, 123). To date, specific LQT3-associated *SCN5A* mutations which display enhanced temperature sensitivities have not been described (121). In general, increased temperature does not affect the ratio between late and peak I_{Na} (124), but enhances the transmural repolarization dispersion thus facilitating the occurrence of torsade de pointes (TdP) during LQTS (125). While these observations suggest an increased sensitivity for LQT3 during fever, evidence for this is as yet lacking.

Diabetes

Patients with diabetes are more vulnerable for the development of arrhythmias, independent of other risk factors like hypertension and atherosclerosis (126). QT-interval prolongation is more

often observed in diabetic patients as compared to non-diabetic individuals (127). QT prolongation, due to downregulation of K_V4 channels, is also observed in rat and mouse models of diabetes (126, 128). Interestingly, diabetic mice also show an enhanced late I_{Na} (126). It is therefore plausible that diabetes increases disease severity in LQT3 patients, but evidence for such a modulatory effect is currently lacking. On the other hand, a decrease in $Na_V1.5$ expression and I_{Na} has been reported in rabbit and rat models of diabetes (129, 130), which may have important implications for BrS.

Obesity

Obesity, marked by excessive fat accumulation and weight gain, may result in various chronic disorders such as dyslipidemia, insulin resistance, hypertension, hyperglycemia, and type 2 diabetes (131). Thus, it has multiple similarities with a number of other topics discussed in this review. Therefore, it is not unexpected that obesity can lead to various cardiac electrical disorders including AF, (supra)ventricular arrhythmias (128, 132), and LQTS (133). At this moment, it is not known whether obesity impacts on disease expressivity and/or severity in *SCN5A*-related channelopathies. However, given its QT-prolonging effect through an increase in $I_{Ca,L}$ and a decrease of various K_V channels (132), it is conceivable that obesity may exacerbate LQT3-associated features. Direct effects of obesity on peak and late I_{Na} have only been investigated in limited fashion, with contrasting results (for review, see (132), and primary references cited therein). Nevertheless, since the number of obese individuals is steadily rising, further studies are essential to elucidate potential obesity-related ion channel remodeling and consequences for arrhythmogenesis in the setting of ion channelopathies.

Hypertension

Hypertension may lead to progressive myocardial remodeling, ultimately resulting in the development of cardiac hypertrophy and associated electrical, homeostatic and structural alterations (134). The latter may act synergistically with biophysical alterations secondary to a *SCN5A* mutation resulting in an enhanced pro-arrhythmogenic substrate (135). Due to its progressive nature, the impact of hypertension-induced pro-arrhythmic remodeling is expected to increase with age. Indeed, we have recently demonstrated that co-existing hypertension increased arrhythmia risk and reduced the efficacy of pacemaker treatment in carriers of the *SCN5A*-1795insD mutation above the age of 40 years. Enhanced late I_{Na}, a known consequence of hypertrophy, was shown to be at least partly involved and may constitute a promising therapeutic strategy by additionally preventing intracellular sodium/calcium dysregulation (51, 136, 137). Other studies have shown a similar interaction between hypertension and disease severity and outcome, for example in hypertrophic cardiomyopathy (138). Hence, careful monitoring of hypertension and hypertrophy in addition to aggressive anti-hypertensive treatment should be considered in *SCN5A* mutation carriers.

Coronary Artery Disease

Coronary artery disease may enhance the risk for cardiac events in BrS and LQTS patients. Co-existence of BrS and coronary spasm has been observed in Japanese patients (139–142), but not in European patients (143). The relation to *SCN5A* mutations were not mentioned in these studies, but van Hoorn and colleagues found that the prevalence of coronary artery disease was significantly higher among BrS patients with *SCN5A* mutations than among BrS patients without *SCN5A* mutations (144). Interestingly, Kujime and coworkers reported that coronary artery vasospasm could be a risk factor for cardiac events in patients with BrS (145). Coronary artery disease was reported to augment the risk for LQTS-related cardiac events in LQTS patients over age the age of 40 years (146), but no subdivision into the various types of LQTS was performed. The exact reason for such an augmentation is not known, but may be related to longer QTc intervals in patients with coronary artery disease (147, 148). Alternatively, it may be consequent to alterations in the tissue substrate (e.g., ischemia, scar formation, reduced ejection fraction) which may lower the threshold for afterdepolarizations in LQTS, a critical factor in the initiation of torsade de pointes that is thought to be the arrhythmogenic mechanism in LQTS-related cardiac events [see (146)]. Thus, it appears that coronary artery disease may enhance the risk for cardiac events in both BrS and LQTS patients, but further clinical studies are required to substantiate these observations.

CONCLUSIONS

Genetic modifiers, (common) co-morbidities, environmental influences, and life style factors including diet and exercise may modify disease expressivity and severity, and as such significantly modulate the risk for arrhythmia occurrence and survival in *SCN5A* channelopathy. Importantly, the impact of modulatory factors may differ between distinct mutations, but may also vary with age and gender. Hence, clinical management of patients with *SCN5A* mutations should include careful and continuous assessment of co-existing diseases and other modulatory factors, in addition to rigorous treatment of relevant co-morbidities. Identification of disease modifiers will be an essential step in further research related to *SCN5A* channelopathies and may help to design better risk stratification algorithms and to improve development of novel diagnostic and therapeutic strategies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Tan HL, Bezzina CR, Smits JP, Verkerk AO, Wilde AAM. Genetic control of sodium channel function. *Cardiovasc Res.* (2003) 57:961–73. doi: 10.1016/S0008-6363(02)00714-9
2. Remme CA. Cardiac sodium channelopathy associated with SCN5A mutations: electrophysiological, molecular and genetic aspects. *J Physiol.* (2013) 591:4099–116. doi: 10.1113/jphysiol.2013.256461
3. Wilde AAM, Amin AS. Clinical apectrum of SCN5A mutations: long QT syndrome, Brugada syndrome, and cardiomyopathy. *J Am Coll Cardiol Clin Electrophysiol.* (2018) 4:569–79. doi: 10.1016/j.jacep.2018.03.006
4. Remme CA, Wilde AAM. SCN5A overlap syndromes: no end to disease complexity? *Europace* (2008) 10:1253–5. doi: 10.1093/europace/eun267
5. Remme CA, Verkerk AO, Hoogaars WM, Aanhaanen WT, Scicluna BP, Annink C, et al. The cardiac sodium channel displays differential distribution in the conduction system and transmural heterogeneity in the murine ventricular myocardium. *Basic Res Cardiol.* (2009) 104:511–22. doi: 10.1007/s00395-009-0012-8
6. Weidmann S. The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. *J Physiol.* (1955) 127:213–24. doi: 10.1113/jphysiol.1955.sp005250
7. Zygmunt AC, Eddlestone GT, Thomas GP, Nesterenko VV, Antzelevitch C. Larger late sodium conductance in M cells contributes to electrical heterogeneity in canine ventricle. *Am J Physiol Heart Circ Physiol.* (2001) 281:H689–97. doi: 10.1152/ajpheart.2001.281.2.H689
8. Baruscotti M, DiFrancesco D, Robinson RB. Na^+ current contribution to the diastolic depolarization in newborn rabbit SA node cells. *Am J Physiol Heart Circ Physiol.* (2000) 279:H2303–09. doi: 10.1152/ajpheart.2000.279.5.H2303
9. Kokubun S, Nishimura M, Noma A, Irisawa H. Membrane currents in the rabbit atrioventricular node cell. *Pflugers Arch.* (1982) 393:15–22. doi: 10.1007/BF00582385
10. Lei M, Jones SA, Liu J, Lancaster MK, Fung SS, Dobrzynski H, et al. Requirement of neuronal- and cardiac-type sodium channels for murine sinoatrial node pacemaking. *J Physiol.* (2004) 559:835–48. doi: 10.1113/jphysiol.2004.068643
11. Verstraelen TE, Ter Bekke RM, Volders PG, Masclee AA, Kruimel JW. The role of the SCN5A-encoded channelopathy in irritable bowel syndrome and other gastrointestinal disorders. *Neurogastroenterol Motil.* (2015) 27:906–13. doi: 10.1111/nmo.12569
12. Parisi P, Oliva A, Coll Vidal M, Partemi S, Campuzano O, Iglesias A, et al. Coexistence of epilepsy and Brugada syndrome in a family with SCN5A mutation. *Epilepsy Res.* (2013) 105:415–8. doi: 10.1016/j.eplepsyres.2013.02.024
13. Balser JR. Structure and function of the cardiac sodium channels. *Cardiovasc Res.* (1999) 42:327–38. doi: 10.1016/S0008-6363(99)00031-0
14. Berecki G, Wilders R, de Jonge B, van Ginneken ACG, and Verkerk AO. Re-evaluation of the action potential upstroke velocity as a measure of the Na^+ current in cardiac myocytes at physiological conditions. *PLoS ONE* (2010) 5:e15772. doi: 10.1371/journal.pone.0015772
15. Remme CA, Verkerk AO, Nuyens D, van Ginneken ACG, van Brunschot S, Belterman CN, et al. Overlap syndrome of cardiac sodium channel disease in mice carrying the equivalent mutation of human SCN5A-1795insD. *Circulation* (2006) 114:2584–94. doi: 10.1161/CIRCULATIONAHA.106.653949
16. Lieve KV, Verkerk AO, Podliesna S, van der Werf C, Tanck MW, Hofman N, et al. Gain-of-function mutation in SCN5A causes ventricular arrhythmias and early onset atrial fibrillation. *Int J Cardiol.* (2017) 236:187–93. doi: 10.1016/j.ijcard.2017.01.113
17. Savio-Galimberti E, Gollob MH, Darbar D. Voltage-gated sodium channels: biophysics, pharmacology, and related channelopathies. *Front Pharmacol.* (2012) 11:124. doi: 10.3389/fphar.2012.00124
18. Meregalli PG, Wilde AAM, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? *Cardiovasc Res.* (2005) 67:367–78. doi: 10.1016/j.cardiores.2005.03.005
19. Schwartz PJ. The congenital long QT syndromes from genotype to phenotype: clinical implications. *J Intern Med.* (2006) 259:39–47. doi: 10.1111/j.1365-2796.2005.01583.x
20. Savio-Galimberti E, Darbar D. Atrial fibrillation and SCN5A variants. *Card Electrophysiol Clin.* (2014) 6:741–8. doi: 10.1016/j.ccep.2014.07.006
21. Ziyadeh-Isleem A, Clatot J, Duchatelet S, Gandjbakhch E, Denjoy I, Hidden-Lucet F, et al. A truncated SCN5A mutation combined with genetic variability causes sick sinus syndrome and early atrial fibrillation. *Heart Rhythm.* (2014) 11:1015–23. doi: 10.1016/j.hrthm.2014.02.021
22. Ellinor PT, Nam EG, Shea MA, Milan DJ, Ruskin JN, MacRae CA. Cardiac sodium channel mutation in atrial fibrillation. *Heart. Rhythm.* (2008) 5, 99–105. doi: 10.1016/j.hrthm.2007.09.015
23. Verkerk AO, Wilders, R. Pacemaker activity of the human sinoatrial node: an update on the effects of mutations in HCN4 on the hyperpolarization-activated current. *Int J Mol Sci.* (2015) 16, 3071–3094. doi: 10.3390/ijms16023071
24. Hayashi H, Sumiyoshi M, Nakazato Y, Daida H. Brugada syndrome and sinus node dysfunction. *J Arrhythmia.* (2018) 34, 216–221. doi: 10.1002/joa3.12046
25. Wilders R. Sinus bradycardia in carriers of the SCN5A-1795insD mutation: unraveling the mechanism through computer simulations. *Int J Mol Sci.* (2018) 19:E634. doi: 10.3390/ijms19020634
26. Wilders R, Verkerk AO. Long QT syndrome and sinus bradycardia - a mini review. *Front Cardiovasc Med.* (2018) 5:106. doi: 10.3389/fcvm.2018.00106
27. Opdal SH, Rognum TO. The sudden infant death syndrome gene: does it exist? *Pediatrics* (2004) 114:e506–12. doi: 10.1542/peds.2004-0683
28. Klaver EC, Versluijs GM, Wilders R. Cardiac ion channel mutations in the sudden infant death syndrome. *Int J Cardiol.* (2011) 152:162–70. doi: 10.1016/j.ijcard.2010.12.051
29. Ioakeimidis NS, Papamitsou T, Meditskou S, Zafiroula Iakovidou-Kritsi Z. Sudden infant death syndrome due to long QT syndrome: a brief review of the genetic substrate and prevalence. *J Biol Res(Thessalon)* (2017) 24:6. doi: 10.1186/s40709-017-0063-1
30. Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: the complexity of a diverse genetic architecture. *Nat Rev Cardiol.* (2013) 10:531–47. doi: 10.1038/nrcardio.2013.105
31. McNair WP, Ku L, Taylor MR, Fain PR, Dao D, Wolfel E, et al. SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. *Circulation* (2004) 110:2163–7. doi: 10.1161/01.CIR.0000144458.58660.BB
32. Bezzina CR, Remme CA. Dilated cardiomyopathy due to sodium channel dysfunction: what is the connection? *Circ Arrhythm Electrophysiol.* (2008) 1:80–2. doi: 10.1161/CIRCEP.108.791434
33. Veerman CC, Wilde AAM, Lodder EM. The cardiac sodium channel gene SCN5A and its gene product $Na_V1.5$: role in physiology and pathophysiology. (2015) *Gene* 573, 177–187. doi: 10.1016/j.gene.2015.08.062
34. Basso C, Corrado D, Marcus FI, Nava A, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. *Lancet* (2009) 373:1289–300. doi: 10.1016/S0140-6736(09)60256-7
35. Kapplinger JD, Landstrom AP, Salisbury BA, Callis TE, Pollevick GD, Tester DJ, et al. Distinguishing arrhythmogenic right ventricular cardiomyopathy/dysplasia-associated mutations from background genetic noise. *J Am Coll Cardiol.* (2011) 57:2317–27. doi: 10.1016/j.jacc.2010.12.036
36. Basso C, Corrado D, Bauce B, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. *Circ Arrhythm Electrophysiol.* (2012) 5:1233–46. doi: 10.1161/CIRCEP.111.962035
37. Yu J, Hu J, Dai X, Cao Q, Xiong Q, Liu X, et al. SCN5A mutation in Chinese patients with arrhythmogenic right ventricular dysplasia. *Herz* (2014) 39:271–5. doi: 10.1007/s00059-013-3998-5
38. Sato PY, Musa H, Coombs W, Guerrero-Serna G, Patiño GA, Taffet SM, et al. Loss of plakophilin-2 expression leads to decreased sodium current and slower conduction velocity in cultured cardiac myocytes. *Circ Res.* (2009) 105:523–6. doi: 10.1161/CIRCRESAHA.109.201418
39. Rizzo S, Lodder EM, Verkerk AO, Wolswinkel R, Beekman L, Pilichou K, et al. Intercalated disc abnormalities, reduced Na^+ current density, and conduction slowing in desmoglein-2 mutant mice prior to cardiomyopathic changes. *Cardiovasc Res.* (2012) 95:409–18. doi: 10.1093/cvr/cvs219

40. Wilders R. Arrhythmogenic right ventricular cardiomyopathy: considerations from *in silico* experiments. *Front Physiol.* (2012) 3:168. doi: 10.3389/fphys.2012.00168
41. Probst V, Wilde AAM, Barc J, Sacher F, Babuty D, Mabo P, et al. SCN5A mutations and the role of genetic background in the pathophysiology of Brugada syndrome. *Circ Cardiovasc Genet.* (2009) 2:552–7. doi: 10.1161/CIRCGENETICS.109.853374
42. Meregalli PG, Tan HL, Probst V, Koopmann TT, Tanck MW, Bhuiyan ZA, et al. Type of SCN5A mutation determines clinical severity and degree of conduction slowing in loss-of-function sodium channelopathies. *Heart Rhythm.* (2009) 6:341–8. doi: 10.1016/j.hrthm.2008.11.009
43. Bezzina C, Veldkamp MW, van den Berg MP, Postma AV, Rook MB, Viersma JW, et al. A single Na^+ channel mutation causing both long-QT and Brugada syndromes. *Circ Res* (1999) 85:1206–13. doi: 10.1161/01.RES.85.12.1206
44. Postema PG, Wolpert C, Amin AS, Probst V, Borggrefe M, Roden DM, et al. Drugs and Brugada syndrome patients: review of the literature, recommendations, and an up-to-date website (www.brugadadrugs.org). *Heart Rhythm.* (2009) 6:1335–41. doi: 10.1016/j.hrthm.2009.07.002
45. Hoogendijk MG, Potse M, Vinet A, de Bakker JMT, Coronel R. ST segment elevation by current-to-load mismatch: an experimental and computational study. *Heart Rhythm.* (2011) 8:111–8. doi: 10.1016/j.hrthm.2010.09.066
46. Paci M, Passini E, Severi S, Hyttinen J, Rodriguez B. Phenotypic variability in LQT3 human induced pluripotent stem cell-derived cardiomyocytes and their response to antiarrhythmic pharmacologic therapy: an *in silico* approach. *Heart Rhythm.* (2017) 14:1704–12. doi: 10.1016/j.hrthm.2017.07.026
47. Kyndt F, Probst V, Potet F, Demolombe S, Chevallier JC, Baro I, et al. Novel SCN5A mutation leading either to isolated cardiac conduction defect or Brugada syndrome in a large French family. *Circulation* (2001) 104:3081–6. doi: 10.1161/hc5001.100834
48. van den Berg MP, Wilde AAM, Viersma TJW, Brouwer J, Haaksma J, van der Hout AH, et al. Possible bradycardic mode of death and successful pacemaker treatment in a large family with features of long QT syndrome type 3 and Brugada syndrome. *J Cardiovasc Electrophysiol.* (2001) 12:630–6. doi: 10.1046/j.1540-8167.2001.00630.x
49. Kolder IC, Tanck MW, Bezzina CR. Common genetic variation modulating cardiac ECG parameters and susceptibility to sudden cardiac death. *J Mol Cell Cardiol.* (2012) 52:620–9. doi: 10.1016/j.yjmcc.2011.12.014
50. Remme CA, Scicluna BP, Verkerk AO, Amin AS, van Brunschot S, Beekman L, et al. Genetically determined differences in sodium current characteristics modulate conduction disease severity in mice with cardiac sodium channelopathy. *Circ Res.* (2009) 104:1283–92. doi: 10.1161/CIRCRESAHA.109.194423
51. Rivaud MR, Baartscheer A, Verkerk AO, Beekman L, Rajamani S, Belardinelli L, et al. Enhanced late sodium current underlies pro-arrhythmic intracellular sodium and calcium dysregulation in murine sodium channelopathy. *Int J Cardiol.* (2018) 263:54–62. doi: 10.1016/j.ijcard.2018.03.044
52. Scicluna BP, Tanck MW, Remme CA, Beekman L, Coronel R, Wilde AAM, et al. Quantitative trait loci for electrocardiographic parameters and arrhythmia in the mouse. *J Mol Cell Cardiol.* (2011) 50:380–9. doi: 10.1016/j.yjmcc.2010.09.009
53. Lodder EM, Scicluna BP, Milano A, Sun AY, Tang H, Remme CA, et al. Dissection of a quantitative trait locus for PR interval duration identifies Tnni3k as a novel modulator of cardiac conduction. *PLoS Genet.* (2012) 8:e1003113. doi: 10.1371/journal.pgen.1003113
54. Ackerman MJ, Splawski I, Makielski JC, Tester DJ, Will ML, Timothy KW, et al. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. *Heart Rhythm.* (2004) 1:600–7. doi: 10.1016/j.hrthm.2004.07.013
55. Viswanathan PC, Benson DW, Balser JR. A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation. *J Clin Invest.* (2003) 111:341–6. doi: 10.1172/JCI200316879
56. Ye B, Valdivia CR, Ackerman MJ, Makielski JC. A common human SCN5A polymorphism modifies expression of an arrhythmia causing mutation. *Physiol Genomics* (2003) 12:187–93. doi: 10.1152/physiolgenomics.00117.2002
57. Poelzing S, Forleo C, Samodell M, Dudash L, Sorrentino S, Anaclerio M, et al. SCN5A polymorphism restores trafficking of a Brugada syndrome mutation on a separate gene. *Circulation* (2006) 114:368–76. doi: 10.1161/CIRCULATIONAHA.105.601294
58. Gui J, Wang T, Trump D, Zimmer T, Lei M. Mutation-specific effects of polymorphism H558R in SCN5A-related sick sinus syndrome. *J Cardiovasc Electrophysiol.* (2010) 21:564–73. doi: 10.1111/j.1540-8167.2010.01762.x
59. Marangoni S, Di Resta C, Rocchetti M, Barile L, Rizzetto R, Summa A, et al. A Brugada syndrome mutation (p.S216L) and its modulation by p.H558R polymorphism: standard and dynamic characterization. *Cardiovasc Res.* (2011) 91:606–16. doi: 10.1093/cvr/cvr142
60. Shinlapawittayatorn K, Du XX, Liu H, Ficker E, Kaufman ES, Deschênes I. A common SCN5A polymorphism modulates the biophysical defects of SCN5A mutations. *Heart Rhythm.* (2011) 8:455–62. doi: 10.1016/j.hrthm.2010.11.034
61. Bezzina CR, Shimizu W, Yang P, Koopmann TT, Tanck MW, Miyamoto Y, et al. Common sodium channel promoter haplotype in asian subjects underlies variability in cardiac conduction. *Circulation.* (2006) 113:338–44. doi: 10.1161/CIRCULATIONAHA.105.580811
62. Groenewegen WA, Firouzi M, Bezzina CR, Vliex S, van Langen IM, Sandkuijl L, et al. A cardiac sodium channel mutation cosegregates with a rare connexin40 genotype in familial atrial standstill. *Circ Res.* (2003) 92:14–22. doi: 10.1161/01.RES.0000050585.07097.D7
63. Makielski JC, Ye B, Valdivia CR, Pagel MD, Pu J, Tester DJ, et al. A ubiquitous splice variant and a common polymorphism affect heterologous expression of recombinant human SCN5A heart sodium channels. *Circ Res.* (2003) 93:821–8. doi: 10.1161/01.RES.0000096652.14509.96
64. Tan BH, Valdivia CR, Song C, Makielski JC. Partial expression defect for the SCN5A missense mutation G1406R depends on splice variant background Q1077 and rescue by mexiletine. *Am J Physiol Heart Circ Physiol.* (2006) 291:H1822–8. doi: 10.1152/ajpheart.00101.2006
65. Cheng J, Morales A, Siegfried JD, Li D, Norton N, Song J, et al. SCN5A rare variants in familial dilated cardiomyopathy decrease peak sodium current depending on the common polymorphism H558R and common splice variant Q1077del. *Clin Transl Sci.* (2010) 3:287–94. doi: 10.1111/j.1752-8062.2010.00249.x
66. Matsuo K, Akahoshi M, Nakashima E, Suyama A, Seto S, Hayano M, et al. The prevalence, incidence and prognostic value of the Brugada-type electrocardiogram: a population-based study of four decades. *J Am Coll Cardiol.* (2001) 38:765–70. doi: 10.1016/S0735-1097(01)01421-8
67. Wilde AAM, Moss AJ, Kaufman ES, Shimizu W, Peterson DR, Benhorin J, et al. Clinical aspects of type 3 long-QT syndrome: an international multicenter study. *Circulation* (2016) 134:872–8. doi: 10.1161/CIRCULATIONAHA.116.021823
68. Verkerk AO, Wilders R, de Geringel W, Tan HL. Cellular basis of sex disparities in human cardiac electrophysiology. *Acta Physiol.* (2006) 187:459–77. doi: 10.1111/j.1748-1716.2006.01586.x
69. Barajas-Martinez H, Haufe V, Chamberland C, Roy MJ, Fecteau MH, Cordeiro JM, et al. Larger dispersion of I_{Na} in female dog ventricle as a mechanism for gender-specific incidence of cardiac arrhythmias. *Cardiovasc Res.* (2009) 81:82–9. doi: 10.1093/cvr/cvn255
70. Postema PG, Wilde AAM. Aging in brugada syndrome: what about risks? *JACC Clin Electrophysiol.* (2017) 3:68–70. doi: 10.1016/j.jacep.2016.05.014
71. Postema PG, Tan HL, Wilde AAM. Ageing and Brugada syndrome: considerations and recommendations. *J Geriatr Cardiol.* (2013) 10:75–81. doi: 10.3969/j.issn.1671-5411.2013.01.012
72. Beaufort-Krol GC, van den Berg MP, Wilde AAM, van Tintelen JP, Viersma JW, Bezzina C. R, et al. Developmental aspects of long QT syndrome type 3 and Brugada syndrome on the basis of a single SCN5A mutation in childhood. *J Am Coll Cardiol.* (2005) 46:331–337. doi: 10.1016/j.jacc.2005.03.066
73. Baba S, Dun W, Hirose M, Boyden PA. Sodium current function in adult and aged canine atrial cells. *Am J Physiol Heart Circ Physiol.* (2006) 291:H756–61. doi: 10.1152/ajpheart.00063.2006

74. Signore S, Sorrentino A, Borghetti G, Cannata A, Meo M, Zhou Y, et al. Late Na^+ current and protracted electrical recovery are critical determinants of the aging myopathy. *Nat Commun.* (2015) 6:8803. doi: 10.1038/ncomms9803
75. Chadda KR, Ahmad S, Valli H, den Uijl I, Al-Hadithi AB, Salvage SC, et al. The effects of ageing and adrenergic challenge on electrocardiographic phenotypes in a murine model of long QT syndrome type 3. *Sci Rep.* (2017) 7:11070. doi: 10.1038/s41598-017-11210-3
76. Probst V, Kyndt F, Potet F, Trochu JN, Mialet G, Demolombe S, et al. Haploinsufficiency in combination with aging causes SCN5A-linked hereditary Lene'gre disease. *J Am Coll Cardiol.* (2003) 41:643–52. doi: 10.1016/S0735-1097(02)02864-4
77. Royer A, van Veen TAB, Le Bouter S, Marionneau C, Griol-Charhbili V, Léoni AL, et al. Mouse model of SCN5A-linked hereditary Lenègre's disease: age-related conduction slowing and myocardial fibrosis. *Circulation* (2005) 111:1738–46. doi: 10.1161/01.CIR.0000160853.19867.61
78. Postema PG, Neville J, de Jong JSSG, Romero K, Wilde AAM, Woosley RL. Safe drug use in long QT syndrome and Brugada syndrome: comparison of website statistics. *Europace* (2013) 15:1042–9. doi: 10.1093/europace/eut018
79. Yap YG, Behr ER, Camm AJ. Drug-induced Brugada syndrome. *Europace* (2009) 11:989–94. doi: 10.1093/europace/eup114
80. Fazio G, Vernuccio F, Grutta G, Lo Re G. Drugs to be avoided in patients with long QT syndrome: vocus on the anaesthesiological management. *World J Cardiol.* (2013) 5:87–93. doi: 10.4330/wjc.v5.i4.87
81. Diness JG, Hansen RS, Nissen JD, Jespersen T, Grunnet M. Antiarrhythmic effect of I_{Kr} activation in a cellular model of LQT3. *Heart Rhythm.* (2009) 6:100–6. doi: 10.1016/j.hrthm.2008.10.020
82. Achaiah A, Andrews N. Intoxication with alcohol: an underestimated trigger of Brugada syndrome? *JRSM Open* (2016) 7:2054270416640153. doi: 10.1177/2054270416640153
83. Klein G, Gardiwal A, Schaefer A, Panning B, Breitmeier D. Effect of ethanol on cardiac single sodium channel gating. *Forensic Sci Int.* (2007) 171:131–5. doi: 10.1016/j.forsciint.2006.10.012
84. Bebarova M, Matejovic P, Pasek M, Simurdova M, Simurda J. Dual effect of ethanol on inward rectifier potassium current I_{K1} in rat ventricular myocytes. *J Physiol Pharmacol.* (2014) 65:497–509.
85. Uyarel H, Ozdol C, Gencer AM, Okmen E, Cam N. Acute alcohol intake and QT dispersion in healthy subjects. *J Stud Alcohol.* (2005) 66:555–558. doi: 10.15288/jsa.2005.66.555
86. O'Leary ME, Hancox JC. Role of voltage-gated sodium, potassium and calcium channels in the development of cocaine-associated cardiac arrhythmias. *Br J Clin Pharmacol.* (2010) 69:427–42. doi: 10.1111/j.1365-2125.2010.03629.x
87. Sartiani L, Cerbai E, Lonardo G, DePaoli P, Tattoli M, Cagiano R, et al. Prenatal exposure to carbon monoxide affects postnatal cellular electrophysiological maturation of the rat heart: a potential substrate for arrhythmogenesis in infancy. *Circulation* (2004) 109:419–23. doi: 10.1161/01.CIR.0000109497.73223.4D
88. Ton AT, Biet M, Delabre J-F, Morin N, Dumaine R. *In-utero* exposure to nicotine alters the development of the rabbit cardiac conduction system and provides a potential mechanism for sudden infant death syndrome. *Arch Toxicol.* (2017) 91:3947–60. doi: 10.1007/s00204-017-2006-x
89. Dallas ML, Yang ML, Boyle Z, Boycott JP, Scragg HE, Milligan JL, et al. Carbon monoxide induces cardiac arrhythmia via induction of the late Na current. *Am J Respir Crit Care Med.* (2012) 186:648–56. doi: 10.1164/rccm.201204-0688OC
90. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* (2000) 102:1178–85. doi: 10.1161/01.CIR.102.10.1178
91. Potet F, Beckermann TM, Kunic JD, George AL Jr. Intracellular calcium attenuates late current conducted by mutant human cardiac sodium channels. *Circ Arrhythm Electrophysiol.* (2015) 8:933–41. doi: 10.1161/CIRCEP.115.002760
92. Mascia G, Arbelo E, Hernández-Ojeda J, Solimene F, Brugada R, Brugada J. Brugada syndrome and exercise practice: current knowledge, shortcomings and open questions. *Int J Sports Med.* (2017) 38:573–81. doi: 10.1055/s-0043-107240
93. Mascia G, Arbelo E, Solimene F, Giaccardi M, Brugada R, Brugada J. The long-QT syndrome and exercise practice: the never-ending debate. *J Cardiovasc Electrophysiol.* (2018) 29:489–96. doi: 10.1111/jce.13410
94. D'Souza A, Bucchi A, Johnsen A-B, Logantha SJRJ, Monfredi O, Yanni J, et al. Exercise training reduces resting heart rate via downregulation of the funny channel HCN4. *Nat Commun.* (2014) 5:3775. doi: 10.1038/ncomms4775
95. Salem KA, Qureshi MA, Sydorenko V, Parekh K, Jayaprakash P, Iqbal T, Singh J, et al. Effects of exercise training on excitation-contraction coupling and related mRNA expression in hearts of Goto-Kakizaki type 2 diabetic rats. *Mol Cell Biochem.* (2013) 380:83–96. doi: 10.1007/s11010-013-1662-2
96. Stones R, Billeter R, Zhang H, Harrison S, White E. The role of transient outward K^+ current in electrical remodelling induced by voluntary exercise in female rat hearts. *Basic Res Cardiol.* (2009) 104:643–52. doi: 10.1007/s00395-009-0030-6
97. Xiao YF, Kang JX, Morgan JP, Leaf A. Blocking effects of polyunsaturated fatty acids on Na^+ channels in neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA.* (1995) 92:11000–4. doi: 10.1073/pnas.92.24.11000
98. Yagi S, Soeki T, Aihara KI, Fukuda D, Ise T, Kadota M, et al. Low serum levels of eicosapentaenoic acid and docosahexaenoic acid are risk factors for cardiogenic syncope in patients with Brugada syndrome. *Int Heart J.* (2017) 58:720–3. doi: 10.1536/ihj.16-278
99. Macleod JC, Macknight ADC, Rodrigo GC. The electrical andmechanical response of adult guinea pig and rat ventricular myocytes to omega-3 polyunsaturated fatty acids. *Eur J Pharmacol.* (1998) 356:261–70. doi: 10.1016/S0014-2999(98)00528-7
100. Wu CC, Su MJ, Chi JF, Wu MH, Lee YT. Comparison of aging and hypercholesterolemic effects on the sodium inward currents in cardiac myocytes. *Life Sci.* (1997) 61:1539–51. doi: 10.1016/S0024-3205(97)00733-9
101. Ikeda T, Abe A, Yusu S, Nakamura K, Ishiguro H, Mera H, et al. The full stomach test as a novel diagnostic technique for identifying patients at risk of Brugada syndrome. *J Cardiovasc Electrophysiol.* (2006) 17:602–7. doi: 10.1111/j.1540-8167.2006.00424.x
102. Ozyilmaz I, Akyol B, Ergul Y. Sudden cardiac arrest while eating a hot dog: a rare presentation of Brugada syndrome in a child. *Pediatrics* (2017) 140:e20162485. doi: 10.1542/peds.2016-2485
103. Chanavirut R, Leelayuwat N. Brugada syndrome and carbohydrate metabolism. *J Cardiol Curr Res.* (2017) 8:00294. doi: 10.15406/jccr.2017.08.00294
104. Hansen PS, Buhagiar KA, Gray DF, Rasmussen HH. Voltage-dependent stimulation of the Na^+-K^+ pump by insulin in rabbit cardiac myocytes. *Am J Physiol Cell Physiol.* (2000) 278:C546–53. doi: 10.1152/ajpcell.2000.278.3.C546
105. Aulbach F, Simm A, Maier S, Langenfeld H, Walter U, Kersting U, et al. Insulin stimulates the L-type Ca^{2+} current in rat cardiac myocytes. *Cardiovasc Res.* (1999) 42:113–20. doi: 10.1016/S0008-6363(98)00307-1
106. Shi YQ, Yan M, Liu LR, Zhang X, Wang X, Geng HZ, et al. High glucose represses hERG K^+ channel expression through trafficking inhibition. *Cell Physiol Biochem.* (2015) 37:284–96. doi: 10.1159/000430353
107. Wu M, Obara Y, Norota I, Nagasawa Y, Ishii K. Insulin suppresses I_{Ks} (KCNQ1/KCNE1) currents, which require β-subunit KCNE1. *Pflügers Arch.* (2014) 466:937–46. doi: 10.1007/s00424-013-1352-7
108. Gastaldelli A, Emdin M, Conforti F, Camastra S, Ferrannini E. Insulin prolongs the QTc interval in humans. *Am J Physiol Regul Integr Comp Physiol.* (2000) 279:R2022–5. doi: 10.1152/ajpregu.2000.279.6.R2022
109. Skinner JR, Marquis-Nicholson R, Luangpraseuth A, Cutfield R, Crawford J, Love DR. Diabetic dead-in-bed syndrome: a possible link to a cardiac ion channelopathy. *Case Rep Med.* (2014) 2014:47252. doi: 10.1155/2014/647252
110. Li J, Hu D, Song X, Han T, Gao Y, Xing Y. The role of biologically asctive ingredients from natural drug treatments for arrhythmias in different mechanisms. *Biomed Res Int.* (2017) 2017:4615727. doi: 10.1155/2017/4615727

111. Heart Rhythm Society of the Chinese Society of Biomedical Engineering, and Nao Xin Tong Zhi Committee of the Chinese Association of Integrative Medicine. Expert consensus on Wenxin granule for treatment of cardiac arrhythmias. *Chin Med J.* (2017) 130, 203–10. . doi: 10.4103/0366-6999.198003
112. Minoura Y, Panama BK, Nesterenko VV, Betzenhauser M, Barajas-Martínez H, Hu D, et al. Effect of Wenxin Keli and quinidine to suppress arrhythmogenesis in an experimental model of Brugada syndrome. *Heart Rhythm.* (2013) 10:1054 −62. doi: 10.1016/j.hrthm.2013.03.011
113. Hou JW, Li W, Guo K, Chen XM, Chen YH, Li CY, et al. Antiarrhythmic effects and potential mechanism of WenXin KeLi in cardiac Purkinje cells. *Heart Rhythm*. (2016) 13:973–82. doi: 10.1016/j.hrthm.2015.12.023
114. Xue X, Guo D, Sun H, Wang D, Li J, Liu T, et al. Wenxin Keli suppresses ventricular triggered arrhythmias via selective inhibition of late sodium current. *Pacing Clin Electrophysiol.* (2013) 36:732–40. doi: 10.1111/pace.12109
115. Fish JM, Welchons DR, Kim YS, Lee SH, Ho WK, Antzelevitch C. Dimethyl lithospermate B, an extract of Danshen, suppresses arrhythmogenesis associated with the Brugada syndrome. *Circulation* (2006) 113:1393–400. doi: 10.1161/CIRCULATIONAHA.105.601690
116. Shah SR, Park K. Long QT syndrome: a comprehensive review of the literature and current evidence. *Curr Probl Cardiol.* (2018). doi: 10.1016/j.cpcardiol.2018.04.002. [Epub ahead of print].
117. Mordukhovich I, Kloog I, Coull B, Koutrakis P, Vokonas P, Schwartz J. Association between particulate air pollution and QT interval duration in an elderly cohort. *Epidemiology* (2016) 27:284–90. doi: 10.1097/EDE.0000000000000424
118. Hahad O, Beutel M, Gori T, Schulz A, Blettner M, Pfeiffer N, Rostock T, et al. Annoyance to different noise sources is associated with atrial fibrillation in the Gutenberg Health Study. *Int J Cardiol.* (2018) 264:79–84. doi: 10.1016/j.ijcard.2018.03.126
119. Münzel T, Sørensen M, Schmidt F, Schmidt E, Steven S, Kröller-Schön S., et al. The adverse effects of environmental noise exposure on oxidative stress and cardiovascular risk. *Antioxid Redox Signal.* (2018) 28:873–908. doi: 10.1089/ars.2017.7118
120. Amin AS, Klemens CA, Verkerk AO, Meregalli PG, Asghari-Roodsari A, de Bakker JMT, et al. Fever-triggered ventricular arrhythmias in Brugada syndrome and type 2 long-QT syndrome. *Neth Heart J.* (2010) 18:165–9. doi: 10.1007/BF03091755
121. Egri C, Ruben PC. A hot topic. *Channels* (2012) 6:75–85. doi: 10.4161/chan.19827
122. Amin AS, Verkerk AO, Bhuiyan ZA, Wilde AAM, Tan HL. Novel Brugada syndrome-causing mutation in ion-conducting pore of cardiac Na^+ channel does not affect ion selectivity properties. *Acta Physiol Scand.* (2005) 185:291–301. doi: 10.1111/j.1365-201X.2005.01496.x
123. Dumaine R, Towbin JA, Brugada P, Vatta M, Nesterenko DV, Nesterenko VV, et al. Ionic mechanisms responsible for the electrocardiographic phenotype of the Brugada syndrome are temperature dependent. *Circ Res.* (1999) 85:803–9. doi: 10.1161/01.RES.85.9.803
124. Nagatomo T, Fan Z, Ye B, Tonkovich GS, January CT, Kyle JW, et al. Temperature dependence of early and late currents in human cardiac wild-type and long QT Delta KPQ Na channels. *Am J Physiol Heart Circ Physiol.* (1998) 275:2016–24. doi: 10.1152/ajpheart.1998.275.6.H2016
125. Burashnikov A, Shimizu W, Antzelevitch C. Fever accentuates transmural dispersion of repolarization and facilitates development of early afterdepolarizations and torsade de pointes under long-QT Conditions. *Circ Arrhythm Electrophysiol.* (2008) 1:202–8. doi: 10.1161/CIRCEP.107.691931
126. Lu Z, Jiang YP, Wu CY, Ballou LM, Liu S, Carpenter ES, et al. Increased persistent sodium current due to decreased PI3K signalling contributes to QT prolongation in the diabetic heart. *Diabetes* (2013) 62:4257–65. doi: 10.2337/db13-0420
127. Veglio M, Borra M, Stevens LK, Fuller JH, Perin PC. The relation between QTc interval prolongation and diabetic complications. *Diabetologia* (1999) 42:68–75. doi: 10.1007/s001250051115
128. Chapalamadugu K, Panguluri SK, Miranda A, Sneed KB, Tipparaju SM. Pharmacogenomics of cardiovascular complications in diabetes and obesity. *Recent Pat Biotechnol.* (2014) 8:123–35. doi: 10.2174/1872208309666140904123023
129. Stables CL, Musa H, Mitra A, Bhushal S, Deo M, Guerrero-Serna G, et al. Reduced Na^+ current density underlies impaired propagation in the diabetic rabbit ventricle. *J Mol Cell Cardiol.* (2015) 69:24–31. doi: 10.1016/j.yjmcc.2013.12.031
130. Yu P, Hu L, Xie J, Chen S, Huang L, Xu Z, et al. O-GlcNAcylation of cardiac Nav1.5 contributes to the development of arrhythmias in diabetic hearts. *Int J Cardiol* (2018) 260, 74–81. doi: 10.1016/j.ijcard.2018.02.099
131. Pi-Sunyer FX. The obesity epidemic: pathophysiology and consequences of obesity. *Obes Res.* (2002) 10:97S−104S. doi: 10.1038/oby.2002.202
132. Aromolaran AS, Boutjdir M. Cardiac ion channel regulation in obesity and the metabolic syndrome: relevance to long QT syndrome and atrial fibrillation. *Front. Physiol.* (2017) 8:431. doi: 10.3389/fphys.2017.00431
133. Huang H, Amin V, Gurin M, Wan E, Thorp E, Homma S, et al. Diet-induced obesity causes long QT and reduces transcription of voltage-gated potassium channels. *J Mol Cell Cardiol.* (2013) 59:151–8. doi: 10.1016/j.yjmcc.2013.03.007
134. Nadruz W. Myocardial remodeling in hypertension. *J Hum Hypertens.* (2015) 29:1–6. doi: 10.1038/jhh.2014.36
135. Coronel R, Casini S, Koopmann TT, Wilms-Schopman FJG, Verkerk AO, de Groot JR, et al. Right ventricular fibrosis and conduction delay in a patient with clinical signs of Brugada syndrome: a combined electrophysiological, genetic, histopathologic, and computational study. *Circulation* (2005) 112:2769–77. doi: 10.1161/CIRCULATIONAHA.105.532614
136. Remme CA, Wilde AAM. Late sodium current inhibition in acquired and inherited ventricular (dys)function and arrhythmias. *Cardiovasc Drugs Ther.* (2013) 27:91–101. doi: 10.1007/s10557-012-6433-x
137. Rivaud MR, Jansen JA, Postema PG, Nannenberg EA, Mizusawa Y, van der Nagel, R., et al A common co-morbidity modulates disease expression and treatment efficacy in inherited cardiac sodium channelopathy. *Eur Heart J.* (2018) 39:2898–907. doi: 10.1093/eurheartj/ehy247
138. Claes GR, van Tienen FH, Lindsey P, Krapels IP, Helderman-van den Enden AT, Hoos MB, et al. Hypertrophic remodelling in cardiac regulatory myosin light chain (MYL2) founder mutation carriers. *Eur Heart J.* (2016) 37:1815–1822. doi: 10.1093/eurheartj/ehv522
139. Noda T, Shimizu W, Taguchi A, Satomi K, Suyama K, Kurita T, et al. ST-segment elevation and ventricular fibrillation without coronary spasm by intracoronary injection of acetylcholine and/or ergonovine maleate in patients with Brugada syndrome. *J Am Coll Cardiol.* (2002) 40:1841–7. doi: 10.1016/S0735-1097(02)02494-4
140. Sasaki T, Niwano S, Kitano Y, Izumi T. Two cases of Brugada syndrome associated with spontaneous clinical episodes of coronary vasospasm. *Intern Med.* (2006) 45:77–80. doi: 10.2169/internalmedicine.45.1404
141. Nishizaki M, Fujii H, Ashikaga T, Yamawake N, Sakurada H, Hiraoka M. ST-T wave changes in a patient complicated with vasospastic angina and Brugada syndrome: differential responses to acetylcholine in right and left coronary artery. *Heart Vessels.* (2008) 23:201–5. doi: 10.1007/s00380-007-1036-3
142. Ohkubo K, Watanabe I, Okumura Y, Kofune M, Nagashima K, Mano H, et al. Brugada syndrome in the presence of coronary artery disease. *J Arrhyth.* (2013) 29:211–6. doi: 10.1016/j.joa.2012.09.008
143. Ong P, Bastiaenen R, Batchvarov VN, Athanasiadis A, Raju H, Kaski JC, et al. Prevalence of the type 1 Brugada electrocardiogram in Caucasian patients with suspected coronary spasm. *Europace* (2011) 13:1625–31. doi: 10.1093/europace/eur205
144. van Hoorn F, Campian ME, Spijkerboer A, Blom MT, Planken RN, van Rossum AC, et al. SCN5A mutations in Brugada syndrome

are associated with increased cardiac dimensions and reduced contractility. *PLoS ONE* (2012) 7:e42037. doi: 10.1371/journal.pone.0042037

145. Kujime S, Sakurada H, Saito N, Enomoto Y, Ito N, Nakamura K, et al. Outcomes of Brugada syndrome patients with coronary artery vasospasm. *Intern Med.* (2017) 56:129–35. doi: 10.2169/internalmedicine.56.7307
146. Sze E, Moss AJ, Goldenberg I, McNitt S, Jons C, Zareba W, et al. Long QT syndrome in patients over 40 years of age: increased risk for LQTS-related cardiac events in patients with coronary disease. *Ann Noninvasive Electrocardiol.* (2008) 13:327–31. doi: 10.1111/j.1542-474X.2008.00250.x
147. Dekker JM, Schouten EG, Klootwijk P, Pool J, Kromhout D. Association between QT interval and coronary heart disease in middle-aged and elderly men. *Zutphen Study Circ.* (1994) 90:779–85. doi: 10.1161/01.CIR.90.2.779
148. Korantzopoulos P, Letsas KP, Christogiannis Z, Kalantzi K, Massis I, Milionis HJ, et al. Exercise-induced repolarization changes in patients with stable coronary artery disease. *Am J Cardiol.* (2011) 107:37–40. doi: 10.1016/j.amjcard.2010.08.038

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Higher Dispersion Measures of Conduction and Repolarization in Type 1 Compared to Non-type 1 Brugada Syndrome Patients: An Electrocardiographic Study From a Single Center

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Background: Brugada syndrome (BrS) is a cardiac ion channelopathy that predisposes affected individuals to sudden cardiac death (SCD). Type 1 BrS is thought to take a more malignant clinical course than non-type 1 BrS. We hypothesized that the degrees of abnormal repolarization and conduction are greater in type 1 subjects and these differences can be detected by electrocardiography (ECG).

Methods: Electrocardiographic data from spontaneous type 1 and non-type 1 BrS patients were analyzed. ECG parameters were measured from leads V1 to V3. Values were expressed as median [lower quartile-upper quartile] and compared using Kruskal-Wallis ANOVA.

Results: Compared to non-type 1 BrS patients ($n = 29$), patients with spontaneous type 1 patterns ($n = 22$) showed similar ($P > 0.05$) heart rate (73 [64–77] vs. 68 [62–80] bpm), QRS duration (136 [124–161] vs. 127 [117–144] ms), uncorrected QT (418 [393–443] vs. 402 [386–424] ms) and corrected QT intervals (457 [414–474] vs. 430 [417–457] ms), JT_{peak} intervals (174 [144–183] vs. 174 [150–188] ms), $T_{peak} - T_{end}$ intervals (101 [93–120] vs. 99 [90–105] ms), $T_{peak} - T_{end}$/QT ratios (0.25 [0.23–0.27] vs. 0.24 [0.22–0.27]), $T_{peak} - T_{end}$/QRS (0.77 [0.62–0.87] vs. 0.77 [0.69–0.86]), $T_{peak} - T_{end}$/(QRS × QT) (0.00074 [0.00034–0.00096] vs. 0.00073 [0.00048–0.00012] ms^{-1}), index of Cardiac Electrophysiological Balance (iCEB, QT/QRS, marker of wavelength: 3.14 [2.56–3.35] vs. 3.21 [2.85–3.46]) and corrected iCEB (QTc/QRS: 3.25 [2.91–3.73]

vs. 3.49 [2.99–3.78]). Higher QRS dispersion was seen in type 1 subjects (QRSd: 34 [24–66] vs. 24 [12–34] ms) but QT dispersion (QTd: 48 [39–71] vs. 43 [22–94] ms), QTc dispersion (QTcd: 52 [41–79] vs. 46 [23–104] ms), JT_{peak} dispersion (44 [23–62] vs. 45 [30–62] ms), $T_{peak} - T_{end}$ dispersion (28 [15–34] vs. 29 [22–53] ms) or $T_{peak} - T_{end}$/QT dispersion (0.06 [0.03–0.08] vs. 0.08 [0.04–0.12]) did not differ between the two groups. Type 1 subjects showed higher (QRSd × $T_{peak} - T_{end}$)/QRS (25 [19–44] vs. 19 [9–30] ms) but similar iCEB dispersion (0.83 [0.49–1.14] vs. 0.61 [0.34–0.92]) and iCEBc dispersion (0.93 [0.51–1.15] vs. 0.65 [0.39–0.96]).

Conclusion: Higher levels of dispersion in conduction and repolarization are found in type 1 than non-type 1 BrS patients, potentially explaining the higher incidence of ventricular arrhythmias in the former group.

Keywords: electrocardiography, conduction, repolarization, wavelength, Brugada syndrome

INTRODUCTION

Brugada syndrome (BrS) is a cardiac ion channelopathy that predisposes affected individuals to ventricular tachyarrhythmias and sudden cardiac death (SCD). Type 1 BrS is thought to take a more malignant clinical course than non-type 1 BrS (1). Abnormalities in both conduction and repolarization processes contribute to ventricular tachyarrhythmias in BrS (2). For instance, slow and discontinuous conduction of action potentials through working myocardium, due to reduced sodium channel activity, may lead to higher degrees of spatial and temporal dispersion in conduction (3). These could potentially be detected as prolonged QRS intervals (4) and higher QRS dispersion (5). Moreover, heterogeneous time-course in full repolarization between the different myocardial layers, due to regional difference in transient outward potassium channel activity, leads to increased transmural repolarization gradients that can be measured electrographically using QT dispersion (QT_d) (6, 7), interval from the peak to the end of the T-wave (8) [$T_{peak} - T_{end}$, reflecting transmural dispersion of repolarization, TDR (9)], ($T_{peak} - T_{end}$)/QT ratio (10, 11) and $T_{peak} - T_{end}$ dispersion. However, the present electrocardiographic indices do not incorporate parameters on dispersion and these may play important roles in producing the reentrant substrate for arrhythmogenesis (12). In this study, we hypothesized that the degree of abnormal repolarization and conduction is greater in spontaneous type 1 subjects and these differences can be detected by electrocardiographic indices incorporating spatial dispersion of conduction and repolarization.

METHODS

Study Subjects

This retrospective study received ethics approval from the NTEC-CUHK Clinical Research Ethics Committee. Inclusion criteria include subjects diagnosed with Brugada Syndrome presented to the Prince of Wales Hospital, a tertiary level teaching hospital in Hong Kong, China. Age, sex, type of Brugada pattern (spontaneous type 1 or otherwise), syncopal symptoms and spontaneous VT or VF were recorded.

Electrocardiographic Measurements

The following parameters were obtained from 12-lead electrocardiograms of spontaneous type 1 (**Data Sheet 1**) and non-type 1 (**Data Sheet 2**) Brugada subjects. Measurements were made from the right precordial leads (V1–V3) with mean values calculated. They were measured together by GT and CL using Phillips ECGVue (Standard Edition). The first ten measurements were validated by clinical electrophysiologists of our centers (KPL and JC). The end of the T-wave was determined using the return to the baseline method. Dispersion was defined as the difference between the maximum and minimum value detected from V1 to V3.

Repolarization parameters including QT interval (onset of the QRS complex to the end of the T wave at T-P baseline; If U waves are present, the QT interval will be taken to the nadir of the curve between the T and U waves), QTc (correction using Bazett's formula), QT dispersion, $T_{peak} - T_{end}$ (peak of T-wave to end of T-wave), $T_{peak} - T_{end}$ dispersion, $T_{peak} - T_{end}$/QT ratio, $T_{peak} - T_{end}$/QT dispersion, and JT_{peak} (J point to peak of T-wave), and JT_{peak} dispersion. Conduction parameters include QRS duration (onset of Q-wave to the terminal portion of S-wave) and QRS dispersion. Conduction-repolarization indices include index of Cardiac Electrophysiological Balance (iCEB, QT/QRS, a surrogate marker of excitation wavelength), iCEBc (QTc/QRS), their dispersion parameters, ($T_{peak} - T_{end}$)/QRS, $T_{peak} - T_{end}$/(QT × QRS) and $QRS_d \times (T_{peak} - T_{end})$/QRS.

Statistical Analysis

Data were expressed as median [lower quartile to upper quartile]. Categorical data were analyzed by Fisher's exact test. Differences between study groups were tested using Kruskal-Wallis ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical Characteristics

This study included a total of 51 Brugada syndrome patients. The baseline demographic and clinical characteristics are shown in **Table 1**. The mean age was 56 ± 2 years and 90% of the subjects were male. A type 1 pattern was observed in 22 patients (43%)

TABLE 1 | Demographic and clinical characteristics of Brugada syndrome patients included in this study ($n = 51$).

Characteristics	Type 1 BrS ($n = 22$)	Non-type 1 BrS ($n = 29$)	*P*-value
Male sex	20 (91%)	26 (92%)	0.6298
Age (years)$^{\Psi}$	58.5 (51.5–67.0)	57.0 (36.0–70.0)	0.6343
ICD insertion	15 (68%)	6 (21%)	0.0005
Appropriate ICD shocks	3 (14%)	1 (3%)	0.2966
Syncope	15 (68%)	10 (34%)	0.0245
Spontaneous VT	5 (23%)	2 (17%)	0.2163

Data were presented as number (%), $^{\Psi}$median (lower quartile to upper quartile). P-value were obtained from Fisher's exact test (for frequency data) or Kruskal-Wallis ANOVA (for continuous data).

and a non-type 1 pattern was observed in 29 patients (57%). Implantable cardioverter-defibrillators were inserted in 21 (71%) subjects. 25 (49%) subjects had syncope, and spontaneous VT was observed in 7 patients. Compared to non-type 1 subjects, type 1 subjects were more likely to have ICD implanted (68 vs. 21%, $P = 0.0005$) and suffer from syncope (68 vs. 34%, $P = 0.02$). However, no difference in age, appropriate ICD shocks or spontaneous VT was observed between the groups ($P > 0.05$). Resting heart rate was similar between type 1 and non-type 1 subjects (73 [64–77] vs. 68 [62–80] bpm, respectively; $P = 0.78$). The different electrocardiographic parameters were measured from the precordial leads V1–V3 and mean values were calculated. Dispersion was defined as the difference in the maximum and minimum values observed in leads V1–V3. Example screenshots of the ECG measurement system, a spontaneous Type 1 Brugada pattern and non-Type 1 Brugada pattern are shown in **Figures 1A–C**, respectively. The positions of the onset of the QRS complex and the end of the T-wave are represented by the vertical lines.

Traditional Conduction or Repolarization Markers: QRS, QT, QTc, and JT_{Peak} Intervals

Compared to non-type 1 BrS subjects, those with type 1 BrS had statistically indistinguishable QRS duration (136 [124–161] vs. 127 [117–144] ms; $P = 0.14$; **Figure 2A**), uncorrected QT (418 [393–443] vs. 402 [386–424] ms; $P = 0.17$; **Figure 2B**) and corrected QT intervals using Bazett's formula (457 [414–474] vs. 430 [417–457] ms; $P = 0.15$; **Figure 2C**). Moreover, JT_{peak} intervals, which are useful for assessing repolarization duration in the context of slowed ventricular conduction (13), were not significantly different between type 1 and non-type 1 BrS patients (174 [144–183] vs. 174 [150–188] ms; $P = 0.52$; **Figure 2D**).

Markers of Repolarization or Conduction Dispersion

The conduction dispersion marker, QRS dispersion, was significantly higher in type 1 subjects (QRSd: 34 [24–66] vs. 24 [12–34] ms; $P = 0.03$; **Figure 3A**). By contrast, the repolarization dispersion markers, QT dispersion (QTd: 48 [39–71] vs. 43 [22–94] ms; $P = 0.98$ **Figure 3B**), QTc dispersion (QTcd: 52 [41–79] vs. 46 [23–104] ms; $P = 0.98$; **Figure 3C**), JT_{peak} dispersion (44 [23–62] vs. 45 [30–62] ms; $P = 0.77$; **Figure 3D**) were statistically indistinguishable between both groups.

Moreover, $T_{peak} - T_{end}$ indices reflecting global or transmural dispersion of repolarization were studied. $T_{peak} - T_{end}$ intervals (101 [93–120] vs. 99 [90–105] ms; $P = 0.28$; **Figure 4A**), $T_{peak} - T_{end}$ dispersion (28 [15–34] vs. 29 [22–53] ms; $P = 0.18$; **Figure 4B**), $T_{peak} - T_{end}$/QT ratios (0.25 [0.23–0.27] vs. 0.24 [0.22–0.27]; $P = 0.56$; **Figure 4C**), or $T_{peak} - T_{end}$/QT dispersion (0.06 [0.03–0.08] vs. 0.08 [0.04–0.12]; $P = 0.09$; **Figure 4D**) did not differ between both groups.

Markers of Excitation Wavelength and Indices Incorporating Conduction and Repolarization Dispersion

Recently, the index of Cardiac Electrophysiological Balance (iCEB, QT/QRS) was proposed as a marker of excitation wavelength (14, 15). However, iCEB (3.14 [2.56–3.35] vs. 3.21 [2.85–3.46]; $P = 0.45$; **Figure 5A**) or iCEB corrected for heart rate (QTc/QRS: 3.25 [2.91–3.73] vs. 3.49 [2.99–3.78]; $P = 0.48$; **Figure 5B**) did not significantly differ between type 1 and non-type 1 BrS patients. Moreover, markers incorporating both repolarization and conduction dispersion, such as $(T_{peak} - T_{end})$/QRS, $T_{peak} - T_{end}$/(QT × QRS) and $QRS_d \times (T_{peak} - T_{end})$/QRS were proposed for risk stratification (16, 17). However, type 1 and non-type 1 BrS patients showed similar $T_{peak} - T_{end}$/QRS (0.77 [0.62–0.87] vs. 0.77 [0.69–0.86]; $P = 0.89$; **Figure 5C**) and $T_{peak} - T_{end}$/(QRS × QT) (0.00074 [0.00034–0.00096] vs. 0.00073 [0.00048–0.00012] ms^{-1}; $P = 0.44$; **Figure 5D**).

In this study, we calculated dispersion of iCEB and iCEBc for the first time. This is based on the physiological findings that reentrant tachycardia may be due to higher spatial dispersion of excitation wavelength, which can predispose to unidirectional conduction block and reentry (18). Moreover, we quantified $(QRSd \times T_{peak} - T_{end})$/QRS for the first time, a parameter combining both dispersion of conduction and of repolarization. The present analysis found that type 1 BrS patients showed statistically indistinguishable iCEB dispersion (0.83 [0.49–1.14] vs. 0.61 [0.34–0.92]; $P = 0.09$; **Figure 6A**), iCEBc dispersion (0.93 [0.51–1.15] vs. 0.65 [0.39–0.96]; $P = 0.08$; **Figure 6B**) but significantly higher mean $(QRSd \times T_{peak} - T_{end})$/QRS (25 [19–44] vs. 19 [9–30] ms; $P = 0.03$; **Figure 6C**) compared to non-type 1 subjects.

DISCUSSION

The most important findings of this study are that parameters that measured the dispersion of conduction, repolarization or both processes across the three precordial leads, V1–V3, can distinguish patients with spontaneous type 1 Brugada from those with non-type 1 Brugada patterns. By contrast, the same parameters measured from a single lead only or their mean values were not significantly different between both groups.

Sudden cardiac death (SCD), frequently due to ventricular tachyarrhythmias, is a significant problem globally (19). Patients

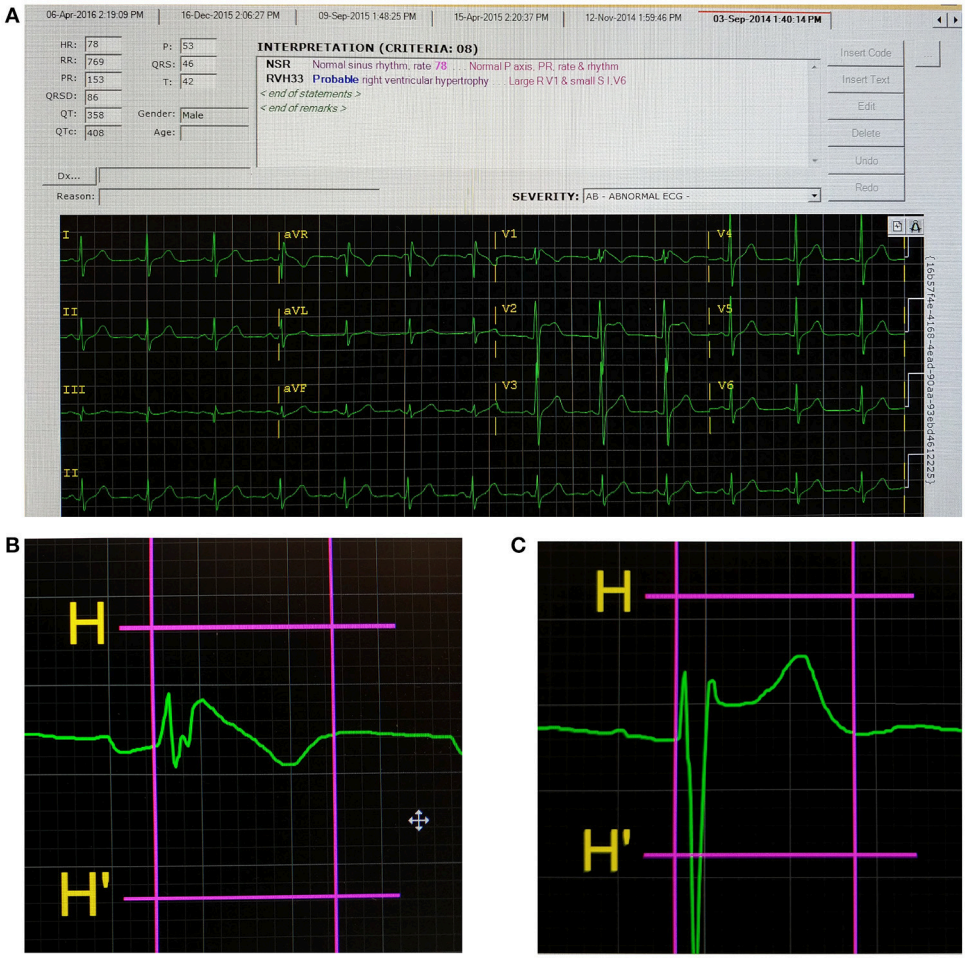

FIGURE 1 | Screenshot of the ECG analysis program **(A)**, a Type 1 Brugada pattern **(B),** and non-Type 1 Brugada pattern **(C)**. The first and second vertical lines indicate the onset of the QRS complex and end of the T-wave, respectively, for **(B,C)**.

with Brugada syndrome (BrS) have an increased risk of developing SCD (20, 21). However, it remains difficult to identify patients at the highest risk for developing these arrhythmias (22). Those with a type 1 pattern are thought to have higher risk of developing such adverse events compared with those with non-type 1 patterns (23–26). However, some investigators have reported that those with non-type 1 patterns, which can be converted to a type 1 pattern using drug challenge, are also at higher risks of ventricular arrhythmias (27).

Depolarization and Repolarization Hypotheses and Their ECG Markers

Generally, the mechanism of arrhythmogenesis in BrS have been broadly divided into the depolarization and repolarization hypotheses (12, 28–31). The depolarization hypothesis posits that delayed propagation of action potentials through the right ventricular outflow tract, can lead to reduction of excitation wavelength to induce reentry. By contrast, the repolarization hypothesis posits that differences in repolarization time-course either locally or across the myocardial wall, can create electrotonic currents during phase 2 of the cardiac action potential, leading to reentry (32, 33). It is likely that both mechanisms co-exist and contribute to arrhythmogenesis in BrS.

These findings provide insights into the different electrocardiographic markers that can be used for risk stratification (34, 35). Traditionally, repolarization markers such as QT interval (corrected, QT_c) have been widely used for this purpose. However they have a low sensitivity and specificity (36), given that ventricular arrhythmias can occur in the presence of a normal or even reduced QT interval (37). By contrast, depolarization or conduction markers such as QRS duration can also predict arrhythmic outcomes in BrS (4, 38).

Wavelength and Dispersion-Based Markers: Traditional and Novel Indices

Given the limitations of the above markers, recent interests have focused on the role of dispersion-based indices (18). Other markers include QT dispersion (QT_d) (6, 7), interval from the peak to the end of the T wave (8, 39, 40) [T_{peak} – T_{end},

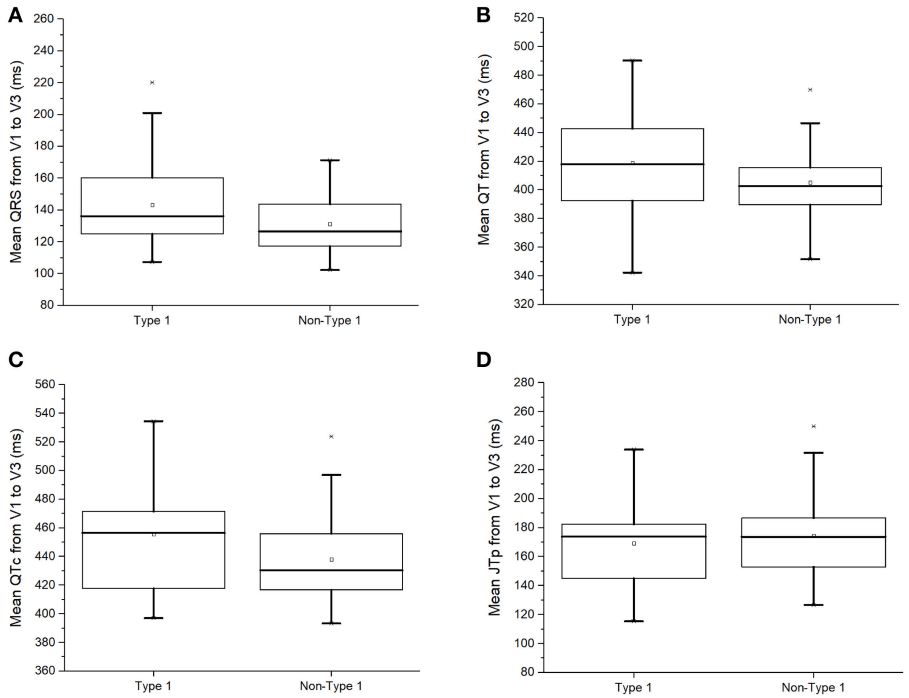

FIGURE 2 | QRS duration **(A)**, uncorrected QT interval **(B)**, corrected QT interval **(C),** or JT_{peak} interval **(D)** in type 1 and non-type 1 Brugada syndrome patients.

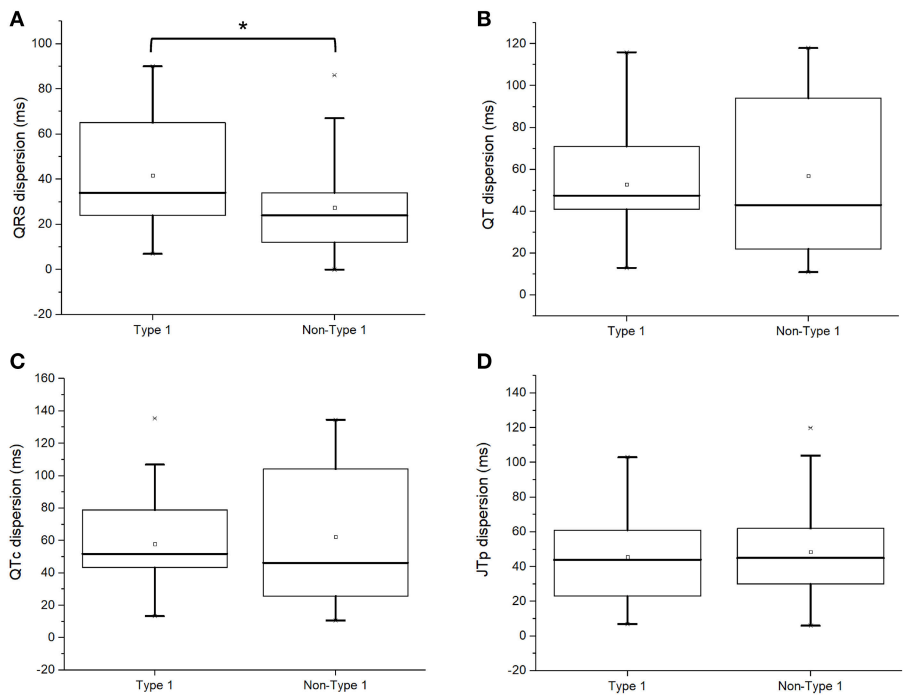

FIGURE 3 | QRS dispersion **(A)**, uncorrected QT dispersion **(B)**, corrected QT dispersion **(C)**, or JT_{peak} dispersion **(D)** in type 1 and non-type 1 Brugada syndrome patients. *Denotes significant difference between the two groups.

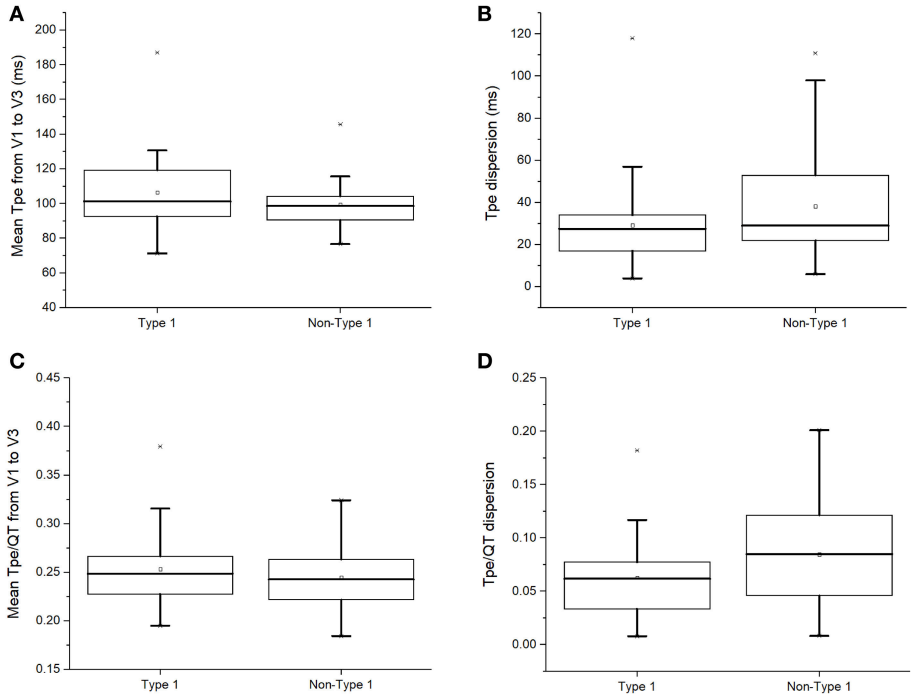

FIGURE 4 | $T_{peak} - T_{end}$ intervals **(A)**, $T_{peak} - T_{end}$ dispersion **(B)**, $T_{peak} - T_{end}$/QT ratios **(C),** or $T_{peak} - T_{end}$/QT dispersion **(D)** in type 1 and non-type 1 Brugada syndrome patients.

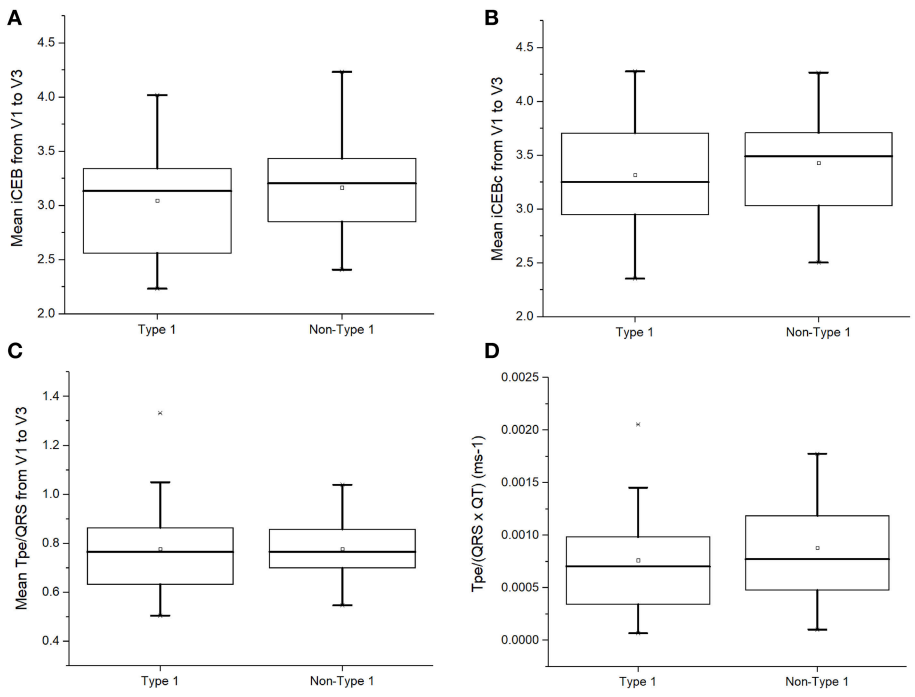

FIGURE 5 | Index of Cardiac Electrophysiological Balance (iCEB, QT/QRS) **(A)**, iCEB corrected for heart rate (QTc/QRS) **(B)**, $T_{peak} - T_{end}$/QRS **(C),** or $T_{peak} - T_{end}$/(QRS × QT) **(D)** in type 1 and non-type 1 Brugada syndrome patients.

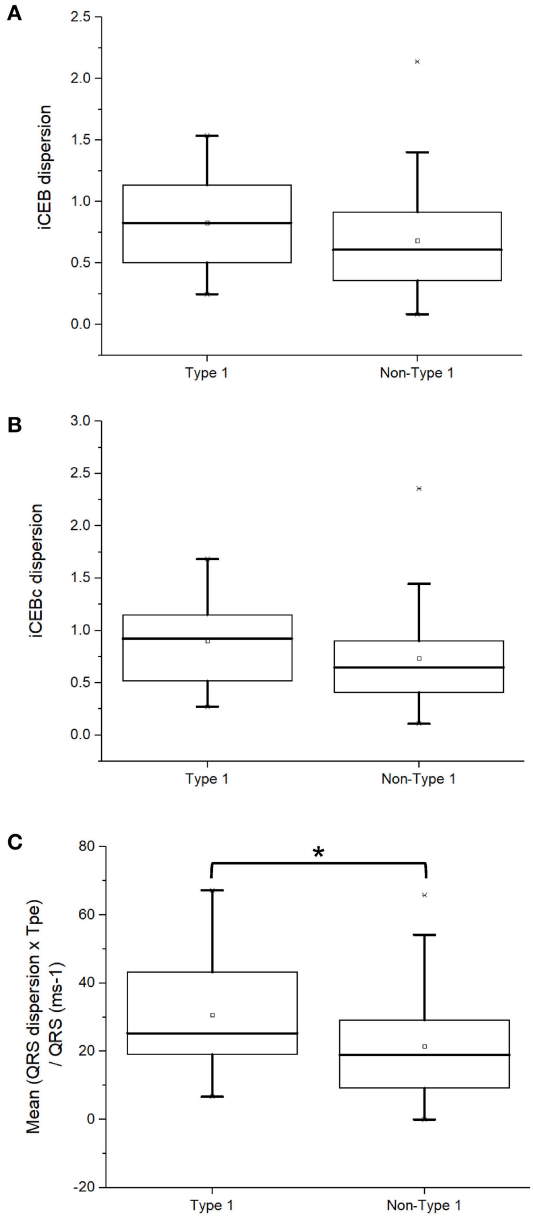

FIGURE 6 | iCEB dispersion **(A)**, iCEBc dispersion **(B)**, or (QRSd × T_{peak} – T_{end})/QRS **(C)** in type 1 and non-type 1 Brugada syndrome patients. *Denotes significant difference between the two groups.

reflecting transmural dispersion of repolarization, TDR (9)], and (T_{peak} – T_{end})/QT ratio (10). These markers stemmed from pre-clinical findings that higher spatial dispersion of repolarization can predispose to phase 2 reentry (41, 42). Although individual studies have reported the value for risk stratification, a recent study of 448 patients, which is the largest cohort to date, found no difference in this interval between subjects with VF/SCD and those who were asymptomatic (43). By contrast, QRS dispersion reflects spatial dispersion of CVs, increases in which can lead to unidirectional conduction block and reentry (44). Higher QRS dispersion (5) and increased fragmentation of the QRS complex (45, 46), have been associated with pro-arrhythmic outcomes in BrS patients.

Experiments from animal studies have demonstrated the importance of excitation wavelength, λ, given by the product of CV and refractory period, in determining arrhythmogenicity (47, 48). Thus, a decrease in either parameter reduces the length of the excitation wave, meaning that a higher number of re-entrant circuits can be accommodated in a given volume of myocardial tissue. However, λ must be determined by invasively with electrophysiological testing (49). This prompted Lu and colleagues to propose iCEB, the first electrocardiographic marker that serves as a good approximate of λ (14). This was subsequently shown to be decreased in BrS patients (15). Our study extends these findings by demonstrating that iCEB and iCEBc were similar between type 1 and non-type 1 BrS patients.

Given the observations that dispersion-based markers could provide additional value for arrhythmic risk stratification (36, 50), a number of indices incorporating repolarization and conduction dispersion have been proposed, namely T_{peak} – T_{end}/QRS, T_{peak} – T_{end}/(QT × QRS) and QRS_d × T_{peak} – T_{end}/QRS (16, 17). Recently, Robyns and colleagues found that T_{peak} – T_{end}/QRS or T_{peak} – T_{end}/(QT × QRS), like iCEB, were significantly different between control, BrS and long QT syndrome patients (51). However, data from Germany found no difference in either index between asymptomatic and symptomatic BrS patients (52). In our study, we found that both parameters did not significantly differ between type 1 and non-type 1 BrS patients. By contrast, we found significantly higher mean QRS_d × T_{peak} – T_{end}/QRS but similar iCEB and iCEBc dispersion parameters in type 1 compared to non-type 1 BrS patients. These findings therefore provide the evidence that higher dispersion of repolarization and conduction are found in type 1 BrS patients, which can potentially explain the higher incidence of ventricular arrhythmias and SCD than non-type 1 patients.

Limitations

Several limitations of this study are recognized. Firstly, this included a small cohort from a single center. These findings therefore need to be explored in larger cohorts. Secondly, this was a retrospective study that did not examine hard endpoints such as arrhythmic or mortality outcomes. It should be noted that our work is hypothesis-generating. Future studies can explore whether these novel dispersion-based electrocardiographic markers are useful for risk stratification in terms of arrhythmic or mortality outcomes.

CONCLUSIONS

This study provides electrocardiographic evidence that higher levels of dispersion in conduction and repolarization are found in type 1 than non-type 1 BrS patients. This may potentially explain the higher incidence of ventricular arrhythmias in the former group. Indices reflecting cumulative conduction and repolarization abnormalities may provide additional value for risk stratification.

AUTHOR CONTRIBUTIONS

GT: study conception, data acquisition, data analysis, statistical analysis, data interpretation, drafting of manuscript, critical revision of manuscript, creation of figures; response to reviewer comments. KHCL: data acquisition. WKKW and KPL: study conception and supervision. YX: revision of manuscript and response to reviewer comments. All authors: data analysis and interpretation, critical revision of manuscript.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Honarbakhsh S, Providencia R, Lambiase PD. Risk stratification in Brugada syndrome: current status and emerging approaches. *Arrhythm Electrophysiol Rev*. (2018) 7:79–83. doi: 10.15420/aer.2018.2.2
2. Lambiase PD, Ahmed AK, Ciaccio EJ, Brugada R, Lizotte E, Chaubey S, et al. High-density substrate mapping in Brugada syndrome: combined role of conduction and repolarization heterogeneities in arrhythmogenesis. *Circulation* (2009) 120:106–17, 101–104. doi: 10.1161/CIRCULATIONAHA.108.771401
3. Postema PG, Van Dessel PF, De Bakker JM, Dekker LR, Linnenbank AC, Hoogendijk MG, et al. Slow and discontinuous conduction conspire in Brugada syndrome: a right ventricular mapping and stimulation study. *Circ Arrhythm Electrophysiol*. (2008) 1:379–86. doi: 10.1161/CIRCEP.108.790543
4. Ohkubo K, Watanabe I, Okumura Y, Ashino S, Kofune M, Nagashima K, et al. Prolonged QRS duration in lead V2 and risk of life-threatening ventricular arrhythmia in patients with Brugada syndrome. *Int Heart J*. (2011) 52:98–102. doi: 10.1536/ihj.52.98
5. Hunuk B, Mugnai G, De Asmundis C, Velagic V, Saitoh Y, Cioconte G, et al. P2324A novel high risk ECG feature in Brugada syndrome probands: localized QRS prolongation on right precordial leads. *Eur Heart J*. (2017) 38:ehx502.P2324. doi: 10.1093/eurheartj/ehx502.P2324
6. Linker NJ, Colonna P, Kekwick CA, Till J, Camm AJ, Ward DE. Assessment of QT dispersion in symptomatic patients with congenital long QT syndromes. *Am J Cardiol*. (1992) 69:634–8. doi: 10.1016/0002-9149(92)90155-R
7. Elming H, Holm E, Jun L, Torp-Pedersen C, Kober L, Kircshoff M, et al. The prognostic value of the QT interval and QT interval dispersion in all-cause and cardiac mortality and morbidity in a population of Danish citizens. *Eur Heart J*. (1998) 19:1391–400. doi: 10.1053/euhj.1998.1094
8. Xia Y, Liang Y, Kongstad O, Holm M, Olsson B, Yuan S. Tpeak-tend interval as an index of global dispersion of ventricular repolarization: evaluations using monophasic action potential mapping of the epi- and endocardium in swine. *J Interv Card Electrophysiol*. (2005) 14:79–87. doi: 10.1007/s10840-005-4592-4
9. Gupta P, Patel C, Patel H, Narayanaswamy S, Malhotra B, Green JT, et al. T(p-e)/QT ratio as an index of arrhythmogenesis. *J Electrocardiol*. (2008) 41:567–74. doi: 10.1016/j.jelectrocard.2008.07.016
10. Castro Hevia J, Antzelevitch C, Tornes Barzaga F, Dorantes Sanchez M, Dorticos Balea F, Zayas Molina R, et al. Tpeak-Tend and Tpeak-Tend dispersion as risk factors for ventricular tachycardia/ventricular fibrillation in patients with the Brugada syndrome. *J Am Coll Cardiol*. (2006) 47:1828–34. doi: 10.1016/j.jacc.2005.12.049
11. Lambiase PD. Tpeak-Tend interval and Tpeak-Tend/QT ratio as markers of ventricular tachycardia inducibility in subjects with Brugada ECG phenotype. *Europace* (2010) 12:158–9. doi: 10.1093/europace/eup424
12. Meregalli PG, Wilde AA, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? *Cardiovasc Res*. (2005) 67:367–78. doi: 10.1016/j.cardiores.2005.03.005
13. Zareba W, Mcnitt S, Polonsky S, Couderc JP. JT interval: What does this interval mean? *J Electrocardiol*. (2017) 50:748–51. doi: 10.1016/j.jelectrocard.2017.07.019
14. Lu HR, Yan GX, Gallacher DJ. A new biomarker–index of cardiac electrophysiological balance (iCEB)–plays an important role in drug-induced cardiac arrhythmias: beyond QT-prolongation and Torsades de Pointes (TdPs). *J Pharmacol Toxicol Methods* (2013) 68:250–9. doi: 10.1016/j.vascn.2013.01.003
15. Robyns T, Lu HR, Gallacher DJ, Garweg C, Ector J, Willems R, et al. Evaluation of index of cardio-electrophysiological balance (iCEB) as a new biomarker for the identification of patients at increased arrhythmic risk. *Ann Noninvasive Electrocardiol*. (2016) 21:294–304. doi: 10.1111/anec.12309
16. Tse G, Wong CW, Gong MQ, Meng L, Letsas KP, Li GP, et al. Meta-analysis of T-wave indices for risk stratification in myocardial infarction. *J Geriatr Cardiol*. (2017) 14:776–9. doi: 10.11909/j.issn.1671-5411.2017.12.009
17. Tse G, Yan BP. Novel arrhythmic risk markers incorporating QRS dispersion: QRSd x (Tpeak - Tend)/QRS and QRSd x (Tpeak - Tend)/(QT x QRS). *Ann Noninvas Electrocardiol*. (2017) 22:e12397. doi: 10.1111/anec.12397
18. Robert E, Aya AGM, Coussaye JEDL, Péray P, Juan JM, Brugada J, et al. Dispersion-based reentry: mechanism of initiation of ventricular tachycardia in isolated rabbit hearts. *Am J Physiol Heart Circ Physiol*. (1999) 276:H413–23. doi: 10.1152/ajpheart.1999.276.2.H413
19. Zipes DP, Wellens HJ. Sudden cardiac death. *Circulation* (1998) 98:2334–51. doi: 10.1161/01.CIR.98.21.2334
20. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J Am Coll Cardiol*. (1992) 20:1391–6. doi: 10.1016/0735-1097(92)90253-J
21. Brugada J, Brugada R, Brugada P. Determinants of sudden cardiac death in individuals with the electrocardiographic pattern of brugada syndrome and no previous cardiac arrest. *Circulation* (2003) 108:3092–6. doi: 10.1161/01.CIR.0000104568.13957.4F
22. Tse G, Lai ET, Yeo JM, Yan BP. Electrophysiological mechanisms of Bayés syndrome: insights from clinical and mouse studies. *Front Physiol*. (2016) 7:88. doi: 10.3389/fphys.2016.00188
23. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm*. (2013) 10:1932–63. doi: 10.1016/j.hrthm.2013.05.014
24. Letsas KP, Liu T, Shao Q, Korantzopoulos P, Giannopoulos G, Vlachos K, et al. Meta-analysis on risk stratification of asymptomatic individuals with the brugada phenotype. *Am J Cardiol*. (2015) 116:98–103. doi: 10.1016/j.amjcard.2015.03.044
25. Bayoumy A, Gong MQ, Christien Li KH, Wong SH, Wu WK, Li GP, et al. Spontaneous type 1 pattern, ventricular arrhythmias and sudden cardiac death in Brugada syndrome: an updated systematic review and meta-analysis. *J Geriatr Cardiol*. (2017) 14:639–43. doi: 10.11909/j.issn.1671-5411.2017.10.010
26. Letsas KP, Asvestas D, Baranchuk A, Liu T, Georgopoulos S, Efremidis M, et al. Prognosis, risk stratification, and management of asymptomatic individuals with Brugada syndrome: a systematic review. *Pacing Clin Electrophysiol*. (2017) 40:1332–45. doi: 10.1111/pace.13214
27. Delise P, Allocca G, Sitta N, Migliore F, Dagradi F, Spazzolini C, et al. Cardiac arrest and Brugada syndrome: Is drug-induced type 1 ECG pattern always a marker of low risk? *Int J Cardiol*. (2018) 254:142–5. doi: 10.1016/j.ijcard.2017.10.118
28. Wilde AA, Postema PG, Di Diego JM, Viskin S, Morita H, Fish JM, et al. The pathophysiological mechanism underlying Brugada syndrome:

depolarization versus repolarization. *J Mol Cell Cardiol.* (2010) 49:543–53. doi: 10.1016/j.yjmcc.2010.07.012
29. Tokioka K, Kusano KF, Morita H, Miura D, Nishii N, Nagase S, et al. Electrocardiographic parameters and fatal arrhythmic events in patients with Brugada syndrome: combination of depolarization and repolarization abnormalities. *J Am Coll Cardiol.* (2014) 63:2131–8. doi: 10.1016/j.jacc.2014.01.072
30. Tse G, Wong ST, Tse V, Yeo JM. Depolarization vs. repolarization: what is the mechanism of ventricular arrhythmogenesis underlying sodium channel haploinsufficiency in mouse hearts? *Acta Physiol.* (2016) 218:234–5. doi: 10.1111/apha.12694
31. Nademanee K, Wilde AAM. Repolarization versus depolarization defects in Brugada syndrome. a tale of two different electrophysiologic settings? *JACC Clin Electrophysiol.* (2017) 3:364–66. doi: 10.1016/j.jacep.2017.01.011
32. Antzelevitch C. Ion channels and ventricular arrhythmias: cellular and ionic mechanisms underlying the Brugada syndrome. *Curr Opin Cardiol.* (1999) 14:274–9. doi: 10.1097/00001573-199905000-00013
33. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. *Circulation* (1999) 100:1660–6. doi: 10.1161/01.CIR.100.15.1660
34. Tse G, Yan BP. Traditional and novel electrocardiographic conduction and repolarization markers of sudden cardiac death. *Europace* (2017) 19:712–21. doi: 10.1093/europace/euw280
35. Asvestas D, Tse G, Baranchuk A, Bazoukis G, Liu T, Saplaouras A, et al. High risk electrocardiographic markers in Brugada syndrome. *IJC Heart Vasculat.* (2018) 18:58–64. doi: 10.1016/j.ijcha.2018.03.001
36. Castro-Torres Y, Carmona-Puerta R, Katholi RE. Ventricular repolarization markers for predicting malignant arrhythmias in clinical practice. *World J Clin Cases* (2015) 3:705–20. doi: 10.12998/wjcc.v3.i8.705
37. Hondeghem LM. QTc prolongation as a surrogate for drug-induced arrhythmias: fact or fallacy? *Acta Cardiol.* (2011) 66:685–9. doi: 10.1080/AC.66.6.2136950
38. Morita H, Miyamoto M, Watanabe A, Tsukuda S, Morimoto Y, Kawada S, et al. Progression of ECG abnormalities associated with initial ventricular fibrillation in asymptomatic patients with Brugada Syndrome. *Heart Rhythm.* (2018). doi: 10.1016/j.hrthm.2018.06.035. [Epub ahead of print].
39. Tse G, Gong M, Wong WT, Georgopoulos S, Letsas KP, Vassiliou VS, et al. The Tpeak - Tend interval as an electrocardiographic risk marker of arrhythmic and mortality outcomes: a systematic review and meta-analysis. *Heart Rhythm.* (2017) 14:1131–7. doi: 10.1016/j.hrthm.2017.05.031
40. Tse G, Gong M, Li CKH, Leung KSK, Georgopoulos S, Bazoukis G, et al. Tpeak – Tend, Tpeak – Tend/QT ratio and Tpeak – Tend dispersion for risk stratification in Brugada Syndrome: a systematic review and meta-analysis. *J Arrhythmia* (2018). doi: 10.1002/joa3.12118. [Epub ahead of print].
41. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, et al. Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation* (2005) 111:659–70. doi: 10.1161/01.CIR.0000152479.54298.51
42. Antzelevitch C, Sicouri S, Di Diego JM, Burashnikov A, Viskin S, Shimizu W, et al. Does Tpeak-Tend provide an index of transmural dispersion of repolarization? *Heart Rhythm.* (2007).4:1114–6. doi: 10.1016/j.hrthm.2007.05.028
43. Mugnai G, Hunuk B, Hernandez-Ojeda J, Stroker E, Velagic V, Ciconte G, et al. Role of electrocardiographic Tpeak-Tend for the prediction of ventricular arrhythmic events in the brugada syndrome. *Am J Cardiol.* (2017) 120:1332–7. doi: 10.1016/j.amjcard.2017.07.014
44. Tse G, Yeo JM. Conduction abnormalities and ventricular arrhythmogenesis: the roles of sodium channels and gap junctions. *Int J Cardiol Heart Vasc.* (2015) 9:75–82. doi: 10.1016/j.ijcha.2015.10.003
45. Meng L, Letsas KP, Baranchuk A, Shao Q, Tse G, Zhang N, et al. Meta-analysis of Fragmented QRS as an electrocardiographic predictor for arrhythmic events in patients with brugada syndrome. *Front Physiol.* (2017) 8:678. doi: 10.3389/fphys.2017.00678
46. Morita H, Watanabe A, Kawada S, Miyamoto M, Morimoto Y, Nakagawa K, et al. Identification of electrocardiographic risk markers for the initial and recurrent episodes of ventricular fibrillation in patients with Brugada syndrome. *J Cardiovasc Electrophysiol.* (2018) 29:107–14. doi: 10.1111/jce.13349
47. Choy L, Yeo JM, Tse V, Chan SP, Tse G. Cardiac disease and arrhythmogenesis: Mechanistic insights from mouse models. *Int J Cardiol Heart Vascul.* (2016) 12:1–10. doi: 10.1016/j.ijcha.2016.05.005
48. Tse G, Wong ST, Tse V, Yeo JM. Determination of action potential wavelength restitution in Scn5a(+/-) mouse hearts modelling human Brugada syndrome. *J Geriatr Cardiol.* (2017) 14:595–6. doi: 10.11909/j.issn.1671-5411.2017.09.011
49. Tse G. Both transmural dispersion of repolarization and of refractoriness are poor predictors of arrhythmogenicity: a role for iCEB (QT/QRS)? *J Geriatr Cardiol.* (2016) 13:813–4. doi: 10.11909/j.issn.1671-5411.2016.09.007
50. Tse G, Wong ST, Tse V, Yeo JM. Variability in local action potential durations, dispersion of repolarization and wavelength restitution in aged wild-type and Scn5a+/- mouse hearts modeling human Brugada syndrome. *J Geriatr Cardiol.* (2016) 13:930–1. doi: 10.1-1909/j.issn.1671-5411.2016.11.009
51. Robyns T, Lu HR, Gallacher DJ, Garweg C, Ector J, Willems R, et al. Response of Robyns to the Tse's letter to editor. *Ann Noninvas Electrocardiol.* (2017) 22:e12515. doi: 10.1111/anec.12515
52. Zumhagen S, Stallmeyer B, Eckardt L, Schulze-Bahr E. (Tpeak – Tend)/QRS and (Tpeak – Tend)/(QT × QRS) as risk markers in Brugada syndrome: authors' reply. *EP Europace* (2017) 19:696–7. doi: 10.1093/europace/euw210

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REVIEW
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A New Cardiac Channelopathy: From Clinical Phenotypes to Molecular Mechanisms Associated With $Na_V1.5$ Gating Pores

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Voltage gated sodium channels (Na_V) are broadly expressed in the human body. They are responsible for the initiation of action potentials in excitable cells. They also underlie several physiological processes such as cognitive, sensitive, motor, and cardiac functions. The $Na_V1.5$ channel is the main Na_V expressed in the heart. A dysfunction of this channel is usually associated with the development of pure electrical disorders such as long QT syndrome, Brugada syndrome, sinus node dysfunction, atrial fibrillation, and cardiac conduction disorders. However, mutations of $Na_V1.5$ have recently been linked to the development of an atypical clinical entity combining complex arrhythmias and dilated cardiomyopathy. Although several $Na_V1.5$ mutations have been linked to dilated cardiomyopathy phenotypes, their pathogenic mechanisms remain to be elucidated. The gating pore may constitute a common biophysical defect for all $Na_V1.5$ mutations located in the channel's VSDs. The creation of such a gating pore may disrupt the ionic homeostasis of cardiomyocytes, affecting electrical signals, cell morphology, and cardiac myocyte function. The main objective of this article is to review the concept of gating pores and their role in structural heart diseases and to discuss potential pharmacological treatments.

Keywords: gating pore current, SCN5A, $Na_V1.5$, dilated cardiomyopathy, cardiomyocytes, cardiac arrhythmias

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INTRODUCTION

Cardiovascular diseases are the single most common cause of death worldwide, and sudden deaths due to cardiac arrhythmias account for ∼50% of these deaths (1). Heart failure (HF) is a major public health problem in industrialized countries, in particular because of its frequency and its consequences in terms of morbidity and mortality (2). The financial costs of heart failure (HF) are substantial and are increasing constantly due to higher healthcare costs, improved therapies that extend life expectancy, and an aging population.

Dilated cardiomyopathy (DCM) is the most common cause of HF in North America. It induces the dilatation of cardiac cavities and impairs contractility and systolic function (3). It accounts for over 90% of all cardiomyopathy cases referred to specialized centers and is collectively the most common reason for heart transplants in the young (4, 5). Familial or genetically related DCM make up 20 to 30% of DCM cases. Most genes associated with DCM encode structural

proteins involved in contractile function and the cytoskeletal matrix. Mutations in genes encoding these proteins are believed to diminish the overall structural integrity of cells, leading to myocyte disarray, the development of fibrosis characteristic of DCM, and myocyte death (3, 6). Sodium (SCN5A), and potassium (ABCC9, K_{ATP}) channel regulation defects have also been associated with the development of DCM, which argues for an alternative disease mechanism of dilatation-induced remodeling that is mainly driven by a dysfunction in an electrical excitability component rather than a primary structural defect (6, 7).

The development of effective drugs has markedly improved the prognosis of patients with HF. Four therapeutic classes have demonstrated efficacy in the management of HF. These include angiotensin-converting enzyme inhibitors such as enalapril, Angiotensin Receptor blockers such as losartan, aldosterone inhibitors such as spironolactone, and beta-blockers such as carvedilol, metoprolol, and bisoprolol (8). A combination of different pharmacological treatments may help limit the pathological remodeling responsible for the evolution of the disease. In addition to the long-term treatment of HF, diuretics are prescribed to limit the appearance of edema (9).

The purpose of this review is to explore the mechanisms involved in *SCN5A* mutations linked to DCM, with a focus on their role in generating gating pore currents as a potentially unifying molecular mechanism.

VOLTAGE-GATED Na^+ CHANNELS

Voltage-gated Na^+ channels are transmembrane proteins that play a critical role in action potential (AP) initiation and propagation in many excitable cells and thus constitute the driving force for generating electrical impulses. The dysfunction of voltage-gated Na^+ channels has been reported to affect activity in skeletal muscle, the heart, and the nervous system, causing a variety of diseases such as paralysis, cardiac arrhythmic disorders such as disturbances in cardiac conduction (10), type 3 long QT syndrome (11), Brugada syndrome (BrS) (12), cardiac conduction defect (CCD) (13), pain, and epilepsy (14). Na^+ channels are composed one α-subunit (260 kDa) associated with one or more accessory β-subunits (β_1-β_4) (15). Channel function and kinetics are primarily driven by pore-forming α-subunits and are modulated by β-subunits. All Na^+ channel α-subunits comprise four homologous domains (DI-DIV), each of which contains six transmembrane segments (S1-S6). S1-S4 form the voltage sensor domain and S5-S6 form the pore domain, with a hairpin-like P-loop located between S5 and S6 (16, 17). The short linkers connecting S5 and S6 form the outer narrow mouth of the pore and the selectivity filter, while the inner wider pore is formed by the S5 and S6 segments. The S4 segments in each voltage sensor domain contain positively charged amino acid residues that act as gating charges and move across the membrane to trigger channel activation in response to membrane depolarization (18). The short intracellular cytoplasmic loop connecting homologous domains III and IV acts as the inactivation gate, which bends back into the channel and blocks the pore from the intracellular side during sustained depolarization of the membrane. The inactivation gate is located in the center of a three-amino-acid stretch consisting of isoleucine, phenylalanine, and methionine (IFM) (19). Residues of the S6 segments in each domain provide the binding site for local anesthetics and link the internal vestibule (20). The α-subunit is the major component of the channel. In a heterologous expression system, it recapitulates all the wilde type channel's main biophysical properties (16).

CARDIAC MUSCLE Na^+ CHANNEL LEGACY

The *SCN5A* gene encodes the cardiac Na^+ channel known as $Na_V1.5$, a member of an evolutionarily highly conserved family of voltage-gated ion channels. The *SCN5A* gene is located on chromosome 3p21 and was initially called hH1 for human heart Na^+ channel 1 (16). $Na_v1.5$ is the main Na^+ channel expressed in the heart. It is also present at high levels in the piriform cortex (larger part of the olfactory system) and subcortical limbic nuclei (21). $Na_V1.5$ is much more TTX-resistant than skeletal muscle or central nervous system sodium channels, requiring much higher concentrations of TTX (micromolar concentrations) to be inhibited. This relative resistance is due to the presence of certain amino acid residues, in particular a cysteine instead of an aromatic residue in the P-region of DI (22, 23). On the other hand, $Na_v1.5$ is more sensitive to inhibition by local anesthetics such as lidocaine and antiarrhythmic agents than peripheral nervous system (PNS) channels and has a more negative voltage-dependence of inactivation than PNS channels (16, 24). Mutations in *SCN5A* have been primarily associated with pure arrhythmic disorders such as long QT syndromes (LQTs), Brugada syndrome (BrS), atrial fibrillation (AFib), progressive cardiac conduction defect (PCCD), and sinus node dysfuction (SND), all of which are inherited cardiac diseases. The most common phenotypes caused by mutations in *SCN5A* are LQTS type 3 (LQT3) (25) and BrS (26). Both syndromes are diagnosed by irregularities on surface ECGs, with no apparent structural heart abnormalities, and can lead to malignant ventricular arrhythmias or even sudden cardiac death (SCD). The different clinical and ECG phenotypes of LQT3 and BrS arise from opposing specific alterations in the biophysical mechanisms associated with cardiac Na^+ channel dysfunction. LQT3 is caused by *SCN5A* mutations that result in a gain of channel function, a disruption in fast inactivation, and the appearance of a persistent Na^+ current. A gain of function consists of a higher quantity of Na^+ flowing through the channel during a stimulation. In contrast, BrS is caused by a loss of channel function, and thus a lower amount of Na^+ flowing through the channel during a stimulation (27, 28). In addition to BrS and LQT3, *SCN5A* variants have also been associated with PCCD. Like BrS, PCCD variants result in a loss of function of Na^+ channels. PCCD and BrS loss-of-function phenotypes are closely related as shown by the fact that three of the six known PCCD variants are also associated with BrS. Only a few *SCN5A* mutations are known to cause such mixed phenotypes, which are

purely electrical in nature, with no structural abnormalities (29, 30). Bezzina et al. described the first *SCN5A* mutation (1795insD) that caused both BrS and LQTS in the same affected individuals of a large family (29). The biophysical characterization revealed balanced defects, with mutated channels displaying both gain and loss of function.

However, mutations in *SCN5A* do not just lead to pure arrhythmic disorders. They can also be associated with structural heart diseases. Distinct cardiac phenotypes caused by *SCN5A* mutations have also been described, including SND and conduction disorder associated with DCM. It is not well understood how a dysfunction in electrical excitability through altered Na^+ channel function may underlie the manifestation of dilatation remodeling and DCM.

The first report linking Na^+ channel dysregulation to the etiology of DCM was published in 2004 by McNair and coworkers, while the same mutation was previously published in 2003 but without any cardiac dilatation phenotype (7, 31). A missense mutation in *SCN5A* (D1275N) was associated with a dilatation phenotype in a pedigree characterized by cardiac arrhythmias and sudden death (7). Echocardiographic data indicated cardiac dilatation in the carriers. Of note, among the 8 affected family members, 3 also demonstrated allelic variations in the promoter region and first exon of the *Cx40 gene*. In 2003, the electrophysiological characterization of the mutant Na^+ channels using the *Xenopus* oocyte expression system revealed enhanced channel activation (31). In 2005, letters from both teams further hypothesized that the dilation observed could also have been caused by a combination of modifier genes, or environmental or unknown factors acting in conjunction with the primary Na^+ ion conduction defect (32). In a more recent study of a cohort of 338 DCM patients, McNair et al. estimated that a dysfunction of $Na_v1.5$ proteins causes 1.7% of familial DCM cases (33). Indeed, the *SCN5A* gene is ranked as the sixth most common cause of familial DCM (3). To date, 12 *SCN5A* mutations have been linked to complex arrhythmia disorders and DCM, including the R219H mutation recently reported by our group (34). Interestingly, nine of these mutations involve highly conserved residues on the VSD, mainly on the S3 and S4 transmembrane segments, which play a pivotal role in channel activation (33). VSD mutations have been implicated in generating leak currents known as gating pore currents or omega currents in neuromuscular disorders (35). Intriguingly, it has recently been shown that *SCN5A* mutations in patients with DCM combined with complex arrhythmias have either gain and/or loss of function biophysical phenotypes when explored in a heterologous expression system (36) (see **Figure 1** for a summary of the locations and biophysical properties of these mutants). However, at this juncture, it is unclear which mechanism is involved in the *SCN5A*-linked pathogenesis of DCM. Gating pore currents are cation currents that selectively flow through the mutated VSDs of Na^+ channels and their biophysical properties are directly related to the movement of the voltage sensor. These currents do not reflect pore activity since pore blockers such as tetrodotoxin (TTX) do not affect them. Similar H^+ channels can be formed by replacing the most positively charged arginine residue of the *Drosophila Shaker* voltage-gated K^+ channel with a histidine (37). Our central hypothesis is that mutation-induced gating pore currents through the $Na_v1.5$ VSD may underlie the biophysical phenotype in DCM.

To better understand the complex relationship of *SCN5A*-linked DCM mutations, Watanabe et al. created a humanized mouse model that harbors the D1275N *SCN5A* mutation. They concluded that the D1275N variant is a pathological mutation that causes conduction slowing, arrhythmias, and DCM phenotypes (38). However, this is not representative of *SCN5A*-linked DCM mutations that are present on different $Na_V1.5$ domains, and it fails to explain the molecular mechanisms underlying DCM phenotypes.

WHAT IS A GATING PORE?

The very first instance of ions flowing directly through the VSD of a voltage sensitive ion channel was reported in 2004 by Starace and Bezanilla (37). At the time, the authors were focused on describing the structure and function of VSDs (39). They showed that the substitution of the first arginine (R1) of the S4 segment (R1/S4) by a histidine led to an aberrant H^+-specific current (37). As this H^+ flow was not sensitive to the block of the physiological pore of the protein, the authors concluded that ions did not pass through this structure but rather directly through the VSD (37). This concept was rapidly extended to the substitution of R1/S4 by other amino acids such as alanine (A), cysteine (C), serine (S), and valine (V) (40). The newly created current was not specific to H^+ but was cation selective. The location of the permeation pathway was further refined using the combination of the R1C mutation and MTSET. The gating pore current was blocked by the addition of MTSET, which forms disulfide bonds with cysteines, thus confirming the location of the permeation pathway (40). At this point, the newly created permeation pathway was called an omega pore in opposition to the physiological alpha pore of the channel protein. The initial arginines of the S4 segment were thought to naturally obstruct this unusual permeation pathway. Their substitution with "smaller" amino acids would thus leave a gap, allowing the permeation of ions. All these experiments were performed using the *Drosophila Shaker* voltage-gated K^+ channel. In contrast, a gating pore current in a mammalian voltage gated Na^+ channel ($Na_v1.2$) was first observed in the setting of a double S4-R1G/R2G substitution in DII (41). Besides the double substitution, the resulting current was of small amplitude. Indeed, due to the monomeric nature of Na_v channels, each channel presents only a single gating pore. On the other hand, the tetrameric nature of K_v channels leads to four identical gating pores for each functional channel.

The biophysical properties of gating pores influence the ion flow. Most of the gating pores that have been described are created by the substitution of S4 arginines. Due to their location, the biophysical properties of gating pores are intimately linked to the function of VSDs. As previously mentioned, VSDs are made up of four transmembrane segments (S1-S4) containing two structures: the positively charged S4 segment and the surrounding stabilizing S1-S3 segments (16, 17, 42, 43).

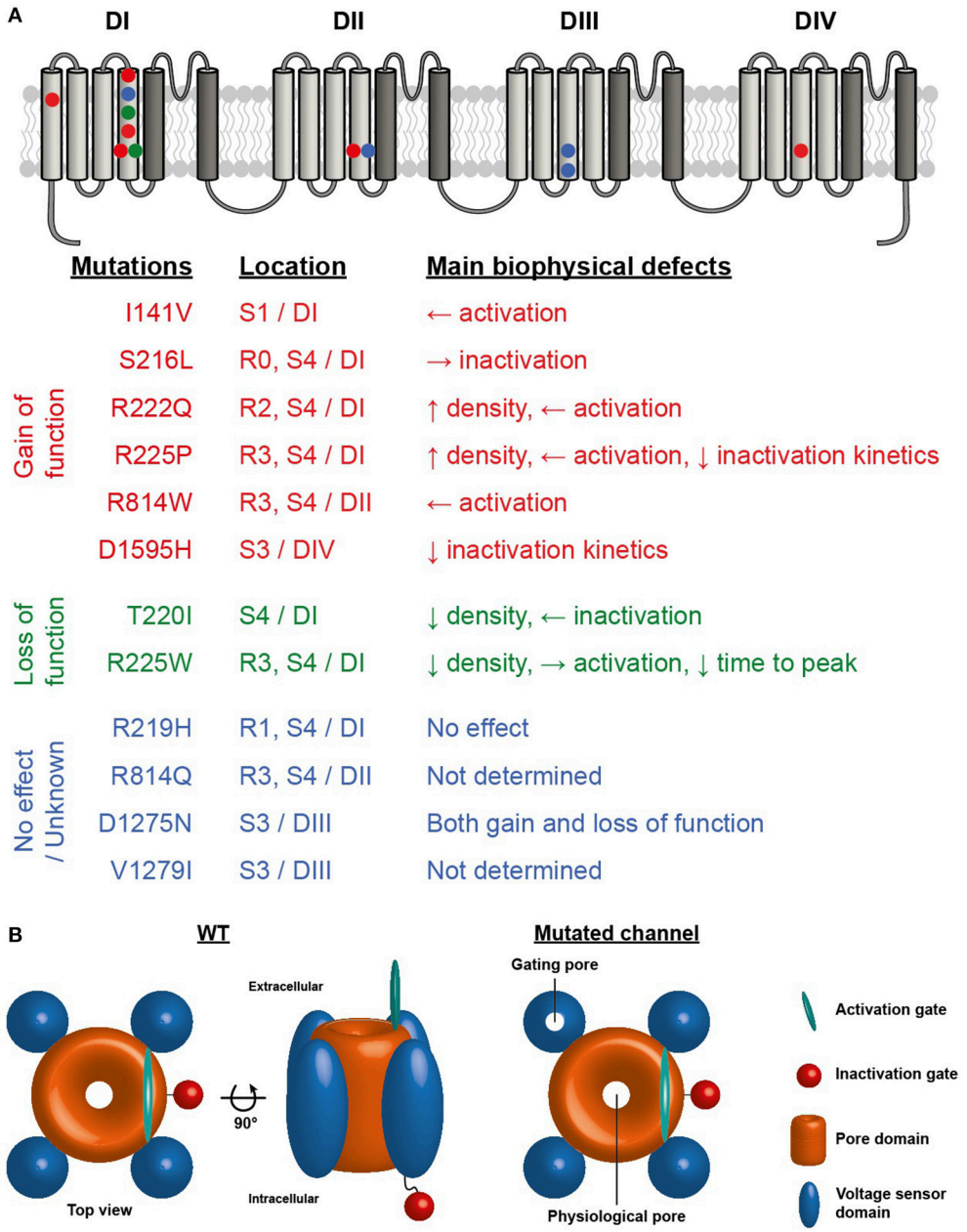

FIGURE 1 | (A) Structure of $Na_V1.5$ voltage-gated sodium channel illustrating the four homologous domains of the channel. The VSD formed by the S1-S4 segments of each domain are represented by light gray segments. The dark gray segments represent the pore domain of the channel. Symbols indicate the locations of the $Na_V1.5$ VSD mutations associated with the development of cardiac arrhythmias and DCM. Red symbols mutation reported to cause gain of function, green symbols loss of function and blue symbols no effect or no known effect on the channel. **(B)** Schematic representation of $Na_V1.5$ sodium channel illustrating the central pore and the presence of a gating pore in one of the VSDs.

Stabilization is notably ensured by the gating charge transfer center (GCTC), a specific arrangement of two negatively charged residues on S2 and S3 and an aromatic amino acid on S2 (43). In voltage gated ion channels, the VSD is the structure responsible for sensing changes in membrane potential. During a depolarization, the S4 segment undergoes a large outward movement in which each S4 arginine sequentially interacts with the GCTC (44–46). This charge movement can be monitored as a function of membrane voltage and gives the Q-V curve, which describes the two main stable states of the VSD: the resting and the activated states. In the resting state (hyperpolarized voltages), the S4 uppermost arginines interact with the GCTC while, in the activated state (depolarized voltages), the S4 innermost arginines preferentially interact with the GCTC (44–46). In WT VSDs,

tight interactions between the S4 arginines and the GCTC create hydrophobic septa that isolate water crevices on both sides of the membrane, ensuring a non-permeable VSD (47–49). Gating pores are created by the disruption of interactions between the S4 and the GCTC, leading to the junction of the water crevices (50–52). The recently reported crystal structure of WT and mutated bacterial Na_vAb channels have provided support for this experimental and molecular dynamic simulation-based hypothesis (49). In their study, after measuring the gating pores created by mutating the second and third S4 arginines, Jiang et al. created and studied the corresponding crystal structures (49). Their study thus provides a strong basis for explaining the atomic mechanism underlying the creation of gating pores. As such, mutations affecting the uppermost S4 arginines disrupt interactions when the VSD is in its resting state, and the permeation pathway allows ions to flow at hyperpolarized voltages (34, 49, 50). On the other hand, mutations affecting the innermost S4 arginines disrupt interactions in the activated state, leading to gating pore currents at depolarized voltages (49, 52, 53). Opening probabilities for gating pores thus depend on the Q-V of the mutated VSD.

Gating pores are permeation pathways located directly inside VSDs, a usually non-conductive structure. Consequently, unlike physiological alpha pores, gating pores do not benefit from dedicated specific selectivity filters. However, two main subtypes of gating pores can be distinguished: (i) cation-selective and (ii) H^+-specific gating pores (34, 35, 37, 39–41, 50, 54–61). Cation-selective gating pores are created by the substitution of S4 arginines for amino acids other than histidine. In this setting, based on published selectivity sequences, large cations (below the exclusion size) preferentially flow through the gating pore (35, 40, 52, 60–62). Anions are excluded because of the lack of a positive charge due to the arginine substitution (63, 64). H^+-specific gating pore currents can be considered as special cases as they are related to the substitution of S4 arginines by histidines. Histidine is the only natural amino acid with a pKa of 6.5. Consequently, at physiological pH, histidine can link and release H^+. Interestingly, in two independent studies, half of the maximal measured gating pore current was observed at pH 6.48 and 6.5, values very close to the pKa of histidine (34, 37). In the specific case of R-to-H substitutions, H^+ permeation occurs through a "*Grotthus hopping*" mechanism where a H^+ is linked to histidine while another is released at the opposite side (37).

Lastly, gating pores can display different voltage dependence and ion selectivity depending on the nature of the mutation and its location in the VSD. S1 and S3 mutations such as I141V (S1/DI), D1275N (S3/DIII), V1279I (S3/DIII), and D1595H (S3/DIV) would be expected to open a permeation pathway since creating a gating pore relies on the disruption of interactions between S4 and the GCTC. However, very few gating pores generated by mutations outside the S4 segment have been described (54, 65). Further work on S1-S3 mutations is clearly required to better understand the biophysical properties of potentially novel permeation pathways.

DOWNSTREAM CONSEQUENCES OF GATING PORES

The cardiac consequences of gating pores remain a matter of debate given that no specific studies have explored this issue to date. Cardiac defects potentially caused by an omega current remain hypothetical. Such studies would require a mutation that does not affect the alpha pore properties of the channel in order to properly isolate defects linked solely to the gating pore. Due to the low amplitude of gating pore currents, their pathological nature is often questioned. However, two major observations argue in favor of gating pores being truly deleterious: (i) gating pores in $Na_v1.4$ and $Ca_v1.1$ are commonly accepted as the cause of hypokalemic/normokalemic periodic paralysis (35, 55, 58, 59, 66–68), and (ii) despite an amplitude that is comparable to omega currents, persistent currents related to $Na_v1.5$ LQT3 mutations are also commonly recognized as the original cause of LQT3 syndrome (69, 70). In fact, the small amplitude of gating pore currents appears to be compensated by the time during which the aberrant permeation pathway is in a conductive state. $Na_v1.5$/S216L, R219H, and T220I mutations affect the outermost S4 residues and have been associated with the development of arrhythmias and DCM (34, 51, 71, 72). Based on their location ($Na_v1.5$/S216L, T220I) and on experimental results ($Na_v1.5$/ R219H), these mutations open (or are expected to open) a gating pore at hyperpolarized voltages. Na^+ or H^+ ions (depending on the mutation) would thus flow during diastole as soon as the VSDs are in their resting state (34, 50). On the other hand, $Na_v1.5$ S4 mutations associated with DCM such as $Na_v1.5$/R222Q, R225W, R225P, R225Q, R814Q, and R814W affect the intermediate or innermost S4 residues (52, 53). The resulting gating pore is mainly opened (or expected to be open) under depolarized conditions (52, 53). However, the biophysical properties of gating pores that open at depolarized voltages appear slightly more complex. Indeed, ions flow as soon as the VSDs are in their activated state (during systole) but, due to the relaxation process of the S4 segment after prolonged depolarizations (several hundred of milliseconds), the gating pores remain temporarily conductive at hyperpolarized voltages. This leads to a major K^+ outflow at depolarized potentials and a transient Na^+ inflow at hyperpolarized voltages (52, 53, 62).

Gating pores in all configurations are thought to induce a global Na^+ overload. This process has been observed directly in patients suffering from HypoPP by Na^+ magnetic resonance imaging (67). In the case of H^+-specific gating pore currents, the Na^+ overload relies on the Na^+/H^+ exchanger working to attenuate the intracellular acidification caused by the increased H^+ concentration. The Na^+ overload is thought to lead to a Ca^{2+} overload by way of the Na^+/Ca^{2+} exchanger, suggesting that it has a major impact on cellular ionic homeostasis (73). Such Ca^{2+} overload could also be pro-arrhythmogenic by it-self. This ionic unbalance is known to block inward rectifier potassium channels (K_{ir} channels). K_{ir} have been described to play a major role in setting the resting membrane potential (V_{Rest}) and also affect the duration of APs (74, 75). While the effect of their blockade should be further studied, it could depolarize the

V_{Rest} and potentially lengthen APs, establishing a highly pro-arrhythmogenic substrate. Furthermore, despite its limited effect, Ca^{2+} has also been described as modulating the rectification of I_K channels thus potentially participating to the AP lengthening (76). K_{ir} blockade most probably resulting in depolarized V_{Rest} has also been described in the pathogenic process of HypoPP and has been attributed to a gating pore current (58, 67, 77, 78). Both cellular acidification and a Ca^{2+} overload impair connexin coupling and thus cell-cell conduction (79–81), further decreasing the conduction velocity. Furthermore, most of $Na_v1.5$ mutations linked to DCM also demonstrate primary biophysical defects (gain or loss of function). Taken together, these primary biophysical defects and gating pores most probably explain the conduction disorders that are often observed in patients carrying $Na_v1.5$ mutations and suffering from complex arrhythmias associated with DCM (33, 34, 82–84). Cellular acidification is strongly suspected in the case of H^+-specific gating pore currents. Based on studies performed in different contexts, cytoplasm acidification has been reported to lengthen APs (85). Interestingly, the opposite process (increase in intracellular pH) has recently been reported to induce AP shortening (86). The proposed cardiac consequences likely constitute a highly pro-arrhythmic substrate most probably participating in the development of electrical dysfunctions reported in affected patients. Interestingly, to get further insights in cardiac electrical effect of a gating pore *in-silico* modeling experiments could reveal to be highly valuable. However, so far, despite the availability of several models of cardiac cellular electrophysiology, further studies are required to develop such insightful adaptations.

In addition to electrical dysfunctions, ionic homeostasis imbalances have been reported to dramatically affect structural protein function. For example, intracellular acidosis decreases the affinity of troponin C for calcium, resulting in excitation-contraction impairment (87). Furthermore, a Ca^{2+} overload leads to partial cardiomyocyte decrease in force contraction and impaired myofilament function (88, 89). Taken together, the consequences of ionic homeostasis imbalances weaken the heart structure against a background of unchanging blood pressure, progressively leading to heart chamber dilatation. It was initially proposed that dilatation in patients carrying $Na_v1.5$ mutations might rely on deleterious adaptive heart remodeling. However, the report of the $Na_v1.5$/R225W mutation in a 1-year-old child who died from severe arrhythmias and DCM ruled out potential remodeling in this patient (83). In a nutshell, gating pore currents are expected to affect V_{Rest}, AP parameters, cellular conduction, and cardiomyocyte structure, all of which might act together to cause multiple arrhythmias associated with cardiac dilatation.

As previously mentioned, $Na_v1.5$ mutations in the VSD that are outside the S4 segment such as $Na_v1.5$/I141V, D1275N, V1279I, and D1595H should be treated with caution. D1275N was the first $Na_v1.5$ mutation associated with the development of arrhythmias and DCM (7). It is also one of the most studied $Na_v1.5$ DCM-linked mutations (7, 38, 71, 90–93). Besides the marked interest in this mutation and the many ensuing experimental models, the molecular mechanism linking the $Na_v1.5$/D1275N mutation and its pathological expression remains unclear. The clinical phenotype is variable, and heart defects include atrial and ventricular arrhythmias and conduction system defects and, most of the time, cardiac dilatation (7, 38, 71, 90–93). Strikingly, $Na_v1.5$/D1275N is also one of the only $Na_v1.5$ mutations that have been linked to cerebro-vascular strokes (7, 90, 93, 94). In heterologous expression systems, only mild defects, mostly shifts in activation/inactivation parameters and current decreases, have been described (91). Further investigations using a humanized mouse model indicated that there is a large reduction in current amplitude (38). The lengthened cardiac conduction time (lengthened PR interval) in transgenic $Na_v1.5$/D1275N zebrafish appear to support this hypothesis. However, in this case, the Na_v current was not measured (95). Lastly, a human cardiac cellular model of the cardiomyopathy linked to the $Na_v1.5$/D1275N mutation has been recently proposed based on patient-specific induced pluripotent stem cells (hiPSC) (93). In this study, the authors only report a decrease in the Na^+ current amplitude and a mild shift of activation leading to a decrease in the maximal depolarization velocity of APs (93). Unfortunately, they did not study intracellular ionic homeostasis and sarcomeric arrangements. Taken together, these studies identified a biophysical defect that does not provide a complete picture of the clinical expression of this mutation. Interestingly, given the location of the D1275N mutation (S3/DIII), a gating pore could be created and participate in the pathological mechanism. However, this hypothesis has never been extensively explored. Furthermore, as previously mentioned, due to the structure and function of the S1–S3 segments, gating pores created by mutations in these segments are not expected to behave in the same way as gating pores created by mutations in the S4 segment. Characterizing and understanding them will thus require thorough studies.

Finally, a specific gating pore blocker would be expected to be ideal to efficiently treat gating pore pathological consequences. Unfortunately, so far, such universal blocker has never been described. Consequently, the only option available is to alleviate the cardiac dilatation and use anti-arrhythmic or implantable devices to slow down the pathology progress. In a research optic, few studies already proposed potential blockers such as divalent (Mg^{2+}), trivalent (Y^{3+}, Yb^{3+}, Lu^{3+}, Ti^{3+}) and quadrivalent (Hf^{4+}) cations (40, 59). In WT channels, gating pores are naturally obstructed by the arginine side, notably made of guanidine (49, 59). Guanidinium compounds have recently been shown to bind to the VSD in the place of the missing side chain of the original arginine (49). Still supporting this hypothesis, the 1-(2,4-xylyl)guanidine, a guanidinium derivative has been shown to partially block gating pores (59). While obstructing gating pores is an ongoing challenge, other approaches could be valuable. Since gating pores properties intimately depends on the VSD characteristics, VSD modulation through the use of toxins has been demonstrated to modulate the voltage dependence of gating pores (96).

In this review, we describe the history of $Na_v1.5$ associated channelopathies with a clear focus on the history of $Na_v1.5$ mutations more recently associated with the development of multiple arrhythmias and DCM. The biophysical function of the VSDs, the creation of a gating pore and their biophysical properties have been described. Finally, the potential cardiac cellular effects of gating pores and their blockers are also

presented here. The increasing knowledge regarding gating pores and their pathologic implication, potentially highlights novel biophysical defects and consequently novel channelopathies. In the current dynamic toward more precise and personalized medicine, this increasing knowledge could in the future orientate clinicians in their day to day practice in the management of cardiac channelopathies. This could also help to develop specifically targeted novel medication to accurately and precisely block gating pores, finally benefitting patients. The increased interest in gating pores highlights VSDs as highly valuable druggable sites with potential impact far beyond field of gating pore currents. So far, most ions channel modulators only target the physiological pore of the channel. Modulating the VSD would thus offer a wide range of benefits and be an approach to consider in most channelopathies.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Estes NA III. Predicting and preventing sudden cardiac death. *Circulation* (2011) 124:651–6. doi: 10.1161/CIRCULATIONAHA.110.974170
2. Savarese G, Lund LH. Global public health burden of heart failure. *Card Fail Rev.* (2017) 3:7–11. doi: 10.15420/cfr.2016:25:2
3. Hershberger RE, Siegfried JD. Update 2011: clinical and genetic issues in familial dilated cardiomyopathy. *J Am Coll Cardiol.* (2011) 57:1641–9. doi: 10.1016/j.jacc.2011.01.015
4. Murphy RT, Starling RC. Genetics and cardiomyopathy: where are we now? *Cleve Clin J Med.* (2005) 72:465–466, 469–70, 472–63 passim.
5. Taylor MR, Carniel E, Mestroni L. Cardiomyopathy, familial dilated. *Orphanet J Rare Dis.* (2006) 1:27. doi: 10.1186/1750-1172-1-27
6. Bienengraeber M, Olson TM, Selivanov VA, Kathmann EC, O'cochlain F, Gao F, et al. ABCC9 mutations identified in human dilated cardiomyopathy disrupt catalytic KATP channel gating. *Nat Genet.* (2004) 36:382–7. doi: 10.1038/ng1329
7. Mcnair WP, Ku L, Taylor MR, Fain PR, Dao D, Wolfel E, et al. SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. *Circulation* (2004) 110:2163–7. doi: 10.1161/01.CIR.0000144458.58660.BB
8. Parikh R, Kadowitz PJ. A review of current therapies used in the treatment of congestive heart failure. *Exp Rev Cardiovasc Ther.* (2013) 11:1171–8. doi: 10.1586/14779072.2013.816478
9. Habal MV, Garan AR. Long-term management of end-stage heart failure. *Best Pract Res Clin Anaesthesiol.* (2017) 31:153–66. doi: 10.1016/j.bpa.2017.07.003
10. Cummins TR, Dib-Hajj SD, Waxman SG. Electrophysiological properties of mutant Nav1.7 sodium channels in a painful inherited neuropathy. *J.Neurosci.* (2004) 24:8232–6. doi: 10.1523/JNEUROSCI.2695-04.2004
11. Bennett PB, Yazawa K, Makita N, George AL Jr. Molecular mechanism for an inherited cardiac arrhythmia. *Nature* (1995) 376:683–5. doi: 10.1038/376683a0
12. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. *J Am Coll Cardiol.* (1992) 20:1391–6. doi: 10.1016/0735-1097(92)90253-J
13. Veldkamp MW, Viswanathan PC, Bezzina C, Baartscheer A, Wilde AM, Balser JR. Two distinct congenital arrhythmias evoked by a multidysfunctional Na^+ channel. *Circ.Res.* (2000) 86:E91–7. doi: 10.1161/01.RES.86.9.e91
14. Meisler MH, Kearney JA. Sodium channel mutations in epilepsy and other neurological disorders. *J Clin Invest.* (2005) 115:2010–7. doi: 10.1172/JCI25466
15. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* (2000) 26:13–25. doi: 10.1016/S0896-6273(00)81133-2
16. Gellens ME, George AL Jr, Chen LQ, Chahine M, Horn R, Barchi RL, et al. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. *Proc Natl Acad Sci USA.* (1992) 89:554–8. doi: 10.1073/pnas.89.2.554
17. Terlau H, Stuhmer W. Structure and function of voltage-gated ion channels. *Naturwissenschaften* (1998) 85:437–44. doi: 10.1007/s001140050527
18. Stühmer W, Conti F, Suzuki H, Wang XD, Noda M, Yahagi N, et al. Structural parts involved in activation and inactivation of the sodium channel. *Nature* (1989) 339:597–603. doi: 10.1038/339597a0
19. West JW, Patton DE, Scheuer T, Wang Y, Goldin AL, Catterall WA. A cluster of hydrophobic amino acid residues required for fast Na^+-channel inactivation. *Proc Natl Acad Sci USA.* (1992) 89:10910–4. doi: 10.1073/pnas.89.22.10910
20. Ragsdale DS, Mcphee JC, Scheuer T, Catterall WA. Molecular determinants of state-dependent block of Na^+ channels by local anesthetics. *Science* (1994) 265:1724–8. doi: 10.1126/science.8085162
21. Hartmann HA, Colom LV, Sutherland ML, Noebels JL. Selective localization of cardiac SCN5A sodium channels in limbic regions of rat brain. *Nat Neurosci.* (1999) 2:593–5. doi: 10.1038/10147
22. Backx PH, Yue DT, Lawrence JH, Marban E, Tomaselli GF. Molecular localization of an ion-binding site within the pore of mammalian sodium channels. *Science* (1992) 257:248–51. doi: 10.1126/science.1321496
23. Heinemann SH, Terlau H, Imoto K. Molecular basis for pharmacological differences between brain and cardiac sodium channels. *Pflügers Arch.* (1992) 422:90–2. doi: 10.1007/BF00381519
24. Bean BP, Cohen CJ, Tsien RW. Lidocaine block of cardiac sodium channels. *J Gen Physiol.* (1983) 81:613–42. doi: 10.1085/jgp.81.5.613
25. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, et al. Spectrum of mutations in long-QT syndrome genes. *KVLQT1*, *HERG*, *SCN5A*, *KCNE1*, and *KCNE2*. *Circulation* (2000) 102:1178–85. doi: 10.1161/01.CIR.102.10.1178
26. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature* (1998) 392:293–6. doi: 10.1038/32675
27. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* (1995) 80:805–11. doi: 10.1016/0092-8674(95)90359-3
28. Herbert E, Chahine M. Clinical aspects and physiopathology of Brugada syndrome: review of current concepts. *Can J Physiol Pharmacol.* (2006) 84:795–802. doi: 10.1139/y06-038
29. Bezzina C, Veldkamp MW, Van Den Berg MP, Postma AV, Rook MB, Viersma JW, et al. A single Na^+ channel mutation causing both long-QT and Brugada syndromes. *Circ Res.* (1999) 85:1206–13. doi: 10.1161/01.RES.85.12.1206
30. Ter Bekke RMA, Isaacs A, Barysenka A, Hoos MB, Jongbloed JDH, Hoorntje JCA, et al. Heritability in a SCN5A-mutation founder population with increased female susceptibility to non-nocturnal ventricular

tachyarrhythmia and sudden cardiac death. *Heart Rhythm* (2017) 14:1873–81. doi: 10.1016/j.hrthm.2017.07.036
31. Groenewegen WA, Firouzi M, Bezzina CR, Vliex S, Van Langen IM, Sandkuijl L, et al. A cardiac sodium channel mutation cosegregates with a rare connexin40 genotype in familial atrial standstill. *Circ Res.* (2003) 92:14–22. doi: 10.1161/01.RES.0000050585.07097.D7
32. Groenewegen WA, Wilde AA. Letter regarding article by McNair et al, "SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia". *Circulation* (2005) 112:e9. doi: 10.1161/CIRCULATIONAHA.104.531475
33. Mcnair WP, Sinagra G, Taylor MR, Di Lenarda A, Ferguson DA, Salcedo EE, et al. SCN5A mutations associate with arrhythmic dilated cardiomyopathy and commonly localize to the voltage-sensing mechanism. *J Am Coll Cardiol.* (2011) 57:2160–8. doi: 10.1016/j.jacc.2010.09.084
34. Gosselin-Badaroudine P, Keller DI, Huang H, Pouliot V, Chatelier A, Osswald S, et al. A proton leak current through the cardiac sodium channel is linked to mixed arrhythmia and the dilated cardiomyopathy phenotype. *PLoS ONE* (2012) 7:e38331. doi: 10.1371/journal.pone.0038331
35. Sokolov S, Scheuer T, Catterall WA. Gating pore current in an inherited ion channelopathy. *Nature* (2007) 446:76–8. doi: 10.1038/nature05598
36. Nguyen TP, Wang DW, Rhodes TH, George ALJr. Divergent biophysical defects caused by mutant sodium channels in dilated cardiomyopathy with arrhythmia. *Circ Res.* (2008) 102:364–71. doi: 10.1161/CIRCRESAHA.107.164673
37. Starace DM, Bezanilla F. A proton pore in a potassium channel voltage sensor reveals a focused electric field. *Nature* (2004) 427:548–53. doi: 10.1038/nature02270
38. Watanabe H, Yang T, Stroud DM, Lowe JS, Harris L, Atack TC, et al. Striking In vivo phenotype of a disease-associated human SCN5A mutation producing minimal changes *in vitro*. *Circulation* (2011) 124:1001–11. doi: 10.1161/CIRCULATIONAHA.110.987248
39. Starace DM, Bezanilla F. Histidine scanning mutagenesis of basic residues of the S4 segment of the shaker k+ channel. *J Gen Physiol.* (2001) 117:469–90. doi: 10.1085/jgp.117.5.469
40. Tombola F, Pathak MM, Isacoff EY. Voltage-sensing arginines in a potassium channel permeate and occlude cation-selective pores. *Neuron* (2005) 45:379–88. doi: 10.1016/j.neuron.2004.12.047
41. Sokolov S, Scheuer T, Catterall WA. Ion permeation through a voltage-sensitive gating pore in brain sodium channels having voltage sensor mutations. *Neuron* (2005) 47:183–9. doi: 10.1016/j.neuron.2005.06.012
42. Banerjee A, Mackinnon R. Inferred motions of the S3a helix during voltage-dependent K+ channel gating. *J Mol Biol.* (2008) 381:569–80. doi: 10.1016/j.jmb.2008.06.010
43. Tao X, Lee A, Limapichat W, Dougherty DA, Mackinnon R. A gating charge transfer center in voltage sensors. *Science* (2010) 328:67–73. doi: 10.1126/science.1185954
44. Delemotte L, Tarek M, Klein ML, Amaral C, Treptow W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *Proc Natl Acad Sci USA.* (2011) 108:6109–14. doi: 10.1073/pnas.1102724108
45. Amaral C, Carnevale V, Klein ML, Treptow W. Exploring conformational states of the bacterial voltage-gated sodium channel NavAb via molecular dynamics simulations. *Proc Natl Acad Sci USA.* (2012) 109:21336–41. doi: 10.1073/pnas.1218087109
46. Tarek M, Delemotte L. Omega currents in voltage-gated ion channels: what can we learn from uncovering the voltage-sensing mechanism using md simulations? *Acc Chem Res.* (2013) 46:2755–62. doi: 10.1021/ar300290u
47. Pless SA, Galpin JD, Niciforovic AP, Ahern CA. Contributions of counter-charge in a potassium channel voltage-sensor domain. *Nat Chem Biol.* (2011) 7:617–23. doi: 10.1038/nchembio.622
48. Delemotte L, Klein ML, Tarek M. Molecular dynamics simulations of voltage-gated cation channels: insights on voltage-sensor domain function and modulation. *Front Pharmacol.* (2012) 3:97. doi: 10.3389/fphar.2012.00097
49. Jiang D, Gamal El-Din TM, Ing C, Lu P, Pomes R, Zheng N, et al. Structural basis for gating pore current in periodic paralysis. *Nature* (2018) 557:590–4. doi: 10.1038/s41586-018-0120-4
50. Gosselin-Badaroudine P, Delemotte L, Moreau A, Klein ML, Chahine M. Gating pore currents and the resting state of Nav1.4 voltage sensor domains. *Proc Natl Acad Sci USA.* (2012) 109:19250–5. doi: 10.1073/pnas.1217990109
51. Moreau A, Gosselin-Badaroudine P, Chahine M. Biophysics, pathophysiology, and pharmacology of ion channel gating pores. *Front Pharmacol.* (2014) 5:53. doi: 10.3389/fphar.2014.00053
52. Moreau A, Gosselin-Badaroudine P, Delemotte L, Klein ML, Chahine M. Gating pore currents are defects in common with two Nav1.5 mutations in patients with mixed arrhythmias and dilated cardiomyopathy. *J Gen Physiol.* (2015) 145:93–106. doi: 10.1085/jgp.201411304
53. Moreau A, Gosselin-Badaroudine P, Boutjdir M, Chahine M. Mutations in the voltage sensors of domains I and II of Nav1.5 that are associated with arrhythmias and dilated cardiomyopathy generate gating pore currents. *Front Pharmacol.* (2015) 6:301. doi: 10.3389/fphar.2015.00301
54. Campos FV, Chanda B, Roux B, Bezanilla F. Two atomic constraints unambiguously position the S4 segment relative to S1 and S2 segments in the closed state of Shaker K channel. *Proc Natl Acad Sci USA.* (2007) 104:7904–9. doi: 10.1073/pnas.0702638104
55. Struyk AF, Cannon SC. A Na+ channel mutation linked to hypokalemic periodic paralysis exposes a proton-selective gating pore. *J Gen Physiol.* (2007) 130:11–20. doi: 10.1085/jgp.200709755
56. Klassen TL, Spencer AN, Gallin WJ. A naturally occurring omega current in a Kv3 family potassium channel from a platyhelminth. *BMC Neurosci.* (2008) 9:52. doi: 10.1186/1471-2202-9-52
57. Struyk AF, Cannon SC. Paradoxical depolarization of BA2+- treated muscle exposed to low extracellular K+: insights into resting potential abnormalities in hypokalemic paralysis. *Muscle Nerve* (2008) 37:326–37. doi: 10.1002/mus.20928
58. Struyk AF, Markin VS, Francis D, Cannon SC. Gating pore currents in DIIS4 mutations of NaV1.4 associated with periodic paralysis: saturation of ion flux and implications for disease pathogenesis. *J Gen Physiol.* (2008) 132:447–64. doi: 10.1085/jgp.200809967
59. Sokolov S, Scheuer T, Catterall WA. Ion permeation and block of the gating pore in the voltage sensor of NaV1.4 channels with hypokalemic periodic paralysis mutations. *J Gen Physiol.* (2010) 136:225–36. doi: 10.1085/jgp.201010414
60. Francis DG, Rybalchenko V, Struyk A, Cannon SC. Leaky sodium channels from voltage sensor mutations in periodic paralysis, but not paramyotonia. *Neurology* (2011) 76:1635–41. doi: 10.1212/WNL.0b013e318219fb57
61. Groome JR, Lehmann-Horn F, Fan C, Wolf M, Winston V, Merlini L, et al. Nav1.4 mutations cause hypokalaemic periodic paralysis by disrupting IIIS4 movement during recovery. *Brain* (2014) 137:998–1008. doi: 10.1093/brain/awu015
62. Moreau A, Gosselin-Badaroudine P, Chahine M. Molecular biology and biophysical properties of ion channel gating pores. *Q Rev Biophys.* (2014) 47:364–88. doi: 10.1017/S0033583514000109
63. Delemotte L, Treptow W, Klein ML, Tarek M. Effect of sensor domain mutations on the properties of voltage-gated ion channels: molecular dynamics studies of the potassium channel Kv1.2. *Biophys J.* (2010) 99:L72–4. doi: 10.1016/j.bpj.2010.08.069
64. Khalili-Araghi F, Tajkhorshid E, Roux B, Schulten K. Molecular dynamics investigation of the omega-current in the Kv1.2 voltage sensor domains. *Biophys J.* (2012) 102:258–67. doi: 10.1016/j.bpj.2011.10.057
65. Fuster C, Perrot J, Berthier C, Jacquemond V, Charnet P, Allard B. Na leak with gating pore properties in hypokalemic periodic paralysis V876E mutant muscle Ca channel. *J Gen Physiol.* (2017) 149:1139–48. doi: 10.1085/jgp.201711834
66. Jurkat-Rott K, Lehmann-Horn F. Do hyperpolarization-induced proton currents contribute to the pathogenesis of hypokalemic periodic paralysis, a voltage sensor channelopathy? *J Gen Physiol.* (2007) 130:1–5. doi: 10.1085/jgp.200709834
67. Jurkat-Rott K, Weber MA, Fauler M, Guo XH, Holzherr BD, Paczulla A, et al. K+-dependent paradoxical membrane depolarization and Na+ overload, major and reversible contributors to weakness by ion channel leaks. *Proc Natl Acad Sci USA.* (2009) 106:4036–41. doi: 10.1073/pnas.0811277106
68. Jurkat-Rott K, Groome J, Lehmann-Horn F. Pathophysiological role of omega pore current in channelopathies. *Front Pharmacol.* (2012) 3:112. doi: 10.3389/fphar.2012.00112

69. Ruan Y, Liu N, Priori SG. Sodium channel mutations and arrhythmias. *Nat Rev Cardiol.* (2009) 6:337–48. doi: 10.1038/nrcardio.2009.44
70. Moreau A, Krahn AD, Gosselin-Badaroudine P, Klein GJ, Christe G, Vincent Y, et al. Sodium overload due to a persistent current that attenuates the arrhythmogenic potential of a novel LQT3 mutation. *Front Pharmacol.* (2013) 4:126. doi: 10.3389/fphar.2013.00126
71. Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ, et al. Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. *JAMA* (2005) 293:447–54. doi: 10.1001/jama.293.4.447
72. Olesen MS, Yuan L, Liang B, Holst AG, Nielsen N, Nielsen JB, et al. High prevalence of long QT syndrome-associated SCN5A variants in patients with early-onset lone atrial fibrillation. *Circulation Cardiovascu Genet.* (2012) 5:450–9. doi: 10.1161/CIRCGENETICS.111.962597
73. Pieske B, Houser SR. [Na+]i handling in the failing human heart. *Cardiovasc Res.* (2003) 57:874–86. doi: 10.1016/S0008-6363(02)00841-6
74. Miake J, Marban E, Nuss HB. Functional role of inward rectifier current in heart probed by Kir2.1 overexpression and dominant-negative suppression. *J Clin Invest.* (2003) 111:1529–1536. doi: 10.1172/JCI200317959
75. Bett GC, Kaplan AD, Lis A, Cimato TR, Tzanakakis ES, Zhou Q, et al. Electronic "expression" of the inward rectifier in cardiocytes derived from human-induced pluripotent stem cells. *Heart Rhythm* (2013) 10:1903–10. doi: 10.1016/j.hrthm.2013.09.061
76. Zaza A, Rocchetti M, Brioschi A, Cantadori A, Ferroni A. Dynamic Ca2+-induced inward rectification of K+ current during the ventricular action potential. *Circ Res.* (1998) 82:947–56. doi: 10.1161/01.RES.82.9.947
77. Cannon SC. Voltage-sensor mutations in channelopathies of skeletal muscle. *J Physiol.* (2010) 588:1887–95. doi: 10.1113/jphysiol.2010.186874
78. Tricarico D, Camerino DC. Recent advances in the pathogenesis and drug action in periodic paralyses and related channelopathies. *Front Pharmacol.* (2011) 2:8. doi: 10.3389/fphar.2011.00008
79. Stergiopoulos K, Alvarado JL, Mastroianni M, Ek-Vitorin JF, Taffet SM, Delmar M. Hetero-domain interactions as a mechanism for the regulation of connexin channels. *Circ Res.* (1999) 84:1144–55. doi: 10.1161/01.RES.84.10.1144
80. Bukauskas FF, Bukauskiene A, Bennett MV, Verselis VK. Gating properties of gap junction channels assembled from connexin43 and connexin43 fused with green fluorescent protein. *Biophys J.* (2001) 81:137–52. doi: 10.1016/S0006-3495(01)75687-1
81. Duffy HS, Ashton AW, O'donnell P, Coombs W, Taffet SM, Delmar M, et al. Regulation of connexin43 protein complexes by intracellular acidification. *Circ Res.* (2004) 94:215–22. doi: 10.1161/01.RES.0000113924.06926.11
82. Benson DW, Wang DW, Dyment M, Knilans TK, Fish FA, Strieper MJ, et al. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). *J Clin Invest.* (2003) 112:1019–28. doi: 10.1172/JCI200318062
83. Bezzina CR, Rook MB, Groenewegen WA, Herfst LJ, Van Der Wal AC, Lam J, et al. Compound heterozygosity for mutations (W156X and R225W) in SCN5A associated with severe cardiac conduction disturbances and degenerative changes in the conduction system. *Circ Res.* (2003) 92:159–68. doi: 10.1161/01.RES.0000052672.97759.36
84. Laurent G, Saal S, Amarouch MY, Beziau DM, Marsman RF, Faivre L, et al. Multifocal ectopic Purkinje-related premature contractions: a new SCN5A-related cardiac channelopathy. *J Am Coll Cardiol.* (2012) 60:144–56. doi: 10.1016/j.jacc.2012.02.052
85. Saegusa N, Moorhouse E, Vaughan-Jones RD, Spitzer KW. Influence of pH on Ca(2)(+) current and its control of electrical and Ca(2)(+) signaling in ventricular myocytes. *J Gen Physiol.* (2011) 138:537–59. doi: 10.1085/jgp.201110658
86. Thorsen K, Dam VS, Kjaer-Sorensen K, Pedersen LN, Skeberdis VA, Jurevicius J, et al. Loss-of-activity-mutation in the cardiac chloride-bicarbonate exchanger AE3 causes short QT syndrome. *Nat Commun.* (2017) 8:1696. doi: 10.1038/s41467-017-01630-0
87. Fabiato A, Fabiato F. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiace and skeletal muscles. *J Physiol.* (1978) 276:233–55. doi: 10.1113/jphysiol.1978.sp012231
88. Chandra M, Montgomery DE, Kim JJ, Solaro RJ. The N-terminal region of troponin T is essential for the maximal activation of rat cardiac myofilaments. *J Mol Cell Cardiol.* (1999) 31:867–80. doi: 10.1006/jmcc.1999.0928
89. Zhang Z, Feng HZ, Jin JP. Structure of the NH2-terminal variable region of cardiac troponin T determines its sensitivity to restrictive cleavage in pathophysiological adaptation. *Arch Biochem Biophys.* (2011) 515:37–45. doi: 10.1016/j.abb.2011.08.013
90. Laitinen-Forsblom PJ, Makynen P, Makynen H, Yli-Mayry S, Virtanen V, Kontula K, et al. SCN5A mutation associated with cardiac conduction defect and atrial arrhythmias. *J Cardiovasc Electrophysiol.* (2006) 17:480–5. doi: 10.1111/j.1540-8167.2006.00411.x
91. Gui J, Wang T, Jones RP, Trump D, Zimmer T, Lei M. Multiple loss-of-function mechanisms contribute to SCN5A-related familial sick sinus syndrome. *PLoS ONE* (2010a) 5:e10985. doi: 10.1371/journal.pone.0010985
92. Gui J, Wang T, Trump D, Zimmer T, Lei M. Mutation-specific effects of polymorphism H558R in SCN5A-related sick sinus syndrome. *J Cardiovasc Electrophysiol.* (2010) 21:564–73. doi: 10.1111/j.1540-8167.2010.01762.x
93. Hayano M, Makiyama T, Kamakura T, Watanabe H, Sasaki K, Funakoshi S, et al. Development of a patient-derived induced pluripotent stem cell model for the Investigation of SCN5A-D1275N-related cardiac sodium channelopathy. *Circ J.* (2017) 81:1783–91doi: 10.1253/circj.CJ-17-0064
94. Moreau A, Janin A, Millat G, Chevalier P. Cardiac voltage-gated sodium channel mutations associated with left atrial dysfunction and stroke in children. *EP Europace*, euy041-euy041. (2018). doi: 10.1093/europace/euy041
95. Huttner IG, Trivedi G, Jacoby A, Mann SA, Vandenberg JI, Fatkin D. A transgenic zebrafish model of a human cardiac sodium channel mutation exhibits bradycardia, conduction-system abnormalities and early death. *J Mol Cell Cardiol.* (2013) 61:123–32. doi: 10.1016/j.yjmcc.2013.06.005
96. Xiao Y, Blumenthal K, Cummins TR. Gating-pore currents demonstrate selective and specific modulation of individual sodium channel voltage-sensors by biological toxins. *Mol Pharmacol.* (2014) 86:159–67. doi: 10.1124/mol.114.092338

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MINI REVIEW
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Recent Advances in Short QT Syndrome

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Short QT syndrome is a highly malignant inherited cardiac disease characterized by ventricular tachyarrhythmias leading to syncope and sudden cardiac death. It is responsible of lethal episodes in young people, mainly infants. International guidelines establish diagnostic criteria with the presence of a QTc $\leq$ 340 ms in the electrocardiogram despite clinical diagnostic values remain controversial. In last years, clinical diagnosis, risk stratification as well as preventive therapies have been improved due to identification of pathophysiological mechanisms. The only effective option is implantation of a defibrillator despite Quinidine may be at times an effective option. Currently, a limited number of rare variants have been identified in seven genes, which account for nearly 20–30% of families. However, some of these variants are associated with phenotypes showing a shorter QT interval but no conclusive diagnosis of Short QT syndrome. Therefore, an exhaustive interpretation of each variant and a close genotype-phenotype correlation is necessary before clinical translation. Here, we review the main clinical and genetic hallmarks of this rare entity.

Keywords: sudden cardiac death, arrhythmias, short QT syndrome, genetics, QT interval variability

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INTRODUCTION

In 2000, a new cardiac channelopathy was described in one family with history of sudden cardiac death (SCD) (1). All relatives analyzed showed idiopathic persistent short QT interval in the electrocardiogram (ECG); one of them also showed paroxysmal atrial fibrillation (AF). Due to its main feature in the ECG, it was called Short QT Syndrome (SQTS). In 2003, two additional unrelated families were described, with mutiple relatives across several generations showing history of SCD. Several relatives showed the same ECG previously reported 3 years before, complaining from palpitations, and syncope; this fact highlighted the familial nature of this novel disease and the malignant arrhythmogenic events associated (2). Currently, more than 350 publications (PubMed: short QT syndrome) have been reported but a low number of cases (almost 250 cases) and families (nearly 150 families) have been diagnosed worldwide; therefore it is classified as a rare disease. This lethal condition may be underdiagnosed because some affected individuals never experience symptoms. In most cases, highly malignant arrhythmias and a lethal outcome is identified, mainly at young age. Nowadays, a reduced number of genes has been associated with the disease. Hence, a comprehensive genetic testing has a diagnostic yield of nearly 20–30% (3). The short QT interval on the ECG is due to an accelerated cardiac repolarization (and shorter refractory periods) serving as substrate for ventricular arrhythmias leading to syncope and even SCD, sometimes the first manifestation of the disease.

CLINICAL FINDINGS

Prevalence

The limited number of cases wordwide difficult establish the real prevalence in global population. However, recent studies suggest a prevalence between 0.02 and 0.1% in adults while in pediatric population the prevalence is 0.05% (4). Arrhyhtmogenic events associated with SQTS have been recorded in all ages from infants to 80-year-old patients, but the first year of life appears to be the most alarming with a 4% rate of cardiac arrest (5). The probability of a first syncope, even SCD, is nearly 40% by 40 years old. One-third of cases with SCD as their first manifestation, and up to 80% of cases showed a personal or family history of SCD (6). Lethal events may occur in both genders, but a slight male predominance seems to exist, although no conclusive data exist concerning this point. A recent study suggests that male predominance may be due to higher testosterone levels and genes located on the X chromosome could be involved in QTc interval regulation (7).

Clinical Assessment

Clinical manifestations associated with SQTS may range from asymptomatic (up to 40% of cases) to dizziness, AF, ventricular arrhythmias, syncope and even SCD. Clinical manifestations can be particularly severe, especially in children, and may cause SCD in infants (sudden infant death syndrome—SIDS). As abovementioned, it is a familial disease; therefore, diagnostic patients usually have a family history of syncope or SCD in a young first or second-degree relative. Recent studies suggest that there are two high-risk peaks of SCD: in the first year of life and from 20 to 40 years old (8). Consequently, clinical assessment is recommended in all family members. Asymptomatic patients who carry a pathogenic variant associated with the disease are also at high risk because the first manifestation of the disease could be the SCD (9).

Diagnosis

The main problem in clinical diagnosis is the definition of cut off value at the lower end of the QTc. In 2011 (10) and 2013 (11), guidelines/consensus documents defined the SQTS as: "*a genetic arrhythmogenic disorder characterized by a short and uniform QT/QTc intervals (<330 ms) on the ECG, with absent or minimal ST segments, with an interval from J point to T wave peak (Jp-Tp) measured in the precordial lead with the T wave of greatest amplitude <120 ms, possible tall T waves with narrow base similar to the T wave of moderate hyperkalemia ("desert tent T waves"), frequent early repolarization pattern, prolongation of T peak-T end interval, and possible presence of prominent U waves in the absence of structural heart disease and others disturbances that cause repolarization abnormalities*" (**Figures 1**, **2**). In addition to a short QT interval, Tülümen et al. suggested the PQ segment depression as a novel marker for SQTS (12) despite further studies should be performed in order to clarify this point. Current guidelines (13) suggest the following diagnostic criteria:

(1) QTc $\leq$ 340 ms (Class IC); or.
(2) QTc $\leq$ 360 ms; and one or more of the following: (a) A confirmed pathogenic mutation. (b) Family history of SQTS. (c) Family history of sudden death at 40 years of age. (d) Survival from a VT/VF episode in the absence of heart diseases (Class IIaC).

Resting 12-lead ECG should be performed at a heart rate within normal limits when the diagnosis of SQTS is suspected (14). Recent studies suggest that a QT/HR relationship slope under —0.9 ms/beat/min in the exercise test could be a useful tool in order to distinguish affected subjects from healthy individuals (15). Authors showed that the QT/HR slope is significantly flatter in the subgroup of males who carry a pathogenic variant in the *KCNH2* gene, which shows the shortest QT intervals, as compared with the subgroup with unknown genotype. In addition, the QT adaptation to standing is reduced, as compared to the values for a normal population. It has been reported that tissue Doppler imaging (TDI) and speckle tracking echocardiography (STE), could be part of the clinical assessment because of systolic function may also be affected and patients presented a dispersion of contraction in myocardium (16). In contrast, invasive electrophysiological study (EPS) with programmed ventricular stimulation is not recommended for SCD risk stratification (13).

Risk Stratification and Treatment

Due to the low number of patients with SQTS, risk stratification represents indeed the main current challenge in clinical characterization. Electrophysiological testing has not been useful in predicting cardiac arrest but patients showing QTc intervals <340 ms should be considered at highest risk of SCD despite no conclusive results have been published, so far. The only predictor of cardiac arrest in a patient with SQTS found so far has been a previous history of cardiac arrest. Therefore, the optimum strategy for primary prevention of cardiac arrest in these patients is still a matter of argue. Nowadays, current guidelines recommend an implantable cardiac defibrillator (ICD) as the first and more effective therapeutic measure in patients who have experienced sustained VT/VF episodes or survivors of an aborted cardiac arrest (17). Inappropriate shock is a common complication in patients carrying an ICD. In asymptomatic patients showing a SQTS, due to lack of definite studies, the implantation or not of an ICD remains to be clarified (18). Recent reports suggest that an ICD might be considered in SQTS patients with a strong family history of SCD and previous evidence of short QTc. However, an alternative therapy is necessary, especially in small children and in adults in whom ICD cannot be a therapeutical option.

Therefore, an alternative treatment to ICD is the pharmacological approach. In last years, several drugs such as Ibutilide, Flecainide, Sotalol, Disopyramide, Nifekalant, Propafenone, Carvedilol, Metoprolol, and Amiodarone have been used but none have undergone conclusive clinical studies, primarily due to the lack of families as wel as low event rate in SQTS patients. Nowadays, SQTS patients are treated with administration of Sotalol or Quinidine, which prolong cardiac repolarization and consequently QT interval due to inhibition of repolarization (19). Sotalol is ineffective in patients carrying pathogenic variants in the *KCNH2* gene (SQTS type 1), the most common gene associated with SQTS. Quinidine, which

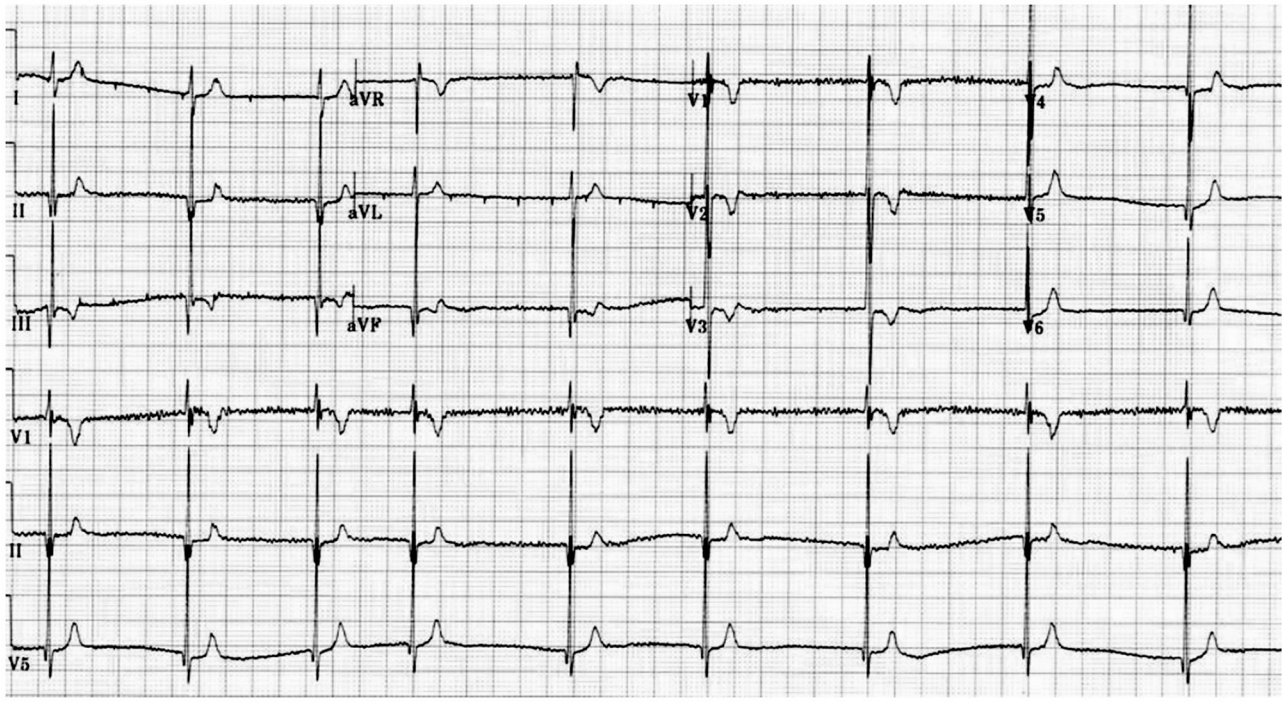

FIGURE 1 | Electrocardiogram showing a short QT interval.

has similar affinity to the open and inactivated states of IKr, is effective therapy for type 1 SQTS (20). Unfortenatelly, Quinidine has been removed from the market in several countries and often has intolerable side-effects. In a recent study, treatment with Hydroxyquinidine was associated with a lower incidence of arrhythmic events in SQTS patients as it prolongs the QT interval (5). Pharmacological treatment in symptomatic patients is recommended, particularly if ICD is not implanted, and in asymptomatic patients with a family history of SCD.

Genetic Basis

This malignant entity can have a congenital origin or acquired. The first genetic alteration associated with the disease was reported in 2004, located in the *KCNH2* gene (21). Currently, more than 30 rare variants have been identified in 8 genes (*CACNA1C, CACNA2D1, CACNB2, KCNH2, KCNJ2, KCNQ1, SCN5A,* and *SLC4A3*), and follow an autosomal dominant pattern of inheritance with high phenotype penetrance (**Table 1**). SQTS is associated with gain-of-function alterations in genes encoding outward K^+ channels and loss-of-function mutations in genes encoding different subunits of cardiac L-type Ca^{2+} channel (22). A reduction in inward repolarizing currents and/or an increase in outward repolarizing currents will favor early repolarization, leading to action potential duration (APD) shortening (reduced QT interval). It predisposes to reentrant mechanisms, which can lead to AF and VF (23).

Rare variants identified in genes encoding potassium channels (*KCNH2, KCNJ2, KCNQ1*) and the *SLC4A3* gene, have been associated with SQTS. In contrast, rare variants located in genes encoding calcium (*CACNA1C, CACNA2D1,* and *CACNB2*) and the *SCN5A* gene, have been associated with Brugada syndrome (BrS) concomitant with shortened QT intervals, but without a conclusive diagnosis of SQTS (24). A comprehensive genetic analysis of all known genes identifies a potential damaging variant in nearly 30% of cases. However, this percentage may be misleading due to the low number of reported families (25). Current guidelines recommend a genetic analysis of only five genes (*KCNH2, KCNQ1, KCNJ2, CACNA1C,* and *CACNB2b*) in all clinically diagnosed or suspected SQTS cases due to high lethality (13).

The mechanism of arrhythmogenesis in SQTS is not well understood. Several pre-clinical studies focused on unravel the pathofisiological mechanism involved in SQTS have been performed. Most part of these studies are *in vitro* analysis of potential pathogenic variants. Additionally, *in vivo* approaches, mainly transgenic animal models, have been also performed focused on selected variants identified in families showing highly malignat phenotypes, even SCD, in most part of relatives. In last years, *in silico* approaches have been also performed, using algorythms reproducing biological systems similar to SQTS (26). Despite these molecular advances, of nearly 30 potential pathogenic variants associated with SQTS, only a slight number can be classified as definitely pathogenic following recent recommendations of American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) (27). In recent years, it has been developed the induced pluripotent stem cell–derived cardiomyocytes

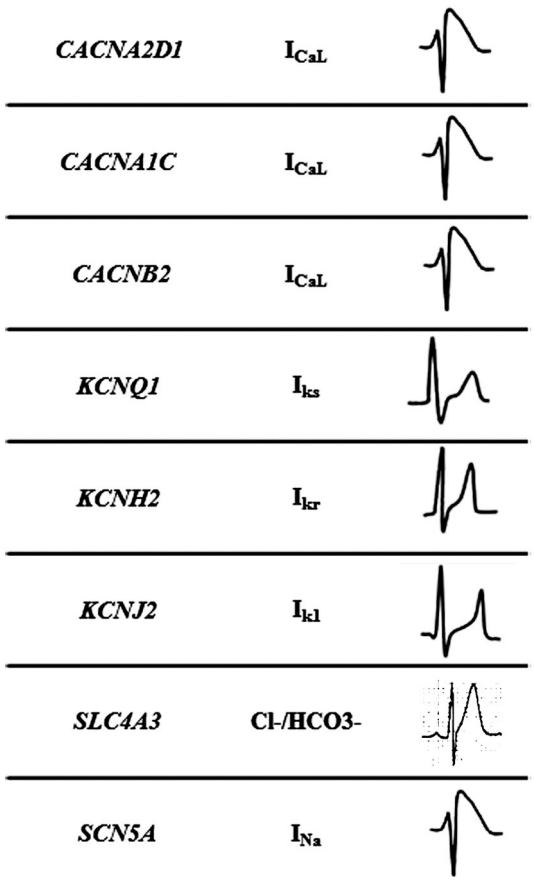

FIGURE 2 | Mechanisms of Short QT syndrome. Gain-of-function mutations of potassium and loss of- function mutations of calcium and sodium channels result in an abbreviated repolarisation phase during action potential and shortening of the QT interval.

(hiPSC-CMs) which allow a most exhaustive study of cellular mechanism of diseases. In 2018, and for the first time, it has been generated a hiPSC-CMs model from a SQTS patient carrying a pathogenic variant in the *KCNH2* gene. Patient-specific hiPSC-CMs are able to recapitulate single-cell phenotype features of SQTS and provide novel opportunities to further elucidate the cellular disease mechanism and test drug effects (28).

Potassium Channels

The main gene is *KCNH2* (ID: 3757) which encodes a voltage-activated potassium channel belonging to the ether-a-go-go (EAG) family-potassium voltage-gated channel, subfamily H (EAG-related), member 2 (Kv 11.1 α subunit/hERG). It mediates the rapidly activating component of the delayed rectifying potassium current in heart (IKr) (29). It is the main gene, associated with the so-called SQTS type 1, and responsible of 15% of all cases. Generally, cardiac events are associated with adrenergic in situations such as noise or exercise, but it may also occur at rest. In 2017, Hu et al. performed an exhaustive study of the phenotypic and functional expression of the highly frequent rare variant associated with SQTS worldwide (30). The hotspot variant (p.T618I) causes a major gain of function in IKr, leading to acceleration of repolarization, which underlies the abbreviation of the QT interval. Other seven rare variants have been also reported in SQTS and associated with SQTS with a potential pathogenic role -p.N588K (c.1764C>A), N588K (c.1764C>G), p.I560T, p.E50D, p.W927G, p.R1135H, and p.R164C- despite further studies should be done in order to clarify their definite role in SQTS. This gene is also associated with other cardiac channelopathies, mainly Long QT syndrome (LQTS), and BrS (**Figure 3**). Therefore, genetic interpretation of variants in *KCNH2* should be done with caution despite most part of current reported variants seems to play a deleterious role. The second gene associated with SQTS is *KCNQ1* (ID: 3784). It encodes a voltage-gated potassium channel (Kv7.1 α subunit) required for repolarization. This protein can form complexes associated with MinK (the *KCNE1* gene) and MiRP2 (the *KCNE3* gene), both also potassium channel proteins. When it is associated with KCNE1, forms the (IKs current) cardiac potassium current and induces a rapid activation of potassium-selective outward current. The Kv7.1 protein may be also associated with MiRP2 protein to form the potassium channel (31). It is associated with the so-called SQTS type 2, and responsible of nearly 5% of all cases. Currently, five rare variants has been identified potentially associated with SQTS (p.Phe279Ile, p.Val307Leu, p.Val141Met, p.Ile274Val, and p.Arg259His). This gene is also associated with other cardiac channelopathies, mainly LQTS (**Figure 3**). The third potassium gene associated with SQTS is *KCNJ2* (ID: 37591). This gene encodes an integral membrane protein and inward-rectifier type potassium channel (Kir2.1 α subunit). Inward rectifier potassium channels are characterized by a greater tendency to allow potassium to flow into the cell rather than out of it (IK_1 current). It is associated with the so-called SQTS type 3, and responsible of nearly 5% of all cases (32). Currently, four rare variants has been identified potentially associated with SQTS (p.Asp172Asn, p.Glu299Val, p.Met301Lys, and p.Lys346Thr). This gene has been also associated with other channelopathies, mainly Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT; **Figure 3**).

Calcium Channels

Concerning calcium channels, the main gene is *CACNA1C* (ID: 775). This gene encodes an alpha-1 subunit of a voltage-dependent calcium channel (calcium channel, voltage-dependent, L type, alpha 1C subunit) (Cav1.2 α subunit). All variants identified so far decrease inward currents at early phases of cell repolarization ($I_{Ca,L}$), induce transmural and epicardial dispersion of repolarization leading to a combined phenotype of BrS and short QTc interval. It is associated with the so-called SQTS type 4, and responsible of <1% of all cases (22). Currently, 10 rare variants have been potentially associated with SQTS despite further studies should be done in order to confirm their certain pathogenic role (p.Ala39Val, p.Gly490Arg, p.Asn547Ser, p.Arg632Arg, p.Glu1115Lys, p.Arg1780His, p.E1829_Q1833dup, p.Arg1880Gln, p.Val2014Ile, and p.Asp2130Asn). This gene has been also associated with other channelopathies, mainly LQTS (**Figure 3**). The second calcium gene is *CACNB2* (ID: 783).

TABLE 1 | Genes associated with Short QT Syndrome or Shorter than normal QT interval.

Gene	Protein	Phenotype	Prevalence
CACNA2D1	Calcium Voltage-Gated Channel Auxiliary Subunit α2/δ1	BrS + Short QT interval	<1%
CACNA1C	Calcium Voltage-Gated Channel Subunit Alpha1 C (Cav1.2)	BrS + Short QT interval	<1%
CACNB2	Calcium Voltage-Gated Channel Auxiliary Subunit Beta 2 (CavB2)	BrS + Short QT interval	<1%
KCNQ1	Potassium Voltage-Gated Channel Subfamily Q Member 1 (Kv7.1 or Kv1.9)	SQTS	<5%
KCNH2	Potassium Voltage-Gated Channel Subfamily H Member 2 (hERG or Kv11.1)	SQTS	15%
KCNJ2	Potassium Voltage-Gated Channel Subfamily J Member 2 (Kv2.1 or Kir2.1)	SQTS	<5%
SLC4A3	Solute Carrier Family 4 Member 3	SQTS	<1%
SCN5A	Sodium channel, voltage gated, type V α subunit (Nav1.5)	BrS + Short QT interval	<1%

BrS, Brugada Syndrome; SQTS, Short QT Syndrome.

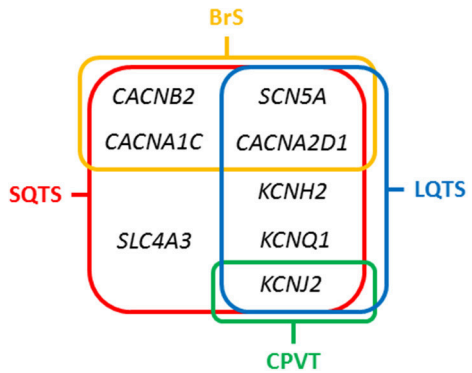

FIGURE 3 | Overlapping genes in Short QT Syndrome. LQTS, Long QT Syndrome; BrS, Brugada Syndrome; CPVT, Catecholaminergic Polymorphic Ventricular Tachycardia.

This gene encodes a subunit of a voltage-dependent calcium channel protein, a member of the voltage-gated calcium channel superfamily (Cav1.2 β subunit). The beta subunit of voltage-dependent calcium channels contributes to the calcium channel function by increasing peak calcium current, shifting the voltage dependencies of activation and inactivation, modulating G protein inhibition and controlling the alpha-1 subunit membrane targeting. Nowadays, only on rare variant has been associated with SQTS (p.Ser481Leu). As also occurs in the *CACNA1C* gene, variants identified in *CACNB2* were associated with BrS and shortened QT interval. It is associated with the so-called SQTS type 5, and responsible of <1% of all cases (22). This gene has been also associated with other channelopathies, mainly LQTS (**Figure 3**). The third calcium gene is *CACNA2D1* (ID: 781). It encodes a member of the alpha-2/delta subunit family, a protein in the voltage-dependent calcium channel complex ($Ca_v1.2$ α2/δ1 subunit). The protein regulates calcium current density and activation/inactivation kinetics of the calcium channel ($I_{Ca,L}$). Only one rare variant has been reported in this gene associated with SQTS (p.Ser755Thr). No conclusive data exist about the association of this gene and SQTS. Therefore, additional studies should be performed in order to clarify a conclusive association. It is associated with the so-called SQTS type 6, and responsible of <1% of all cases (22). This gene has been also associated with other channelopathies, mainly LQTS (**Figure 3**).

Sodium Channel

In 2012, it was identified the p.R689H variant in the *SCN5A* gene (ID:6331) (33). This gene encodes the sodium channel protein type 5-subunit alpha (Nav1.5) which mediates the voltage-dependent sodium ion permeability of myocite membranes. This gene has been also associated with other familial channelopathies, mainly BrS (**Figure 3**). Concerning SQTS, the reported patient was an asymptomatic 40-year-old male with family history of SD of unknown origin who had a Brugada-like ECG with short QT intervals. This variant has been identified in global databases despite in low frequencies (ExAC: 0.011% and gnomAD: 0.01). Thererfore, no conclusive data exist concerning the association of this variant with SQTS. Despite this fact, it is so-called SQTS type 7, and potentially responsible of <1% of all cases. At our point of view, genetic translation of *SCN5A* variants in SQTS patients should be done with caution due to its ambiguous role.

The *SLC4A3* Gene

In 2017, the *SLC4A3* gene (ID:6508) was associated with SQTS (34). This gene (Solute Carrier Family 4 Member 3) encodes a plasma membrane anion exchange protein 3 (AE3). It mediates a part of the Cl-/HCO3- exchange in cardiac myocytes. To date, only one rare variant (p.R370H) have been identified in the *SLC4A3* gene associated with SQTS. It follows an autosomal dominant pattern of inheritance. The pathogenic variant leads to a trafficking defect, decreased Cl,HCO3-exchange over the cell membrane and increased intracellular pH, shortened the APD and it reduces QT interval. This variant has not been identified in global databases, reinforcing its potential deleterious role. However, no conclusive data exist concerning the association of this variant with SQTS. It is associated with the so-called SQTS type 8, and responsible, so far, for <1% of all cases (**Figure 3**). At our point of view, genetic translation of variants in this gene should be done with caution due to its ambiguous role in SQTS.

CONCLUSIONS

Nearly 20 years ago, SQTS was reported as a familial arrhyhtmogenic entity. Nowadays, low number of families have been reported but with a high lethality. This lack of families impedes the stablishment of a conclusive risk stratification scale, particulary in asymptomatic cases carrying a genetic alteration. New development of hiPSC-CMs from patients may allow unraveling pathophysiological mechanism, helping to understand or treat the disease. Patients suffering of SQTS are at high risk of syncope and SCD. Implantation of an ICD remains the most effective preventive measure after aborted SCD and malignant ventricular arrhythmia although pharmacological therapies may be used in certain cases, especially in children. Currently, nevertheless improving advances in genetics, almost 70–80% of families remain without a genetic cause identified after a comprehensive analysis. Genotype-phenotype analysis are necessary in order to improve current guidelines in early identification as well as prevention in families suffering of SQTS.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Gussak I, Brugada P, Brugada J, Wright RS, Kopecky SL, Chaitman BR, et al. Idiopathic short QT interval: a new clinical syndrome? *Cardiology* (2000) 94:99–102. doi: 10.1159/000047299
2. Gaita F, Giustetto C, Bianchi F, Wolpert C, Schimpf R, Riccardi R, et al. Short QT Syndrome: a familial cause of sudden death. *Circulation* (2003) 108:965–70. doi: 10.1161/01.CIR.0000085071.28695.C4
3. Mazzanti A, Underwood K, Nevelev D, Kofman S, Priori SG. The new kids on the block of arrhythmogenic disorders: short QT syndrome and early repolarization. *J Cardiovasc Electrophysiol*. (2017) 28:1226–36. doi: 10.1111/jce.13265
4. Guerrier K, Kwiatkowski D, Czosek RJ, Spar DS, Anderson JB, Knilans TK. Short QT interval prevalence and clinical outcomes in a pediatric population. *Circ Arrhythm Electrophysiol*.(2015) 8:1460–4. doi: 10.1161/CIRCEP.115.003256
5. Mazzanti A, Kanthan A, Monteforte N, Memmi M, Bloise R, Novelli V, et al. Novel insight into the natural history of short QT syndrome. *J Am Coll Cardiol.* (2014) 63:1300–8. doi: 10.1016/j.jacc.2013.09.078
6. Ferrero-Miliani L, Holst AG, Pehrson S, Morling N, Bundgaard H. Strategy for clinical evaluation and screening of sudden cardiac death relatives. *Fundam Clin Pharmacol.* (2010) 24:619–35. doi: 10.1111/j.1472-8206.2010.00864.x
7. Jørgensen IN, Skakkebaek A, Andersen NH, Pedersen LN, Hougaard DM, Bojesen A, et al. Short QTc interval in males with klinefelter syndrome-influence of CAG repeat length, body composition, and testosterone replacement therapy. *Pacing Clin Electrophysiol*.(2015) 38:472–82. doi: 10.1111/pace.12580
8. Brugada J, Gussak I, Brugada P. Short QT syndrome: a predictable story. *Cardiology* (2014) 128:231–3. doi: 10.1159/000359995
9. Suzuki H, Hoshina S, Ozawa J, Sato A, Minamino T, Aizawa Y, et al. Short QT syndrome in a boy diagnosed on screening for heart disease. *Pediatr Int*. (2014) 56:774–6. doi: 10.1111/ped.12308
10. Gollob MH, Blier L, Brugada R, Champagne J, Chauhan V, Connors S, et al. Recommendations for the use of genetic testing in the clinical evaluation of inherited cardiac arrhythmias associated with sudden cardiac death: canadian cardiovascular society/canadian heart rhythm society joint position paper. *Can J Cardiol.* (2011) 27:232–45. doi: 10.1016/j.cjca.2010.12.078
11. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm* (2013) 10:1932–63. doi: 10.1016/j.hrthm.2013.05.014
12. Tülümen E, Giustetto C, Wolpert C, Maury P, Anttonen O, Probst V, et al. PQ segment depression in patients with short QT syndrome: a novel marker for diagnosing short QT syndrome? *Heart Rhythm* (2014) 11:1024–30. doi: 10.1016/j.hrthm.2014.02.024
13. Priori SG, Blomstrom-Lundqvist C. 2015 European Society of Cardiology Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death summarized by co-chairs. *Eur Heart J.* (2015) 36:2757–9. doi: 10.1093/eurheartj/ehv316
14. Giustetto C, Di Monte F, Wolpert C, Borggrefe M, Schimpf R, Sbragia P, et al. Short QT syndrome: clinical findings and diagnostic-therapeutic implications. *Eur Heart J.* (2006) 27:2440–7. doi: 10.1093/eurheartj/ehl185
15. Giustetto C, Scrocco C, Schimpf R, Maury P, Mazzanti A, Levetto M, et al. Usefulness of exercise test in the diagnosis of short QT syndrome. *Europace* (2015) 17:628–34. doi: 10.1093/europace/euu351
16. Frea S, Giustetto C, Capriolo M, Scrocco C, Fornengo C, Benedetto S, et al. New echocardiographic insights in short QT syndrome: more than a channelopathy? *Heart Rhythm* (2015) 12:2096–105. doi: 10.1016/j.hrthm.2015.05.024
17. Tristani-Firouzi M. The long and short of it: insights into the short QT syndrome. *J Am Coll Cardiol*. (2014) 63:1309–10. doi: 10.1016/j.jacc.2013.11.021
18. Behere SP, Weindling SN. Inherited arrhythmias: the cardiac channelopathies. *Ann Pediatr Cardiol.* (2015) 8:210–20. doi: 10.4103/0974-2069.164695
19. Bjerregaard P, Nallapaneni H, Gussak I. Short QT interval in clinical practice. *J Electrocardiol.* (2010) 43:390–5. doi: 10.1016/j.jelectrocard.2010.06.004
20. Giustetto C, Schimpf R, Mazzanti A, Scrocco C, Maury P, Anttonen O, et al. Long-term follow-up of patients with short QT syndrome. *J Am Coll Cardiol.* (2011) 58:587–95. doi: 10.1016/j.jacc.2011.03.038
21. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M.Brugada R, et al. Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation* (2004) 109:30–5. doi: 10.1161/01.CIR.0000109482.92774.3A
22. Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, et al. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation* (2007) 115:442–9. doi: 10.1161/CIRCULATIONAHA.106.668392
23. Templin C, Ghadri JR, Rougier JS, Baumer A, Kaplan V, Albesa M, et al. Identification of a novel loss-of-function calcium channel gene mutation in short QT syndrome (SQTS6). *Eur Heart J.* (2011) 32:1077–88. doi: 10.1093/eurheartj/ehr076
24. Hong K, Hu J, Yu J, Brugada R. Concomitant Brugada-like and short QT electrocardiogram linked to SCN5A mutation. *Eur J Hum Genet*. (2012) 20:1189–92. doi: 10.1038/ejhg.2012.63

25. Fernandez-Falgueras A, Sarquella-Brugada G, Brugada J, Brugada R, Campuzano O. Cardiac channelopathies and sudden death: recent clinical and genetic advances. *Biology* (2017) 6:E7. doi: 10.3390/biology6010007
26. Cunjin Luo , Kuanquan Wang , Henggui Zhang .Cunjin L, Kuanquan W, Henggui Z. Effects of island-distribution of mid-cardiomyocytes on ventricular electrical excitation associated with the KCNQ1-linked short QT syndrome. Conference proceedings. In: *Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Annual Conference 2017*. (Seogwipo) (2017). p. 3684–7.
27. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, 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) 17:405–24. doi: 10.1038/gim.2015.30
28. El-Battrawy I, Lan H, Cyganek L, Zhao Z, Li X, Buljubasic F, et al. Modeling short QT syndrome using human-induced pluripotent stem cell-derived cardiomyocytes. *J Am Heart Assoc*. (2018) 7. doi: 10.1161/JAHA.117.007394
29. Schimpf R, Wolpert C, Gaita F, Giustetto C, Borggrefe M. Short QT syndrome. *Cardiovasc Res.* (2005) 67:357–66. doi: 10.1016/j.cardiores.2005.03.026
30. Hu D, Li Y, Zhang J, Pfeiffer R, Gollob MH, Healey J, et al. The phenotypic spectrum of a mutation hotspot responsible for the short QT syndrome. *JACC Clin Electrophysiol.* (2017) 3:727–43. doi: 10.1016/j.jacep.2016.11.013
31. Bellocq C, van Ginneken AC, Bezzina CR, Alders M, Escande D, Mannens MM, et al. Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. *Circulation* (2004) 109:2394–7. doi: 10.1161/01.CIR.0000130409.72142.FE
32. Priori SG, Pandit SV, Rivolta I, Berenfeld O, Ronchetti E, Dhamoon A, et al. A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. *Circ Res*. (2005) 96:800–7. doi: 10.1161/01.RES.0000162101.76263.8c
33. Refsgaard L, Holst AG, Sadjadieh G, Haunso S, Nielsen JB, Olesen MS. High prevalence of genetic variants previously associated with LQT syndrome in new exome data. *Eur J Hum Genet*. (2012) 20:905–8. doi: 10.1038/ejhg.2012.23
34. Thorsen K, Dam VS, Kjaer-Sorensen K, Pedersen LN, Skeberdis VA, Jurevičius J. et al. Loss-of-activity-mutation in the cardiac chloride-bicarbonate exchanger AE3 causes short QT syndrome. *Nat Commun*. (2017) 8:1696. doi: 10.1038/s41467-017-01630-0

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REVIEW
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Electrophysiological Basis for Early Repolarization Syndrome

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During last centuries, Early Repolarization pattern has been interpreted as an ECG manifestation not linked to serious cardiovascular events. This view has been challenged on the basis of sporadic clinical observations that linked the J-wave with ventricular arrhythmias and sudden cardiac death. The particular role of this characteristic pattern in initiating ventricular fibrillation has been sustained by clinical descriptions of a marked and consistent J-wave elevation preceding the onset of the ventricular arrhythmia. Until now, Early Repolarization syndrome patients have been evaluated using ECG and theorizing different interpretations of the findings. Nonetheless, ECG analysis is not able to reveal all depolarization and repolarization properties and the explanation for this clinical events. Recent studies have characterized the epicardial substrate in these patients on the basis of high-resolution data, in an effort to provide insights into the substrate properties that support arrhythmogenicity in these patients. An overview for the current evidence supporting different theories explaining Early Repolarization Syndrome is provided in this review. Finally, future developments in the field directed toward individualized treatment strategies are examined.

Keywords: early repolarization, idiopathic ventricular fibrillation, mapping, sudden cardiac death, Brugada syndrome, ventricular fibrillation

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INTRODUCTION

The concept early repolarization (ER) points out a J-point elevation and terminal QRS abnormalities that might have a relative high prevalence in the population. In the last two decades, it has been proposed that early repolarization pattern (ERP) may be associated with an increased risk of ventricular fibrillation (1–4).

When ERP is associated with ventricular tachycardia or ventricular fibrillation in the absence of organic heart disease, ERP is referred to as early repolarization syndrome (ERS). Some studies evaluated different parameters to distinguish benign ERP from the malignant type (ERS), based on its electrocardiographic appearance (3, 5–10). The evaluation of ERP has been difficult in the past due to the absence of a clear definition. It is because of this problem that a high variability in the incidence of ERP has been described.

EPIDEMIOLOGY

ER pattern is significantly more common in blacks than in Caucasians. ER pattern seems to be more common in Aboriginal Australians than in Caucasian Australians (11). In the general population, the prevalence of an ER pattern in the lateral and/or inferior leads with a J point elevation of ≥ 0.1 mV ranges between 1b and 24%, and between 0b.6 and 6.4% for J point elevation of > 0.2 mV (11–13). These population-based and case control studies have provided some clinical evidence for an increased risk of suffering sudden cardiac death and life-threatening arrhythmic events in this population of patients presenting an ER pattern, particularly in inferior and infero-lateral leads (4, 10, 14).

Several epidemiological studies have tried to evaluate the risk of sudden cardiac death related to ER. A study from Finland evaluated ER in more than 10,000 patients. They described that the inferior ER pattern of 0.1 mV was present in 3.5% and lateral in 2.4% of the study population. J-Point Elevation in inferior or lateral leads was associated with death from cardiac arrhythmias. Interestingly, it was not the case in patients with J-point elevation in both inferior and lateral leads ($p =$ ns) (2). Another study of more than 29000 patients in the USA evaluated resting ambulatory ECG. They found that J waves or other common patterns of ST segment elevation was not associated to cardiovascular death (11). A recent meta-analysis has described a modest increase risk in arrhythmic death 1.70 (95% CI: 1.19–2.42; $p = 0.003$) and no significative risk in cardiac death or non-cardiac death (13). Rosso et al. have calculated that an ER pattern in a young patient from 35 to 45 years would increase the probability of suffering an episode of VF from 3.4 to 11 in 100,000 patients (15). Other authors have argued that ER would be a marker or vulnerability more than a disease by itself. Some epidemiological studies have proposed that ER would increase the risk of VF in the context of myocardial ischemia (16). Future studies will clarify the clinical implications of ERP in a population without history of cardiac arrhythmias.

ELECTROCARDIOGRAPHIC PATTERN

The presence of J waves in the electrocardiogram (ECG) have previously been reported in cases of healthy individuals, particularly in young males, black individuals and athletes. Prominent J waves have been described in hypercalcemia, hypothermia and ischemia (17–21).

For decades, an early ERP, consisting of a J point elevation, a slur or notch of the terminal part of the QRS with and without an ST segment elevation, was considered as benign (22, 23).

The benign nature of this pattern was challenged in 2001 on the basis of experimental laboratory data (in coronary-perfused wedge preparations) showing that this ECG morphology is linked to the development of polymorphic ventricular tachycardia and ventricular fibrillation (19, 24–26). The clinical validation of this hypothesis was provided less than a decade later (1, 15, 27).

Sometimes, the J wave can be so tall and broad to mimic an ST segment elevation. In humans, the normal J wave often appears as a J point elevation, with part of the J wave hidden inside the QRS. A horizontal/descending ST-segment morphology has been associated with an increased arrhythmic risk in the population with inferolateral ER (3, 28–30).

The evaluation of the ST segment in cases of ER is complex and sometimes suited to different interpretations. Also, there is a lack of consensus regarding whether only the leads with a J wave should be evaluated, or if only the revelation of a compatible morphology in a single lead is enough to make a diagnosis. In this situation, some research projects have started to evaluate the T wave and its relation to the R wave. Recently, a study has evaluated the characteristics of the T wave of 92 malignant inferolateral ER syndrome versus a group of 247 controls (30). The study has revealed that ERS patients present a lower amplitude of T waves, a lower T/R ratio in lead II or V5 and also al prolonged QTc interval. The data revealed that the combination of ERS and a QTc in the upper normal limit had an ominous prognosis.

Before considering the J-wave amplitude as a marker of risk, some limitations should be acknowledge. First, as demonstrated in a population study of more than 10,000 individuals, the prevalence in the general population of a J-point elevation in the inferior leads > 0.2 mV is very low (0.3%) (2). Second, J-point elevation is dynamic. 18.3% of the population with >0.1 mV didn't present this pattern in the follow up (1). Also, it has been shown that J wave increases preceding episodes of ventricular arrhythmias (1).

Some studies have tried to identify other risk factors associated with SCD (14). Pause dependent augmentation of the J wave has been proposed as a possible marker of risk (31). Twenty patients out of forty idiopathic ventricular fibrillation presented a pause dependent augmentation of the J wave. This characteristic had a lower sensitivity (55%) but a high specificity (100%) for ERS (31). New prospective observational data is needed to confirm these findings. Other authors have identified different ST-morphology variations linked with different phenotypes of ER (3). Descending or horizontal ST segment after J-point patients present an increased risk of sudden cardiac death. Also, ascending ST segments is not associated with sudden cardiac death (32).

DIAGNOSIS OF ERS

A great number of discussions regarding the diagnosis and identification relative to ER pattern have taken place in the past. The diagnosis criteria, based on consensus papers on ERP, have gradually changed from the initial focus on ST-segment elevation toward the abnormalities of the terminal QRS (slurring or notching), J wave and the evaluation of the T wave (10, 19, 32–34).

For that reason, an expert consensus report focused on the definition of ER stated that to diagnose an ER pattern, the peak of an end QRS notch and/or the onset of an end QRS slur be designated as Jp. This point should exceed 0.1 mV in ≥ 2 contiguous inferior and/or lateral leads of a standard 12-lead ECG. The QRS duration should be less than 120 ms measured in leads without a notch or slur (10).

In 2009, a consensus defined the ER ECG pattern as "J-point elevation and rapidly upsloping or normal ST segment" as "a normal variant" (35). Six years later, another consensus defined ERP as a slur or an end-QRS notch on the downslope of a prominent R wave (10). The slur/notch should be above the baseline and the QRS duration should be <120 ms. A peak of the J wave of 0.1 mV in >2 contiguous leads of the 12-lead EC (excluding leads V1–V3).

In 2016 Based on the existence of higher incidence of some specific patterns in the ECG of patients who have suffered idiopathic VF, a statement proposed two criteria that increase the risk of presenting an episode of idiopathic ventricular fibrillation. J waves in II, III, aVF (inferior leads) and a descending/horizontal pattern of ST following the J point (36).

Lastly, in 2017, an international consensus document added some ECG criteria for the diagnosis of ER including a threshold of 0.2 mV for the amplitude of the J wave in two inferior or lateral ECG leads and also the existence of dynamic changes of the J-point (Shanghai ERS Score). In this context, a benign pattern has been identified that is characterized by an upsloping ST segment after the J point (37). The Shanghai ERS diagnosis Score presented in **Table 1** is based on literature data and expert opinion. Due to the lack of large scale data or randomized controlled studies, rigorous weighted coefficients are lacking (34). However, the utilization of the scale can be regarded as a tool to orientate the clinician. Future studies will test this scale before using it in clinical practice.

As a resume, ERS is diagnosed in patients who present "ER in the inferior and/or lateral leads presenting with aborted cardiac arrest, documented VF, or polymorphic VT" (15, 32).

ER is identified if all these criteria are met "(a) There is an end-QRS notch or slur on the downslope of a prominent R-wave. If there is a notch, it should lie entirely above the baseline. The onset of a slur must also be above the baseline; (b) the peak of the notch or J wave ≥0.1 mV in ≥2 contiguous leads of the 12-lead ECG, excluding leads V1–V3; and (c) QRS duration <120 ms" (15, 32).

Due to the difficult diagnosis of this syndrome, the differential diagnosis has a major importance. The differential diagnosis of ERP is presented in **Table 2**.

TABLE 1 | Proposed Shanghai Score System for diagnosis of early repolarization syndrome.

	Points
I. Clinical History	
• Unexplained cardiac arrest, documented ventricular fibrillation or polymorphic ventricular tachycardia	3
• Suspected arrhythmic syncope	2
• Syncope of unclear mechanism/unclear etiology	1
II. Twelve-Lead ECG	
• Pattern A: ER ≥ 0.2 mV in ≥ 2 inferior and/or lateral ECG leads with horizontal/ descending ST segment.	2
• Pattern B: Dynamic changes in J-point elevation (≥0.1 mV) in ≥ 2 inferior and/or lateral ECG leads.	1.5
• Pattern C: ≥ 0.1 mV J-point elevation in at least 2 inferior and/or lateral ECG leads.	1
III. Ambulatory ECG Monitoring	
• Short-coupled premature ventricular contractions with R on ascending limb or peak of T wave	2
IV. Family History	
• Relative with definite ERS	2
• ≥2 first-degree relatives with a II.A. ECG pattern	2
• First-degree relative with a II.A. ECG pattern	1
• Unexplained sudden cardiac death ,45 years in a first- or second-degree relative	0.5
V. Genetic Test Result	
• Probable pathogenic ERS susceptibility mutation	0.5
Score (requires at least 1 ECG finding)	
• ≥ 5 points: Probable/ definite early repolarization syndrome	
• 3–4.5 points: Possible early repolarization syndrome	
• < 3 points: Nondiagnostic	

TABLE 2 | Differential diagnosis of early repolarization pattern.

- Metabolic disorders: Hypothermia, hyperthermia, hypocalcemia, hyperpotassemia
- Hypertensive heart disease
- Athlete's heart
- Myocardial ischemia (e.g., anteroseptal acute myocardial infarction)
- Thymoma
- Aortic dissection
- Arrhythmogenic right ventricular cardiomyopathy
- Takotsubo cardiomyopathy
- Intracerebral bleeding, acute cerebrovascular accident
- Pericardial disease, Myocardial tumor, and Myocarditis
- Chagas disease
- Cocaine intoxication

DIFFERENCES AND SIMILARITIES BETWEEN BRUGADA SYNDROME AND ERS

Notwithstanding some difficulties to make a diagnosis of ERS, some publications have tried to differentiate both entities (38–41). The region affected of both entities appears to be different, RVOT in BrS vs. inferior or lateral left ventricle in ERS (42). Both entities exhibit different incidence of late potentials in signal-averaged ECGs.

In BrS, 60% of patients present late potentials vs. 7% in the case of ERS (43). The effect of sodium channel blockers is different in both entities, the elevation of the ST segment is higher in BrS than in ERS (44). The incidence of other arrhythmias is higher in BrS than in ERS (45). Lastly, some articles have described diverse structural alterations in BrS that are not present in ERS (46). **Table 3** presents some differences between both entities.

GENETICS

Variants in 7 genes have been associated with both ER pattern and ERS (47–49). Variants in ABCC9 and KCNJ8, responsible for the pore forming and ATP-sensing subunits of the IK-ATP channel, have been reported in patients with ERS (12, 49, 50).

TABLE 3 | Differences between ERS and BrS.

	BrS	ERS
Region most involved	RVOT	Inferior LV wall
Leads affected	V_1–V_3, V5, V6, II, III, aVF (inferior and lateral repolarization cases)	II, III, aVF, V_4, V_5, V_6; I, aVL
Global Incidence	Asia BrS > ERS	Europe BrS = ERS (not confirmed)
Incidence of late potential in signal- averaged ECG	Higher	Lower
Prevalence of atrial fibrillation	Higher	Unknown
Effect of sodium channel blockers on surface ECG	Increased J-wave	Reduced J-wave
Structural changes, including mild fibrosis and reduced expression of Cx43 in RVOT	Higher in severe forms of the syndrome	Unknown

These findings are in the same direction of the experimental models showing that IK-ATP activation can generate an ER pattern in canine ventricular wedge preparations. Loss-of-function variations in subunits of the cardiac L-type calcium channel (CACNA1C, CACNB2, and CACNA2D1) and the sodium channel (SCN5A and SCN10A) have been also reported linked to ERS (51–53).

It is important to note that only a fraction of identified variants have been evaluated using functional expression studies to clarify causality and also pathogenesis. A few have been studied in native or induced pluripotent stem cell derived from ERS patients. The limitation of the genetic test interpretation is based mainly in the lack of biological or functional validation (54). **Table 4** shows the genes that have been associated with ER patterns and ERS.

PATHOPHYSIOLOGIC EVIDENCE AND ANIMALS MODELS

The pathophysiologic basis of the ER pattern is currently not entirely understood. A predominant theory states that J-point elevation appears in the context of a marked increase phase 1 notch of the epicardial action potential in relation to that of the endocardium. The consequence is an enhancement of ventricular transmural voltage gradient (**Figure 1**), which is illustrated as J-point elevation (55). Regarding the ionic mechanisms underlying an increased transmural voltage gradient, a marked increase in the epicardial action potential notch has been linked to a higher epicardial potassium current relative to the endocardium (55). Other ionic currents, including sodium, calcium and potassium-ATP dependent channels have also been described as involved in the repolarization variability in ER (26).

ER patients may also present a dynamic J-point variation. It has been described that this elevation is more obvious during period of bradycardia (56). The justification of this finding is that during episodes of increased vagal tone, the potassium currents (IK-ATP and IKACh) are increased and also there is a slow restoration of the Ito current (56). It should be note that in ERS, bradycardia mediated J-point elevation is more pronounced during episodes of high vagal tone than normal human beings.

TABLE 4 | Gene defects associated with the early repolarization syndrome (ERS).

	Locus	Gene	Ion channel	Percent of probands
ERS1	12*p*11.23	KCNJ8	$\uparrow I_{K-ATP}$	<1%
ERS2	12*p*13.3	CACNA1C	$\downarrow I_{Ca}$	4.1%
ERS3	10*p*12.33	CACNB2b	$\downarrow I_{Ca}$	8.3%
ERS4	7*q*21.11	CACNA2D1	$\downarrow I_{Ca}$	4.1%
ERS5	12*p*12.1	ABCC9	$\uparrow I_{K-ATP}$	<1%
ERS6	3*p*21	SCN5A	$\downarrow I_{Na}$	<1%
ERS7	3*p*22.2	SCN10A	$\downarrow I_{Na}$	<1%

Regarding the mechanism of ventricular arrhythmias, it seems that the dispersion of repolarization associated with ER enhances susceptibility to phase 2 reentry arrhythmias (55). As a consequence, a premature ventricular complex would interact with an predisposed ventricular substrate to trigger ventricular arrhythmias (57).

Recently, in an experimental model of canine ventricular preparation, an increment in vagal tone increase J-point elevation and induces phase 2 reentry. In addition, the authors showed that the intrinsic potassium current (Ito) was higher in the inferior wall of the left ventricle that could justify an increase sensitivity of this region for ventricular arrhythmias (58).

NON INVASIVE MAPPING IN PATIENTS SUFFERING ER SYNDROME

A recent publication has analyzed the electrophysiological substrate in 29 patients, 17 with the malignant form of ER syndrome and compared to 7 normal individuals (59). Body-surface ECG potentials were obtained simultaneously from 256 electrodes. Later, the patients underwent a CT scan with ECG gating to obtain the epicardial geometry and the electrode positions. The EP mapping data were evaluated for electrogram repolarization (measured by recovery time and activation recovery interval, the epicardial dispersion of repolarization was calculated based on the previous values), conduction (measured by activation time and activation duration) and morphology (J wave on local epicardial electrogram).

Epicardial J-wave was observed in EGMs from all ERS patients and in none of the controls. The study revealed in the ERS population, that the distribution of J waves was not localized to the inferior or lateral wall of the left ventricle, pointing that the substrate might not be limited to a specific region of the heart. The distribution of the epicardial J-wave was heterogeneous. Twenty seven percentage of the patients presented J-wave in the anterior wall, sixty five percentage in the lateral wall and seventy nine percentage in the inferior wall. There was an absence of

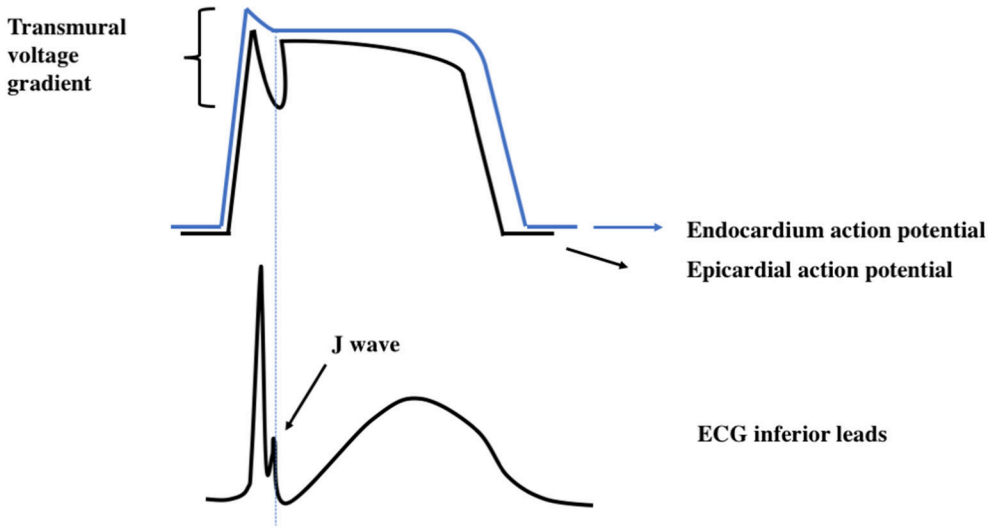

FIGURE 1 | Proposed electrophysiological mechanisms of J waves. The upper panel shows the possible origin of the voltage gradient. These transmural differences of action potential are supposed to generate the ERP.

fractionated electrograms. Also, there was not any data pointing to a delay activation in this group of ERS patients.

The data also showed that action potential duration in areas with J waves was shorter than the control group. In addition, the shortening of action potential duration was heterogeneous within the ventricle. The result was the creation of sharp repolarization gradients in comparison with controls. This characteristic is presented as the pathophysiologic basis of ERS and a differentiation with BrS. In BsS patients, a prolongation in the activation recovery interval has also been described. The latter has been studied also using ECGi (60). In BsS patients, both steep dispersion of repolarization and slow discontinuous conduction were present in the right ventricular outflow tract (60).

As a resume, this study has evaluated a cohort of ERS patients using noninvasive ECGi mapping, the arrhythmogenic abnormal substrate has been characterized by a heterogeneous shortening of the action potential duration and as a result, creation of steep repolarization gradients (59). Both mechanism could provide a substrate for re-entrant arrhythmias. These findings are different to those of BrS, where also a delay in ventricular activation has been described (60).

CLINICAL IMPLICATIONS

Although in the last two decades, a great number of articles have improved our understanding of ERS, there is an important knowledge gap regarding the pathophysiology, the clinical manifestations and management of ERS. ICD implantation is recommended for secondary prevention of SCD. In the field of primary prevention, ICD has been suggested by some authors as an option in case of "high risk" pattern, strong and unequivocal family history of SCD (37). It should be noted that the implantation of an ICD in the primary prevention setting is not free of complications (inappropriate shocks, infections). In this situation, the benefit of an implantation should be balanced against the risk of the complications. A clear definition of the syndrome and the risk of SCD in the primary prevention setting should be clarified before promoting the implantation of an ICD.

FUTURE DIRECTIONS

There are a lot of areas of uncertainty regarding the diagnosis, epidemiology, biological substrate, associations, prognosis and treatment of ERS. First, to avoid confusions regarding the diagnosis of the disease, ERS diagnosis should be based on prevalent and relevant variant patterns. Second, in order to identify the groups at risk and define the triggers, prospective studies should be performed in populations at risk. Third, due to the fact that the biological substrate is not completely understood, and the mechanism of ventricular arrhythmias are not fully elucidated, genetics and research in basic research would help to clarify the factors that promote arrhythmogenesis. Forth, there is still a lack of data to quantify predictive values and the number needed to treat in primary prevention. Fifth, the etiologic fraction of this pattern is probably still low and should be assessed. Sixth, due to the previous statements, an effective cost-effective treatment or preventive therapy are lacking. Large scale registries analyzing different cost-effective approaches will be necessary to deliver the best therapy for the population.

CONCLUSION

ER is a frequent ECG characteristic in the general population. In a very small number of cases, ER is the unique apparent cause of SCD in and individual or family. Also, a complex genetic pattern favors the idea that ER is probably a disease-modifying factor than a standalone disease.

Therefore, proper identification of ER high risk patterns is critical to improve assessment and prevention. More research is needed to better understand the electrophysiological basis and clinical significance, prognosis and prevention of ER. The design of algorithms to integrate the stratification of risk of ER is a key topic for future research in the field of cardiac arrhythmias.

AUTHOR CONTRIBUTIONS

RC: conception or design of the work, drafting the work, provide approval for publication of the content. JS, MK, DL, SM, PB: reviewers of the article and critical corrections, providing approval for publication of the content.

REFERENCES

1. Haissaguerre M, Derval N, Sacher F, Jesel L, Deisenhofer I, de Roy L, et al. Sudden cardiac arrest associated with early repolarization. *N Engl J Med.* (2008) 358:2016–23. doi: 10.1056/NEJMoa071968
2. Tikkanen JT, Anttonen O, Junttila MJ, Aro AL, Kerola T, Rissanen HA, et al. Long-term outcome associated with early repolarization on electrocardiography. *N Engl J Med.* (2009) 361:2529–37. doi: 10.1056/NEJMoa0907589
3. Tikkanen JT, Junttila MJ, Anttonen O, Aro AL, Luttinen S, Kerola T, et al. Early repolarization: electrocardiographic phenotypes associated with favorable long-term outcome. *Circulation* (2011) 123:2666–73. doi: 10.1161/circulationaha.110.014068
4. Haissaguerre M, Sacher F, Nogami A, Komiya N, Bernard A, Probst V, et al. Characteristics of recurrent ventricular fibrillation associated with inferolateral early repolarization role of drug therapy. *J Am Coll Cardiol.* (2009) 53:612–9. doi: 10.1016/j.jacc.2008.10.044
5. Noseworthy PA, Tikkanen JT, Porthan K, Oikarinen L, Pietila A, Harald K, et al. The early repolarization pattern in the general population: clinical correlates and heritability. *J Am Coll Cardiol.* (2011) 57:2284–9. doi: 10.1016/j.jacc.2011.04.003
6. Tikkanen JT. The phenomenon of early repolarization: a false alarm? *Circ Arrhythm Electrophysiol.* (2014) 7:368–9. doi: 10.1161/circep.114.001792
7. Tikkanen JT, Huikuri HV. Characteristics of "malignant" vs. "benign" electrocardiographic patterns of early repolarization. *J Electrocardiol.* (2015) 48:390–4. doi: 10.1016/j.jelectrocard.2014.12.020
8. Junttila MJ, Sager SJ, Tikkanen JT, Anttonen O, Huikuri HV, Myerburg RJ. Clinical significance of variants of J-points and J-waves: early repolarization patterns and risk. *Eur Heart J.* (2012) 33:2639–43. doi: 10.1093/eurheartj/ehs110
9. Tikkanen JT, Huikuri HV. Early repolarization ECG pattern in the Finnish general population. *J Electrocardiol.* (2013) 46:439–41. doi: 10.1016/j.jelectrocard.2013.06.012
10. Macfarlane PW, Antzelevitch C, Haissaguerre M, Huikuri HV, Potse M, Rosso R, et al. The Early repolarization pattern: a consensus paper. *J Am Coll Cardiol.* (2015) 66:470–7. doi: 10.1016/j.jacc.2015.05.033
11. Uberoi A, Jain NA, Perez M, Weinkopff A, Ashley E, Hadley D, et al. Early repolarization in an ambulatory clinical population. *Circulation* (2011) 124:2208–14. doi: 10.1161/CIRCULATIONAHA.111.047191
12. Barajas-Martinez H, Hu D, Ferrer T, Onetti CG, Wu Y, Burashnikov E, et al. Molecular genetic and functional association of Brugada and early repolarization syndromes with S422L missense mutation in KCNJ8. *Heart Rhythm* (2012) 9:548–55. doi: 10.1016/j.hrthm.2011.10.035
13. Sinner MF, Porthan K, Noseworthy PA, Havulinna AS, Tikkanen JT, Muller-Nurasyid M, et al. A meta-analysis of genome-wide association studies of the electrocardiographic early repolarization pattern. *Heart Rhythm* (2012) 9:1627–34. doi: 10.1016/j.hrthm.2012.06.008
14. Aizawa Y, Chinushi M, Hasegawa K, Naiki N, Horie M, Kaneko Y, et al. Electrical storm in idiopathic ventricular fibrillation is associated with early repolarization. *J Am Coll Cardiol.* (2013) 62:1015–9. doi: 10.1016/j.jacc.2013.05.030
15. Rosso R, Kogan E, Belhassen B, Rozovski U, Scheinman MM, Zeltser D, et al. J-point elevation in survivors of primary ventricular fibrillation and matched control subjects: incidence and clinical significance. *J Am Coll Cardiol.* (2008) 52:1231–8. doi: 10.1016/j.jacc.2008.07.010
16. Tikkanen JT, Wichmann V, Junttila MJ, Rainio M, Hookana E, Lappi OP, et al. Association of early repolarization and sudden cardiac death during an acute coronary event. *Circ Arrhythm Electrophysiol.* (2012) 5:714–8. doi: 10.1161/CIRCEP.112.970863
17. Federman NJ, Mechulan A, Klein GJ, Krahn AD. Ventricular fibrillation induced by spontaneous hypothermia in a patient with early repolarization syndrome. *J Cardiovasc Electrophysiol.* (2013) 24:586–8. doi: 10.1111/jce.12030
18. Bastiaenen R, Hedley PL, Christiansen M, Behr ER. Therapeutic hypothermia and ventricular fibrillation storm in early repolarization syndrome. *Heart Rhythm* (2010) 7:832–4. doi: 10.1016/j.hrthm.2010.02.037
19. Antzelevitch C, Yan GX. J wave syndromes. *Heart Rhythm* (2010) 7:549–58. doi: 10.1016/j.hrthm.2009.12.006
20. Sridharan MR, Horan LG. Electrocardiographic J wave of hypercalcemia. *Am J Cardiol.* (1984) 54:672–3.
21. Clements SD, Hurst JW. Diagnostic value of electrocardiographic abnormalities observed in subjects accidentally exposed to cold. *Am J Cardiol.* (1972) 29:729–34.
22. Wasserburger RH, Alt WJ. The normal RS-T segment elevation variant. *Am J Cardiol.* (1961) 8:184–92.
23. Mehta MC, Jain AC. Early repolarization on scalar electrocardiogram. *Am J Med Sci.* (1995) 309:305–11.
24. Shu J, Zhu T, Yang L, Cui C, Yan GX. ST-segment elevation in the early repolarization syndrome, idiopathic ventricular fibrillation, and the Brugada syndrome: cellular and clinical linkage. *J Electrocardiol.* (2005) 38:26–32. doi: 10.1016/j.jelectrocard.2005.06.006
25. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. *Circulation* (1999) 100:1660–6.
26. Gussak I, Antzelevitch C. Early repolarization syndrome: clinical characteristics and possible cellular and ionic mechanisms. *J Electrocardiol.* (2000) 33:299–309. doi: 10.1054/jelc.2000.18106
27. Nam GB, Kim YH, Antzelevitch C. Augmentation of J waves and electrical storms in patients with early repolarization. *N Engl J Med.* (2008) 358:2078–9. doi: 10.1056/NEJMc0708182
28. Rosso R, Adler A, Halkin A, Viskin S. Risk of sudden death among young individuals with J waves and early repolarization: putting the evidence into perspective. *Heart Rhythm* (2011) 8:923–9. doi: 10.1016/j.hrthm.2011.01.037
29. Rollin A, Maury P, Bongard V, Sacher F, Delay M, Duparc A, et al. Prevalence, prognosis, and identification of the malignant form of early repolarization pattern in a population-based study. *Am J Cardiol.* (2012) 110:1302–8. doi: 10.1016/j.amjcard.2012.06.033
30. Roten L, Derval N, Maury P, Mahida S, Pascale P, Leenhardt A, et al. Benign vs. malignant inferolateral early repolarization: Focus on the T wave. *Heart Rhythm* (2016) 13:894–902. doi: 10.1016/j.hrthm.2015.11.020
31. Aizawa Y, Sato A, Watanabe H, Chinushi M, Furushima H, Horie M, et al. Dynamicity of the J-wave in idiopathic ventricular fibrillation with a special reference to pause-dependent augmentation of the J-wave. *J Am Coll Cardiol.* (2012) 59:1948–53. doi: 10.1016/j.jacc.2012.02.028
32. Antzelevitch C, Yan GX, Ackerman MJ, Borggrefe M, Corrado D, Guo J, et al. J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *J Arrhythm.* (2016) 32:315–39. doi: 10.1016/j.joa.2016.07.002
33. Antzelevitch C, Yan GX. J-wave syndromes: Brugada and early repolarization syndromes. *Heart Rhythm* (2015) 12:1852–66. doi: 10.1016/j.hrthm.2015.04.014
34. Priori SG, Napolitano C. J-Wave Syndromes: electrocardiographic and clinical aspects. *Card Electrophysiol Clin.* (2018) 10:355–69. doi: 10.1016/j.ccep.2018.02.009

35. Rautaharju PM, Surawicz B, Gettes LS, Bailey JJ, Childers R, Deal BJ, et al. AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram: part IV: the ST segment, T and U waves, and the QT interval: a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society. Endorsed by the International Society for Computerized Electrocardiology. *J Am Coll Cardiol.* (2009) 53:982–91. doi: 10.1016/j.jacc.2008.12.014
36. Patton KK, Ellinor PT, Ezekowitz M, Kowey P, Lubitz SA, Perez M, et al. Electrocardiographic early repolarization: a scientific statement from the American Heart Association. *Circulation* (2016) 133:1520–9. doi: 10.1161/cir.0000000000000388
37. Antzelevitch C, Yan GX, Ackerman MJ, Borggrefe M, Corrado D, Guo J, et al. J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Europace* (2017) 19:665–94. doi: 10.1093/europace/euw235
38. Sieira J, Ciconte G, Conte G, de Asmundis C, Chierchia GB, Baltogiannis G, et al. Long-term prognosis of drug-induced Brugada syndrome. *Heart Rhythm* (2017) 14:1427–33. doi: 10.1016/j.hrthm.2017.04.044
39. Sieira J, Ciconte G, Conte G, Chierchia GB, de Asmundis C, Baltogiannis G, et al. Asymptomatic Brugada syndrome: clinical characterization and long-term prognosis. *Circ Arrhythm Electrophysiol.* (2015) 8:1144–50. doi: 10.1161/CIRCEP.114.003044
40. Casado-Arroyo R, Berne P, Rao JY, Rodriguez-Mañero M, Levinstein M, Conte G, et al. Long-term trends in newly diagnosed Brugada syndrome: implications for risk stratification. *J Am Coll Cardiol.* (2016) 68:614–23. doi: 10.1016/j.jacc.2016.05.073
41. Sieira J, Conte G, Ciconte G, Chierchia GB, Casado-Arroyo R, Baltogiannis G, et al. A score model to predict risk of events in patients with Brugada Syndrome. *Eur Heart J.* (2017) 38:1756–63. doi: 10.1093/eurheartj/ehx119
42. Nademanee K, Raju H, de Noronha SV, Papadakis M, Robinson L, Rothery S, et al. Fibrosis, connexin-43, and conduction abnormalities in the Brugada syndrome. *J Am Coll Cardiol.* (2015) 66:1976–86. doi: 10.1016/j.jacc.2015.08.862
43. Olesen MS, Jensen NF, Holst AG, Nielsen JB, Tfelt-Hansen J, Jespersen T, et al. A novel nonsense variant in Nav1.5 cofactor MOG1 eliminates its sodium current increasing effect and may increase the risk of arrhythmias. *Can J Cardiol.* (2011) 27:523.e17–23. doi: 10.1016/j.cjca.2011.01.003
44. Kawata H, Noda T, Yamada Y, Okamura H, Satomi K, Aiba T, et al. Effect of sodium-channel blockade on early repolarization in inferior/lateral leads in patients with idiopathic ventricular fibrillation and Brugada syndrome. *Heart Rhythm* (2012) 9:77–83. doi: 10.1016/j.hrthm.2011.08.017
45. Junttila MJ, Tikkanen JT, Kentta T, Anttonen O, Aro AL, Porthan K, et al. Early repolarization as a predictor of arrhythmic and nonarrhythmic cardiac events in middle-aged subjects. *Heart Rhythm* (2014) 11:1701–6. doi: 10.1016/j.hrthm.2014.05.024
46. Di Diego JM, Antzelevitch C. J wave syndromes as a cause of malignant cardiac arrhythmias. *Pacing Clin Electrophysiol.* (2018) 41:684–99. doi: 10.1111/pace.13408
47. Reinhard W, Kaess BM, Debiec R, Nelson CP, Stark K, Tobin MD, et al. Heritability of early repolarization: a population-based study. *Circ Cardiovasc Genet.* (2011) 4:134–8. doi: 10.1161/CIRCGENETICS.110.958298
48. Nunn LM, Bhar-Amato J, Lowe MD, Macfarlane PW, Rogers P, McKenna WJ, et al. Prevalence of J-point elevation in sudden arrhythmic death syndrome families. *J Am Coll Cardiol.* (2011) 58:286–90. doi: 10.1016/j.jacc.2011.03.028
49. Haissaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Loussouarn G, et al. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. *J Cardiovasc Electrophysiol.* (2009) 20:93–8. doi: 10.1111/j.1540-8167.2008.01326.x
50. Medeiros-Domingo A, Tan BH, Crotti L, Tester DJ, Eckhardt L, Cuoretti A, et al. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac K(ATP) channel Kir6.1 as a pathogenic substrate for J-wave syndromes. *Heart Rhythm* (2010) 7:1466–71. doi: 10.1016/j.hrthm.2010.06.016
51. Watanabe H, Nogami A, Ohkubo K, Kawata H, Hayashi Y, Ishikawa T, et al. Electrocardiographic characteristics and SCN5A mutations in idiopathic ventricular fibrillation associated with early repolarization. *Circ Arrhythm Electrophysiol.* (2011) 4:874–81. doi: 10.1161/CIRCEP.111.963983
52. Burashnikov E, Pfeiffer R, Barajas-Martinez H, Delpon E, Hu D, Desai M, et al. Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. *Heart Rhythm* (2010) 7:1872–82. doi: 10.1016/j.hrthm.2010.08.026
53. Hu D, Barajas-Martínez H, Pfeiffer R, Dezi F, Pfeiffer J, Buch T, et al. Mutations in SCN10A are responsible for a large fraction of cases of Brugada syndrome. *J Am Coll Cardiol.* (2014) 64:66–79. doi: 10.1016/j.jacc.2014.04.032
54. Schwartz PJ, Ackerman MJ, George AL, Wilde AAM. Impact of genetics on the clinical management of channelopathies. *J Am Coll Cardiol.* (2013) 62:169–80. doi: 10.1016/j.jacc.2013.04.044
55. Yan GX, Antzelevitch C. Cellular basis for the electrocardiographic J wave. *Circulation* (1996) 93:372–9.
56. Nam GB, Ko KH, Kim J, Park KM, Rhee KS, Choi KJ, et al. Mode of onset of ventricular fibrillation in patients with early repolarization pattern vs. Brugada syndrome. *Eur Heart J.* (2010) 31:330–9. doi: 10.1093/eurheartj/ehp423
57. Meregalli PG, Wilde AAM, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? *Cardiovasc Res.* (2005) 67:367–78. doi: 10.1016/j.cardiores.2005.03.005
58. Koncz I, Gurabi Z, Patocskai B, Panama BK, Szel T, Hu D, et al. Mechanisms underlying the development of the electrocardiographic and arrhythmic manifestations of early repolarization syndrome. *J Mol Cell Cardiol.* (2014) 68:20–8. doi: 10.1016/j.yjmcc.2013.12.012
59. Zhang J, Hocini M, Strom M, Cuculich PS, Cooper DH, Sacher F, et al. The electrophysiological substrate of early repolarization syndrome: noninvasive mapping in patients. *JACC Clin Electrophysiol.* (2017) 3:894–904. doi: 10.1016/j.jacep.2016.12.017
60. Zhang J, Sacher F, Hoffmayer K, O'Hara T, Strom M, Cuculich P, et al. Cardiac electrophysiological substrate underlying the ECG phenotype and electrogram abnormalities in Brugada syndrome patients. *Circulation* (2015) 131:1950–9. doi: 10.1161/CIRCULATIONAHA.114.013698

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Early Repolarization Syndrome: Diagnostic and Therapeutic Approach

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An early repolarization pattern can be observed in 1% up to 13% of the overall population. Whereas, this pattern was associated with a benign outcome for many years, several more recent studies demonstrated an association between early repolarization and sudden cardiac death, so-called early repolarization syndrome. In early repolarization syndrome patients, current imbalances between epi- and endo-cardial layers result in dispersion of de- and repolarization. As a consequence, J waves or ST segment elevations can be observed on these patients' surface ECGs as manifestations of those current imbalances. Whereas, an early repolarization pattern is relatively frequently found on surface ECGs in the overall population, the majority of individuals presenting with an early repolarization pattern will remain asymptomatic and the isolated presence of an early repolarization pattern does not require further intervention. The mismatch between frequently found early repolarization patterns in the overall population, low incidences of sudden cardiac deaths related to early repolarization syndrome, but fatal, grave consequences in affected patients remains a clinical challenge. More precise tools for risk stratification and identification of this minority of patients, who will experience events, remain a clinical need. This review summarizes the epidemiologic, pathophysiologic and diagnostic background and presents therapeutic options of early repolarization syndrome.

Keywords: early repolarization syndrome, sudden cardiac death, J wave, ICD implantation, idiopathic ventricular fibrillation

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INTRODUCTION

Up to 10% of all sudden cardiac deaths are caused by primary electrical disorders or ion channel diseases. As consequence, the identification of genetic mutations affecting these ion channels has opened a new area of translational research in cardiac electrophysiology (1, 2). Over the last decades, an early repolarization pattern had been considered as a benign finding, it is frequently observed on surface ECGs, characterized by J-point and ST segment elevation in 2 or more contiguous leads. More recently, the early repolarization pattern has increasingly attracted attention as it has been reported as a risk to idiopathic ventricular fibrillation and sudden cardiac death in case-control studies, characterized as early repolarization syndrome. This review provides a historic, epidemiologic and pathophysiologic background and describes diagnostic and therapeutic approaches in the treatment of early repolarization syndrome.

HISTORIC PERSPECTIVE

In 1936, Shipley and Hallaran firstly described an early ST segment elevation in ECGs which they derived in a population of 200 young, healthy individuals. They observed this phenomenon in 25% of male and in 16% of female study participants (3). Two years later, in 1938, similar findings were observed in the surface ECG of an individual who died from hypothermia (4). In 1951, the term "Early Repolarization" was firstly used by Grant in a study on vector electrocardiography (5). Osborn described the classic J-wave in experimental hypothermia in 1953, presumably resulting from an increased dispersion of repolarization caused by a disproportionate abbreviation of the epicardial action potential compared to the endocardium (6).

Historically, early repolarization was described as a normal variant phenotype. Since the past decade, growing evidence exists about the association between an early repolarization phenotype and the incidence of sudden cardiac death (7). In 2008, Haïssaguerre et al. reported a significantly increased prevalence of early repolarization in patient cohort with history of idiopathic ventricular fibrillation (8).

EPIDEMIOLOGY

Early repolarization can be observed in 1% up to 13% of the overall population, with a higher incidence in the more recent studies and in populations of athletes and adolescents. For a long time, early repolarization was associated with benign outcome (9–13). Recently, an association between early repolarization in the inferolateral leads and sudden cardiac death has been described in several studies. There may be two potential reasons for the observed variation in incidence of early repolarization between single studies: Firstly, the definition and interpretation of early repolarization used in different studies varies. Secondly, there are significant differences in baseline characteristics of studied populations. Studies which showed a correlation of early repolarization and sudden cardiac death included mainly Caucasian and Asian and less African or American individuals. It is noticeable that among all studied patients showing early repolarization, 75% were males (13–15). J point elevation is found more frequently in patients with idiopathic ventricular fibrillation than in healthy individuals (11). In concordance, in sudden cardiac deaths related to early repolarization, male gender is found in 75% (8, 13).

In a case-control study of Haïssaguerre et al. the prevalence of early repolarization was documented in 31% of patients with a history of idiopathic ventricular fibrillation, whereas only in 5% of healthy controls (8). In a meta-analysis, the absolute risk for sudden cardiac death in individuals with early repolarization syndrome is estimated to be 0.07% (16).

PATHOPHYSIOLOGY AND GENETICS

In early repolarization syndrome patients, current imbalances between epi- and endo-cardial layers result in a dispersion of de- and repolarization. These imbalances manifest as J wave or ST segment elevation on the surface ECG. In the epicardium, a larger transient-outward K^+ (I_{to}) and Adenosine triphosphate-sensitive current (I_{KATP}), and a reduced inward sodium (I_{Na}) and inward calcium (I_{CaL}) current than in the endocardium result in greater net repolarizing outward current flow during the early phase of the myocardial action potential. In early repolarization patients, a further increase in epicardial net outward current results in an increase of differences of action potential between epi- and endo-cardium. The resulting prominent notch in the action potential of ventricular epicardium but not endocardium induces a transmural voltage gradient during ventricular activation and manifests as J wave on the surface ECG. The electrical heterogeneity results in so-called phase 2 reentries, which produce closely coupled PVCs capable of initiating circus movement reentry and ventricular fibrillation. (**Figures 1A,B**, **3A,B**). The amplitude of the J waves, representing a disequilibrium between epi- and endo-cardial currents, increases during bradycardia phases and vagotonia, short-long sequences are then more likely to trigger ventricular fibrillation.

Experimental studies showed that testosterone increases outward potassium currents, including the rapidly activating and the slowly activating component of the delayed rectifier potassium current, and decreased the inward L-type calcium current (17, 18). As the maintenance of the action potential dome is determined by a precise balance of currents, any agents that increase outward currents or decrease inward currents may increase the magnitude of the action potential notch, increasing the voltage gradient across the endo- and epi-cardium, thus augmenting ST-segment and J-point elevation.

The previously described male predominance in the prevalence of an early repolarization ECG pattern (13–15) may be attributable to the higher testosterone levels of males, resulting in an increased outward potassium current and an increased J point elevation.

Early repolarization is more frequently found in genetic relatives of patients with a history of arrhythmic sudden cardiac death, what suggests pro-arrhythmic genetic mutations and in several recent studies, early repolarization syndrome has been described as a kind of heritable disease. Population-based studies suggested some degree of inheritance of early repolarization ECG patterns (19–21). However, only a few genes have related to early repolarization syndrome have been identified by now. Mutations of KCNJ8, which encodes a subunit of the ATP-sensitive potassium channel, of the L-type calcium channel genes (CACNA1C, CACNB2B, CACNA2D1) as well as loss-of-function mutations of SCN5A are related to early repolarization and idiopathic VF (22–25). Most of the early repolarization syndrome causing gene mutations were detected in the sporadic cases and the current understanding of the genetic basis of early repolarization syndrome remains limited. It has recently been proposed that early repolarization syndrome may be a near-Mendelian or oligogenic inheritance disease (21).

DIAGNOSIS

An "early repolarization pattern" is diagnosed on the surface ECG as a sharp, positive deflection at the onset of the ST segment, following immediately after a positive QRS complex. Also, as

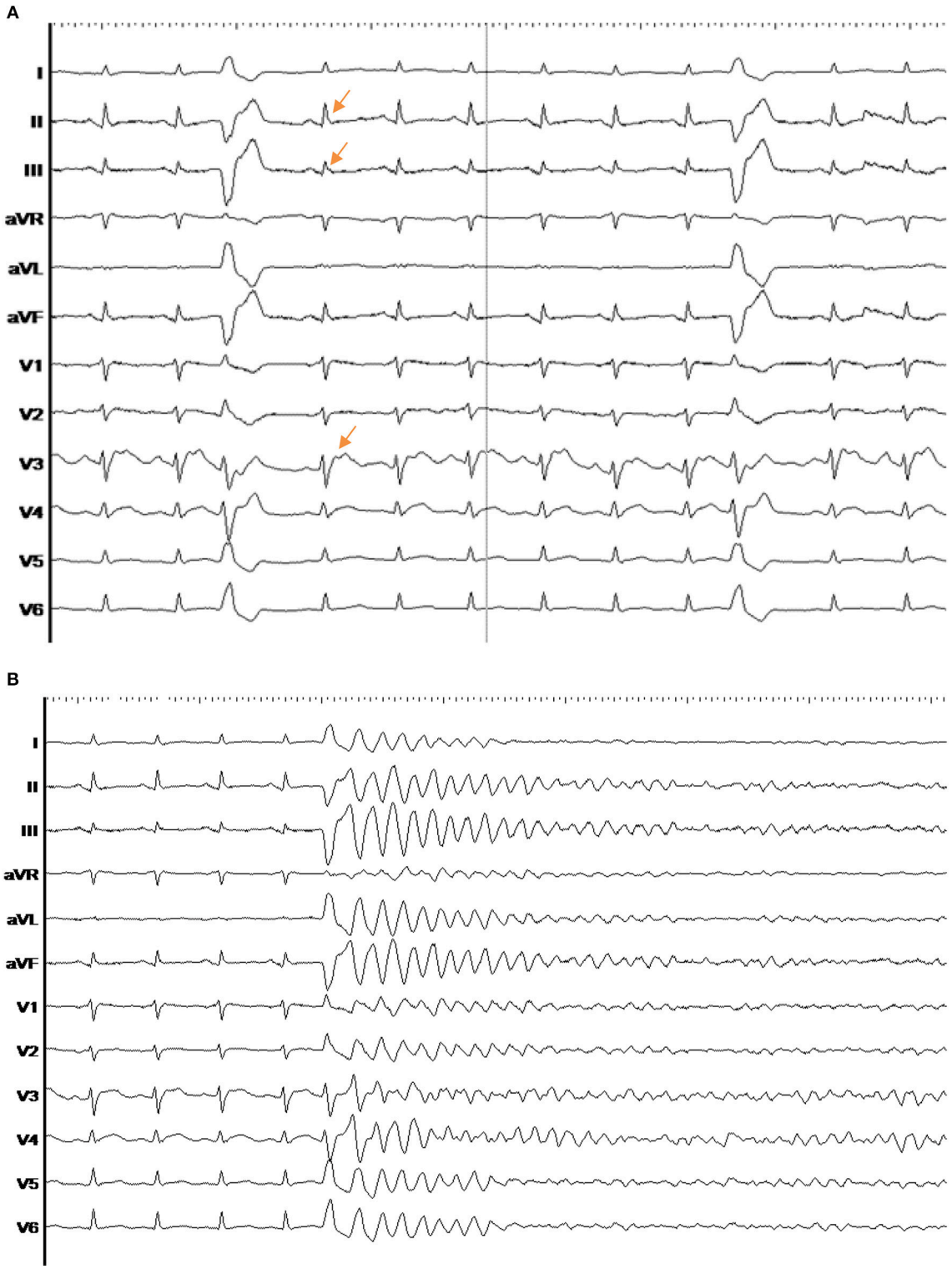

FIGURE 1 | (A) ECG example of a patient Nr. 1 with early repolarization syndrome. Two frequent clinical PVCs, arising from the septal basal RV, are documented. **(B)** The clinical PVC of early repolarization syndrome patient Nr. 1 induces an episode of ventricular fibrillation.

an increased J point level may be hidden in the terminal QRS complex, early repolarization may be indicated by a slurring of the terminal QRS complex. A J point elevation, exceeding 0.1 mV, has to be present in two or more contiguous inferior and/or lateral leads. The onset of the QRS slurring has to be entirely above the ECG baseline level, and the angle between the tangents of the slurring and the initial R downslope has to be $>10°$ (26). An "early repolarization syndrome" can be diagnosed, if the early repolarization pattern is found in a patient with a history of idiopathic ventricular fibrillation or polymorphic ventricular tachycardia. It is important to emphasize that an "early repolarization pattern" on its own is not a cardiac arrhythmic disease. The 2013 Expert Consensus Statement by Heart Rhythm Society (HRS), the European Heart Rhythm Association (EHRA), and the Asia Pacific Heart Rhythm Society (APHRS) summarizes diagnostic criteria: (27)

- Early repolarization syndrome **is diagnosed** by the presence of J-point elevation $\geq$1 mm in $\geq$2 contiguous inferior and/or lateral leads of a standard 12-lead ECG in a patient resuscitated from otherwise unexplained ventricular fibrillation/ polymorphic ventricular tachycardia.
- Early repolarization syndrome **can be diagnosed** in a SCD victim with a negative autopsy and medical chart review with a previous ECG demonstrating J-point elevation $\geq$1 mm in $\geq$2 contiguous inferior and/or lateral leads of a standard 12-lead ECG.
- Early repolarization pattern **can be diagnosed** by the presence of J-point elevation $\geq$1 mm in $\geq$2 contiguous inferior and/or lateral leads of a standard 12-lead ECG.

As an early repolarization pattern can be relatively frequently observed in the overall population, but also growing evidence exists that early repolarization is linked to sudden cardiac death, there is a specific need for risk stratification.

Life-threatening arrhythmias are often the first, unexpected clinical manifestation of early repolarization syndrome. An increase of J wave/ST segment amplitude has been described before the onset of ventricular fibrillation in early repolarization patients. Ventricular fibrillation is often initiated by short-long-short QRS sequences, with a single PVC falling into the T-wave of the preceding QRS complex (28).

ECG examples of early repolarization patients are presented in **Figures 1–4**.

RISK STRATIFICATION

Based on different studies linking early repolarization pattern to the incidence of sudden cardiac death, the majority of patients will remain asymptomatic, while arrhythmic events and sudden cardiac death will only occur in few patients. Thus, more precise tools for risk stratification and the identification of this minority of patients, who will experience events, remain a clinical need and challenge.

Observational studies subcategorized early repolarization ECG patterns to distinguish benign and malignant ECG variations. Tikkanen et al. classified ST segments as either horizontal/descending ($\leq$0.1 mV within 100 ms after the J point) or upsloping/ascending ($>$0.1 mV elevation throughout the ST segment). The ascending early repolarization pattern

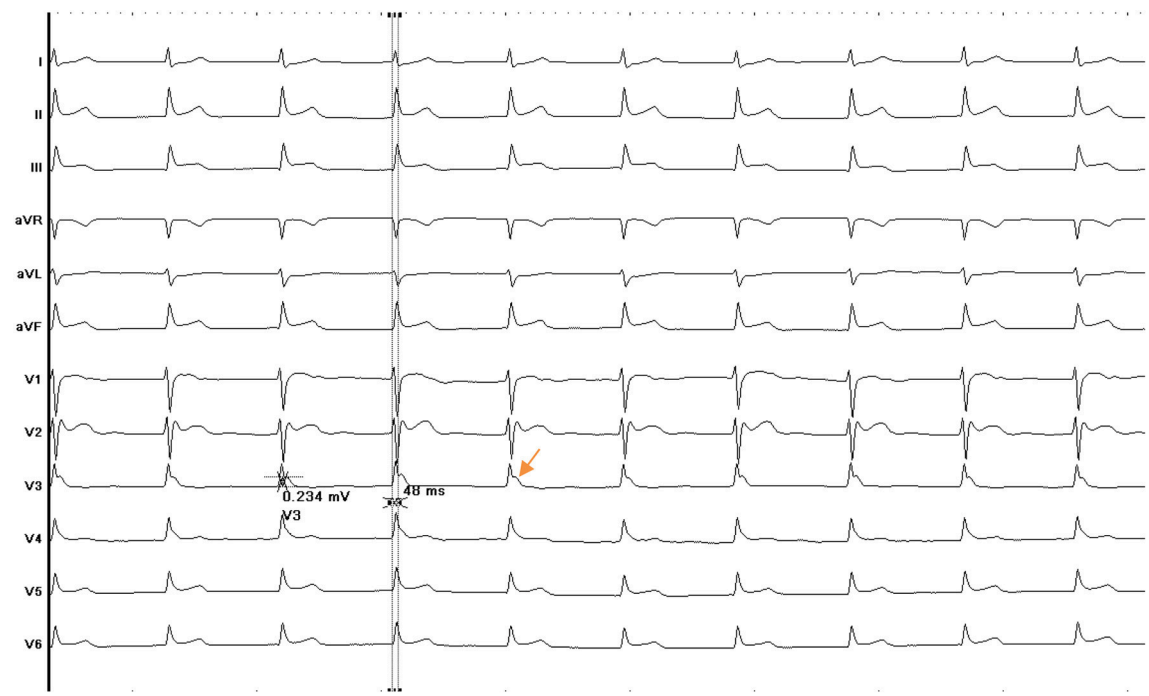

FIGURE 2 | ECG of patient Nr. 2 presenting with early repolarization syndrome. J point elevation is predominantly present in lead v3.

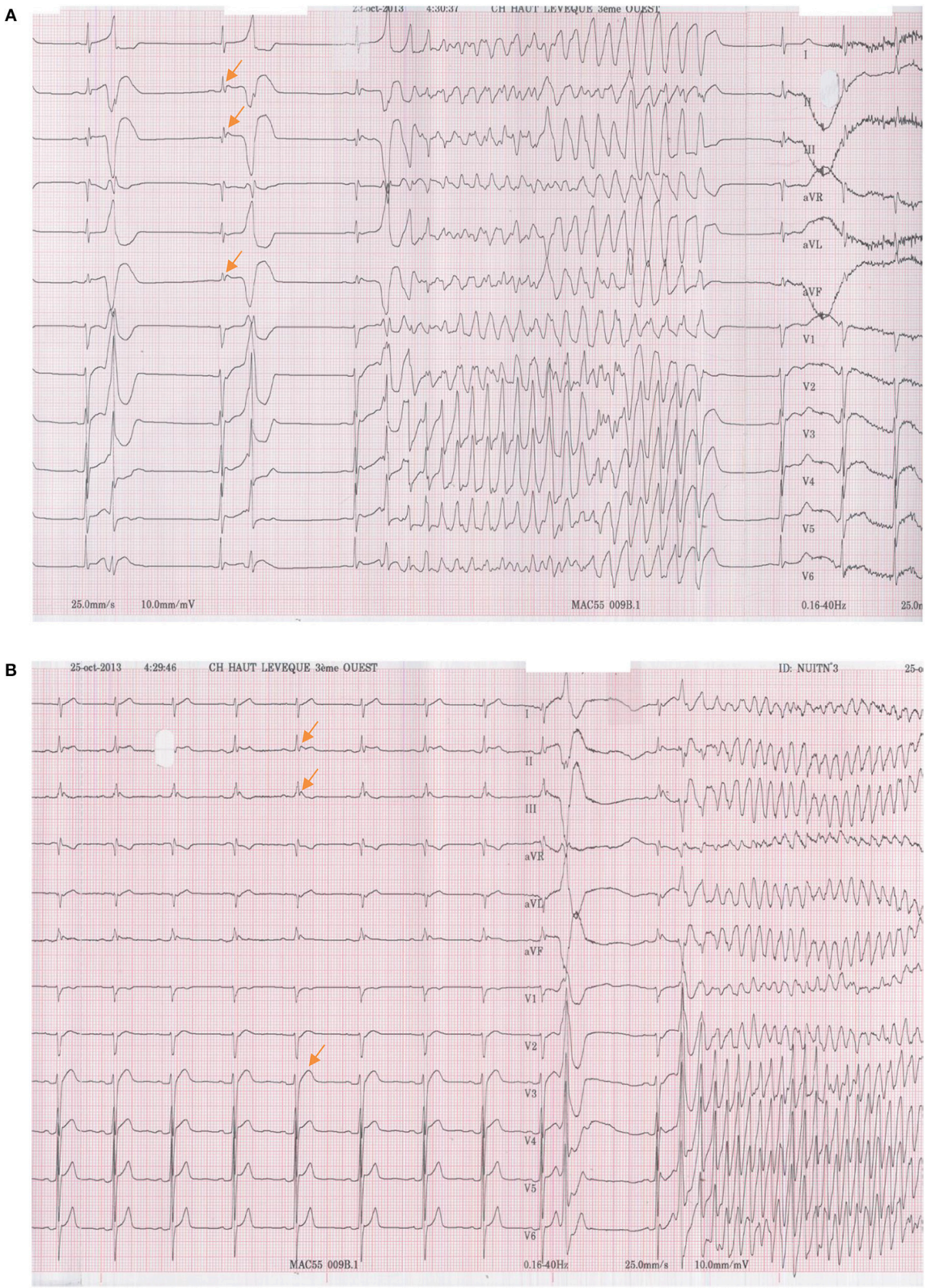

FIGURE 3 | (A) An episode of non-sustained ventricular fibrillation, which occurred during baseline ECG recording of early repolarization syndrome patient Nr. 3. The VF episode was induced by the patient's early clinical PVC. **(B)** ECG recording of patient Nr. 3. The clinical PVC now induces an episode of sustained ventricular fibrillation.

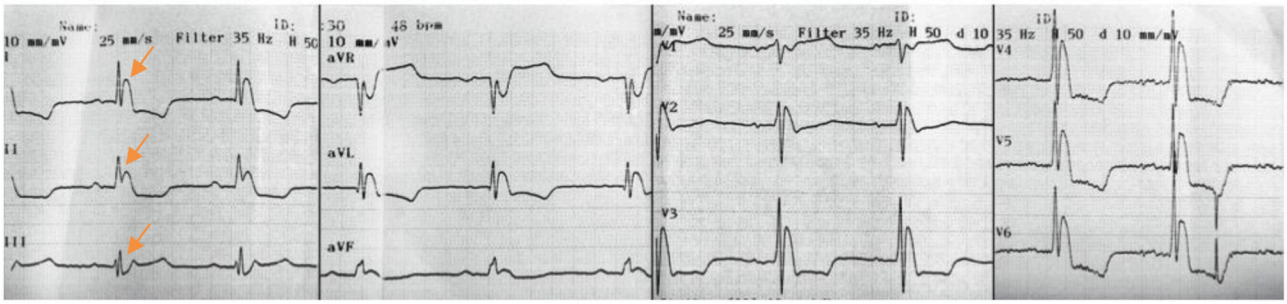

FIGURE 4 | ECG recording of patient Nr. 4 shows a predominant early repolarization pattern in the inferior leads.

was found to be more frequent (>85%) within the studied population of young athletes. In a large overall population, the horizontal/descending type was strongly associated with sudden cardiac death when compared to patients without early repolarization pattern, whereas the ascending pattern did not show significant association with sudden cardiac death (29, 30).

Roten et al. compared ECGs of healthy, asymptomatic patients with an early repolarization pattern ECG to patients with an early repolarization pattern ECG and history of ventricular fibrillation. The latter group had significantly longer QTc intervals, J waves and higher J wave amplitudes, low-amplitude T waves were found more frequently, and a lower T/R ratio. A low T/R ratio showed the strongest association with a malignant early repolarization variant among all studied parameters (31).

Bastiaenen et al. demonstrated the usefulness of exercise tolerance testing and Ajmaline provocation testing to unmask more malignant variants of early repolarization patterns. They conducted a study on 229 patients with a history of survived sudden cardiac death, ventricular arrhythmias, unexplained syncopes and a positive family history of sudden cardiac death. In 11% of studied patients, an early repolarization pattern was present in the baseline ECG. Exposing these patients with early repolarization pattern to exercise tolerance testing and Ajmaline provocation testing resulted in the disappearance of all ascending early repolarization patterns and patterns in the lateral leads. Sixty percent of horizontal/descending early repolarization patterns disappeared during Ajmaline provocation testing and only 25% disappeared during exercise tolerance testing. Patients with persistent early repolarization pattern showed a significantly higher frequency of arrhythmia related symptoms than patients in which early repolarization pattern had disappeared during testing (32–34).

Studies showed that patients with early repolarization pattern, found in the inferior leads, have a higher risk of mortality and sudden cardiac death. In patients aged older than 50 years, mortality between groups with and without early repolarization pattern additionally separates. Early repolarization may be interpreted to especially increase the risk of sudden cardiac death in the presence of additional triggers, such as acute ischemic events (10, 11, 13).

In asymptomatic patients, an electrophysiology study including programmed ventricular stimulation was not predictive for later ventricular arrhythmia in early repolarization syndrome patients (35). However in near future, advanced invasive and non-invasive electrophysiology technologies may improve risk stratification in patients with early repolarization pattern: Electrocardiographic imaging is a novel, non-invasive imaging technology which is based on a combination of cardiac electrical data recorded on the body surface and cardiac CT imaging. This technique can be used to create epicardial potential maps, and maps of epicardial activation and repolarization. Using this technology, Ghosh et al. recently demonstrated abnormally short activation-recovery intervals in inferior and lateral ventricular regions in patients with early repolarization syndrome, suggesting augmented repolarization and repolarization gradients in these areas that may represent substrates for ventricular arrhythmias. In the future, electrocardiographic imaging could prove to be a valuable tool for differentiating between malignant and benign forms of ER syndrome, thereby enhancing risk stratification (36).

Another novel technology that could potentially enhance risk stratification is a catheter equipped with monophasic action potential (MAP) electrodes. MAP catheters facilitate direct measurements of action potential characteristics and has the potential to characterize transmural repolarization gradients in detail. This may provide a more detailed characterization of the arrhythmogenic substrate in early repolarization syndrome patients. Invasive studies using this technique may be valuable in further investigating early repolarization patients who are at intermediate or high risk of sudden death (37).

THERAPY

As an early repolarization pattern is relatively frequent ECG-finding in the general population and the incidence of idiopathic ventricular fibrillation or polymorphic ventricular tachycardia is relatively low, the majority of individuals presenting an early repolarization pattern ECG will remain asymptomatic and the isolated presence of an early repolarization pattern does not require further intervention.

ICD-implantation

Conversely, in patients with an early repolarization pattern who survived sudden cardiac death (early repolarization syndrome), the implantation of an implantable cardioverter defibrillator is indicated (27). The 2013 HRS/EHRA/APHRS Expert Consensus

TABLE 1 | 2013 HRS/EHRA/APHRS Expert Consensus Statement recommendations for ICD implantation in early repolarization syndrome patients.

Class I	1. ICD implantation **is recommended** in patients with a diagnosis of early repolarization syndrome who have survived a cardiac arrest.
Class IIa	2. Isoproterenol infusion **can be useful** in suppressing electrical storms in patients with a diagnosis of early repolarization syndrome. 3. Quinidine in addition to an ICD **can be useful** for secondary prevention of VF in patients with a diagnosis of early repolarization syndrome.
Class IIb	4. ICD implantation **may be considered** in symptomatic family members of early repolarization syndrome patients with a history of syncope in the presence of ST segment elevation >1mm in 2 or more inferior or lateral leads. 5. ICD implantation **may be considered** in asymptomatic individuals who demonstrate a high-risk early repolarization ECG pattern (high J-wave amplitude, horizontal/descending ST-segment) in the presence of a strong family history of juvenile unexplained sudden death with or without a pathogenic mutation.
Class III	6. ICD implantation **is not recommended** in asymptomatic patients with an isolated early repolarization ECG pattern.

Statement gives recommendations for therapeutic interventions (**Table 1**):

In the setting of survived sudden cardiac death, a familial screening is recommended. In a case of isolated, asymptomatic early repolarization ECG pattern, there is no recommendation for familial screening.

Drug Therapy

The incidence of ventricular fibrillation episodes and electrical storm is relatively common in early repolarization syndrome patients after ICD implantation. Regarding drug therapy in this situation, there is evidence that isoproterenol infusion acutely suppresses recurrent ventricular fibrillation in these patients. The dose can be initiated with 1.0 μg/min and should target a 20% in baseline heart rate or an absolute heart rate > 90 bpm, adapted to hemodynamic conditions and suppression of arrhythmia. The adrenergic activation with isoproterenol is likely effective by augmenting inward currents (particularly L-type Ca^{2+}) which offset the net outward K^{+} current excess. Also the use of Quinidine or Hydroquinidine has been described to achieve a long-term suppression of ventricular fibrillation and ventricular tachycardia in early repolarization syndrome (38, 39). Quinidine, which inhibits outward currents, mainly I_{to}, reduces the amplitude of the J wave and ST segment. The targeted range of Hydroquinidine serum levels should be 2–5 μg/ml, therefore a daily dose of 600 mg Hydroquinidine was applied in studies. In addition, Quinidine may reduce the early repolarization pattern or even restore a normal ECG in patients. The administration of Cilostazol, an oral phosphodiesterase III inhibitor which has been shown to be an effective drug therapy in Brugada syndrome, has also been described to successfully terminate ventricular fibrillation episodes, which were refractory to Quinidine therapy in early repolarization syndrome. Cilostazol has been shown to have a significant effect to block I_{to} and to augment I_{Ca}, reducing the occurrence of phase 2 reentry phenomenon (40, 41).

CONCLUSION

Whereas, an early repolarization pattern is frequently found in the overall population, the incidence of idiopathic ventricular fibrillation and the risk of early repolarization syndrome is relatively low. Several studies distinguished benign from more malignant early repolarization patterns. However, the mismatch between frequently found early repolarization ECG patterns, low incidences of early repolarization syndrome related sudden cardiac deaths, but fatal, grave consequences in affected patients remains a clinical challenge.

AUTHOR CONTRIBUTIONS

FB, AD, GC, AL, KV, MT, TK, AF, JD, TP, NK, ND, FS, PJ, MiH and MéH Review of articles, preparation of manuscript, revision of article.

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REFERENCES

1. Chugh SS, Kelly KL, Titus JL. Sudden cardiac death with apparently normal heart. *Circulation* (2000) 102:649–54. doi: 10.1161/01.CIR.102.6.649
2. Wever EF, Robles de Medina EO. Sudden death in patients without structural heart disease. *J Am Coll Cardiol.* (2004) 43:1137–44. doi: 10.1016/j.jacc.2003.10.053
3. Shipley RA, Hallaran WR. The four-lead electrocardiogram in two hundred normal men and women. *Am Heart J.* (1936) 11:325–45. doi: 10.1016/S0002-8703(36)90417-9
4. Tomaszewski W. Changement electrocardiographiques observes chez un homme mort de froid [Electrocardiographic changes observed in a man frozen to death]. *Arch Mal Coeur.* (1938) 31:525–8.
5. Grant RP, Estes HE, Doyle JT. Spatial vector electrocardiography; the clinical characteristics of S-T and T vectors. *Circulation* (1951) 3:182–97. doi: 10.1161/01.CIR.3.2.182
6. Osborn JJ. Experimental hypothermia: respiratory and blood pH changes in relation to cardiac function. *Am J Physiol.* (1953) 175:389–98. doi: 10.1152/ajplegacy.1953.175.3.389
7. Derval N, Shah A, Jais P. Definition of early repolarization: a tug of war. *Circulation* (2011) 124:2185–6. doi: 10.1161/CIRCULATIONAHA.111.064063
8. Haïssaguerre M, Derval N, Sacher F, Jesel L, Deisenhofer I, de Roy L, et al. Sudden cardiac arrest associated with early repolarization. *N Engl J Med.* (2008) 358:2016–23. doi: 10.1056/NEJMoa071968
9. Jones RL, Rubal B, Jones S, Steel K, Davenport E, Nasir J. Prevalence of early repolarization in a large cohort of young adults. *J Am Coll Cardiol.* (2015) 65:A371. doi: 10.1016/S0735-1097(15)60371-0
10. Haruta D, Matsuo K, Tsuneto A, Ichimaru S, Hida A, Sera N, et al. Incidence and prognostic value of early repolarization pattern in the 12-lead electrocardiogram. *Circulation* (2011) 123:2931–7. doi: 10.1161/CIRCULATIONAHA.110.006460

11. Sinner MF, Reinhard W, Müller M, Beckmann BM, Martens E, Perz S, et al. Association of early repolarization pattern on ECG with risk of cardiac and all-cause mortality: a population-based prospective cohort study (MONICA/KORA). *PLoS Med*. (2010) 7:e1000314. doi: 10.1371/journal.pmed.1000314
12. Rosso R, Kogan E, Belhassen B, Rozovski U, Scheinman MM, Zeltser D, et al. J-point elevation in survivors of primary ventricular fibrillation and matched control subjects. *J Am Coll Cardiol*. (2008) 52:1231–8. doi: 10.1016/j.jacc.2008.07.010
13. Tikkanen JT, Anttonen O, Junttila MJ, Aro AL, Kerola T, Rissanen HA, et al. Long-term outcome associated with early repolarization on electrocardiography. *N Engl J Med*. (2009) 361:2529–37. doi: 10.1056/NEJMoa0907589
14. Benito B, Guasch E, Rivard L, Nattel S. Clinical and mechanistic issues in early repolarization. *J Am Coll Cardiol*. (2010) 56:1177–786. doi: 10.1016/j.jacc.2010.05.037
15. Antzelevitch C, Yan GX. J wave syndromes. *Heart Rhythm* (2010) 7:549–58. doi: 10.1016/j.hrthm.2009.12.006
16. Wu SH, Lin XX, Cheng YJ, Qiang CC, Zhang J. Early repolarization pattern and risk for arrhythmia death: a meta-analysis. *J Am Coll Cardiol*. (2013) 61:645–50. doi: 10.1016/j.jacc.2012.11.023
17. Liu XK, Katchman A, Whitfield BH, Wan G, Janowski EM, Woosley RL, Ebert SN. *In vivo* androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits. *Cardiovasc Res*. (2003) 57:28–36. doi: 10.1016/S0008-6363(02)00673-9
18. Bai CX, Kurokawa J, Tamagawa M, Nakaya H, Furukawa T. Non-transcriptional regulation of cardiac repolarization currents by testosterone. *Circulation* (2005) 112:1701–10. doi: 10.1161/CIRCULATIONAHA.104.523217
19. Nunn LM, Bhar-Amato J, Lowe MD, Macfarlane PW, Rogers P, McKenna WJ, et al. Prevalence of J-point elevation in sudden arrhythmic death syndrome families. *J Am Coll Cardiol*. (2011) 58:286–90. doi: 10.1016/j.jacc.2011.03.028
20. Noseworthy PA, Tikkanen JT, Porthan K, Oikarinen L, Pietilä A, Harald K, et al. The early repolarization pattern in the general population: clinical correlates and heritability. *J Am Coll Cardiol*. (2011) 57:2284–9. doi: 10.1016/j.jacc.2011.04.003
21. Reinhard W, Kaess BM, Debiec R, Nelson CP, Stark K, Tobin MD, et al. Heritability of early repolarization: a population-based study. *Circ Cardiovasc Genet*. (2011) 4:134–8. doi: 10.1161/CIRCGENETICS.110.958298
22. Haïssaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Loussouarn G, et al. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. *J Cardiovasc Electrophysiol*. (2009) 20:93–8. doi: 10.1111/j.1540-8167.2008.01326.x
23. Medeiros-Domingo A, Tan BH, Crotti L, Tester DJ, Eckhardt L, Cuoretti A, et al. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac K(ATP) channel Kir6.1 as a pathogenic substrate for J-wave syndromes. *Heart Rhythm* (2010) 7:1466–71. doi: 10.1016/j.hrthm.2010.06.016
24. Burashnikov E, Pfeiffer R, Barajas-Martinez H, Delpón E, Hu D, Desai M, et al. Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. *Heart Rhythm* (2010) 7:1872–82. doi: 10.1016/j.hrthm.2010.08.026
25. Watanabe H, Nogami A, Ohkubo K, Kawata H, Hayashi Y, Ishikawa T, et al. Electrocardiographic characteristics and SCN5A mutations in idiopathic ventricular fibrillation associated with early repolarization. *Circ Arrhythm Electrophysiol*. (2011) 4:874–81. doi: 10.1161/CIRCEP.111.963983
26. Macfarlane PW, Antzelevitch C, Haissaguerre M, Huikuri HV, Potse M, Rosso R, et al. The early repolarization pattern. *J Am Coll Cardiol*. (2015) 66:470–77. doi: 10.1016/j.jacc.2015.05.033
27. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm* (2013) 10:1932–63. doi: 10.1016/j.hrthm.2013.05.014
28. Nam GB, Ko KH, Kim J, Park KM, Rhee KS, Choi KJ, et al. Mode of onset of ventricular fibrillation in patients with early repolarization pattern vs. Brugada syndrome. *Eur Heart J*. (2010) 31:330–9. doi: 10.1093/eurheartj/ehp423
29. Tikkanen JT, Junttila MJ, Anttonen O, Aro AL, Luttinen S, Kerola T, et al. Early repolarization: electrocardiographic phenotypes associated with favorable long-term outcome. *Circulation* (2011) 123:2666–73. doi: 10.1161/CIRCULATIONAHA.110.014068
30. Viskin S, Havakuk O, Antzelevitch C, Rosso R. Malignant early repolarization: it's the T-wave, stupid. *Heart Rhythm* (2016) 13:903–4. doi: 10.1016/j.hrthm.2015.12.017
31. Roten L, Derval N, Maury P, Mahida S, Pascale P, Leenhardt A, et al. Benign vs malignant inferolateral early repolarization: focus on the T wave. *Heart Rhythm* (2016) 13:894–902. doi: 10.1016/j.hrthm.2015.11.020
32. Rautaharju PM, Surawicz B, Gettes LS, Bailey JJ, Childers R, Deal BJ, et al. AHA/ACCF/HRS recommendations for the standardization and interpretation of the electrocardiogram: part IV: the ST segment, T and U waves, and the QT interval: a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society: endorsed by the international society for computerized electrocardiology. *J Am Coll Cardiol*. (2009) 53:982–91. doi: 10.1016/j.jacc.2008.12.014
33. Bastiaenen R, Raju H, Sharma S, Papadakis M, Chandra N, Muggenthaler M, et al. Characterization of early repolarization during ajmaline provocation and exercise tolerance testing. *Heart Rhythm* (2013) 10:247–54. doi: 10.1016/j.hrthm.2012.10.032
34. Refaat MM, Hotait M, Tseng ZH. Utility of the exercise electrocardiogram testing in sudden cardiac death risk stratification. *Ann Noninvasive Electrocardiol*. (2014) 19:311–8. doi: 10.1111/anec.12191
35. Mahida S, Derval N, Sacher F, Leenhardt A, Deisenhofer I, Babuty D, et al. Role of electrophysiological studies in predicting risk of ventricular arrhythmia in early repolarization syndrome. *J Am Coll Cardiol*. (2015) 65:151–9. doi: 10.1016/j.jacc.2014.10.043
36. Ghosh S, Cooper DH, Vijayakumar R, Zhang J, Pollak S, Haissaguerre M. Early repolarization associated with sudden death: insights from noninvasive electrocardiographic imaging. *Heart Rhythm* (2010) 7:534–7. doi: 10.1016/j.hrthm.2009.12.005
37. Mahida S, Derval N, Sacher F, Berte B, Yamashita S, Hooks DA. History and clinical significance of early repolarization syndrome. *Heart Rhythm* (2015) 12:242–9. doi: 10.1016/j.hrthm.2014.09.048
38. Nam GB, Kim YH, Antzelevitch C. Augmentation of J waves and electrical storms in patients with early repolarization. *N Engl J Med*. (2008) 358:2078–9. doi: 10.1056/NEJMc0708182
39. Haïssaguerre M, Sacher F, Nogami A, Komiya N, Bernard A, Probst V, et al. Characteristics of recurrent ventricular fibrillation associated with inferolateral early repolarization role of drug therapy. *J Am Coll Cardiol*. (2009) 53:612–9. doi: 10.1016/j.jacc.2008.10.044
40. Iguchi K, Noda T, Kamakura S, Shimizu W. Beneficial effects of cilostazol in a patient with recurrent ventricular fibrillation associated with early repolarization syndrome. *Heart Rhythm* (2013) 10:604–6. doi: 10.1016/j.hrthm.2012.11.001
41. Patocskai B, Barajas-Martinez H, Hu D, Gurabi Z, Koncz I, Antzelevitch C. Cellular and ionic mechanisms underlying the effects of cilostazol, milrinone, and isoproterenol to suppress arrhythmogenesis in an experimental model of early repolarization syndrome. *Heart Rhythm* (2016) 13:1326–34. doi: 10.1016/j.hrthm.2016.01.024

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REVIEW
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Calmodulinopathy: A Novel, Life-Threatening Clinical Entity Affecting the Young

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Sudden cardiac death (SCD) in the young may often be the first manifestation of a genetic arrythmogenic disease that had remained undiagnosed. Despite the significant discoveries of the genetic bases of inherited arrhythmia syndromes, there remains a measurable fraction of cases where in-depth clinical and genetic investigations fail to identify the underlying SCD etiology. A few years ago, 2 cases of infants with recurrent cardiac arrest episodes, due to what appeared to be as a severe form of long QT syndrome (LQTS), came to our attention. These prompted a number of clinical and genetic research investigations that allowed us to identify a novel, closely associated to LQTS but nevertheless distinct, clinical entity that is now known as *calmodulinopathy*. Calmodulinopathy is a life-threatening arrhythmia syndrome, affecting mostly young individuals, caused by mutations in any of the 3 genes encoding calmodulin (CaM). Calmodulin is a ubiquitously expressed Ca^{2+} signaling protein that, in the heart, modulates several ion channels and participates in a plethora of cellular processes. We will hereby provide an overview of CaM's structure and function under normal and disease states, highlighting the genetic etiology of calmodulinopathy and the related disease mechanisms. We will also discuss the phenotypic spectrum of patients with calmodulinopathy and present state-of-the art approaches with patient-derived induced pluripotent stem cells that have been thus far adopted in order to accurately model calmodulinopathy *in vitro*, decipher disease mechanisms and identify novel therapies.

Keywords: CALM, calmodulin, long QT syndrome, sudden cardiac death, catecholaminergic polymorphic ventricular tachycardia

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INTRODUCTION

Sudden cardiac death (SCD) is an unexpected natural death due to cardiac causes that is responsible for up to 25% of all deaths in the Western world (1). While SCD as a term describes a final common clinical outcome, it does not by itself relay information on the pathophysiological mechanisms underlying its occurrence, which are quite distinct, especially when considering the age of the SCD victim. Indeed, in the young (<35 years), SCD is mainly the adverse outcome of inherited cardiac diseases, such as cardiomyopathies and channelopathies, with ventricular fibrillation (VF) being mostly the culprit arrhythmia (2). Unfortunately, SCD

may afflict even those in the perinatal, neonatal and early childhood period. We and others have shown that when post-mortem clinical investigations fail to identify the underlying causes ("mors sine materia" or "normal heart" SD) (3), channelopathies, and the long QT syndrome (LQTS) in particular, may contribute significantly to cases of sudden infant death syndrome (4), as well as intrauterine fetal death and stillbirth (5). In these cases, early-onset and highly malignant arrhythmias caused by penetrant genetic mutations in ion channel genes, or their accessory protein partners, often arising *de novo*, largely dictate SCD occurrence.

A few years ago, two infants having experienced recurrent VF episodes and suffering from what appeared to be as an unusually severe form of LQTS (QTc > 600 ms, intermittent 2:1 atrioventricular block and T wave alternans) came to our attention. Both were born to healthy parents and genetic testing of the major LQTS genes was negative. In order to identify what we presumed to be a novel underlying genetic cause of these severe arrhythmia manifestations, we performed whole-exome sequencing in both infants (as part of parent-child trios) and identified novel mutations in two of the three genes encoding the Ca^{2+}-signaling protein calmodulin (*CALM1*-p.D130G and *CALM2*-p.D96V) that were shown to have arisen *de novo*, thus explaining the parents' normal phenotype (6). This finding was further validated by expanding our search for calmodulin (CaM) mutations in a pool of unrelated LQTS patients that were genetically negative for mutations in the main LQTS genes. In doing so, we identified the same *CALM1*-p.D130G mutation as well as the novel *CALM1*-p.F142L mutation in two other unrelated LQTS cases with severe disease and recurrent cardiac arrest episodes (6). Shortly after, other investigators identified the same p.D130G mutation, albeit in the *CALM3* gene, as a novel genetic substrate of severe LQTS (7), thus completing the picture of a "genetic trilogy" for a new clinical entity that has been termed *calmodulinopathy* (8).

In the past few years our knowledge on calmodulinopathy has expanded and it is now known to be a severe arrhythmogenic condition that can manifest mainly as LQTS (6), catecholaminergic polymorphic ventricular tachycardia (9) or idiopathic VF (IVF) (10) caused by genetic mutations in any of the 3 calmodulin genes (*CALM1, CALM2, CALM3*). Calmodulin is a ubiquitously expressed protein that participates in a plethora of cellular processes, while acting intracellularly both as a Ca^{2+} sensor and signal transducer. In the heart, CaM is a major player in the modulation of several ion channels such as the L-type calcium channel (LTCC), the sodium channel, different potassium channels, and the ryanodine receptor (RyR) (11).

In this review, we will provide an overview of currently available knowledge on this severe arrhythmogenic syndrome, termed calmodulinopathy. In particular, we will present in detail CaM's sequence, structure and function, the genetic spectrum of CaM mutations and the associated phenotypes, as well as available therapies. We will also overview the underlying disease mechanisms of calmodulinopathy (reviewed in detail in the accompanying article by Badone et al.) and present the thus far used *in vitro* methods for deciphering these disease mechanisms.

CALMODULIN GENES AND PROTEIN

The first complementary DNA (cDNA) clone of human CaM was isolated from a human liver cDNA library in 1984 (12). Within a few years, two more human cDNA clones were isolated and characterized (13, 14) with diverse nucleic acid sequence identity but all encoding an identical CaM protein. These results suggested the existence of a multigene CaM family operating under selective pressure to regulate and express a protein with a prominent cellular role.

Calmodulin in humans is indeed encoded by three different genes (*CALM1*, *CALM2* and *CALM3*; NG_013338.1, NG_042065.1 and NG_051331.1 RefSeqGenes, respectively), located on three different chromosomes (14q32.11, 2p21 and 19q13.32, respectively). Although several splice variants exist, only few transcripts contribute to the full-length CaM protein with the principal ones being NM_006888.4, NM_001743.5 and NM_005184.3, respectively. The three genes have a similar exon-intron structure, with 6 coding exons (14–16). The CaM protein generated from the translation of each of the three main transcripts has a length of 149 amino acids and an identical amino acid sequence. Its evolutionary importance is highlighted not only by the presence of three different genes located on three different chromosomes encoding the same protein, but also by the extent of conservation of its protein sequence that is full across vertebrates and very high across all eukaryotes (17) (**Figure 1A**).

Despite CaM's significant conservation, the respective *CALM* genes share only approximately 80% sequence identity among their coding regions, while they have no significant homology in their non-coding regions (14). This shows that the *CALM* genes have diverged early during evolution (14) and are not the result of gene duplications as is often the case with multigene families, while operating under a strict evolutionary control that aims to maintain their structural integrity.

Although CaM's genetic redundancy may partly serve its ubiquitous nature and support its pivotal role in several processes essential for cell survival and functioning, little is known about *CALM* genes' differential expression. Gene expression analyses have shown that in mammalian cells (16) and human hearts at the fetal, infant and adult stages of development (6), significantly higher transcriptional levels of *CALM3* than *CALM2* and *CALM1* are observed, with the latter being the least transcribed. Data from the Expression Atlas (18) and the Human Protein Atlas (19) indicate that all three CaM genes are ubiquitously expressed in the majority of tissues, with high and medium levels of expression found in the brain and heart, respectively. At the protein level, the relative contribution of each transcript is still unclear. Upon the initial identification of different cDNA clones, suggesting the existence of three different genes, it was hypothesized that one gene could be the housekeeping gene, while the other two could be differentially expressed under conditions of particular stimuli (14). This however seems not to be the case and all transcripts contribute toward CaM's overall expression (20), albeit unclear to which extent.

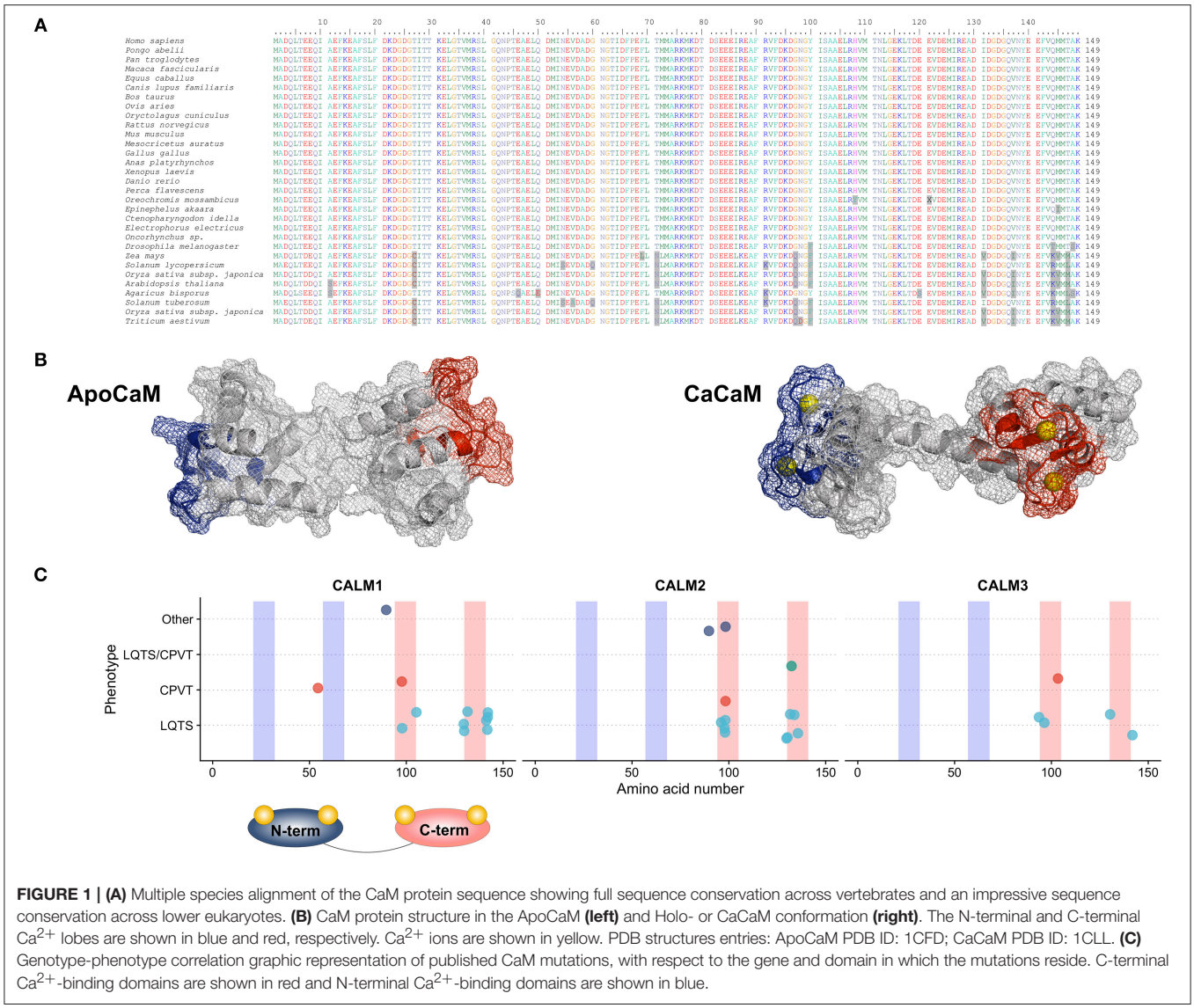

FIGURE 1 | (A) Multiple species alignment of the CaM protein sequence showing full sequence conservation across vertebrates and an impressive sequence conservation across lower eukaryotes. **(B)** CaM protein structure in the ApoCaM **(left)** and Holo- or CaCaM conformation **(right)**. The N-terminal and C-terminal Ca^{2+} lobes are shown in blue and red, respectively. Ca^{2+} ions are shown in yellow. PDB structures entries: ApoCaM PDB ID: 1CFD; CaCaM PDB ID: 1CLL. **(C)** Genotype-phenotype correlation graphic representation of published CaM mutations, with respect to the gene and domain in which the mutations reside. C-terminal Ca^{2+}-binding domains are shown in red and N-terminal Ca^{2+}-binding domains are shown in blue.

CALMODULIN'S STRUCTURE

Human CaM is a small 149 amino acid protein with a length of 65 Angstroms (Å) that could be pictured in the shape of a dumbbell (21). It is comprised of two Ca^{2+}-binding lobes at its N- and C-terminus which are linked by a flexible helix, each containing two EF-hand (helix-loop-helix) structural motifs with Ca^{2+}-binding properties (**Figure 1B**). The lobes display cooperative Ca^{2+}-binding properties (22), with faster Ca^{2+} binding at the N-terminal lobe and higher Ca^{2+}-binding affinity at the C-terminal lobe (23), thus allowing CaM to function either as a rapid or slow Ca^{2+} sensor (24).

Within the cell, CaM may exist in multiple conformations that can be summarized in a six-state folding model, dependent on intracellular Ca^{2+} levels (25). Calmodulin folding and regulation have been evolutionally selected as robust key mediators of multiple Ca^{2+}-based intracellular signals to control the activity of downstream targets in response to a broad range of intracellular Ca^{2+} concentration changes and different states of energetic stability (25). At low intracellular Ca^{2+} concentrations, the empty, Ca^{2+}-free form (ApoCaM) prevails; increasing Ca^{2+} concentration leads to a progressive and cooperative occupation of the four Ca^{2+}-binding sites until the saturated form (CaCaM or *HoloCaM*) is reached. Ca^{2+} binding and folding properties of each domain do influence both target binding and target regulation and confer to CaM the pliability to sense both local and global Ca^{2+} levels (24).

CALMODULIN'S INTERACTION NETWORK

Multiple cardiac ion channels and pumps are targeted and regulated by intracellular Ca^{2+} through Ca^{2+}-sensing proteins, with CaM being the predominant and most widespread. This Ca^{2+}-dependent regulation acts on multiple levels and involves, among others, sarcolemmal ion channels responsible for the

L-type Ca^{2+} current (I_{CaL}, $Ca_v1.2$), the peak sodium current (I_{Na}, $Na_v1.5$), the slow delayed rectifier potassium current (I_{Ks}, $K_v7.1$), the inward rectifier potassium current (I_{K1}, $K_{ir}2.1$) and sarcoplasmic reticulum (SR) proteins such as the cardiac ryanodine receptors (RyR2) and phospholamban (PLB) (11). The regulation of these targets by CaM can be primary, through a direct association of CaM to its target, or mediated by the activity of Ca^{2+}-CaM-dependent protein kinase II (CaMKII).

Different CaM pools do exist within the cell and the net result of this somehow unique protein distribution and equilibrium is that the vast majority (∼99%) of CaMs appear to be already pre-bound to targets (26, 27), thereby assigning a buffering capacity to the remaining tiny fraction of the total CaM pool. This means that the three different CaM genes encode for identical CaM proteins that will be mostly bound to their targets. This scenario dramatically reduces the competition for target binding and constitutes a vulnerable substrate toward genetic mutations.

Under certain conditions, when the ApoCaM configuration is adopted, the N-terminal lobe has higher stability than the C-terminal lobe (25), which allows the binding/pre-association to important biological targets such as the $Na_v1.5$ or $Ca_v1.2$ channels in the ApoCaM form (28, 29). In particular, ApoCaM pre-association to $Ca_v1.2$ is required since CaMs from the cytosolic bulk are unable to adequately access the binding site on $Ca_v1.2$ during Ca^{2+} inflow (28); this property confers to the residual CaMs the ability to finely modulate substrate activities in response to local intracellular Ca^{2+} concentration changes (30, 31) with such a precision and velocity that would not be possible if Ca-CaM would have to be recruited from the cytosolic CaM bulk.

A certain degree of cytosolic CaM–level dependency is nevertheless maintained in the cells as a mechanism of Ca^{2+} homeostasis tuning other ion channels (32). Furthermore, according to modeling data published by Valeyev and coworkers, specificity and selectivity of CaM target regulation relies on target CaM-specific constants of dissociations and on the number of Ca^{2+} ions required for CaM-target complex activation (33), with some interactions requiring a preferential Ca^{2+} binding to the N-terminus (34), C-terminus or both.

Results by multiple groups have converged on deciphering the role of CaM's two main targets that underlie and define the main calmodulinopathy phenotypes, i.e., $Ca_v1.2$ and RyR2, encoded by *CACNA1C* and *RYR2*, genes with a previously established role in LQTS and CPVT pathogenesis, respectively.

GENETICS OF CALMODULINOPATHY

Twenty three mutations in one of the three CaM genes have been identified so far in thirty index calmodulinopathy patients with different arrhythmic phenotypes (6, 7, 9, 10, 35–43) (**Table 1**). The mutations thus far described have mainly arisen *de novo*, as demonstrated by parental genetic screening in 23 families. In only three cases the mutation was inherited (*CALM3*-p.A103V, *CALM1*-p.N54I, *CALM1*-p.F90L (9, 10, 35) and was shown to co-segregate with an arrhythmic phenotype in the respective families.

All 23 mutations described are single nucleotide substitutions, leading to 18 distinct missense amino acid changes and are prevalently distributed in the *CALM1* ($n = 8$) and *CALM2* ($n = 10$) genes, while a few reside in the *CALM3* gene ($n = 5$). Interestingly, the majority of mutations (18/23, 78%) are localized in CaM's C-terminal Ca^{2+}-binding domains (EF-hands III and IV) (**Figure 1C**), and especially in the specific residues directly involved in Ca^{2+} binding (16/23, 70%). Since the C-terminal EF-hands (III and IV) have been demonstrated to have a higher Ca^{2+}-binding affinity than those in the N-terminus (I and II), calmodulinopathy's mutation distribution strongly highlights the importance of Ca^{2+}-binding affinity for proper protein function and indicates specific topological domains that seem to be intolerant to genetic variation.

In support of the latter also come data on CaM genetic variation from the general population (genome aggregation database, gnomAD) (44). Not only thus far described calmodulinopathy-causative variants are absent from the general population, but also, the *CALM* non-synonymous genetic variants that are present ($n = 29$) have a different distribution within the CaM protein compared to the calmodulinopathy-causing mutations. In fact, the former are mainly localized outside the Ca^{2+}-binding domains, in the EF-hand linkers and in the N- and C-terminal ends of the protein. In addition, the C-terminal EF-hands III and IV host recurrent calmodulinopathy-causing mutations, such as the p.N98S, p.D130G, and p.F142L, identified thus far in 6, 4 and 4 index cases, respectively, further highlighting the functional importance and intolerance to variation of these topological domains within the CaM protein.

CALMODULINOPATHY DISEASE MECHANISMS

Among all CaM mutations thus far described (45), 11 have been functionally investigated *in vitro,* mostly by Ca^{2+}-binding assays and/or electrophysiological studies. In our initial description of LQTS-associated calmodulinopathy (6) we provided preliminary evidence that the CaM mutations identified exhibited reduced affinity for Ca^{2+} and were thereby predicted to interfere with CaM's ability to transduce Ca^{2+}-mediated signals. Diminished Ca^{2+}-binding capacity had also been previously demonstrated by *in vitro* overexpression of mutant CaM in mammalian cardiomyocytes (CMs), which resulted in severe action potential prolongation, i.e., a cellular recapitulation of LQTS (46). We have further validated this reduced affinity for Ca^{2+} in the context of other LQTS-related CaM mutations (38, 39). Moreover, we have identified impaired Ca^{2+}-dependent inactivation (CDI) of the L-type Ca^{2+} channel $Ca_v1.2$- with concordant disruption of cellular Ca^{2+} homeostasis- as the prominent mechanism of LQTS-associated calmodulinopathy (39, 47) (**Figure 2A**). Our findings have been in accordance with those of other investigators.

Calcium-dependent inactivation is the mechanism by which conformational changes in CaM, driven by extracellular Ca^{2+} inflow through $Ca_v1.2$, modulate part of the inactivation of the LTCCs. This mechanism acts as a negative feedback loop to precisely control the amount of Ca^{2+} entering the cardiomyocyte at each heartbeat and it is deranged in the presence of certain CaM mutations associated with an LQTS

TABLE 1 | List of published CaM mutations associated with arrhythmic phenotypes.

Gene	Nucleotide change	Amino acid change	Protein domain	N. of probands	Associated phenotype	References
CALM1	c.161A>T	p.N54I	inter-EF hand I-II linker	1	CPVT	(9)
CALM1	c.268T>C	p.F90L	inter-EF hand II-III linker	1	IVF	(10)
CALM2	c.268T>C	p.F90L	inter-EF hand II-III linker	1	SUD	(43)
CALM3	c.281A>C	p.D94A	EF-hand III	1	LQTS	(37)
CALM3	c.286G>C	p.D96H	EF-hand III	1	LQTS	(40)
CALM2	c.287A>T	p.D96V	EF-hand III	1	LQTS	(6)
CALM1	c.293A>G	p.N98S	EF-hand III	2	LQTS, CPVT	(9, 37)
CALM2	c.293A>G	p.N98S	EF-hand III	4	LQTS,CPVT,SUD	(36, 38, 43)
CALM2	c.293A>T	p.N98I	EF-hand III	1	LQTS	(38)
CALM3	c.308C>T	p.A103V	EF-hand III	1	CPVT	(35)
CALM1	c.314A>C	p.E105A	EF-hand III	1	LQTS	(42)
CALM1	c.389A>G	p.D130G	EF-hand IV	2	LQTS	(6)
CALM2	c.389A>G	p.D130G	EF-hand IV	1	LQTS	(41)
CALM3	c.389A>G	p.D130G	EF-hand IV	1	LQTS	(7)
CALM2	c.389A>T	p.D130V	EF-hand IV	1	LQTS	(41)
CALM2	c.394G>C	p.D132H	EF-hand IV	1	LQTS	(39)
CALM1	c.395A>T	p.D132V	EF-hand IV	1	LQTS	(39)
CALM2	c.396T>G	p.D132E	EF-hand IV	1	LQTS/CPVT Overlap	(38)
CALM2	c.400G>C	p.D134H	EF-hand IV	1	LQTS	(38)
CALM2	c.407A>C	p.Q136P	EF-hand IV	1	LQTS	(38)
CALM1	c.422A>G	p.E141G	EF-hand IV	1	LQTS	(41)
CALM1	c.426C>G	p.F142L	C-terminal region	3	LQTS	(6, 41)
CALM3	c.426T>G	p.F142L	C-terminal region	1	LQTS	(40)
Total	23	18		30		

phenotype (LQTS-CaM), (**Figure 2A**). Multiple lines of evidence converge on the fact that LQTS-CaM mutations decrease CaM's affinity for Ca^{2+} and lead to an impaired CDI, resulting in an increased and uncontrolled Ca^{2+} inflow, action potential (AP) prolongation, QT interval prolongation and potentially lethal arrhythmogenic events. Conversely, other CaM mutations do not impair CaM's Ca^{2+}-binding properties, but, rather, they strengthen CaM's binding affinity to RyR2, thus promoting the open conformation of RyR2 and interfering with its fine regulation. When dysfunctional interactions between CaM and RyR2 occur, e.g., due to mutations associated to a CPVT phenotype (CPVT-CaM), SR Ca^{2+} content, along with its feedback mechanism, are dysregulated, leading to premature and spontaneous Ca^{2+} releases from the SR and arrhythmogenic propensity (48) (**Figure 2B**).

The polyhedral nature of CaM's interaction network certainly hampers the identification of straightforward links between clinical manifestations of calmodulinopathy and its underlying molecular bases. Although several hypotheses have been formulated, many of which by our group (6, 49), and calmodulinopathy's main molecular mechanisms delineated, a generalized consensus and in-depth understanding of the reasons leading to such severe clinical phenotypes, are still pending. The current hypothesis to explain the malignancy of the disease manifestations mainly relies on two mechanisms which might synergistically concur to shape the disease phenotype (**Figure 3**). Considering that 99% of CaMs are already pre-bound to their targets, this logically implies that also mutant CaMs will be bound to targets. However, this hypothesis alone, especially in the presence of a plethora of downstream mediators, may not fully explain the funneling of clinical manifestations into two main phenotypes, sometimes even in the presence of identical mutations.

The reason may partly rely also on a second mechanism, which involves the collateral consequences of ApoCaM's molecular interaction with its targets. Given that CaM pre-association to LTCC does not require Ca^{2+}, it is reasonable to speculate that mutations impacting on the Ca^{2+}-binding affinity of CaM should not theoretically alter ApoCaM's substrate-binding ability *per se*, but, on the contrary, they may indirectly enable it since mutant CaMs have a lower affinity for Ca^{2+} in their C-terminus lobe and thus are more likely to be found in the ApoCaM form. The data generated by the Yue lab (50) demonstrated, through elegant FRET assays, that LQTS-CaMs have at least an equal affinity for LTCC in the ApoCaM form, with some LQTS-CaM mutations exhibiting even a higher affinity than WT-CaMs. Therefore, targets that require a pre-association of ApoCaM will be preferentially bound to LQTS-CaM mutants, which would explain both the fact that some targets seem unaffected by these mutations, as well as the dominant expression of the pathogenic phenotype despite the wild type-favoring (5:1) allelic balance (**Figure 3**).

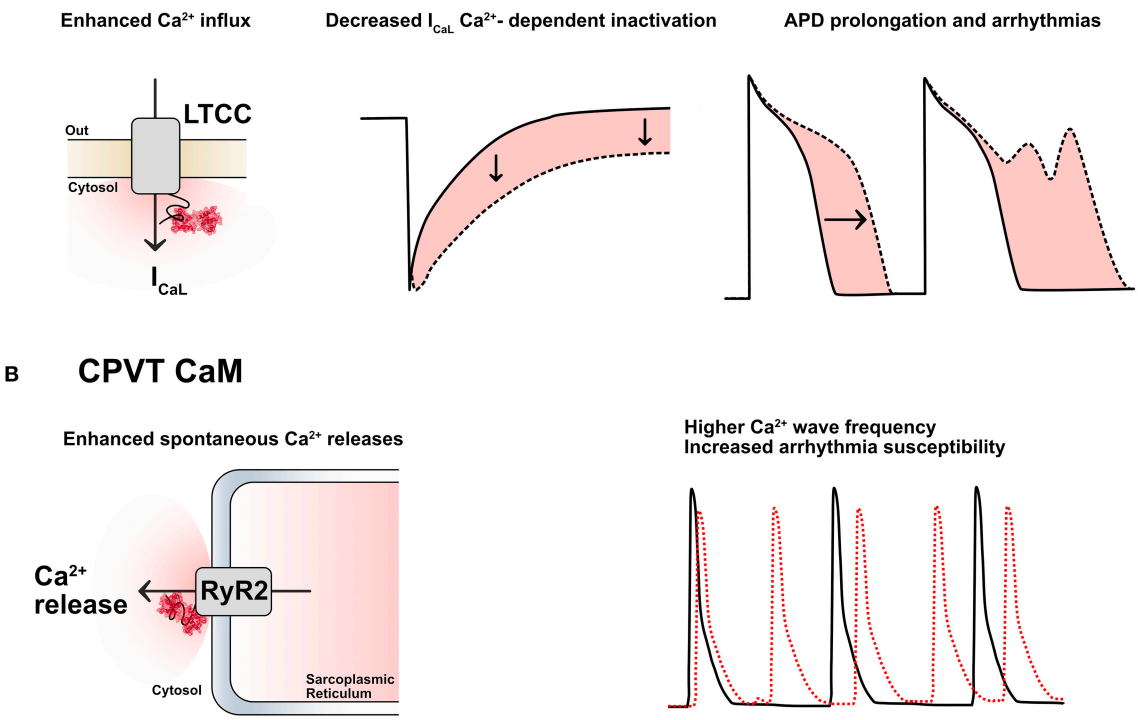

FIGURE 2 | (A) LQTS-CaMs induce an increased Ca^{2+} influx via I_{CaL} by decreasing its Ca^{2+}-dependent inactivation (CDI); this causes an enhanced Ca^{2+} influx, a consequent action potential duration (APD) prolongation and arrhythmias. LTCC: L-type calcium channels. **(B)** CPVT-CaMs promote Ca^{2+}-release from the sarcoplasmic reticulum through the cardiac ryanodine receptors (RyR2). This leads to a higher frequency of Ca^{2+} waves and to a higher arrhythmia susceptibility, especially in the presence of catecholamines.

INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE PLATFORMS FOR CALMODULINOPATHY MODELING *IN VITRO* AND TESTING OF THERAPEUTIC APPROACHES

Most of the studies that have aimed to characterize the relative effects of CaM mutations have used heterologous expression systems such as transient expression of CaM mutants in HEK293 (48, 50), tsA201 cells (41, 47), mammalian CMs (35, 41, 48, 50), or human induced pluripotent stem cells (hiPSCs) from healthy donors (39). Although these studies have provided insight into the prominent pathophysiological mechanisms of calmodulinopathy, they have had the major inherent limitation of not being able to faithfully recapitulate the *in vivo* effects by simply not respecting the native stoichiometry of mutant and wild-type CaM proteins. Having three different genes encoding the same CaM protein implies that in the presence of a mutation in a heterozygous state, there is a 5:1 wild-type:mutant allelic stoichiometry, with the quantitatively underrepresented mutant CaM still being able to confer its devastating effects. The redundancy of the CaM genes hampers also the generation of transgenic animal models, with diverse CaM gene copy numbers among species representing a substantial caveat for *in vivo* disease modeling.

In order to provide a more profound understanding of the effects conferred by CaM mutations in their native CM environment, multiple groups, including ours, generated hiPSCs from patients with CaM mutations (20, 49, 51). With the confidence of working in a physiologically relevant experimental model, CaM mutation rescue strategies have been recently attempted with multiple approaches. Limpitikul and coworkers used an elegant CRISPRi gene silencing approach to precisely suppress the transcription of the mutant *CALM2*^{D130G} allele, allowing a complete rescue of the LQTS phenotype (20). Yamamoto and coworkers, instead, followed a gene editing approach to selectively knockout the *CALM2*^{N98S} allele (51). Although not currently feasible in humans, this procedure demonstrated that the *CALM* loci can be realistic targets for gene correction approaches. Finally, Rocchetti and coworkers rescued the pathogenic LQTS phenotype caused by a *CALM1*^{F142L} mutation investigated in detail in hiPSC-CMs from a calmodulinopathy patient (6) by blocking I_{CaL} with verapamil (49). The field potential (FP) duration, measured with MEA, was rescued with acute exposure to verapamil, holding promise for a rapid implementation of this pharmacological approach in the clinical setting. This observation was immediately translated

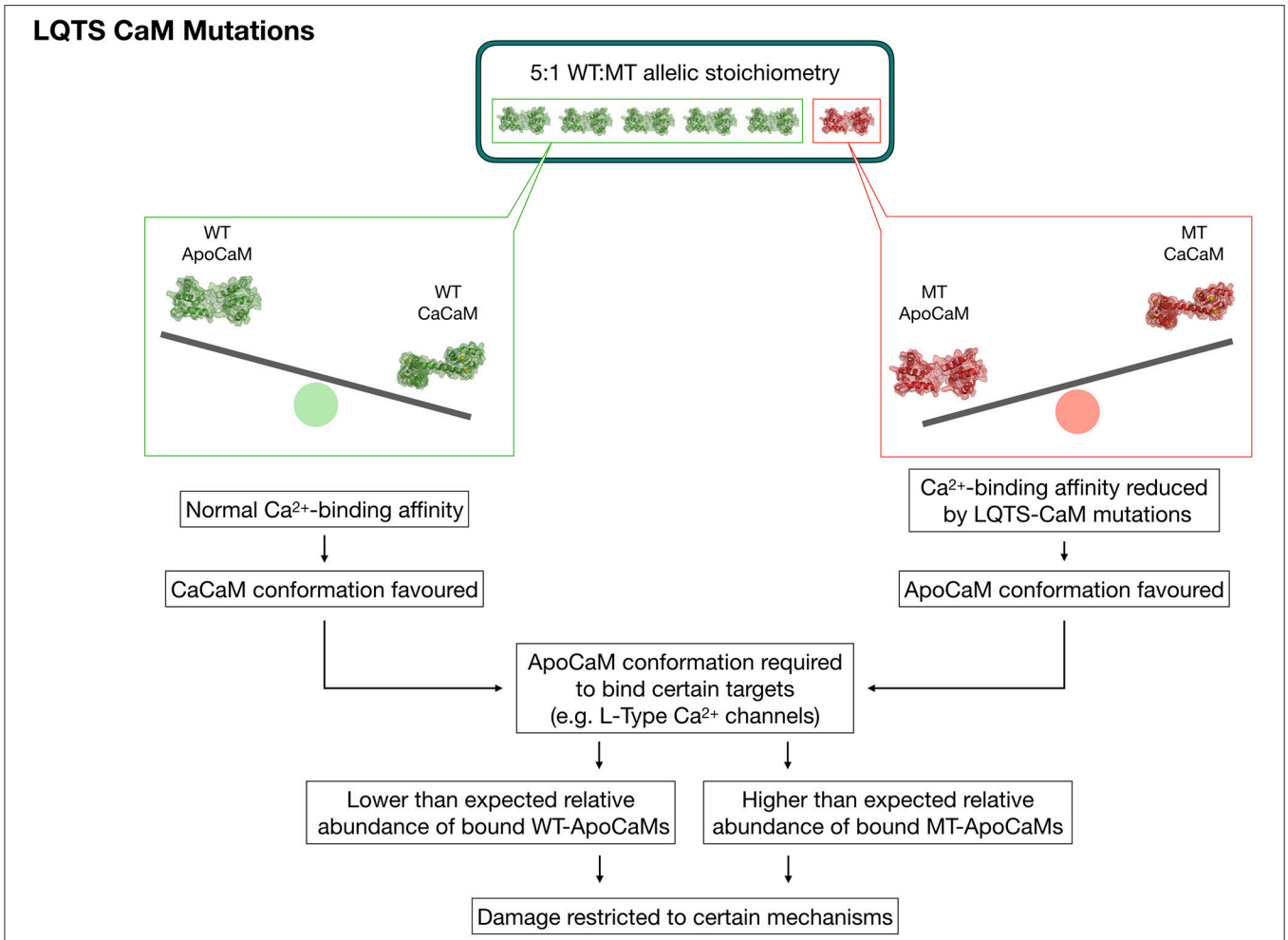

FIGURE 3 | Graphic representation depicting a theory to explain the strong phenotypic effect and allelic dominance of CaM mutants in the setting of heterozygous LQTS-CaM mutations. The lower Ca^{2+} binding affinity of LQTS-CaMs creates a larger than expected pool of LQTS-ApoCaMs, i.e., the conformation that binds LTCCs, with a consequent higher-than-expected abundance of LQTS-CaM bound to LTCCs. Thus, LTCCs will have a high percentage of dysfunctional CaMs bound, which will significantly disrupt their activity despite the 5:1 WT:MT allelic stoichiometry.

by Webster and coworkers in clinical practice and in a single calmodulinopathy patient (52) verapamil showed some promising results.

Overall, these studies demonstrated the importance and feasibility of modeling calmodulinopathy in the setting of its native environment, provided insight into the pathophysiological mechanism underlying the respective life-threatening arrhythmias and validated calmodulinopathy patient-derived iPSC-CMs as a platform for precision medicine investigations.

CALMODULINOPATHY PHENOTYPES AND CLINICAL FEATURES

The rapid emergence of the genetic discoveries related to calmodulinopathy has eclipsed the ability of the field to ascertain and compare in depth all the individual clinical features among different subjects thus far described in the literature. This has naturally led to the "lumping" of cases under existing clinical "umbrella" terms. Indeed, CaM mutations have been associated with a spectrum of arrhythmic phenotypes, including LQTS, CPVT, IVF, as well as LQTS/CPVT overlap and Sudden Unexplained Death (SUD) (6, 7, 9, 10, 35–43). From the thus far available descriptions, however, it seems to emerge that there is a novel constellation of symptoms, arrhythmia types, ECG features and response to therapy that are characteristic of calmodulinopathy as a clinical entity, closely-related, but nevertheless distinct, from other arrhythmia syndromes.

To this end, we have recently established an international registry of subjects with CaM mutations (ICalmR) (45), currently enrolling patients from overall 15 countries, in order to conduct a thorough ascertainment of the clinical spectrum, genotype-phenotype relationship, and treatment responses of these patients. In order to further aid the enrollment of new

cases and facilitate the discussion among experts, ICalmR has become a member of the European Reference Network on rare diseases of the heart (53), while, recently, the registry has been made available to the European Commission for online data submission and patient enrollment, according to EU requirements.

From the descriptions thus far available in the literature, the most prevalent phenotype is LQTS, observed in 73% (22/30) of index cases. All LQTS-associated CaM mutations are localized within CaM's EF-hands III ($n = 7$) and IV ($n = 9$) or in the C-terminal region ($n = 2$). The second most frequent arrhythmic phenotype observed is CPVT, with 4 index cases carrying the *CALM1*-p.N54I, *CALM1*-p.N98S, *CALM2*-p.N98S, or *CALM3*-p.A103V mutations. Much less represented phenotypes are LQTS/CPVT overlap (1 case, *CALM2*-p.D132E), IVF (1 case, *CALM1*-p.F90L), and SUD (2 cases, *CALM2*-p.F90L, *CALM2*-p.N98S). Interestingly, particular hotspot mutations, such as the p.N98S mutation, may give rise to diverse arrhythmic phenotypes (LQTS, CPVT, SUD), regardless of the gene in which the nucleotide substitution resides, whilst others, such as the p.D130G and p.F142L, always cause definite LQTS.

Irrespective of the associated phenotypes, a common feature of all CaM mutations identified so far is the extreme severity of disease manifestations and early occurrence with almost half of subjects having a perinatal presentation (28th week of gestation to 28th postnatal day) (12/28, 43%, with the exclusion of 2 SUDs), mainly among those with an LQTS phenotype. The latter often show 2:1 AV block, T wave alternans and QTc values above 550 ms (45).

As in other inherited arrhythmogenic conditions, SCD can be the first manifestation of the disease, but unfortunately SCD can also occur after diagnosis and establishment of antiarrhythmic therapies. Indeed, to provide some examples, in two patients, both carrying the *CALM1*-p.F142L mutation, the diagnosis of LQTS was made just after birth and beta-blocker therapy was immediately started. Later on, a pacemaker was implanted. Nevertheless, they both later died suddenly, at 2 and 1 years of age (41). Another LQTS subject, carrying the *CALM2*-p.Q136P mutation, came to medical attention at 8 years of age after an episode of syncope associated with a prolonged period of unconsciousness. At that time, she had a prolonged QTc of 500 ms with ventricular bigeminy, she was put on nadolol, but she died suddenly during a dancing competition at 11 years of age (38).

Unfortunately, half of calmodulinopathy patients will experience SCD (45) and may survive only if, often by chance, appropriate resuscitation will be provided in due time. Among those who will survive, many may end up neurologically compromised. Of note, in our original description of LQTS-associated calmodulinopathy (6), all patients presented with some neurodevelopmental/neurological features. However, it is still unclear whether neurological impairment may be an inherent feature of the disease spectrum, since CaM is also highly expressed in the brain, or such an impairment may be only the secondary result of multiple resuscitated cardiac arrests causing brain injury. By means of studying the disease systemically and by jointly examining a larger number of cases through the ICalmR (45), some light may be shed on whether CaM's non-cardiac expression may also give rise to concomitant non-cardiac phenotypes in the setting of calmodulinopathy.

CALMODULINOPATHY THERAPY

Calmodulinopathy is a severe condition for which effective therapies are currently lacking. From the clinical data and descriptions available thus far in the literature, and from our own clinical experience (45), commonly used anti-arrhythmic therapies and procedures (i.e., left cardiac sympathetic denervation) largely fail to treat these young patients. Indeed, β blocker therapy- the mainstay treatment for LQTS and CPVT- seems to offer minimal benefit at controlling the life-threatening arrhythmias, while other antiarrhythmics, such as sodium channel blockers, have also not produced promising results (6, 52). Ca^{2+} channel blockade may seem a rational therapeutic option since impaired CDI of the Ca^{2+} channel $Ca_v1.2$ is the prominent underlying mechanism of LQTS related to CaM mutations (20, 49, 51). However, the Ca^{2+} antagonist verapamil, despite some positive results in one case (52), largely fails to prevent cardiac event recurrences in other clinical cases (6, 45). This may be attributed to the fact that verapamil principally targets peak calcium current rather than modulating channel inactivation (52).

Although implantable cardioverter defibrillators (ICD) would be the treatment of choice for any patient surviving SCD due to VF (54), this type of intervention in these young patients imposes almost impossible clinical dilemmas. On one hand, the risk of complications is higher than in adults, but, on the other hand, these children are at very high risk of dying suddenly or having neurological sequelae due to brain injury secondary to multiple resuscitated cardiac arrests. Unfortunately, we have examples showing a premature death in both scenarios (i.e., death related to ICD complications or death related to VF when the ICD was not implanted).

These real life examples highlight the urgent need to identify appropriate management schemes and therapies for life-threatening calmodulinopathy.

CONCLUSION

Genetic mutations in any of the 3 genes encoding the ubiquitous Ca^{2+} signaling protein CaM cause calmodulinopathy, a recently identified severe arrhythmogenic entity, affecting very young individuals. Although more arrhythmic phenotypes have been associated to CaM mutations, LQTS is the predominant one, with CDI of the L-type Ca^{2+} channel $Ca_v1.2$ and disruption of cellular Ca^{2+} homeostasis being the main underlying mechanism. To date, no therapies exist that may effectively treat the life-threatening arrhythmias and prevent SCD occurrence, while ICD implantation in these young patients is frequently associated with complications.

State-of-the-art technologies such as patient-derived iPSC-CMs have been successfully thus far employed for

calmodulinopathy modeling, not only providing insight into the mechanistic bases but also showing promising results for future precision medicine investigations, e.g., through gene correction approaches.

Systematic clinical evaluation of a large number of patients and identification of new cases prospectively in combination with the most recent technological advancements in hiPSC-CM technology, pharmacological screenings (55), and tridimensional approaches (56, 57), hold promise in identifying effective therapeutic strategies for this devastating disease.

AUTHOR CONTRIBUTIONS

M-CK wrote the manuscript and drew the protein alignments. LS wrote the manuscript and drew the figures. AG wrote the manuscript. BB, CR, GP, and AZ critically reviewed the manuscript. LC wrote and critically reviewed the manuscript.

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REFERENCES

1. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC)endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). *Eur Heart J.* (2015) 36:2793–867. doi: 10.1093/eurheartj/ehv316
2. Crotti L, Kotta MC. The role of genetics in primary ventricular fibrillation, inherited channelopathies and cardiomyopathies. *Int J Cardiol.* (2017) 237:45–8. doi: 10.1016/j.ijcard.2017.03.119
3. Basso C, Carturan E, Pilichou K, Rizzo S, Corrado D, Thiene G. Sudden cardiac death with normal heart: molecular autopsy. *Cardiovasc Pathol.* (2010) 19:321–5. doi: 10.1016/j.carpath.2010.02.003
4. Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, et al. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. *Circulation* (2007) 115:361–7. doi: 10.1161/CIRCULATIONAHA.106.658021
5. Crotti L, Tester DJ, White WM, Bartos DC, Insolia R, Besana A, et al. Long QT syndrome – associated mutations in intrauterine fetal death. *JAMA* (2013) 309:1473–82. doi: 10.1001/jama.2013.3219
6. Crotti L, Johnson CN, Graf E, De Ferrari GM, Cuneo BF, Ovadia M, et al. Calmodulin mutations associated with recurrent cardiac arrest in infants. *Circulation* (2013) 127:1009–17. doi: 10.1161/CIRCULATIONAHA.112.001216
7. Reed GJ, Boczek NJ, Etheridge SP, Ackerman MJ. CALM3 mutation associated with long QT syndrome. *Heart Rhythm* (2015) 12:419–22. doi: 10.1016/j.hrthm.2014.10.035
8. George AL. Calmodulinopathy: a genetic trilogy. *Heart Rhythm* (2015) 12:423–4. doi: 10.1016/j.hrthm.2014.11.017
9. Nyegaard M, Overgaard MT, Søndergaard MT, Vranas M, Behr ER, Hildebrandt LL, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. *Am J Hum Genet.* (2012) 91:703–12. doi: 10.1016/j.ajhg.2012.08.015
10. Marsman RF, Barc J, Beekman L, Alders M, Dooijes D, van den Wijngaard A, et al. A mutation in CALM1 encoding calmodulin in familial idiopathic ventricular fibrillation in childhood and adolescence. *J Am Coll Cardiol.* (2014) 63:259–66. doi: 10.1016/j.jacc.2013.07.091
11. Ben-Johny M, Dick I, Yang W, Issa J, Sang L, Lee S, et al. Towards a unified theory of calmodulin regulation (calmodulation) of voltage-gated calcium and sodium channels. *Curr Mol Pharmacol.* (2015) 8:188–205. doi: 10.2174/1874467208666150507110359
12. Wawrzynczak EJ, Perham RN. Isolation and nucleotide sequence of a cDNA encoding human calmodulin. *Biochem Int.* (1984) 9:177–85.
13. Sen Gupta B, Detera-Wadleigh SD, McBride OW, Friedberg F. A calmodulin pseudogene on human chromosome 17. *Nucleic Acids Res.* (1989) 17:2868. doi: 10.1093/nar/17.7.2868
14. Fischer R, Koller M, Flura M, Mathews S, Strehler-Page MA, Krebs J, et al. Multiple divergent mRNAs code for a single human calmodulin. *J Biol Chem.* (1988) 263:17055–62.
15. Rhyner JA, Ottiger M, Wicki R, Greenwood TM, Strehler EE. Structure of the human CALM1 calmodulin gene and identification of two CALM1-related pseudogenes CALM1P1 and CALM1P2. *Eur J Biochem.* (1994) 225:71–82. doi: 10.1111/j.1432-1033.1994.00071.x
16. Toutenhoofd SL, Foletti D, Wicki R, Rhyner JA, Garcia F, Tolon R, et al. Characterization of the human CALM2 calmodulin gene and comparison of the transcriptional activity of CALM1, CALM2 and CALM3. *Cell Calcium* (1998) 23:323–38. doi: 10.1016/S0143-4160(98)90028-8
17. Halling DB, Liebeskind BJ, Hall AW, Aldrich RW. Conserved properties of individual Ca^{2+}-binding sites in calmodulin. *Proc Natl Acad Sci USA.* (2016) 113:E1216–25. doi: 10.1073/pnas.1600385113
18. Papatheodorou I, Fonseca NA, Keays M, Tang YA, Barrera E, Bazant W, et al. Expression Atlas: gene and protein expression across multiple studies and organisms. *Nucleic Acids Res.* (2018) 46:D246–51. doi: 10.1093/nar/gkx1158
19. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. *Science* (2015) 347:1260419. doi: 10.1126/science.1260419
20. Limpitikul WB, Dick IE, Tester DJ, Boczek NJ, Limphong P, Yang W, et al. A precision medicine approach to the rescue of function on malignant calmodulinopathic long-QT syndrome. *Circ Res.* (2017) 120:39–48. doi: 10.1161/CIRCRESAHA.116.309283
21. Babu YS, Bugg CE, Cook WJ. Structure of calmodulin refined at 2.2 A resolution. *J Mol Biol.* (1988) 204:191–204. doi: 10.1016/0022-2836(88)90608-0
22. Beccia MR, Sauge-Merle S, Lemaire D, Brémond N, Pardoux R, Blangy S, et al. Thermodynamics of calcium binding to the calmodulin N-terminal domain to evaluate site-specific affinity constants and cooperativity.*J Biol Inorg Chem.* (2015) 20:905–19. doi: 10.1007/s00775-015-1275-1
23. VanScyoc WS, Sorensen BR, Rusinova E, Laws WR, Ross JBA, Shea MA. Calcium binding to calmodulin mutants monitored by domain-specific intrinsic phenylalanine and tyrosine fluorescence. *Biophys J.* (2002) 83:2767–80. doi: 10.1016/S0006-3495(02)75286-7
24. Tadross MR, Dick IE, Yue DT. Mechanism of local and global Ca^{2+} sensing by calmodulin in complex with a Ca^{2+} channel. *Cell* (2008) 133:1228–40. doi: 10.1016/j.cell.2008.05.025
25. Stigler J, Rief M. Calcium-dependent folding of single calmodulin molecules. *Proc Natl Acad Sci USA.* (2012) 109:17814–9. doi: 10.1073/pnas.1201801109
26. Wu X, Bers DM. Free and bound intracellular calmodulin measurements in cardiac myocytes. *Cell Calcium* (2007) 41:35364. doi: 10.1016/j.ceca.2006.07.011
27. Maier LS, Ziolo MT, Bossuyt J, Persechini A, Mestril R, Bers DM. Dynamic changes in free Ca-calmodulin levels in adult cardiac myocytes. *J Mol Cell Cardiol.* (2006) 41:451–8. doi: 10.1016/j.yjmcc.2006.04.020
28. Erickson MG, Alseikhan BA, Peterson BZ, Yue DT. Preassociation of calmodulin with voltage-gated Ca(2+) channels revealed by FRET in single living cells. *Neuron* (2001) 31:973–85. doi: 10.1016/S0896-6273(01)00438-X
29. Shah VN, Wingo TL, Weiss KL, Williams CK, Balser JR, Chazin WJ. Calcium-dependent regulation of the voltage-gated sodium channel hH1: intrinsic and extrinsic sensors use a common molecular switch. *Proc Natl Acad Sci USA.* (2006) 103:3592–7. doi: 10.1073/pnas.0507397103

30. Mori MX, Erickson MG, Yue DT. Functional stoichiometry and local enrichment of calmodulin interacting with Ca^{2+} Channels. *Science* (2004) 304:432–5. doi: 10.1126/science.1093490
31. Peterson BZ, DeMaria CD, Adelman JP, Yue DT. Calmodulin is the Ca^{2+} sensor for Ca^{2+}-dependent inactivation of L-type calcium channels. *Neuron* (1999) 22:549–58. doi: 10.1016/S0896-6273(00)80709-6
32. Liu X, Zhang X, Herbert SJ. Feeding China's growing needs for grain. *Nature* (2010) 465:420. doi: 10.1038/465420a
33. Valeyev N V, Bates DG, Heslop-Harrison P, Postlethwaite I, Kotov N V. Elucidating the mechanisms of cooperative calcium-calmodulin interactions: a structural systems biology approach. *BMC Syst Biol.* (2008) 2:48. doi: 10.1186/1752-0509-2-48
34. Boschek CB, Jones TE, Squier TC, Bigelow DJ. Calcium occupancy of N-terminal sites within calmodulin induces inhibition of the ryanodine receptor calcium release channel. *Biochemistry* (2007) 46:10621–8. doi: 10.1021/bi700655h
35. Gomez-Hurtado N, Boczek NJ, Kryshtal DO, Johnson CN, Sun J, Nitu FR, et al. Novel CPVT-associated calmodulin mutation in CALM3 (CALM3-A103V) activates arrhythmogenic Ca waves and sparks. *Circ Arrhythm. Electrophysiol.* (2016) 9:e004161. doi: 10.1161/CIRCEP.116.004161
36. Jiménez-Jáimez J, Doza JP, Ortega Á, Macías-Ruiz R, Perin F, Rodríguez-Vázquez Del Rey MM, et al. Calmodulin 2 mutation N98S is associated with unexplained cardiac arrest in infants due to low clinical penetrance electrical disorders. *PLoS ONE* (2016) 11:e0153851. doi: 10.1371/journal.pone.0153851
37. Daly, A, Johnson, NM, Decker, E, Callis, TE, Tahiliani, J, Garcia, J, et al. Pathogenic variants in calmodulin associated with resuscitated childhood cardiac arrest (Abstract C-AB01-05). *Heart Rhythm* (2017) 14:S2. Available online at: https://www.heartrhythmjournal.com/article/S1547-5271(17)30422-8/pdf
38. Makita N, Yagihara N, Crotti L, Johnson CN, Beckmann B-M, Roh MS, et al. Novel calmodulin mutations associated with congenital arrhythmia susceptibility. *Circ Cardiovasc Genet.* (2014) 7:466–74. doi: 10.1161/CIRCGENETICS.113.000459
39. Pipilas DC, Johnson CN, Webster G, Schlaepfer J, Fellmann F, Sekarski N, et al. Novel calmodulin mutations associated with congenital long QT syndrome affect calcium current in human cardiomyocytes. *Heart Rhythm* (2016) 13:2012–9. doi: 10.1016/j.hrthm.2016.06.038
40. Chaix MA, Koopmann TT, Goyette P, Alikashani A, Latour F, Fatah M, et al. Novel CALM3 mutations in pediatric long QT syndrome patients support a CALM3-specific calmodulinopathy. *Heart Rhythm Case Rep.* (2016) 2:250–4. doi: 10.1016/j.hrcr.2016.02.002
41. Boczek NJ, Gomez-Hurtado N, Ye D, Calvert ML, Tester DJ, Kryshtal D, et al. Spectrum and prevalence of CALM1-, CALM2-, and CALM3-encoded calmodulin variants in long QT syndrome and functional characterization of a novel long QT syndrome-associated calmodulin missense variant, E141G. *Circ Cardiovasc Genet.* (2016) 9:136–46. doi: 10.1161/CIRCGENETICS.115.001323
42. Takahashi K, Ishikawa T, Makita N, Takefuta K, Nabeshima T, Nakayashiro M. A novel *de novo* calmodulin mutation in a 6-year-old boy who experienced an aborted cardiac arrest. *Heart Rhythm Case Rep.* (2017) 3:69–72. doi: 10.1016/j.hrcr.2016.09.004
43. Anderson JH, Tester DJ, Will ML, Ackerman MJ. Whole-exome molecular autopsy after exertion-related sudden unexplained death in the young. *Circ Cardiovasc Genet.* (2016) 9:259–65. doi: 10.1161/CIRCGENETICS.115.001370
44. Lek M, Karczewski KJ, Minikel E V., Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* (2016) 536:285–91. doi: 10.1038/nature19057
45. Crotti L, Spazzolini C, Boczek N, Jimenez Jaimez J, Makita N, Tester D, et al. International Calmodulinopathy Registry (ICaMR). *Circulation* (2016) 134:A14840. doi: 10.1161/circ.134.suppl_1.14840
46. Alseikhan BA, DeMaria CD, Colecraft HM, Yue DT. Engineered calmodulins reveal the unexpected eminence of Ca^{2+} channel inactivation in controlling heart excitation. *Proc Natl Acad Sci USA*. (2002) 99:17185–90. doi: 10.1073/pnas.262372999
47. Yin G, Hassan F, Haroun AR, Murphy LL, Crotti L, Schwartz PJ, et al. Arrhythmogenic calmodulin mutations disrupt intracellular cardiomyocyte Ca^{2+} regulation by distinct mechanisms. *J Am Heart Assoc.* (2014) 3:e000996. doi: 10.1161/JAHA.114.000996
48. Hwang HS, Nitu FR, Yang Y, Walweel K, Pereira L, Johnson CN, et al. Divergent regulation of ryanodine receptor 2 calcium release channels by arrhythmogenic human calmodulin missense mutants. *Circ Res.* (2014) 114:1114–24. doi: 10.1161/CIRCRESAHA.114.303391
49. Rocchetti M, Sala L, Dreizehnter L, Crotti L, Sinnecker D, Mura M, et al. Elucidating arrhythmogenic mechanisms of long-QT syndrome CALM1-F142L mutation in patient-specific induced pluripotent stem cell-derived cardiomyocytes. *Cardiovasc Res.* (2017) 113:531–41. doi: 10.1093/cvr/cvx006
50. Limpitikul WB, Dick IE, Joshi-Mukherjee R, Overgaard MT, George AL, Yue DT. Calmodulin mutations associated with long QT syndrome prevent inactivation of cardiac L-type Ca^{2+} currents and promote proarrhythmic behavior in ventricular myocytes. *J Mol Cell Cardiol.* (2014) 74:115–24. doi: 10.1016/j.yjmcc.2014.04.022
51. Yamamoto Y, Makiyama T, Harita T, Sasaki K, Wuriyanghai Y, Hayano M, et al. Allele-specific ablation rescues electrophysiological abnormalities in a human iPS cell model of long-QT syndrome with a CALM2 mutation. *Hum Mol Genet.* (2017) 26:1670–7. doi: 10.1093/hmg/ddx073
52. Webster G, Schoppen ZJ, George AL. Treatment of calmodulinopathy with verapamil. *BMJ Case Rep.* (2017) 2017:bcr-2017-220568. doi: 10.1136/bcr-2017-220568
53. *European Reference Networks, ERN GUARD-Heart*. Available online at: https://ec.europa.eu/health/ern/overview_en and http://guardheart.ern-net.eu/disease-registries/
54. Zipes DP, Wellens HJJ. Sudden cardiac death. *Circulation* (1998) 98:2334–51. doi: 10.1161/01.CIR.98.21.2334
55. Sala L, Bellin M, Mummery CL. Integrating cardiomyocytes from human pluripotent stem cells in safety pharmacology: has the time come? *Br J Pharmacol.* (2017) 174:3749–65. doi: 10.1111/bph.13577
56. Giacomelli E, Bellin M, Sala L, van Meer BJ, Tertoolen LGJ, Orlova VV, et al. Three-dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. *Development* (2017) 144:1008–17. doi: 10.1242/dev.143438
57. Schaaf S, Shibamiya A, Mewe M, Eder A, Stöhr A, Hirt MN, et al. Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology. *PLoS ONE* (2011) 6:e26397. doi: 10.1371/journal.pone.0026397

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REVIEW
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Calmodulinopathy: Functional Effects of CALM Mutations and Their Relationship With Clinical Phenotypes

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In spite of the widespread role of calmodulin (CaM) in cellular signaling, CaM mutations lead specifically to cardiac manifestations, characterized by remarkable electrical instability and a high incidence of sudden death at young age. Penetrance of the mutations is surprisingly high, thus postulating a high degree of functional dominance. According to the clinical patterns, arrhythmogenesis in CaM mutations can be attributed, in the majority of cases, to either prolonged repolarization (as in long-QT syndrome, LQTS phenotype), or to instability of the intracellular Ca^{2+} store (as in catecholamine-induced tachycardias, CPVT phenotype). This review discusses how mutations affect CaM signaling function and how this may relate to the distinct arrhythmia phenotypes/mechanisms observed in patients; this involves mechanistic interpretation of negative dominance and mutation-specific CaM-target interactions. Knowledge of the mechanisms involved may allow critical approach to clinical manifestations and aid in the development of therapeutic strategies for "calmodulinopathies," a recently identified nosological entity.

Keywords: calmodulin mutations, ion channels, repolarization, Ca^{2+} handling, arrhythmia mechanisms

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INTRODUCTION

As other ions, Ca^{2+} is used as a charge carrier to modulate membrane potential; however, Ca^{2+} also has a central role as a diffusible signaling molecule and as a trigger of diverse cellular functions. While some of these are clearly complementary in achieving a functional goal (e.g., cAMP signaling in functional upregulation) and can coexist, others are devoted to apparently competing aims (e.g., apoptosis pathway) and need to be separated. This requires mechanisms allowing intracellular Ca^{2+} to act on its targets with high specificity. Several strategies are employed by the cell to achieve this goal. Ca^{2+} buffering by intracellular proteins and small molecules, leads to a strictly controlled mobility of the ion. Active Ca^{2+} compartmentalization within organelles allows to keep "resting" Ca^{2+} concentration in the general (or "bulk") cytosolic compartment at very low levels (around 10^{-7} M), i.e., below the threshold required to activate downstream effectors; at the same time, structural organization (e.g., T-tubules) allows very small Ca^{2+} fluxes to achieve high Ca^{2+} concentration in the specific subcellular compartment hosting the target effector (1).

The presence of molecules devoted to detect Ca^{2+} and transduce its concentration changes into specific actions is a further strategy, pivotal to the integrated operation of Ca^{2+}-dependent processes. Perhaps the most diffuse of such "Ca^{2+} sensor" molecules is calmodulin (CaM), a protein present in all cell types and highly conserved throughout evolution (1). Most Ca^{2+}-binding proteins are characterized by "EF hand" domains, which constitute the ion binding site. Whereas, in proteins involved in Ca^{2+} buffering and controlled mobility the EF hand is simply a binding site, in Ca^{2+} sensors the EF domain changes protein conformation in response to Ca^{2+} binding, thus triggering a variety of downstream events (2).

Essential to CaM's targeting role, is its property to stably bind to many of its downstream effectors. This corresponds to the presence on the latter of specific CaM-binding sequences, which make CaM an integral component of the target protein. Thus, CaM can exist as a freely diffusible signal (cytosolic pool), or as a sensor intrinsic to a given effector (bound pool), thus affording either diffuse or highly confined signaling. Furthermore, in various cells types, the cytosolic CaM pool can be redistributed to the nuclear compartment upon a rise in Ca^{2+} levels, thus broadening the targets range (3, 4). Also, except in selected cell types (e.g., mitotic cells), local CaM concentrations may follow Ca^{2+} oscillations, thus generating spatial and temporal patterns which may play a crucial role in biological processes (5).

In keeping with its central and evolutionarily conserved function, CaM is generated in a highly redundant mode. Indeed, an identical amino acid sequence is encoded by 3 CaM genes (CALM 1, 2, and 3) (5). Such redundancy is in apparent contrast with the high penetrance of heterozygous CaM mutations. While the possible role of transcriptional regulation of these genes is discussed in the accompanying article (6), specific molecular mechanisms may contribute to negative dominance in a mutation's effect.

In cardiac muscle cells, Ca^{2+} at the same time contributes as a charge carrier to electrical excitation (the "action potential," AP) and triggers the development of mechanical force; therefore, Ca^{2+} is crucial to excitation-contraction coupling (ECC) (7). Several processes central to beat-to beat control of intracellular Ca^{2+} dynamics are CaM-mediated; furthermore, CaM acts a Ca^{2+} sensor in the control of gene expression, thus playing a role in long-term modulation of cell function and fate (8). This might lead to the expectation that a CaM loss of function should result in general myocyte dysfunction and death. However, this is contradicted by the observation that CaM mutations affect only the function of specific targets, leading to mutation-specific phenotypes with pronounced electrical instability as a common feature (9).

Our objective is to revise the information available on the various aspects of CaM structure/function that we see as potentially relevant to a mechanistic interpretation of CaM mutations phenotypes, with a focus on cardiac ones.

CaM Structure, Ca^{2+}-Sensing and Target Recognition

CaM is composed of 149 amino acid residues to form a 17 kDa protein. The protein is ubiquitous and expressed in all eukaryotic cells, with 100% identity in its amino acid sequence among vertebrates. Three genes (CALM1, CALM2, and CALM3) encode CaM with an identical amino acid sequence, thus resulting in potential redundancy (10).

CaM is formed of two "lobes," named N and C, respectively, according to their position with respect to protein ends, connected by an α-helix "linker" containing a flexible region ("hinge") (**Figure 1**) (12); this allows each lobe to move relative to the other. Each lobe consists of two "EF hand" domains (EF) with one Ca^{2+} binding site each (2 Ca^{2+} binding sites per lobe). All EF hand domains can bind Ca^{2+}; however, while the N-lobe (EF I and EF II) has higher affinity for Mg^{2+}, the C-lobe (EF III and EF IV) binds Ca^{2+} with ten times higher affinity (13). Another functional distinction between the two CaM lobes is the rate of Ca^{2+} binding and unbinding, faster for the N-lobe than for the C-lobe (14).

Knowledge of CaM's 3D structure has evolved since its first description in 1985 (15), with a major contribution provided, 10 years later, by nuclear magnetic resonance (16). Whereas, this technology revealed detail of the linker helix accounting for its flexibility (17, 18), more recent studies added information about recognition of target proteins and how it can be affected by CaM complexing with Ca^{2+} (12, 19).

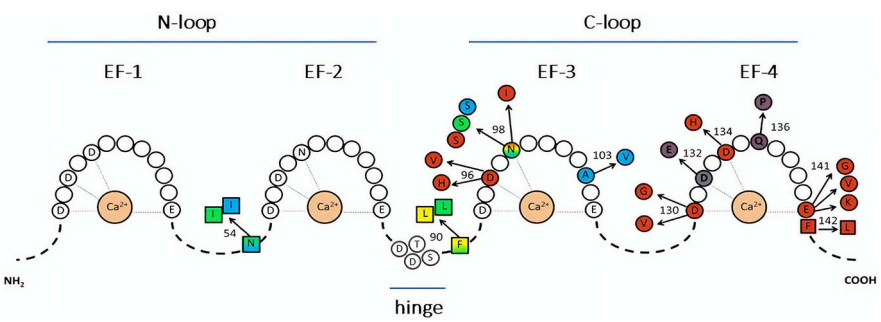

FIGURE 1 | Representation of CaM sequence and relative disease-associated mutations. The letters identify amino acids directly involved in Ca^{2+} binding (within the EF-hands), or in the hinge region. Color-substituted amino acids represent mutations in the EF-hands (circles) or in the linkers (squares); colors correspond to the associated phenotype: catecholaminergic polymorphic ventricular tachycardia (CPVT, light blue), long QT syndrome (LQTS, red), idiopathic ventricular fibrillation (IVF, yellow), other unexplained sudden death (green). LQTS/CPVT overlap mutations are shown in shaded color. Modified from Crotti and Kotta, (11).

The interplay between structure and function is relevant to the three components of CaM signaling: Ca^{2+} binding, target binding, and target modulation.

The first aspect of interest is CaM's interaction with Ca^{2+}. In apo-CaM (the Ca^{2+} unloaded CaM), EF I and EF II (N-lobe) are in a packed conformation, thus with low affinity for Ca^{2+}; EF III and EF IV (C-lobe) are instead in a partially open conformation, more prone to Ca^{2+} binding. At resting Ca^{2+} levels (about 10^{-7} M), all CaM binding sites are typically unoccupied. When Ca^{2+} rises, its binding to the C-lobe sites triggers a conformational change leading the N-lobe to increase its affinity for Ca^{2+}. In other words, Ca^{2+} binding to CaM is "cooperative," i.e., the overall affinity increases when Ca^{2+} concentration exceeds the threshold for C-lobe occupation. CaM can potentially bind 1–4 Ca^{2+} ions; activation of downstream targets requires CaM being loaded with 3 Ca^{2+} ions at least (12), a configuration referred to as "holo-CaM."

Also of interest are the structural aspects of CaM's interaction with target proteins, which are necessary for modulation of their activity. "Anchor" domains on target proteins are characterized by hydrophobic residue sequences flanked by negatively charged ones; the latter provide electrostatic interaction that may orient CaM binding (12). Many CaM targets (e.g., $Ca_v1.2$) are characterized by the presence of a typical basic amino acid sequence called the "IQ motif." The IQ motif is closely preceded by a "preIQ" region, which is a common site of permanent CaM binding (20). CaM interaction with the IQ motif itself is instead more likely responsible for downstream signaling (8, 14); indeed, specificity of the IQ sequence may dictate whether Ca^{2+}-CaM signaling leads to target activation or inhibition (20). A well-studied instance of preIQ-IQ binding is CaM interaction with the voltage-dependent L-type Ca^{2+}-channel ($Ca_v1.2$); the detailed mechanism and Ca^{2+}-dependency of this specific interaction will be described in paragraph Voltage-gated Ca^{2+} channels (Cav1.2).

As for Ca^{2+} sensing, CaM domains mainly involved in target binding are the lobes. Both CaM lobes contain nine methionine residues, playing a key role in target recognition, whose high flexibility provides plasticity crucial for this function (21).

The CaM binding interface is highly structured, but it may dynamically accommodate various binding modes in a metastable equilibrium. Ca^{2+} may stabilize a given interface conformation (12). Ca^{2+}-dependency of CaM binding differs among target proteins. While holo-CaM is strictly required for strong target binding in some cases, in others target interaction occurs preferentially at lower levels of occupancy by Ca^{2+}. This accounts for the finding that CaM may be bound to many targets at resting Ca^{2+} concentrations (8, 12, 22). At low Ca^{2+} the N-lobe has higher stability than the C-lobe (23), which favors its binding in the apo form (pre-association) (8). On the other hand, due to its higher Ca^{2+} affinity, the C-lobe is more often involved in Ca^{2+}-dependent target binding than the N-lobe (12, 24) and both the N- and C-lobes may participate to stabilize the protein-target complex (25).

Ca^{2+} binding changes CaM conformation to expose hydrophobic (methionine-rich) sites, either at the N-lobe or at the C-lobe, suitable to interact with hydrophobic residues in the anchor (18). The large size and flexibility of CaM interaction landscape is essential to accommodate a variety of "anchor" side-chains, thus providing CaM with its extraordinary pleiotropicity. Nonetheless, the observation that single site mutations may selectively affect CaM binding to a specific target suggests high specificity of the binding interface. Less information exists about the target binding mode of apo-CaM. The main differences with holo-CaM binding may concern the N-lobe and a larger involvement of electrostatic interactions (12).

Consistent with the notion that CaM lobes change their conformation when interacting with the target, the relationship between target and Ca^{2+} binding by CaM is mutual: binding to the target may increase CaM affinity for Ca^{2+} (12); this generates cooperativity in Ca^{2+}-dependency of CaM-target interactions. According to modeling data (26), differences among targets in the extent of such cooperativity contribute to specificity of target recognition.

The third aspect of CaM signaling is modulation of target function which, depending on the target, can be either stimulatory or inhibitory. Target modulation can be either enacted by apo-CaM, or require Ca^{2+} binding and its mechanism differs among targets (see below examples for RyR and $Ca_v1.2$ channels). As a general interpretational scheme, CaM binding may stabilize an otherwise short-lived configuration, spontaneously assumed by the target protein and associated with a specific functional state (12).

"Free" and "Pre-Bound" CaM Pools

Many CaM targets have been described so far. These include proteins involved in cell cycle, cell proliferation and autophagy (in healthy cells), tumor progression proteins (in cancerous cells) (5), proteins essential to cell communication and metabolism (27), and a wide number of ion channels (28–30). Most of these targets strictly require CaM pre-association to allow their regulation (31).

To better understand the role of CaM in cells, we need to consider its distribution between the "free" (cytosolic) and "pre-bound" pools. As mentioned previously, such a distribution may vary according to CaM's occupancy by Ca^{2+} (25).

Total CaM concentration is variable among tissues: it usually ranges between 5 and 40 μM (e.g. ,about 6 μM in intact myocytes) (32), but values up to 100 μM have been described in specific cell types (e.g., in the testis) (33). In cardiomyocytes, even at diastolic Ca^{2+} concentration, 99% of total intracellular CaM is bound to cellular proteins, leaving about 50-100 nM of free CaM (1%) in the cell (32). Nonetheless, the proportion of CaM in the pre-bound pool is variable among tissue types (e.g., 11% in testis and 63% in spleen), and it may differ between normal and pathological cells and depend on environmental factors, such as cell density in culture (5). The pre-bound CaM pool includes mainly apo-CaM or CaM with incomplete occupancy, depending on the target (32); its functional relevance may be to increase speed of target response to local Ca^{2+} elevation.

The pre-bound CaM pool localizes to structures in the plasma membrane as well as in intracellular organelles; under resting Ca^{2+} concentrations (e.g., 100 nM), pre-bound CaM may largely exist as apo-CaM. Pre-bound apo-CaM may even be released to the free CaM pool in response to Ca^{2+} elevation,

thus representing a local diffusible CaM store (22); this is true particularly in growing cells, where CaM is highly expressed.

As Ca^{2+} occupancy increases, CaM becomes almost completely bound to targets, thus leaving a very small pool of freely diffusible holo-CaM. Competition among targets for this pool may be of significance for their reciprocal regulation (32). Even if the CaM-target interaction is generally facilitated by Ca^{2+}, the pattern is quite complex. Chin and Means (22) identified at least six CaM target groups, according to their CaM recognition sequences and Ca^{2+}-dependency of binding. Some of them are strongly pre-bound to apo-CaM, for others binding is stronger for holo-CaM, while, finally, apo-CaM binding to a class of targets can be released by Ca^{2+}, thus providing a local reservoir for free CaM.

CAM MODULATION OF VOLTAGE-GATED CHANNELS

Voltage-Gated Ca^{2+} Channels ($Ca_v1.2$)

Ca^{2+} current from $Ca_v1.2$ channels (I_{CaL}) is the most abundant type in cardiomyocytes (34). The symbol "L" recapitulates the main features of this channel (as compared to other Ca^{2+} ones): large conductance, activation at larger depolarizations and long lasting openings. The activation of this channel is driven by the action potential upstroke, with I_{CaL} reaching a peak in 2–7 ms. Thereafter, channels inactivate with time constants in the order of 50–100 ms at plateau potential, due to both Ca^{2+} and voltage-dependent gating (35). Ca^{2+} influx through I_{CaL} leads to a rapid increase of cytosolic Ca^{2+} concentration in the confined space between the sarcolemmal and sarcoplamic reticulum (SR) membranes (also called "dyadic cleft"). This is responsible for the opening of ryanodine receptors (RyRs), Ca^{2+}-activated Ca^{2+}channels clustered in the SR membrane facing $Ca_v1.2$ channels.

I_{CaL} is modulated by two feedback signals that, albeit of opposite sign, are both dependent on the rise of Ca^{2+} concentration close to cytosolic mouth of the channel and involve CaM. Ca^{2+}-dependent inactivation (CDI) is responsible for most of the rapid I_{CaL} decay occurring during sustained depolarization (35). Ca^{2+}-dependent facilitation (CDF), a weaker phenomenon, reflects instead the increase in I_{CaL} peak conductance that may be observed during repetitive activation at high rates (36). Both CDF and CDI depend on Ca^{2+}-CaM complexing (37). Earlier studies (37) reported that, whereas replacement of isoleucine or glutamine to alanine in the IQ motif of the C-terminal region of the $Ca_v1.2$ channel α_{1C} subunit abolished CDI but enhanced CDF, replacement of isoleucine to glutamate in the same region abolished both forms of auto-regulation. This led to the conclusion that CDI and CDF had different mechanisms, but that CaM binding to the IQ motif is involved in both cases.

$Ca_v1.2$ channels are constitutively associated with apo-CaM. Such pre-association is required since CaMs from the cytosolic bulk are unable to adequately access the binding site on $Ca_v1.2$ during Ca^{2+} inflow (38); pre-association to the C-terminal region of $Ca_v1.2$ places CaM within a nanodomain at channel cytosolic mouth. This location confers to the C-lobe of pre-bound CaM the ability to sense Ca^{2+} changes in temporal relation to channel gating (14).

CDI Mechanism

According to a recent interaction model, apo-CaM is constitutively tethered (pre-bound pool) to a pre-IQ motif present on the C-terminus of the α_{1C} channel subunit. Such "pre-association" involves the N-lobe and occurs at resting Ca^{2+} concentrations. Signaling activation by Ca^{2+} elevation (i.e., CDI induction) requires Ca^{2+} binding to the C-lobe, whose affinity for the target IQ motif is thus increased; C-lobe interaction with the IQ motif stabilizes the channel conformational state associated with CDI (8) (**Figure 2**). CDI has been further modeled as transitions between different states: apo-CaM release from the pre-association site, formation of the Ca^{2+}-CaM complex, its subsequent binding to the effector site (14). The novel and most relevant feature of this model is that, using the difference in the kinetics of Ca^{2+} binding and unbinding between the C- and N-lobes (faster for the N-lobe), identifies their respective role in sensing Ca^{2+} at the channel mouth (local sensing, largely insensitive to intracellular Ca^{2+} buffering) vs. global cell Ca^{2+} (sensitive to even weak Ca^{2+} buffering). Unlike in neuronal channel isoforms, $Ca_v1.2$ channels retain robust CDI even in the presence of strong Ca^{2+} buffering; such form of CDI is entirely triggered by Ca^{2+} association with the C-lobe (39). Numerical modeling provides the (counterintuitive) conclusion that, if associated with slow CaM-channel interaction kinetics, fast Ca^{2+} binding/unbinding (typical of the N-lobe) may best support selective sensing of a smaller but sustained Ca^{2+} signal (14). The latter is typical of CDI in non-cardiac channel isoforms (39).

CDF Mechanism

As discussed above, earlier studies indicated that CDF requires an intact anchoring region on the channel C-terminus, thus suggesting that pre-bound CaM is involved (37). Nonetheless, there is now general agreement that, unlike CDI, CDF is operated by Ca^{2+}-CaM dependent activation of calmodulin-kinase II (CaMKII), which then phosphorylates the $Ca_v1.2$ channel at two serine residues close to the EF-hand motif (40) (**Figure 2**); mutation of these two serine residues abolished CDF but did significantly affect CDI (40), hence confirming independent mechanisms for these processes. Notably, CaMKII phosphorylation of nearby serine residues also induces Mode2 gating of the channel (41). Thus, at variance with CDI, CDF is the consequence of protein phosphorylation, dependent on CaM, but not directly operated by it.

Voltage-Gated K^+ Channels ($K_v7.1$)

$K_v7.1$ is a K^+-selective channel which, in association with KCNE subunits, carries the slow component of the delayed-rectifier current (I_{Ks}). I_{Ks} gating is positively regulated by cytosolic Ca^{2+} through a CaM-dependent process (42), with an effect similar to that of the membrane constituent phosphatidylinositol-4,5-bisphosphate (PIP2). Indeed, PIP2 and the N-lobe of CaM competitively interact at the same site on the $K_v7.1$ protein (the helix B on the proximal C terminus). Interpretation of the

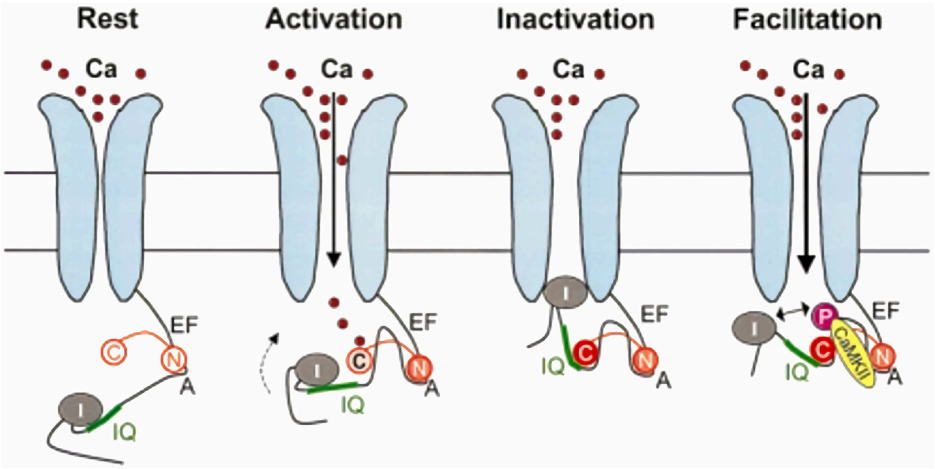

FIGURE 2 | Model for CaM-dependent modulation of $Ca_v1.2$ channels (I_{CaL}). *CDI mechanism*: in the channel closed state (Rest), the N-lobe of apo-CaM (N) is constitutively bound to a pre-IQ region (A) in the channel C-terminus. When the channel opens (Activation), the CaM C-lobe (C) binds to the entering Ca^{2+}, which increases its affinity for the channel IQ-domain; this moves the channel inactivation particle (I) in the permeation path (Inactivation). *CDF mechanism*: holo-CaM binding to CaMKII promotes channel phosphorylation, which results in repulsion of the inactivation particle from the permeation pore (Facilitation). Modified from Maier and Bers (8).

effect of a helix B $K_v7.1$ mutant (K526E) and of interference of Ca^{2+}-CaM with $K_v7.1$ pull-down by PIP2 has led to the following model: at diastolic Ca^{2+} levels, CaM is bound to a non-activating $K_v7.1$ site (helix A) by its apo-C-lobe; the N-lobe is displaced from the helix B site. As cytosolic Ca^{2+} increases, calcification of the C-lobe causes its dissociation from helix A and the N-lobe then interacts with its site on the helix B; this results in stabilization of the channel open state (43). According to an alternative model, at resting Ca^{2+} levels the C- and N-lobes are permanently bound to the channel (at helices A and B, respectively) and limit its open probability; when Ca^{2+} increases, N-lobe binding is reinforced and C-lobe is released thereby relieving the inhibitory effect on channel gating (44).

Both models imply that CaM binding to $K_v7.1$ and positive regulation of I_{Ks} are separate processes. Binding occurs in the apo-CaM form (pre-bound pool), I_{Ks} enhancement requires Ca^{2+} elevation. Furthermore, both CaM lobes are involved and a preserved C-lobe Ca^{2+} affinity is essential for the signaling function.

PIP2 is a membrane phospholipid degraded by phospholipase C (PLC) to produce inositol 3-phosphate (IP3) in response to activation of a number of membrane receptors associated with G_q proteins. Receptor activation may result in PIP2 depletion, which would reduce I_{Ks}; however, IP3-induced Ca^{2+} release from the sarcoplasmic reticulum may compensate PIP2 reduction by activating positive I_{Ks} regulation by Ca^{2+}-CaM. This may represent the main physiological role of I_{Ks} modulation by Ca^{2+}-CaM, which would therefore be of particular relevance during activation of the PLC-IP3 signaling pathway.

CaM integrity may also be necessary for $K_v7.1$ channel trafficking; indeed, mutations disrupting N- and C-lobe integrity reduce channel membrane expression (44).

It has been reported that holo-CaM complexing with KCNE4 (channel β-subunit) inhibits I_{Ks} (45). While this would provide antagonism to direct holo-CaM modulation of the channel α-subunit, the physiological role of CaM-KCNE4 interaction remains unclear.

CaM-mediated I_{Ks} regulation also occurs indirectly by CaMKII-mediated phosphorylation of the channel at serine 484; contrary to direct CaM modulation, phosphorylative modulation is inhibitory and may account for I_{Ks} downregulation upon sustained β-adrenergic receptor activation (46).

Voltage-Gated Na^+ Channels ($Na_v1.5$)

Among all voltage-gated channels involved in arrhythmogenesis, $Na_v1.5$ channels also interact with CaM. CaM binds to an IQ motif on the C-terminus of this channel in a Ca^{2+}-independent manner. Binding reduces CaM's affinity for Ca^{2+} and does not induce the conformational changes that have been observed for $Ca_v1.2$ channels; therefore, similarities in the binding site may not necessarily translate into similarities of channel modulation. Nonetheless, disruption of the CaM binding site (by mutation of the $Na_v1.5$ IQ motif) leads to the enhancement of persistent Na^+ current, thus suggesting a role of CaM in stabilizing the inactivated state (47), possibly by fostering the interaction between the channel C-terminus and the II-IV linker. According to another model, CaM binding to $Na_v1.5$ channels would obstruct their direct modulation by Ca^{2+}; holo-CaM would lose its affinity for the channel, thus unveiling the direct modulatory site (48). In this case, failure of CaM interaction with the channel (as in the case of holo-CaM) causes a "leftward" (negative) shift of the steady-state inactivation curve (48); this would presumably reduce channel availability at diastolic potential as well as the "window" component of I_{Na}.

Overall, while CaM interaction with Na_v and Ca_v channels are somewhat similar, the consequences of CaM-dependent modulation on Na_v function are less defined and, possibly, quantitatively less important.

RYRS MODULATION BY Ca^{2+} AND Ca^{2+}-CAM

RyRs are homotetramers of ∼2,200 kDa (each subunit is >550 kDa), containing ∼5,035 amino acid residues in total, sharing the general structure of the six-transmembrane ion channel superfamily (49). Since RyRs span the SR membrane, they have domains facing both the cytosol and the SR lumen. Of the three isoforms present in nature, RyR2 is the predominant one in cardiac myocytes, where it is organized in large clusters on the SR membrane (50). In T-tubules, RyR2 clusters are separated from sarcolemmal $Ca_v1.2$ channels by a 10–15 nm gap; thus, small Ca^{2+} influx through I_{CaL} exposes them to very high Ca^{2+} concentrations (50, 51). This structural arrangement is generally referred to as "couplon" (52). The RyR/Ca_v ratio in couplons is up to 15-fold higher in cardiac than in skeletal muscle and differs between species (53).

RyRs are strongly regulated by Ca^{2+} in CaM-independent ways. At the same time, they are regulated by CaM in both Ca^{2+}-dependent and -independent ways. This makes investigation of CaM's role in RyRs' regulation very complex.

CaM-Independent RyR Regulation by Ca^{2+}

RyR gating is highly sensitive to Ca^{2+} on both sides of the SR membrane in a CaM-independent way. Although not the focus of the present review, a brief discussion of such "direct" regulation by Ca^{2+} is required to understand the potential difficulty in isolating the CaM-dependent one.

Each of the N- and C-terminal domains of RyR2 contains two EF-hand Ca^{2+} binding motifs (54), similar to those of CaM. These motifs are both on the cytosolic domain of RyR2; they show high (C-terminal) and low (N-terminal) affinity for Ca^{2+} and induce channel opening and inactivation, respectively (55, 56). Since RyR2 inactivation occurs at Ca^{2+} concentrations exceeding the physiological range, Ca^{2+}-dependent activation is the dominant phenomenon and the basis for the Ca^{2+}-induced-Ca^{2+}release (CICR) mechanism (7).

SR luminal Ca^{2+} modulates RyR2 open probability by two CaM-independent mechanisms. The indirect one involves stabilization of the closed state by a macromolecular complex, involving calsequestrin (CASQ) and is disrupted by increases in luminal Ca^{2+} (57). Sensitivity to luminal Ca^{2+} is preserved after CASQ knock out. This stands for the presence of a Ca^{2+}-sensing mechanism on the RyR protein itself, located in a luminal domain also involved in control of Ca^{2+} permeation (58). Direct lumenal Ca^{2+} sensing may be important for channel activation under conditions of SR overload (59).

CaM-Dependent RyR Regulation

CaM-dependent modulation of RyRs differs between channel isoforms, which have only ∼70% of gene homology and contain three divergent regions (60). In general, conductance of all the three RyR isoforms is reduced by CaM when cytosolic Ca^{2+} is above 1 μM. At lower Ca^{2+} concentrations, favoring apo-CaM and more relevant to diastole, CaM increases the open probability of RyR1 and RyR3 (61, 62), but it stabilizes the closed conformation of RyR2 (54).

RyR2 channels have high affinity (nanomolar K_d) for both apo-CaM and holo-CaM, thus resulting in a pre-bound CaM pool (54, 63). Apo-CaM may actually be a stronger inhibitor of RyR2 opening than holo-CaM, as indicated by relief of inhibition at Ca^{2+} concentrations in the μM range (64). Therefore, CaM modulation of RyR2 gating may be largely Ca^{2+}-independent. Increasing Ca^{2+} up to 100 μM has been reported to increase the number of CaM molecules bound to RyR2 (from 1 to 7.5 per RyR2 tetramer) (54); however, it is difficult to relate responses to such an abnormally high Ca^{2+} concentration to physiological function.

Given that CaM binding domains are highly homologous between RyR1 and RyR2 (65), what may explain the Ca^{2+}-dependent discrepancy of CaM effects on RyR opening between RyR1 and RyR2 channels?

Mutations in both the N-terminal and central RyR2 regions similarly destabilize the channel closed state; this suggests that the latter may require interaction between these two regions ("zipping" model of RyR gating). This view is confirmed by the effect of peptides (e.g., DPc-10) interfering with such interaction (66). On the other hand, CaM binds to a domain other than those involved in zipping. According to the "inter-domain hypothesis," CaM binding to RyR2 may induce a protein conformation change that allosterically stabilizes the zipping interaction (and the closed state) (**Figure 3**). Vice versa, agents interfering with the zipping interaction may reduce CaM binding affinity (66).

A different gating model has been proposed for RyR1 channels, in which the channel CaM binding domain is followed, within about 450 residues, by a CaM-like sequence. FRET data indicate that, at high Ca^{2+}, the two channel domains interact with each other in the folded protein; such Ca^{2+}-dependent interaction is required for channel opening. Binding of holo-CaM to the channel may disrupt the activating interdomain interaction, thus explaining CaM-induced RyR1 inhibition at high Ca^{2+} concentrations (68). However, this model does not explain why low Ca^{2+} concentrations (favoring apo-CaM) may produce CaM-dependent RyR1 activation, which remains an open question.

Cryo-EM studies in RyR1 indicate that increasing Ca^{2+} shifts CaM binding to the channel by about 3 nM, corresponding in the 3D structure to two different protein domains. In view of the fact that CaM activates RyR1 at low Ca^{2+} and inhibits it at high Ca^{2+}, the two domains may be seen as activator and inhibitory sites, respectively. Importantly, these studies indicate that the Ca^{2+}-dependent shift in CaM binding site is a consequence of a rearrangement of the binding surface of CaM, rather than of RyR1 conformation (69) (which is also Ca^{2+}-dependent). The same technique shows that in RyR2 CaM binds to the "inhibitory site" (as identified in RyR1) already at low Ca^{2+}; this might explain why RyR2 is inhibited by CaM in a Ca^{2+}-independent way (69). Apparently at odd with these observations, FRET experiments (in the same study), measuring the position of CaM

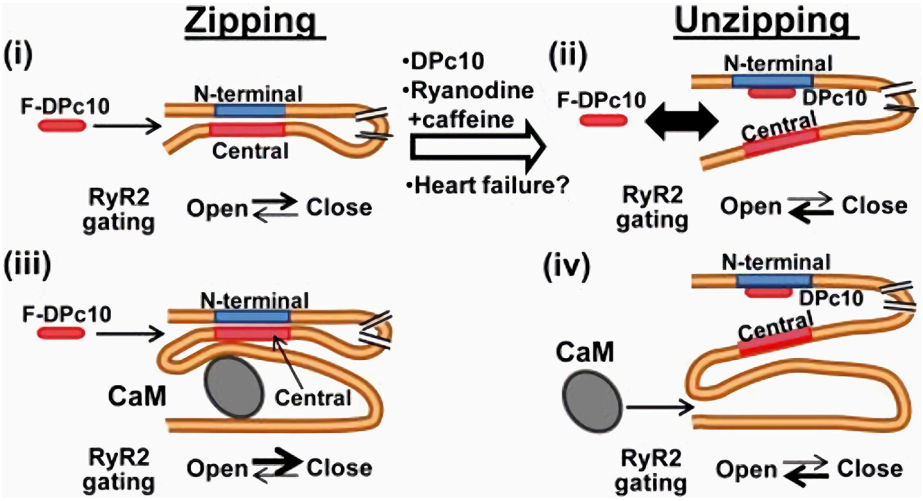

FIGURE 3 | CaM-dependent modulation of RyR2 channels. RyR2 closed state is stabilized by the interaction (zipping) between "terminal" and "central" regions of the N-terminal (cytosolic) tail of the protein. If such interaction is removed (unzipping), the channel closed state is destabilized. Apo-CaM binds to a domain distal to the "zipping" one, but the resulting conformation allosterically facilitates the zipping interaction, thus stabilizing RyR2 closed state. CaM and F-DPc10 (a peptide obstructing the zipping interaction) allosterically "compete" for binding to RyR2. Similarly, the unzipped state, promoted by drugs and reactive oxygen species which facilitate RyR2 opening, reduces RyR2 affinity for CaM (67). F-DPc10 is a peptide fragment designed to prevent the interaction between the central and N-terminal protein domains (a tool in testing the unzipping model). From Oda et al. (66).

relative to that of FKBP in RyR1, did not detect Ca^{2+}-dependent shifts in CaM position of a magnitude compatible with the results of cryo-EM data (69). However, Ca^{2+}-induced structural changes in both probe-carrying proteins are possible and might minimize FRET distances even in the presence of real shifts in binding position.

In conclusion, even if the detailed mechanism remains to be resolved, it is now accepted that direct regulation of RyR gating by CaM is Ca^{2+}-dependent (stimulatory to inhibitory) in RyR1 and Ca^{2+}-independent (always inhibitory) in RyR2. Formation of the Ca^{2+}-CaM complex does affect RyR2 gating significantly, but this occurs through an indirect mechanism, involving CaMKII activation.

CaMKII is a cytosolic serine-threonine kinase activated by Ca^{2+} with a K_d of 20-100 nM, which dramatically decreases (to 60 pM) after enzyme autophosphorylation (8). The Ca^{2+}-CaM complex activates kinase activity by binding to an enzyme regulatory region, located in the central protein domain. Enzyme activation occurs by displacement of an auto-inhibitory segment that occludes access of the substrate to the N-terminal catalytic domain. As for other targets, both N- and C-terminal CaM lobes are involved in activation of kinase activity (25), which is in this case strictly Ca^{2+}-dependent.

CaMKII mediates a number of Ca^{2+}-activated phosphorylations, including that of RyR2 at Ser2814 on the cytosolic surface of the channel (exclusively for CaMKII) and, additional serine residues (49, 70). Most of the evidence converges to show that CaMKII-dependent phosphorylation facilitates opening of RyR2 channels, thus increasing sensitivity of SR Ca^{2+}-release to cytosolic Ca^{2+}. This may be particularly relevant under pathological conditions (70).

CaM binding to RyRs also depends on factors other than Ca^{2+}, such as pH, Mg^{2+} oxidation state (54, 62). In particular, oxidative modifications compromise the normal activity of RyR2 by influencing their luminal Ca^{2+} regulation in a manner similar to that observed in heart failure (71).

Stabilization of RyR2 closed state by CaM is crucial in minimizing spontaneous (non-triggered) Ca^{2+} release from the SR in the form of either "Ca-leak" (random release from individual RyRs) or "Ca^{2+} spark" (synchronous release from a RyR2 cluster), a function pivotal to both contractile and electrical function of cardiomyocytes (63).

PHENOTYPES IN CAM MUTATIONS AND UNDERLYING MECHANISMS

Mutations in one of the three CALM genes, even in the heterozygous form, have been described in patients with a severe cardiac phenotype, characterized by a high propensity to ventricular arrhythmias, syncopal episodes and sudden death at a young age (72).

Despite sharing strong electrical instability, two distinct phenotypes can be identified in carriers of CaM mutations: the long QT syndrome (LQTS) (9, 73), characterized by prolongation of repolarization, and catecholamine-induced ventricular tachycardia (CPVT), characterized instead by exercise-induced ventricular arrhythmias (74). In general, each specific CALM mutation is associated with one of the two phenotypes; nonetheless, mutations with mixed phenotypes have also been described (72). Such confounding complexity contrasts with an apparently sharp separation of the molecular mechanisms underlying the LQTS and CPVT patterns. A third,

less well-defined arrhythmia phenotype, idiopathic ventricular fibrillation (IVF), has also been associated with a CALM mutation (75) and will be addressed here in paragraph Mixed phenotype.

A point of interest in the interpretation of CALM mutations is their extremely high penetrance: 1 mutant allele in 6 encoding for the same amino acid sequence (as in heterozygous mutations) is sufficient to result in marked functional derangements.

Also in view of the multiplicity of functions exerted by CaM in many cell types, all this suggests that, for one reason or another, mutant CaMs must interact with their target with high specificity. In the following paragraphs we will describe the cellular functional derangements associated with the LQTS and CPVT phenotypes and address, as much as current knowledge allows, the mechanisms underlying target specificity of CaM mutations.

LQTS Phenotype

Prolongation of action potential duration (APD), reflected as QT interval prolongation on the ECG, can result from loss of function of outward currents, or gain of function of inward ones; therefore, CaM abnormalities affecting modulation of $K_v7.1$ (I_{Ks}) and $Ca_v1.2$ (I_{CaL}) might theoretically be involved in prolongation of repolarization. Enhancement of the "window" (I_{NaW}) or "late" (I_{NaL}) components of the Na^+ current I_{Na} ($Na_v1.5$ channel) might represent a further potential mechanism.

Nevertheless, gain of $Ca_v1.2$ function (I_{CaL} enhancement) has emerged as the dominant mechanism in CALM gene mutations associated with delayed repolarization.

In 2013, Crotti et al. reported three *de novo* heterozygous missense CALM gene mutations in LQT-infants with recurrent cardiac arrest (73). In particular, the *CALM1*-p.D130G and *CALM2*-p.D96V mutations affect highly conserved aspartic acid residues (C-lobe EF IV and EF III, respectively) involved in Ca^{2+} binding. The *CALM1*-p.F142L (next to C-lobe EF IV), albeit outside the EF-hand, is expected to alter the energetics of the conformational change associated to Ca^{2+} binding (22). The p.D130G mutation, associated with the LQTS phenotype, has also been identified in the *CALM3* gene (76), thus reinforcing the concept that mutation effect may be independent of the gene affected. *In vitro* Ca^{2+} binding studies revealed that all these three mutations are characterized by a 5- to 50-fold reduction in Ca^{2+} binding affinity of the C-lobe, without affecting N-lobe affinity (73). Overexpression of these mutations in guinea-pig myocytes or an engineered cell line showed loss of I_{CaL} CDI, leading to action potential prolongation with enhanced intercellular variability. The amplitude of Ca^{2+} transients and its dispersion were also increased, likely secondary to increased Ca^{2+} influx; notably, spontaneous Ca^{2+} release events were not reported (77). Indeed, consistent with the LQTS phenotype, RyR2 function was unaffected. Binding of CaM mutants to $Ca_v1.2$ was tested by FRET and found to be enhanced for p.F142L and unaffected by the other mutations. On the other hand, titration of WT vs. mutant expression levels showed that a ratio (WT/mutant) of 7 (compatible with heterozygosity) was enough to impair CDI (77). Therefore, selectivity of mutant CaMs in altering $Ca_v1.2$ channel function can be explained by the fact that modulation of this target requires a pre-bound apo-CaM pool (containing mixtures of WT and mutant CaMs) and subsequent Ca^{2+} binding to this pool (impaired in mutants by loss of Ca^{2+} affinity). This interpretation would explain sparing of RyR2, whose modulation may not require Ca^{2+} binding, and of CaMKII, which binds CaM directly in its holo-form (not represented if Ca^{2+} affinity is reduced). Selective CDI impairment by additional mutations reducing C-lobe Ca^{2+} affinity (*CALM2*-p.D132H and *CALM1*-p.D132V) has also been reported in transfection studies on human induced pluripotent stem cell-derived cardiomyocytes (78).

Other heterozygous LQTS mutations, *CALM2*-p.D130V and *CALM1*-p.E141G have been recently identified by Boczek et al. (79). As in p.D130G, the former involves replacement of aspartic acid by a neutral residue; therefore, loss of C-lobe Ca^{2+} affinity is to be expected. *CALM1*-p.E141G has a phenotype closely resembling that of *CALM1*-p.F142L, indicating that residues 141 and 142 are both crucial for C-lobe Ca^{2+} binding. Notably, when transfected in isolation, *CALM1*-p.E141G also enhanced I_{NaL}, but the effect disappeared when mutant and WT constructs were co-expressed. This is consistent with a role of CaM stabilizing $Na_v1.5$ inactivation (see above) and implies lack of functional dominance of the mutation for this target. However, at least according to a current model of CaM-$Na_v1.5$ interaction (48), reduced affinity of mutant CaM for Ca^{2+} would not explain I_{NaL} enhancement.

CaM mutations resulting in downregulation of K^+ currents have not been reported, even if I_{Ks} function has been tested in some cases (*CALM1*-p.F142L) (80). Nonetheless, the $K_v7.1$ α-subunit mutation p.K526E, accounting for a case of LQT1, impairs interaction of the channel's helix B with CaM's N-lobe. This leads to I_{Ks} downregulation and delayed repolarization (43). Considering that the mode of CaM–$K_v7.1$ interaction involves a pre-bound pool and Ca^{2+}-dependent C-lobe signaling (as for $Ca_v1.2$), it is surprising that CaM mutations with loss of C-lobe Ca^{2+} affinity may not affect I_{Ks}. One tentative explanation is compensation of the loss of CaM-dependent regulation by PIP2 signaling; it would be therefore interesting to evaluate the effect of known CaM mutations on I_{Ks} under conditions of PIP2 depletion.

CPVT Phenotype

Catecholaminergic polymorphic ventricular tachycardias are malignant arrhythmias with an ECG pattern suggesting multifocal origin (unlike TdP), typically induced by exercise, or other conditions associated with enhanced adrenergic stimulation (81). The prototypical form of this arrhythmia has been associated with mutations of RyR2 channels, or of the proteins associated with them in a macromolecular complex (junction, triadin, calsequestrin, sorcin etc.). The electrical disturbance at the basis of CPVT originates from "Ca^{2+} waves," i.e., macroscopic surges of cytosolic Ca^{2+} resulting from spontaneous RyR opening at a point site, followed by auto-regenerative propagation (by Ca^{2+}-induced Ca^{2+} release) of the ionic perturbation to the whole cell (82). The mechanism connecting the Ca^{2+} wave to membrane potential is Ca^{2+}-induced activation of the electrogenic Na^+/Ca^{2+} exchanger (NCX), which results in a depolarizing current, also referred

to as "transient inward current" (I_{TI}). While "Ca^{2+} overload" facilitates Ca^{2+} waves (by increasing RyRs open probability), it is neither necessary nor sufficient to induce them; indeed some degree of intrinsic RyR2 instability may be involved even in the prototypical case of digitalis toxicity (83).

CaM mutations associated with the CPVT phenotype are generally characterized by a relatively small impairment of C-lobe Ca^{2+} binding (e.g., *CALM1*-p.N98S, *CALM1*-p.N54I, and *CALM3*-p.A103V) (84, 85), which (as shown for *CALM3*-p.A103V) (85) corresponds to a minor effect on I_{CaL} CDI. Besides this, the relationship between mutation features and the CPVT phenotype is somewhat elusive. Nyegaard and colleagues tested *CALM1*-p.N54I and -p.N98S binding to a small RyR2 segment, found a decrease in *CALM1*-p.N98S affinity only at low Ca^{2+} levels and explained mutation phenotype with loss of CaM-RyR2 complexing. These findings were later contradicted by studies using the entire RyR2 protein (86), that detected an increase in RyR2 affinity for both these mutations (also accounting for dominance of effects). Nonetheless, increased affinity for RyR2 may not be a prerequisite for channel destabilization; indeed the CPVT mutation *CALM3*-p.A103V, which strongly increased Ca^{2+} release events, displayed normal RyR2 affinity (85). Notably, mutations sites N54 and N98, albeit affecting the N- and C-lobes, respectively, are not contained within any known protein-protein interaction sites (84).

In conclusion, the features of CPVT mutations may explain why they are not generally associated with an LQTS phenotype (however see below), but a general mechanism by which they induce RyR2 instability cannot be clearly envisioned. Since CaM interaction with RyR2 is essentially Ca^{2+}-independent, the mechanism must conceivably reside in a change of 3D protein conformations involved in CaM-RyR2 complexing; nonetheless, the nature of this change remains to be clarified.

Mixed Phenotype

Notwithstanding the apparently sharp demarcation of CaM abnormalities affecting $Ca_v1.2$ and RyR2 channels, several mutations have been associated with both LQTS and CPVT phenotypes. CaM mutations were found in five subjects with QT prolongation; nonetheless, two of them (*CALM2*-p.D132E and –p.Q136P) were associated with arrhythmia features strongly suggestive of SR instability and thus were assigned to the CPVT phenotype (72). These mutations affect EF III and EF IV of the C-loop and displayed reduced Ca^{2+} affinity; thus, even if I_{CaL} CDI was not directly tested in this study, it may be tentatively considered responsible for the observed QT prolongation. Intriguingly, two different reports assign LQTS and CPVT phenotypes to the same mutation (p.N98S) occurring in genes *CALM2* (72) and *CALM* (84), respectively; since the CaM amino acid sequence encoded by the 2 genes is identical, other factors should account for the discrepancy.

The reason for SR instability in all these cases is as elusive as the properties of mutations that favor RyR2 dysfunction (see above). Nonetheless, it should be considered that impairment of I_{CaL} CDI and the resulting APD prolongation are obviously stress conditions for intracellular Ca^{2+} homeostasis, requiring robust compensatory mechanisms. Thus, albeit not observed in hiPSC-CMs from an LQTS case (80), SR instability secondary to defective CDI might occur in subjects (or conditions) in which such compensation is less efficient. Thus, assignment to an LQTS or CPVT clinical phenotype may not be always accurate in defining the mutation-induced abnormality accounting for arrhythmogenesis.

Marsman et al. have described the *CALM1*-p.F90L mutation in a patient with IVF, i.e., VF episodes without the features of either LQTS or CPVT (mild QT prolongation only during exercise recovery) (75). The mutation, which resides on the linker between EF III and EF IV, impairs C-lobe Ca^{2+} affinity conformational stability and CaM-RyR2 interaction (87). In heterologous expression experiments, p.F90L also affects small-conductance Ca^{2+}-activated K^+ channels (SK channels) (88); nonetheless, the role of these channels in ventricular electrophysiology is unclear.

EVALUATING CAM MUTATIONS IN PATIENT-DERIVED CARDIOMYOCYTES

Induced pluripotent stem cell-derived cardiomyocytes from mutation carriers (hiPSC-CMs) provide the means to test mutation effects in the context of each patient's genetic background. Recent studies have exploited this cellular model to test the effect of CaM mutations. Yamamoto et al. reported a typical LQTS phenotype for the heterozygous *CALM2*-p.N98S mutation and obtained reversal of the phenotype by knocking out the mutant allele by gene editing (89), thus supporting a causal relationship between mutation and phenotype. Notably, *CALM2*-p.N98S affinity for Ca^{2+} is only mildly reduced, and a CPVT phenotype has also been reported for this mutation (84).

We recently investigated hiPSC-CMs from a patient with LQTS phenotype and heterozygous carrier of the *CALM1*-p.F142L mutation (80) (**Figure 4**). CDI of I_{CaL} was severely impaired, thus accounting for APD prolongation (which was reversed by I_{CaL} blockade) and its failure to shorten adequately at high pacing rates. As expected from the increase in Ca^{2+} influx, the amplitude of V-triggered Ca^{2+} transients was significantly increased; nonetheless, SR Ca^{2+} content was normal and no spontaneous Ca^{2+} release events were observed, thus suggesting preserved homeostatsis of intracellular Ca^{2+}. This argues against SR instability as the arrhythmogenic mechanism in this case and suggests a primary role of prolonged and "stiff" (non-rate-adaptive) repolarization instead (80). Other currents under CaM modulation were also evaluated in this study: I_{Ks} was found to be unaffected and a persistent component of I_{Na} (likely contributed by I_{NaW}) was significantly reduced (80). While this confirms loss of I_{CaL} CDI as the sole mechanism of repolarization abnormality, I_{NaW} reduction was unexpected; indeed, loss of CaM affinity for Ca^{2+} should if anything, increase I_{NaW} (48). Notably, CaMKII activity was preserved and even slightly enhanced, probably secondary to the increase in Ca^{2+} transients amplitude. This supports the view that negative dominance of the mutation only applies to targets, as $Ca_v1.2$, binding CaM in its apo form.

Altogether, these findings clearly confirm loss of I_{CaL} CDI as the mechanism underlying the LQTS phenotype in CaM

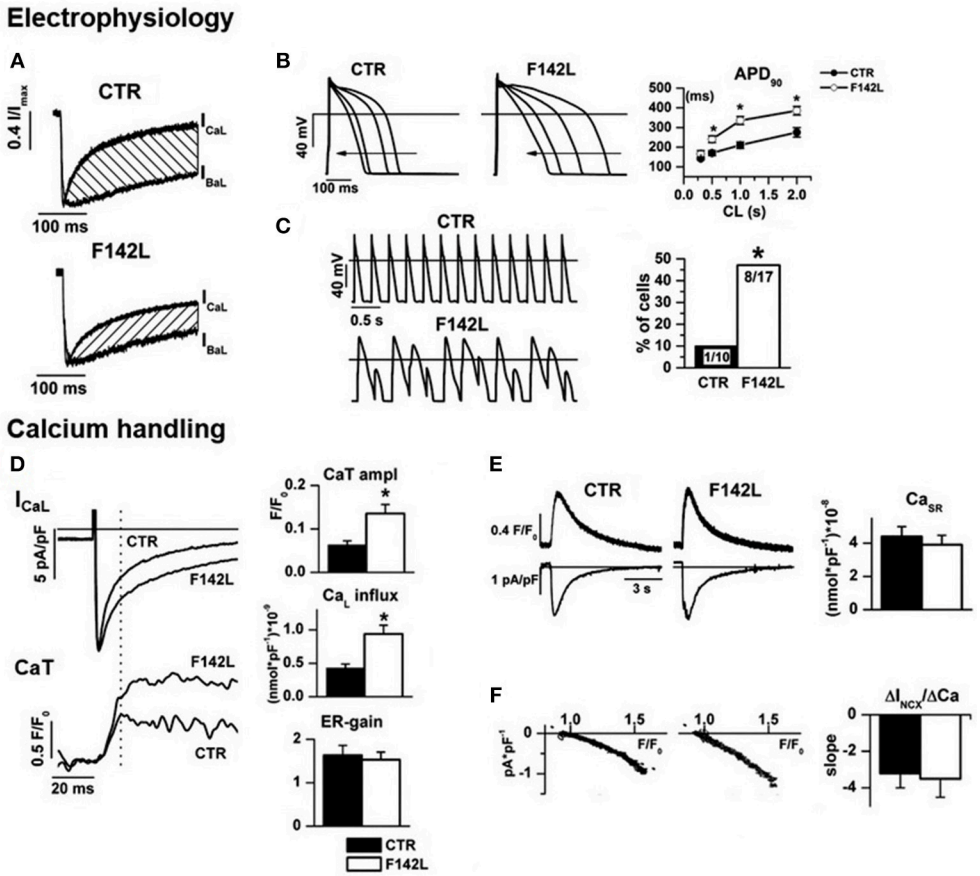

FIGURE 4 | Arrhythmogenic mechanism of CALM1 F142L from experiments in patient-derived hiPSC-CMs. *Electrophysiology:* **(A)** I_{CaL} CDI (hatched area) was reduced; **(B)** CDI impairment led to APD prolongation and inadequate APD shortening at high pacing rate; **(C)** APD abnormalities led to loss of 1:1 response to fast pacing in a large % of F142L cells. *Calcium handling*: **(D)** Impaired I_{CaL} CDI led to matching increments of Ca^{2+} influx and of the amplitude of Ca^{2+} transients (CaT); excitation/release gain (ER-gain) was unchanged, thus suggesting normal RyRs function. **(E)** In spite of enhanced Ca^{2+} influx, SR Ca^{2+} content was unchanged, thus implying compensation by homeostatic mechanisms. **(F)** The slope of the relationship between Na^{+}/Ca^{2+} exchanger current (I_{NCX}) and Ca^{2+} concentration was unchanged, to indicate that homeostatic compensation did not involve changes in the expression of the exchanger. Asterisks denote significance of changes. Modified from ref. Rocchetti et al. (80).

mutations with reduced Ca^{2+} affinity. To our best knowledge, no hiPSC-CMs studies are thus far available for mutations with a clear-cut CPVT phenotype. It should be considered that, due to immaturity of the structures involved in intracellular Ca^{2+} handling (e.g., lack of T-tubules) (90), hiPSC-CMs may be less suitable in evaluating CaM mutations leading to SR instability (CPVT phenotype).

CONCLUSIONS AND THERAPEUTIC IMPLICATIONS

CaM functions as a Ca^{2+} sensor to maintain physiological Ca^{2+} levels in cells. In addition to this homeostatic role, CaM signal targeting is required to transduce fundamental cell processes, for which Ca^{2+}-CaM complexing is not necessarily involved, but may still have a modulatory effect. This is possible because of the presence of CaM-binding sequences suitable to allow CaM to bind multiple targets even in its "apo" form; this generates a quantitatively prevailing "pre-bound" CaM pool. CaM binding to targets occurs with very high specificity, which is required to explain restriction of CaM mutations phenotype to the myocardium and, within it, to specific subcellular targets.

Whereas mutation-induced loss of Ca^{2+} sensing function is crucial in impairing CDI of sarcolemmal Ca^{2+} channels (carrying I_{CaL}), it is not required for mutations associated with RyR2 instability. For the latter, changes in CaM affinity for RyR2 channels are apparently more important; however, the direction and even the need for such changes are still unclear. Possibly, mutations induce complex (3D) modifications in the protein-protein binding interface, of which changes in CaM affinity for the target are just a gross readout. This is a field in which new information is strongly required.

Whereas, "pure" LQTS and CPVT phenotypes suggest abnormal modulation of I_{CaL} and RyR2, respectively, we hypothesize that coexistence of QT prolongation and SR instability (mixed phenotypes) might be accounted for by impaired I_{CaL} CDI, possibly with the complement of (very

common) conditions weakening homeostatic control of intracellular Ca^{2+}.

Based on the information reviewed above, mechanism-guided therapeutic approaches to calmodulinopathies should ideally address the interaction of mutant CaM with its targets. Particularly in the case of LQTS-type mutations, this approach is justified by the role of the high target affinity of mutant CaMs in causing negative dominance of the mutation. Tools for this purpose are not available yet, but possibilities exist and are currently explored.

Therapy of CaM mutations with more classical approaches may depend on the phenotype. I_{CaL} blockade seems a logical approach in the case of I_{CaL} gain of function, resulting from loss of CDI (LQTS phenotype); indeed, verapamil did shorten the QT interval in hiPSC-CMs from *CALM1*-p.F142L carriers (80). Nonetheless, selective inhibition of the sustained I_{CaL} component would be desirable and should be pursued by developing I_{CaL} blockers with such a property; as suggested by availability of selective blockade of I_{Na} sustained component (91), this should be seen as an achievable goal. Pharmacological treatment of CPVT-type CaM mutations may require RyR2 stabilization, or at least, blunting membrane electrical response to spontaneous Ca^{2+} release events. This is a long-pursued goal for which no ideal tool has been thus far identified; while agents as flecainide or carvedilol may provide some protection [for review see Zaza and Rocchetti (82)], the search of clinically usable specific RyR2 blockers is currently ongoing.

Because calmodulinopathies have been recently described, information on the clinical efficacy of therapies is not available yet.

FUTURE CHALLENGES

Calmodulinopathies have undoubtedly attracted high quality, multidisciplinary research; nonetheless, and rather unsurprisingly, many questions have yet to be addressed. The present review highlights a few that, in our view, might deserve particular attention.

The role and mechanism of $Na_V1.5$ dysregulation in CaM mutation-associated phenotypes is elusive. Notably, I_{NaL} enhancement, a target for which therapeutic interventions are available, might have a role in both QT prolongation (LQTS phenotype) and SR instability (CPVT phenotype) (91).

The interplay between CaM- and PIP2-dependent modulation of I_{Ks} suggests that factors affecting membrane PIP2 levels (e.g., phospholipase-C signaling) potentially influence CaM mutation penetrance. If this were the case, such factors might represent easily accessible therapeutic targets.

The ultimate mechanism of arrhythmia facilitation by loss of I_{CaL} CDI, which seems to diverge from what would be expected, has thus far been only superficially addressed.

Finally, the molecular basis of RyR2 dysfunction in the context of CaM mutations remains largely unresolved, thus preventing identification of mechanism-specific targets.

AUTHOR CONTRIBUTIONS

BB wrote a general manuscript draft. CR focused on the section about CaM modulation of voltage-gated channels. M-CK, LS, and AG provided text and discussion for integration with clinical and genetic aspects of calmodulinopathies. LC and AZ supervised the process and edited the manuscript to its final version.

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REFERENCES

1. Clapham DE. Calcium signaling. *Cell* (2007) 131:1047–58. doi: 10.1016/j.cell.2007.11.028
2. Ikura M. Calcium binding and conformational response in EF-hand proteins. *Trends Biochem Sci.* (1996) 21:14–7. doi: 10.1016/S0968-0004(06)80021-6
3. Luby-Phelps K, Hori M, Phelps JM, Won D. Ca^{2+}-regulated dynamic compartmentalization of calmodulin in living smooth muscle cells. *J Biol Chem.* (1995) 270:21532–8. doi: 10.1074/jbc.270.37.21532
4. Deisseroth K, Heist EK, Tsien RW. Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature* (1998) 392:198. doi: 10.1038/32448
5. Berchtold MW, Villalobo A. The many faces of calmodulin in cell proliferation, programmed cell death, autophagy, and cancer. *Biochim Biophys Acta Mol Cell Res.* (2014) 1843:398–435. doi: 10.1016/j.bbamcr.2013.10.021
6. Kotta M-C, Sala L, Ghidoni A, Badone B, Ronchi C, Parati G, Zaza A and Crotti L. Calmodulinopathy: A Novel, Life-Threatening Clinical Entity Affecting the Young. *Front Cardiovasc Med.* (2018). 5:175. doi: 10.3389/fcvm.2018.00175
7. Bers DM. Cardiac excitation–contraction coupling. *Nature* (2002) 415:198. doi: 10.1038/415198a
8. Maier LS, Bers DM. Calcium, calmodulin, and calcium-calmodulin kinase II: heartbeat to heartbeat and beyond. *J Mol Cell Cardiol.* (2002) 34:919–39. doi: 10.1006/jmcc.2002.2038
9. Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: from genetics to management. *Circ Arrhythm Electrophysiol.* (2012) 5:868–77. doi: 10.1161/CIRCEP.111.962019
10. Fischer R, Koller M, Flura M, Mathews S, Strehler-Page MA, Krebs J, et al. Multiple divergent mRNAs code for a single human calmodulin. *J Biol Chem.* (1988) 263:17055–62.
11. Crotti L, Kotta MC. The role of genetics in primary ventricular fibrillation, inherited channelopathies and cardiomyopathies. *Int J Cardiol.* (2017) 237:45–8. doi: 10.1016/j.ijcard.2017.03.119
12. Villarroel A, Taglialatela M, Bernardo-Seisdedos G, Alaimo A, Agirre J, Alberdi A, et al. The ever changing moods of calmodulin: how structural plasticity entails transductional adaptability. *J Mol Biol.* (2014) 426:2717–35. doi: 10.1016/j.jmb.2014.05.016
13. Malmendal A, Linse S, Evenäs J, Forsén S, Drakenberg T. Battle for the EF-hands: magnesium– calcium interference in calmodulin. *Biochemistry* (1999) 38:11844–50. doi: 10.1021/bi9909288
14. Tadross MR, Dick IE, Yue DT. Mechanism of local and global Ca^{2+} sensing by calmodulin in complex with a Ca^{2+} channel. *Cell* (2008) 133:1228–40. doi: 10.1016/j.cell.2008.05.025
15. Babu YS, Sack JS, Greenhough TJ, Bugg CE, Means AR, Cook WJ. Three-dimensional structure of calmodulin. *Nature* (1985) 315:37. doi: 10.1038/315037a0

16. Zhang M, Tanaka T, Ikura M. Calcium-induced conformational transition revealed by the solution structure of apo calmodulin. *Nat Struct Mol Biol.* (1995) 2:758. doi: 10.1038/nsb0995-758
17. Chou JJ, Li S, Klee CB, Bax A. Solution structure of Ca 2+-calmodulin reveals flexible hand-like properties of its domains. *Nat Struct Mol Biol.* (2001) 8:990. doi: 10.1038/nsb1101-990
18. Yamniuk AP, Vogel HJ. Calmodulin's flexibility allows for promiscuity in its interactions with target proteins and peptides. *Mol Biotechnol.* (2004) 27:33–57. doi: 10.1385/MB:27:1:33
19. Marshall CB, Nishikawa T, Osawa M, Stathopulos PB, Ikura M. Calmodulin and STIM proteins: two major calcium sensors in the cytoplasm and endoplasmic reticulum. *Biochem Biophys Res Commun.* (2015) 460:5–21. doi: 10.1016/j.bbrc.2015.01.106
20. Bähler M, Rhoads A. Calmodulin signaling via the IQ motif. *FEBS Lett.* (2002) 513:107–13. doi: 10.1016/S0014-5793(01)03239-2
21. Gellman SH. On the role of methionine residues in the sequence-independent recognition of nonpolar protein surfaces. *Biochemistry* (1991) 30:6633–6. doi: 10.1021/bi00241a001
22. Chin D, Means AR. Calmodulin: a prototypical calcium sensor. *Trends Cell Biol.* (2000) 10:322–8. doi: 10.1016/S0962-8924(00)01800-6
23. Stigler J, Rief M. Calcium-dependent folding of single calmodulin molecules. *Proc Natl Acad Sci USA.* (2012) 109:17814–9. doi: 10.1073/pnas.1201801109
24. O'Neil KT, DeGrado WF. How calmodulin binds its targets: sequence independent recognition of amphiphilic α-helices. *Trends Biochem Sci.* (1990) 15:59–64. doi: 10.1016/0968-0004(90)90177-D
25. Tidow H, Nissen P. Structural diversity of calmodulin binding to its target sites. *FEBS J.* (2013) 280:5551–65. doi: 10.1111/febs.12296
26. Valeyev NV, Bates DG, Heslop-Harrison P, Postlethwaite I, Kotov NV. Elucidating the mechanisms of cooperative calcium-calmodulin interactions: a structural systems biology approach. *BMC Syst Biol.* (2008) 2:48. doi: 10.1186/1752-0509-2-48
27. Beitner R. Calmodulin antagonists and cell energy metabolism in health and disease. *Mol Genet Metab.* (1998) 64:161–8. doi: 10.1006/mgme.1998.2691
28. Schumacher MA, Rivard AF, Bächinger HP, Adelman JP. Structure of the gating domain of a Ca2+-activated K+ channel complexed with Ca2+/calmodulin. *Nature* (2001) 410:1120. doi: 10.1038/35074145
29. Wagner S, Maier LS. Modulation of cardiac Na(+) and Ca(2+) currents by CaM and CaMKII. *J Cardiovasc Electrophysiol.* (2006) 17(Suppl. 1):S26–33. doi: 10.1111/j.1540-8167.2006.00382.x
30. Wang C, Chung BC, Yan H, Wang H-G, Lee S-Y, Pitt GS. Structural analyses of Ca 2+/CaM interaction with Na V channel C-termini reveal mechanisms of calcium-dependent regulation. *Nat Commun.* (2014) 5:4896. doi: 10.1038/ncomms5896
31. Jurado LA, Chockalingam PS, Jarrett HW. Apocalmodulin. *Physiol Rev.* (1999) 79:661–82. doi: 10.1152/physrev.1999.79.3.661
32. Maier LS, Ziolo MT, Bossuyt J, Persechini A, Mestril R, Bers DM. Dynamic changes in free Ca-calmodulin levels in adult cardiac myocytes. *J Mol Cell Cardiol.* (2006) 41:451–8. doi: 10.1016/j.yjmcc.2006.04.020
33. Kakiuchi S, Yasuda S, Yamazaki R, Teshima Y, Kanda K, Kakiuchi R, et al. Quantitative determinations of calmodulin in the supernatant and particulate fractions of mammalian tissues. *J Biochem.* (1982) 92:1041–8. doi: 10.1093/oxfordjournals.jbchem.a134019
34. Bers DM, Perez-Reyes E. Ca channels in cardiac myocytes: structure and function in Ca influx and intracellular Ca release. *Cardiovasc Res.* (1999) 42:339–60. doi: 10.1016/S0008-6363(99)00038-3
35. Lee K, Marban E, Tsien R. Inactivation of calcium channels in mammalian heart cells: joint dependence on membrane potential and intracellular calcium. *J Physiol.* (1985) 364:395–411. doi: 10.1113/jphysiol.1985.sp015752
36. Zygmunt AC, Maylie J. Stimulation-dependent facilitation of the high threshold calcium current in guinea-pig ventricular myocytes. *J Physiol.* (1990) 428:653–71. doi: 10.1113/jphysiol.1990.sp018233
37. Zuehlke RD, Pitt GS, Deisseroth K, Tsien RW, Reuter H. Calmodulin supports both inactivation and facilitation of L-type calcium channels. *Nature* (1999) 399:159. doi: 10.1038/20200
38. Erickson MG, Alseikhan BA, Peterson BZ, Yue DT. Preassociation of calmodulin with voltage-gated Ca(2+) channels revealed by FRET in single living cells. *Neuron* (2001) 31:973–85. doi: 10.1016/S0896-6273(01)00438-X
39. Liang H, DeMaria CD, Erickson MG, Mori MX, Alseikhan BA, Yue DT. Unified mechanisms of Ca2+ regulation across the Ca2+ channel family. *Neuron* (2003) 39:951–60. doi: 10.1016/S0896-6273(03)00560-9
40. Lee T-S, Karl R, Moosmang S, Lenhardt P, Klugbauer N, Hofmann F, et al. Calmodulin kinase II is involved in voltage-dependent facilitation of the L-type cav1. 2 calcium channel identification of the phosphorylation sites. *J Biol Chem.* (2006) 281:25560–67. doi: 10.1074/jbc.M508661200
41. Erxleben C, Liao Y, Gentile S, Chin D, Gomez-Alegria C, Mori Y, et al. Cyclosporin and timothy syndrome increase mode 2 gating of CaV1. 2 calcium channels through aberrant phosphorylation of S6 helices. *Proc Natl Acad Sci USA.* (2006) 103:3932–7. doi: 10.1073/pnas.0511322103
42. Shamgar L, Ma L, Schmitt N, Haitin Y, Peretz A, Wiener R, et al. Calmodulin is essential for cardiac IKS channel gating and assembly: impaired function in long-QT mutations. *Circ Res.* (2006) 98:1055–63. doi: 10.1161/01.RES.0000218979.40770.69
43. Tobelaim WS, Dvir M, Lebel G, Cui M, Buki T, Peretz A, et al. Ca(2+)-Calmodulin and PIP2 interactions at the proximal C-terminus of Kv7 channels. *Channels* (2017) 11:686–95. doi: 10.1080/19336950.2017.1388478
44. Chang A, Abderemane-Ali F, Hura GL, Rossen ND, Gate RE, Minor Jr DL. A calmodulin C-Lobe Ca2+-dependent switch governs Kv7 channel function. *Neuron* (2018) 97:836–52.e836. doi: 10.1016/j.neuron.2018.01.035
45. Ciampa EJ, Welch RC, Vanoye CG, George AL. KCNE4 juxtamembrane region is required for interaction with calmodulin and for functional suppression of KCNQ1. *J Biol Chem.* (2011) 286:4141–9. doi: 10.1074/jbc.M110.158865
46. Shugg T, Johnson DE, Shao M, Lai X, Witzmann F, Cummins TR, et al. Calcium/calmodulin-dependent protein kinase II regulation of IKs during sustained beta-adrenergic receptor stimulation. *Heart Rhythm* (2018) 15:895–904. doi: 10.1016/j.hrthm.2018.01.024
47. Kim J, Ghosh S, Liu H, Tateyama M, Kass RS, Pitt GS. Calmodulin mediates Ca2+ sensitivity of sodium channels. *J Biol Chem.* (2004) 279:45004–12. doi: 10.1074/jbc.M407286200
48. Shah VN, Wingo TL, Weiss KL, Williams CK, Balser JR, Chazin WJ. Calcium-dependent regulation of the voltage-gated sodium channel hH1: intrinsic and extrinsic sensors use a common molecular switch. *Proc Natl Acad Sci USA.* (2006) 103:3592–7. doi: 10.1073/pnas.0507397103
49. Lanner JT, Georgiou DK, Joshi AD, Hamilton SL. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. *Cold Spring Harb Perspect Biol.* (2010) 2:a003996. doi: 10.1101/cshperspect.a003996
50. Soeller C, Crossman D, Gilbert R, Cannell MB. Analysis of ryanodine receptor clusters in rat and human cardiac myocytes. *Proc Natl Acad Sci USA.* (2007) 104:14958–63. doi: 10.1073/pnas.0703016104
51. Louch WE, Stokke MK, Sjaastad I, Christensen G, Sejersted OM. No rest for the weary: diastolic calcium homeostasis in the normal and failing myocardium. *Physiology* (2012) 27:308–23. doi: 10.1152/physiol.00021.2012
52. Franzini-Armstrong C, Protasi F, Ramesh V. Shape, size, and distribution of Ca2+ release units and couplons in skeletal and cardiac muscles. *Biophys J.* (1999) 77:1528–39. doi: 10.1016/S0006-3495(99)77000-1
53. Bers DM, Stiffel VM. Ratio of ryanodine to dihydropyridine receptors in cardiac and skeletal muscle and implications for EC coupling. *Am J Physiol Cell Physiol.* (1993) 264:C1587–93. doi: 10.1152/ajpcell.1993.264.6.C1587
54. Balshaw DM, Xu L, Yamaguchi N, Pasek DA, Meissner G. Calmodulin binding and inhibition of cardiac muscle calcium release channel (ryanodine receptor). *J Biol Chem.* (2001) 276:20144–53. doi: 10.1074/jbc.M010771200
55. Xiong H, Feng X, Gao L, Xu L, Pasek DA, Seok J-H, et al. Identification of a two EF-hand Ca2+ binding domain in lobster skeletal muscle ryanodine receptor/Ca2+ release channel. *Biochemistry* (1998) 37:4804–14. doi: 10.1021/bi971198b
56. Xu L, Gomez AC, Pasek DA, Meissner G, Yamaguchi N. Two EF-hand motifs in ryanodine receptor calcium release channels contribute to isoform-specific regulation by calmodulin. *Cell Calcium* (2017) 66:62–70. doi: 10.1016/j.ceca.2017.05.013
57. Gyorke S, Terentyev D. Modulation of ryanodine receptor by luminal calcium and accessory proteins in health and cardiac disease. *Cardiovasc Res.* (2008) 77:245–55. doi: 10.1093/cvr/cvm038
58. Li P, Chen SW. Molecular basis of Ca^{2+} activation of the mouse cardiac Ca2+ release channel (ryanodine receptor). *J Gen Physiol.* (2001) 118:33–44. doi: 10.1085/jgp.118.1.33

59. Guo W, Sun B, Xiao Z, Liu Y, Wang Y, Zhang L, et al. The EF-hand Ca2+ binding domain is not required for cytosolic Ca^{2+} activation of the cardiac ryanodine receptor. *J Biol Chem.* (2016) 291:2150–60. doi: 10.1074/jbc.M115.693325
60. Zalk R, Lehnart SE, Marks AR. Modulation of the ryanodine receptor and intracellular calcium. *Annu Rev Biochem.* (2007) 76:367–85. doi: 10.1146/annurev.biochem.76.053105.094237
61. Buratti R, Prestipino G, Menegazzi P, Treves S, Zorzato F. Calcium-dependent activation of skeletal-muscle Ca^{2+} release channel (ryanodine receptor) by calmodulin. *Biochem Biophys. Res Commun.* (1995) 213:1082–90. doi: 10.1006/bbrc.1995.2238
62. Tripathy A, Xu L, Mann G, Meissner G. Calmodulin activation and inhibition of skeletal muscle Ca^{2+} release channel (ryanodine receptor). *Biophys J.* (1995) 69:106–19. doi: 10.1016/S0006-3495(95)79880-0
63. Walweel K, Oo YW, Laver DR. The emerging role of calmodulin regulation of RyR2 in controlling heart rhythm, the progression of heart failure and the antiarrhythmic action of dantrolene. *Clin Exp Pharmacol Physiol.* (2017) 44:135–42. doi: 10.1111/1440-1681.12669
64. Xu L, Meissner G. Mechanism of calmodulin inhibition of cardiac sarcoplasmic reticulum Ca2+ release channel (ryanodine receptor). *Biophys J.* (2004) 86:797–804. doi: 10.1016/S0006-3495(04)74155-7
65. Yamaguchi N, Xu L, Pasek DA, Evans KE, Meissner G. Molecular basis of calmodulin binding to cardiac muscle Ca^{2+} release channel (ryanodine receptor). *J Biol Chem.* (2003) 278:23480–6. doi: 10.1074/jbc.M301125200
66. Oda T, Yang Y, Nitu FR, Svensson B, Lu X, Fruen BR, et al. In cardiomyocytes, binding of unzipping peptide activates ryanodine receptor 2 and reciprocally inhibits calmodulin binding. *Circ Res.* (2013) 112:487–97. doi: 10.1161/CIRCRESAHA.111.300290
67. Oda T, Yang Y, Uchinoumi H, Thomas DD, Chen-Izu Y, Kato T, et al. Oxidation of ryanodine receptor (RyR) and calmodulin enhance Ca release and pathologically alter, RyR structure and calmodulin affinity. *J Mol Cell Cardiol.* (2015) 85:240–8. doi: 10.1016/j.yjmcc.2015.06.009
68. Gangopadhyay JP, Ikemoto N. Interaction of the Lys(3614)-Asn(3643) calmodulin-binding domain with the Cys(4114)-Asn(4142) region of the type 1 ryanodine receptor is involved in the mechanism of Ca2+/agonist-induced channel activation. *Biochem J.* (2008) 411:415–23. doi: 10.1042/BJ20071375
69. Huang X, Fruen B, Farrington DT, Wagenknecht T, Liu Z. Calmodulin-binding locations on the skeletal and cardiac ryanodine receptors. *J Biol Chem.* (2012) 287:30328–35. doi: 10.1074/jbc.M112.383109
70. Wehrens XH, Lehnart SE, Reiken SR, Marks AR. Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. *Circ Res.* (2004) 94:e61–70. doi: 10.1161/01.RES.0000125626.33738.E2
71. Terentyev D, Gyorke I, Belevych AE, Terentyeva R, Sridhar A, Nishijima Y, et al. Redox modification of ryanodine receptors contributes to sarcoplasmic reticulum Ca2+ leak in chronic heart failure. *Circ Res.* (2008) 103:1466–72. doi: 10.1161/CIRCRESAHA.108.184457
72. Makita N, Yagihara N, Crotti L, Johnson CN, Beckmann B-M, Roh MS, et al. Novel calmodulin mutations associated with congenital arrhythmia susceptibilityclinical perspective. *Circ Genom Prec Med.* (2014) 7:466–74. doi: 10.1161/CIRCGENETICS.113.000459
73. Crotti L, Johnson CN, Graf E, De Ferrari GM, Cuneo BF, Ovadia M, et al. Calmodulin mutations associated with recurrent cardiac arrest in infants. *Circulation* (2013) 127:1009–17. doi: 10.1161/CIRCULATIONAHA.112.001216
74. Sondergaard MT, Sorensen AB, Skov LL, Kjaer-Sorensen K, Bauer MC, Nyegaard M, et al. Calmodulin mutations causing catecholaminergic polymorphic ventricular tachycardia confer opposing functional and biophysical molecular changes. *FEBS J.* (2015) 282:803–16. doi: 10.1111/febs.13184
75. Marsman RF, Barc J, Beekman L, Alders M, Dooijes D, van den Wijngaard A, et al. A mutation in CALM1 encoding calmodulin in familial idiopathic ventricular fibrillation in childhood and adolescence. *J Am Coll Cardiol.* (2014) 63:259–66. doi: 10.1016/j.jacc.2013.07.091
76. Reed GJ, Boczek NJ, Etheridge SP, Ackerman MJ. CALM3 mutation associated with long QT syndrome. *Heart Rhythm* (2015) 12:419–22. doi: 10.1016/j.hrthm.2014.10.035
77. Limpitikul WB, Dick IE, Joshi-Mukherjee R, Overgaard MT, George AL, Yue DT. Calmodulin mutations associated with long QT syndrome prevent inactivation of cardiac L-type Ca2+ currents and promote proarrhythmic behavior in ventricular myocytes. *J Mol Cell Cardiol.* (2014) 74:115–24. doi: 10.1016/j.yjmcc.2014.04.022
78. Pipilas DC, Johnson CN, Webster G, Schlaepfer J, Fellmann F, Sekarski N, et al. Novel calmodulin mutations associated with congenital long QT syndrome affect calcium current in human cardiomyocytes. *Heart Rhythm* (2016) 13:2012–9. doi: 10.1016/j.hrthm.2016.06.038
79. Boczek NJ, Gomez-Hurtado N, Ye D, Calvert ML, Tester DJ, Kryshtal D, et al. Spectrum and prevalence of CALM1-, CALM2-, and CALM3-encoded calmodulin (CaM) variants in long QT syndrome (LQTS) and functional characterization of a novel LQTS-associated CaM missense variant, E141G. *Circ Cardiovasc Genet.* (2016) 9:136–46. doi: 10.1161/CIRCGENETICS.115.001323
80. Rocchetti M, Sala L, Dreizehnter L, Crotti L, Sinnecker D, Mura M, et al. Elucidating arrhythmogenic mechanisms of long-QT syndrome CALM1-F142L mutation in patient-specific induced pluripotent stem cell-derived cardiomyocytes. *Cardiovasc Res.* (2017) 113:531–41. doi: 10.1093/cvr/cvx006
81. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation* (2001) 103:196–200. doi: 10.1161/01.CIR.103.2.196
82. Zaza A, Rocchetti M. Calcium store stability as an antiarrhythmic endpoint. *Curr Pharm Des.* (2015) 21:1053–61. doi: 10.2174/1381612820666141029100650
83. Nishio M, Ruch SW, Kelly JE, Aistrup GL, Sheehan K, Wasserstrom JA. Ouabain increases sarcoplasmic reticulum calcium release in cardiac myocytes. *J Pharmacol Exp Ther.* (2004) 308:1181–90. doi: 10.1124/jpet.103.060004
84. Nyegaard M, Overgaard MT, Søndergaard MT, Vranas M, Behr ER, Hildebrandt LL, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. *Am J Hum Genet.* (2012) 91:703–12. doi: 10.1016/j.ajhg.2012.08.015
85. Gomez-Hurtado N, Boczek NJ, Kryshtal DO, Johnson CN, Sun J, Nitu FR, et al. Novel CPVT-associated calmodulin mutation in CALM3 (CALM3-A103V) activates arrhythmogenic Ca waves and sparks. *Circ Arrhythm Electrophysiol.* (2016) 9:e004161. doi: 10.1161/CIRCEP.116.004161
86. Hwang H-S, Nitu FR, Yang Y, Walweel K, Pereira L, Johnson CN, et al. Divergent regulation of RyR2 calcium release channels by arrhythmogenic human calmodulin missense mutants. *Circ Res.* (2014) 114:1114–24. doi: 10.1161/CIRCRESAHA.114.303391
87. Nomikos M, Thanassoulas A, Beck K, Vassilakopoulou V, Hu H, Calver BL, et al. Altered RyR2 regulation by the calmodulin F90L mutation associated with idiopathic ventricular fibrillation and early sudden cardiac death. *FEBS Lett.* (2014) 588:2898–902. doi: 10.1016/j.febslet.2014.07.007
88. Yu CC, Ko JS, Ai T, Tsai WC, Chen Z, Rubart M, et al. Arrhythmogenic calmodulin mutations impede activation of small-conductance calcium-activated potassium current. *Heart Rhythm* (2016) 13:1716–23. doi: 10.1016/j.hrthm.2016.05.009
89. Yamamoto Y, Makiyama T, Harita T, Sasaki K, Wuriyanghai Y, Hayano M, et al. Allele-specific ablation rescues electrophysiological abnormalities in a human iPS cell model of long-QT syndrome with a CALM2 mutation. *Hum Mol Genet.* (2017) 26:1670–7. doi: 10.1093/hmg/ddx073
90. Koivumäki JT, Naumenko N, Tuomainen T, Takalo J, Oksanen M, Puttonen KA, et al. Structural immaturity of human iPSC-derived cardiomyocytes: *in silico* investigation of effects on function and disease modeling. *Front Physiol.* (2018) 9:80. doi: 10.3389/fphys.2018.00080
91. Zaza A, Rocchetti M. The late Na+ current–origin and pathophysiological relevance. *Cardiovasc Drugs Ther.* (2013) 27:61–8. doi: 10.1007/s10557-012-6430-0

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Pleiotropic Phenotypes Associated With PKP2 Variants

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Plakophilin-2 (PKP2) is a component of the desmosome complex and known for its role in cell-cell adhesion. Recently, alterations in the *Pkp2* gene have been associated with different inherited cardiac conditions including Arrythmogenic Cardiomyopathy (ACM or ARVC), Brugada syndrome (BrS), and idiopathic ventricular fibrillation to name the most relevant. However, the assessment of pathogenicity regarding the genetic variations associated with *Pkp2* is still a challenging task: the gene has a positive Residual Variation Intolerance Score and the potential deleterious role of several of its variants has been disputed. Limitations in facilitating interpretation and annotations of these variants are seen in the lack of segregation and clinical data in the control population of reference. In this review, we will provide a summary of all the currently available genetic information related to the *Pkp2* gene, including different phenotypes, ClinVar annotations and data from large control database. Our goal is to provide a literature review that could help clinicians and geneticists in interpreting the role of *Pkp2* variants in the context of heritable sudden death syndromes. Limitations of current algorithms and data repositories will be discussed.

Keywords: plakophilin-2, Arrhythmogenic Cardiomyopathy, ARVC, Brugada syndrome, sudden cardiac death, genetic mutation, cardiomyopathies

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INTRODUCTION

The recent expansion of genetic testing in the field of cardiac arrhythmias, due to decreased sequencing costs and increased availability of large comprehensive panels, recently brought up the discoveries that variants in the same genes can be detected in association with different phenotypes. The pleiotropic effect of a gene, i.e., its potential to influence different clinical phenotypes, reflects the new notion that the same protein could exert different and unrelated functions, in addition to the one historically associated with it. On the other hand, the increased number of variants discovered in association with multiple phenotypes and often without robust linkage data raised, in parallel, concerns on the evidence supporting their genotype-phenotype causal relation.

As an example of pleiotropic genes, we are focusing our review on plakophilin-2 (PKP2) coded by the gene *Pkp2*, whose pathogenic role has been recently recognized in different inherited cardiac arrhythmias syndromes, ranging from Arrhythmogenic Cardiomyopathy (ACM or ARVC), Brugada Syndrome (BrS), idiopathic ventricular fibrillation, hypertrophic cardiomyopathy (HCM), and dilated cardiomyopathy (DCM). Evidence has simultaneously emerged of the positive Residual Variation Intolerant Score (RVIS) (1) of *Pkp2* suggesting that its overall variation could be quite tolerated. Along these lines, a study showed that several *Pkp2* variants can be found in a high percentage of ostensibly healthy controls, adding to the complexity of interpretation of the genetic

variability of this protein (2). We will review the spectrum of phenotypes that have been attributed to *Pkp2* variants and discuss clinical and functional evidence, as well as controversial interpretations in light of current data deriving from large statistical analyses and public databases.

INTERPRETATION OF GENETIC VARIANTS: LARGE POPULATION DATABASE

Currently, the adjudication of the clinical significance of genetic variants is among the major limitations of genetic testing (3). The increasing progress in sequencing technology unraveled the complexity of human genetic variability, bringing up the new challenge of distinguishing between variants with a potential deleterious effect and the ones which may only be a bystander.

Several public databases have been created with the goal of facilitating this task. The most cited annotation database is ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) (3). This database is part of the NCBI's Entrez system, and includes information for more than 30,181 genes. This is the first freely available archive of reports from several selected sources which attempts to establish a relation between variants and phenotype. ClinVar accepts direct submissions from genetic laboratories, academic centres, genetic repositories, and scientific societies that are required to share the phenotype details, the methodology used to capture variant calls and finally their clinical interpretation. However, there is a lack of structured determination, since the respective submitter determines the variant adjudication in ClinVar, based on internal criteria and possibly in agreement with the American College of Medical Genetics (ACMG) guidelines. Additional limitations are the lack of a central review process, the scarce if any clinical data provided and the lack of a defined denominator, since the latter is established by each single submitter based on their own screening panel features and on the number of panels and clinical diagnoses encompassing a specific gene. Hence, the integration with additional independent sources is pivotal for an appropriate interpretation of data in ClinVar in the clinical context.

In the past few years, several databases of whole exome data from large populations of ostensibly healthy controls have been made available to the public. The goal of this repositories is to provide information on the expected human variation frequency. But the lack of phenotypical data may create controversial interpretation even when using these sources. The Genome Aggregation database (GnomAD; gnomad.broadinstitute.org) is currently one of the largest available and the most used support for variant interpretation. This freely accessible database includes genetic data from 123,136 exome sequences and 15,496 whole-genome sequences. It is mainly used to discriminate common from rare variants, across eight different ethnic groups. Usually, a Minor Allele Frequency (MAF) >0.001 is considered the threshold to discriminate between common from rare variants, although the value should be adjusted by disease prevalence, which is often a challenge to determine, especially in the case of cardiac channelopathies and cardiomyopathies that show incomplete penetrance and concealed or progressive phenotypes.

A new statistically robust framework to assess disease-specific thresholds has also been recently developed. This support, freely available at http://cardiodb.org/allelefrequencyapp/ (4) allows to calculate the maximum expected allele frequency of a disease-causing variant in the general population in order to classify it according to the phenotype. The algorithm has been created in order to address the arbitrarity of the cutoff determination in mendelian disorders. It combines disease prevalence, genetic and allelic heterogeneity, inheritance mode, penetrance, and sampling variance in reference database to assess the frequency cutoff. In the initial presentation of their algorithm, the authors show a false positive rate<0.001 when applied to HCM-related variants. The application of this new algorithm to *Pkp2* in the future may shed some light of the expected pathogenicity of several unresolved variants at least for the ACM phenotype.

These tools combined together represent a helpful "first-pass" interpretation that should be followed by a detailed analysis from genetic experts taking into account the clinical presentation of the patient and evidence of co-segregation in the family.

PLAKOPHILIN-2: STRUCTURE AND FUNCTIONS

Desmosomes are intercellular junctions of epithelia and cardiac muscle (5), tethering intermediate filaments to the plasma membrane to maintain cell adhesion and tissue integrity. Structurally, they are composed by three major gene families: desmosomal cadherins, armadillos, and plakins (6).

The *Pkp2* gene has been characterized for the first time in 1996 (7) and encodes for the PKP2 protein. The main recognized function of PKP2 is mechanical, i.e., providing a lateral stabilizing force with the desmosomal-intermediate filament assembly facilitating cell-to-cell contact. However, in recent years evidence has been provided of the pleiotropic role of this protein with functions ranging from intracellular signaling regulation to electrophysiologic and trafficking regulation, to the control of transcription processes. Studies oriented to discover the relation between defective PKP2 and arrhythmias showed that PKP2 is necessary to maintain gap junction integrity and formation (8–10): indeed, hearts samples from ACM patients presented loss of gap junction plaques (11). Subsequently, it was demonstrated that loss of PKP2 expression disrupts trafficking of the sodium channel at the intercalated disc, thus decreasing cardiac sodium current and facilitating arrhythmias (12, 13). These effects reflect the interplay between defective PKP2 and the molecular complex at the intercalated disc defined "connexome"(14, 15).

More recently, data on a novel cardiac-specific, tamoxifen activated PKP2 knock-out mouse model linked loss of PKP2 to transcriptional disruption of calcium homeostasis (16). In addition to these electrophysiologic effects, it has been proposed that PKP2 deficiency could activate the Hippo and Wnt pathways, facilitating fibro-adiposis in ACM (17, 18). Dubash et al. (19) showed another mechanism by which PKP2 may facilitate fibrosis formation: in their work, lack of PKP2 expression caused increased expression of TGF-beta1 and activation of the p38-MAPK-dependent pro-fibrotic program.

The different functions and protein-protein interactions that have been demonstrated for PKP2 provide interesting insights on the complexity of proteins functions that often overcome the primary role attributed at the time of initial discovery and could justify some of the pleiotropic phenotypical manifestations of its genetic variants.

PKP2 VARIANTS IN ACM

ACM is a familial disease characterized by ventricular arrhythmias, increased risk of sudden cardiac death (SCD) and progressive fibrofatty replacement of the myocardium, usually starting at the right ventricle and subsequently evolving to biventricular dilation and heart failure (20). The symptoms usually manifest starting from early adulthood and the disease has an estimated prevalence of 1:2,000–1:5,000 in the general population, is more common in males (2:1, which also seems to show higher incidence of fatal arrhythmic events (20, 21). However, the disease phenotype is highly variable and characterized by incomplete penetrance. SCD can be its initial manifestation even before an overt cardiomyopathy is evident (20).

Currently, 14 different genetic loci have been reported for ACM, the majority being desmosomal genes such as *Pkp2*, *Dsp*, and *Dsg2 and Dsc2*. In a minority of the cases, alterations in other structural cardiac genes have been also detected (22). Furthermore, compound/digenic heterozygosity has been identified in about 4% of ACM mutation carriers (21). Data on patients with compound/digenic heterozygosity suggest that they might show an earlier onset of symptoms (21). Genetic alterations in *Pkp2* are the most frequent, accounting for 40–60% of the genotype positive patients (20, 23, 24). These include single amino acids mutations such as missense, non-sense or frameshift variants, but also large genomic rearrangements of several exons (23, 25).

At present, the ClinVar database (accessed June 2018) would return a total of 521 annotated *Pkp2* variants. While the majority (65%) is linked to a cardiac condition, the remaining 35% has been submitted without an associated clinical diagnosis. A search only for "ACM" yielded 290 alterations. Among those, 83 (29%) are classified as pathogenic (P) /likely pathogenic (LP), 66 (24%) are benign (B)/likely benign (LB) and 110 are uncertain significant (VUS) (38%). In addition, about 10% (26) of the total reported variations in ClinVar has a conflicting interpretation based on discordant data among different reports (**Table 1**).

If one focuses on P/LP variants, only 5 of them (6%) are missense, whereas most are non-sense (25%), frameshift variants (46%), splicing alterations ±1 or ±2 (17%) or large deletions (6%). In contrast with this finding, among the ones classified as variant of unknown significance (VUS) 59% are missense, 12% are intron variants located relatively far from the donor or acceptor sites, 3% are synonymous variants and 25% are nucleotide substitutions in the 3′UTR. This data shows that the majority of *Pkp2* variants (∼77%) associated with ACM in ClinVar and annotated as P/LP are radical alterations, hence considered at high probability of causing a disruption of the protein and resulting in extensive transcriptional and posttranslational alterations, while single amino acid changes are of more complex interpretation, especially in light of the known "signal-to-noise ratio" (2).

TABLE 1 | *PKP2* variants reported in ClinVar related to different cardiac conditions.

Condition	P/LP	VUS	B/LB	Conflicting interpretation
ACM	83	110	66	31
CV phenotype	6	19	4	2
Cardiomyopathy	2	1		
HCM		1		1
DCM	1			
VF				1
VT		2		
LVNC		1		

P, Pathogenic; LP, Likely Pathogenic; VUS, Variant with uncertain significance; B, Benign; LB, Likely Benign; ACM, Arrhythmogenic Cardiomyopathy; CV, Cardiovascular; HCM, Hypertrophic Cardiomyopathy; DCM, Dilated Cardiomyopathy; VF, Ventricular Fibrillation; VT, Ventricular Tachycardia; LVNC, Left Ventricular Non Compaction.

However, according to the ACMG and the Association of Molecular Pathology (27) guidelines, the type of variant is only one among the criteria considered useful to adjudicate pathogenicity. Another relevant criterion, is the analysis of the variant frequency in the general population (4). When analyzing the allele frequency of the ACM-related 83 P/LP mutations from ClinVar, only 19 (23%) of these are also present in GnomAD (June 2018), hence in apparently healthy controls. These are all reported with an allele frequency ranging 0.000004–0.00004 as shown in **Table 2**. Considering that ACM is a rare autosomal disease, with adult onset and incomplete penetrance, a very low allele frequency is not unexpected, thus their presence in GnomAD does not necessarily rule out a pathogenic role. The remainig 77% of P/LP ACM variants from ClinVar are not reported in GnomAD, in support of their potential causative disease role. In contrast with the data for P/LP mutations, 61% of *PKP2* VUS annotated in ClinVar are also present in GnomAD and their allele frequency ranges 0.00003–0.001. This higher allele frequency, which encompasses also synonymous and UTRs variants, far from the flanking regions, adds on the controversial interpretation of their effect.

In summary, large population databases support the established relation between *Pkp2* and the ACM phenotype and confirm published evidence that radical mutations are often associated with a more severe clinical presentation (2, 27).

PKP2 AND BRUGADA SYNDROME

Genetic variants in *Pkp2* have also been recently identified in patients affected by BrS (28). This inherited arrhythmia is characterized by ST- segment elevation in the right precordial leads, and increased risk of ventricular fibrillation, without macroscopic structural disease (29). Over 22 different genes have been so far linked to BrS (30). Mutations on the SCN5A

TABLE 2 | Allele frequency of the ACM-related Pathogenic/Likely Pathogenic variants reported in ClinVar according to GnomAD (both database accessed on June 2018).

Variants	#rs	Allele count	Allele frequency
c.2489+1G>A	rs111517471	6	2,17E-05
p.Arg735Ter	rs121434421	1	4,06E-06
c.2146-1G>C	rs193922674	10	3,61E-05
p.Glu667Ter	rs397517015	1	4,06E-06
p.Arg651Ter	rs751288871	2	7,22E-06
p.Gln638Ter	rs397517012	2	7,22E-06
c.1688+1G>A	rs397517003	1	4,07E-06
p.Trp538Ter	rs193922672	3	1,22E-05
c.1378+1G>C	rs397516994	1	4,07E-06
p.Arg413Ter	rs372827156	4	1,44E-05
p.Arg388Trp	rs766209297	1	4,06E-06
p.Gln378Ter	rs397516986	2	7,22E-06
p.Tyr221Ter	rs767987619	2	7,22E-06
p.Trp123Ter	rs774663443	1	4,12E-06
p.Leu771ProfsTer2	rs121434420	1	4,06E-06
p.Arg79Ter	NA	1	4,07E-06
p.His318TrpfsTer10	NA	1	4,07E-06
p.Gln323ArgfsTer12	rs745882420	13	4,71E-05
p.Val280HisfsTer55	rs772220644	2	7,23E-06

gene, coding for the alpha-subunit of the cardiac sodium channel account for ∼25% of cases and all other genes cover altogether only 5–10% of genotyped patients (31). Polygenic inheritance has also been suggested (26). Interestingly, past studies have suggested a possible overlap between the BrS and ACM phenotypes, suggesting that the two diseases could be at the opposite end of a common clinical condition (32). Functionally, *SCN5A* loss of function variants associated with BrS lead to decreased sodium current, through different mechanisms (29, 31).

Following a gene-candidate approach based on the relation between lack of PKP2 expression and decreased sodium current *in vitro*, we reported the first cases of patients with a BrS phenotype in the absence of overt structural cardiomyopathy carrying genetic variants in the *Pkp2* gene (28). We discovered five different *Pkp2* missense variants in five unrelated individuals, and provided genotype/phenotype co-segregation data in one family across two generations. Functional *in vitro* studies in HL1 cardiac cells and in human ips-derived cardiomyocytes demonstrated that these variants could decrease sodium current, an electrophysiologic effect consistent with the BrS phenotype, thus supporting the potential pro-arrhythmic role of the variants. Based on this initial study, followed by other clinical case reports (33), some commercial genetic panels for BrS now include *Pkp2*.

Due to its recent inclusion in commercial testing, ClinVar does not yet report any *Pkp2* variant under the "BrS" diagnosis. At present there have been only 8 PKP2 non-synonymous variants associated with BrS reported in the literature (28, 33, 34). Three of these are absent in ClinVar, and the remaining 5 are VUS or have a "conflicting interpretation" for the diagnoses "ACM" or "cardiovascular phenotype" (**Table 1**). GnomAD offers contrasting interpretation in respect from ClinVar for these 8 variants, whose allele frequency ranges from 0.0002382 to 0.000008127, hence supporting a possible pathogenic role.

However, in this perspective it is important to highlight data from a recent large study that challenged the role for "minor" BrS genes, including *Pkp2*, as causal for BrS (35). The authors used an evidence-based semiquantitative scoring system of genetic and experimental evidence, which showed that only the gene SCN5a could be linked to the disease with a definite evidence.

ADDITIONAL CARDIAC PHENOTYPES LINKED TO PKP2

The availability of large panels and of exome screening has allowed investigators to discover pathogenic variants in genes initially unexpected to be related to the phenotype. In the case of *Pkp2,* variants have been detected in idiopathic ventricular fibrillation and SCD (36, 37), possible catecholaminergic polymorphic ventricular tachycardia (38), HCM, DCM and left ventricular non-compaction (39–41). The association between *Pkp2* mutations and idiopathic SCD is not that surprising, considering that often SCD is the earliest manifestation in ACM athletes before the onset of overt cardiomyopathy. The association between Pkp2 variants and other structural cardiomyopathies aside from ACM remains instead quite controversial, as discussed further. A ClinVar search for PKP2 variants based on following diagnoses: "cardiovascular phenotype," "cardiomyopathy," "LVNC," "Paroxysmal familial ventricular fibrillation," "DCM," "HCM," and "ventricular tachycardia," returns 41 different changes. However, most of them (76%) appear under the generic definition of "cardiovascular phenotype," which is not an informative diagnosis. By narrowing the search to DCM and HCM, ClinVar reports 3 variants only (1 DCM and 2 HCM) annotated as "LP" (H679T), "VUS" (M349V) and "conflicting interpretation" (T526A), respectively (**Table 1**). Among these, the two that are associated with HCM are reported in GnomAD with an allele frequency of 0.00012 and 0.000036, respectively. The relation between non-sarcomeric genes and HCM has been questioned recently in a large statistic-based approach study (42), showing limited evidence that these genes associate with the phenotype. Even if *Pkp2* was not included in the study, the data suggest caution in the evaluation of these reported variants.

No data regarding the variant associated with DCM is reported in GnomAD, supporting its low prevalence. However, if this is a variant linked to a clinical phenotype that initially manifested as ACM and then evolved into DCM is yet to be assessed. Considering that clinically cardiomyopathies are diseases with a progressive course, one cannot exclude that DCM cases carrying *Pkp2* variants could be cases of advanced ACM which were missed in the initial disease phases.

The 2 PKP2 VUS linked to "Ventricular Tachycardia" (Q59L and G327V) have a low allele frequency in GnomAD (0.00002 and 0.00009, respectively). The splice site alteration (c.1974A>G)

for the ClinVar diagnosis of "paroxysmal ventricular fibrillation" (conflicting interpretation) is not present in GnomAD.

CONCLUSIONS

The expansion and increased availability of genetic testing has challenged the concept of "one gene-one disease" and has shown that different phenotypes can be caused by variants on one same gene. The interpretation of these findings in light of human variation data is complex and casts some warning on the clinical application of this information. The evidence of the pleiotropy of a gene suggested by genetic variants and their functional effect *in vitro* has the important value of discovering different protein functions and suggest arrhythmia mechanisms. The use of all this data for clinical diagnosis and risk assessment may not yet be ready for prime time, as shown by recent large statistical studies. Current database and *in silico* tools have known limitations, but the combination of this evidence with increasing collaborative efforts pooling clinical and genetic information together could in the future shed new light on how to interpret these data in lights of patients' care.

AUTHOR CONTRIBUTIONS

VN: Initial preparation of the manuscript and figures; KM: Critical revision of article; MC: Article conception and design, critical revision of manuscript, approval of final version.

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REFERENCES

1. Petrovski S, Wang Q, Heinzen EL, Allen AS, Goldstein, DB. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet.* (2013) 9:e1003709. doi: 10.1371/journal.pgen.1003709
2. Kapplinger JD, Landstrom AP, Salisbury BA, Callis T, Pollevick GD, Tester DJ, et al. Distinguishing arrhythmogenic right ventricular cardiomyopathy/dysplasia-associated mutations from background genetic noise. *J Am Coll Cardiol.* (2011) 57:2317–27. doi: 10.1016/j.jacc.2010.12.036
3. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, et al. (2018). ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res.* 46:D1062–7. doi: 10.1093/nar/gkx1153
4. Whiffin N, Minikel E, Walsh RAH, O'Donnell-Luria, Karczewski K, Ing AY, et al. (2017). Using high-resolution variant frequencies to empower clinical genome interpretation. *Genet Med* 19: 1151-1158. doi: 10.1038/gim.2017.26
5. Garrod D, Chidgey M. Desmosome structure, composition and function. *Biochim Biophys Acta* (2008) 1778:572–87. doi: 10.1016/j.bbamem.2007.07.014
6. Al-Amoudi A, Castano-Diez D, Devos DP, Russell RB, Johnson GT, Frangakis AS. The three-dimensional molecular structure of the desmosomal plaque. *Proc Natl Acad Sci USA*. (2011) 108:6480–5. doi: 10.1073/pnas.1019469108
7. Mertens C, Kuhn C, Franke WW. Plakophilins 2a and 2b: constitutive proteins of dual location in the karyoplasm and the desmosomal plaque. *J Cell Biol.* (1996) 135:1009–25. doi: 10.1083/jcb.135.4.1009
8. Joshi-Mukherjee R, Coombs W, Musa H, Oxford E, Taffet S, Delmar M. Characterization of the molecular phenotype of two arrhythmogenic right ventricular cardiomyopathy (ARVC)-related plakophilin-2 (PKP2) mutations. *Heart Rhythm* (2008) 5:1715–23. doi: 10.1016/j.hrthm.2008.09.009
9. Oxford EM, Danko CG, Kornreich BG, Maass K, Hemsley, SA, Raskolnikov D, et al. Ultrastructural changes in cardiac myocytes from Boxer dogs with arrhythmogenic right ventricular cardiomyopathy. *J Vet Cardiol.* (2011) 13:101–13. doi: 10.1016/j.jvc.2011.03.002
10. Leo-Macias A, Liang FX, Delmar M. Ultrastructure of the intercellular space in adult murine ventricle revealed by quantitative tomographic electron microscopy. *Cardiovasc Res.* (2015) 107:442–52. doi: 10.1093/cvr/cvv182
11. Kaplan SR, Gard JJ, Protonotarios N, Tsatsopoulou A, Spiliopoulou C, Anastasakis A, et al. Remodeling of myocyte gap junctions in arrhythmogenic right ventricular cardiomyopathy due to a deletion in plakoglobin (Naxos disease). *Heart Rhythm* (2004) 1:3–11. doi: 10.1016/j.hrthm.2004.01.001
12. Sato PY, Musa H, Coombs W, Guerrero-Serna G, Patino GA, Taffet SM, et al. Loss of plakophilin-2 expression leads to decreased sodium current and slower conduction velocity in cultured cardiac myocytes. *Circ Res.* (2009) 105:523–6. doi: 10.1161/CIRCRESAHA.109.201418
13. Cerrone M, Noorman M, Lin X, Chkourko H, Liang FX, van der Nagel R, et al. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. *Cardiovasc Res.* (2012) 95:460–8. doi: 10.1093/cvr/cvs218
14. Agullo-Pascual E, Reid DA, Keegan S, Sidhu M, Fenyo D, Rothenberg E, et al. Super-resolution fluorescence microscopy of the cardiac connexome reveals plakophilin-2 inside the connexin43 plaque. *Cardiovasc Res.* (2013) 100:231–40. doi: 10.1093/cvr/cvt191
15. Agullo-Pascual E, Cerrone M, Delmar M. Arrhythmogenic cardiomyopathy and Brugada syndrome: diseases of the connexome. *FEBS Lett.* (2014) 588:1322–30. doi: 10.1016/j.febslet.2014.02.008
16. Cerrone M, Montnach J, Lin X, Zhao YT, Zhang M, Agullo-Pascual E, et al. Plakophilin-2 is required for transcription of genes that control calcium cycling and cardiac rhythm. *Nat Commun.* (2017) 8:106. doi: 10.1038/s41467-017-00127-0
17. Garcia-Gras E, Lombardi R, Giocondo MJ, Willerson JT, Schneider MD, Khoury DS, et al. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. *J Clin Invest.* (2006) 116:2012–21. doi: 10.1172/JCI27751
18. Chen SN, Gurha P, Lombardi R, Ruggiero A, Willerson JT, Marian AJ. The hippo pathway is activated and is a causal mechanism for adipogenesis in arrhythmogenic cardiomyopathy. *Circ Res.* (2014) 114:454–68. doi: 10.1161/CIRCRESAHA.114.302810
19. Dubash AD, Kam CY, Aguado BA, Patel D, Delmar M, Shea LD, et al. Plakophilin-2 loss promotes TGF-beta1/p38 MAPK-dependent fibrotic gene expression in cardiomyocytes. *J Cell Biol.* (2016) 212:425–38. doi: 10.1083/jcb.201507018
20. Groeneweg JA, Bhonsale A, James CA, te Riele AS, Dooijes D, Tichnell C, et al. Clinical presentation, long-term follow-up, and outcomes of 1001 arrhythmogenic right ventricular dysplasia/cardiomyopathy patients and family members. *Circ Cardiovasc Genet.* (2015) 8:437–46. doi: 10.1161/CIRCGENETICS.114.001003
21. Bhonsale A, Groeneweg JA, James CA, Dooijes D, Tichnell C, Jongbloed JD, et al. Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. *Eur Heart J.* (2015) 36:847–55. doi: 10.1093/eurheartj/ehu509
22. Pilichou K, Thiene G, Bauce B, Rigato I, Lazzarini E, Migliore F, et al. Arrhythmogenic cardiomyopathy. *Orphanet J Rare Dis.* (2016) 11:33. doi: 10.1186/s13023-016-0407-1
23. van Tintelen JP, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld AC, Wilde AA, et al. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation* (2006) 113:1650–8. doi: 10.1161/CIRCULATIONAHA.105.609719
24. den Haan AD, Tan BY, Zikusoka MN, Llado L, Jain R, Daly A, et al. Comprehensive desmosome mutation analysis in north americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circ Cardiovasc Genet.* (2009) 2:428–35. doi: 10.1161/CIRCGENETICS.109.858217

25. Pilichou K, Lazzarini E, Rigato I, Celeghin R, De Bortoli M, Perazzolo Marra M, et al. Large genomic rearrangements of desmosomal genes in italian arrhythmogenic cardiomyopathy patients. *Circ Arrhythm Electrophysiol.* (2017) 10:e005324. doi: 10.1161/CIRCEP.117.005324
26. Bezzina CR, Barc J, Mizusawa Y, Remme CA, Gourraud J, Simonet F, et al. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. *Nat Genet.* (2013) 45:1044–9. doi: 10.1038/ng.2712
27. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, 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) 17:405–24. doi: 10.1038/gim.2015.30
28. Cerrone M, Lin X, Zhang M, Agullo-Pascual E, Pfenniger A, Chkourko Gusky H, et al. Missense mutations in plakophilin-2 cause sodium current deficit and associate with a brugada syndrome phenotype. *Circulation* (2014) 129:1092–103. doi: 10.1161/CIRCULATIONAHA.113.003077
29. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, et al. Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation* (2005) 111:659–70. doi: 10.1161/01.CIR.0000152479.54298.51
30. Cerrone M. Controversies in Brugada syndrome. *Trends Cardiovasc Med.* (2017) 28:284–92. doi: 10.1016/j.tcm.2017.11.003
31. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm* (2011) 8:1308–39. doi: 10.1016/j.hrthm.2011.05.020
32. Corrado D, Basso C, Buja G, Nava A, Rossi L, Thiene G. Right bundle branch block, right precordial st-segment elevation, and sudden death in young people. *Circulation* (2001) 103:710–17. doi: 10.1161/01.CIR.103.5.710
33. Forkmann M, Tomala J, Huo Y, Mayer J, Christoph M, Wunderlich C, et al. Epicardial ventricular tachycardia ablation in a patient with brugada ECG pattern and mutation of PKP2 and DSP genes. *Circ Arrhythm Electrophysiol.* (2015) 8:505–7. doi: 10.1161/CIRCEP.114.002342
34. Allegue C, Coll M, Mates J, Campuzano O, Iglesias A, Sobrino B, et al. Genetic analysis of arrhythmogenic diseases in the Era of NGS: the complexity of clinical decision-making in brugada syndrome. *PLoS ONE* (2015) 10:e0133037. doi: 10.1371/journal.pone.0133037
35. Hosseini SM, Kim R, Udupa S, Costain G, Jobling R, Liston E, et al. Reappraisal of reported genes for sudden arrhythmic death: an evidence-based evaluation of gene validity for brugada syndrome. *Circulation* (2018) 138:1195–205. doi: 10.1161/CIRCULATIONAHA.118.035070
36. Zhang M, Tavora F, Oliveira JB, Li L, Franco M, Fowler D, et al. PKP2 mutations in sudden death from arrhythmogenic right ventricular cardiomyopathy (ARVC) and sudden unexpected death with negative autopsy (SUDNA). *Circ J.* (2012) 76:189–94. doi: 10.1253/circj.CJ-11-0747
37. Dewar LJ, Alcaide M, Fornika D, D'Amato L, Shafaatalab S, Stevens C, et al. Investigating the genetic causes of sudden unexpected death in children through targeted next-generation sequencing analysis. *Circ Cardiovasc Genet.* (2017) 10:e001738. doi: 10.1161/CIRCGENETICS.116.001738
38. Anderson JH, Tester DJ, Will ML, Ackerman M. Whole-exome molecular autopsy after exertion-related sudden unexplained death in the young. *Circ Cardiovasc Genet.* (2016) 9:259–65. doi: 10.1161/CIRCGENETICS.115.001370
39. Waldmuller S, Schroeder C, Sturm M, Scheffold T, Imbrich K, Junker S, et al. Targeted 46-gene and clinical exome sequencing for mutations causing cardiomyopathies. *Mol Cell Probes* (2015) 29:308–14. doi: 10.1016/j.mcp.2015.05.004
40. Bainbridge MN, Li L, Tan Y, Cheong BY, Marian A. Identification of established arrhythmogenic right ventricular cardiomyopathy mutation in a patient with the contrasting phenotype of hypertrophic cardiomyopathy. *BMC Med Genet.* (2017) 18:24. doi: 10.1186/s12881-017-0385-8
41. Ramond F, Janin A, Di Filippo S, Chanavat V, Chalabreysse L, Roux-Buisson N, et al. Homozygous PKP2 deletion associated with neonatal left ventricle noncompaction. *Clin Genet.* (2017) 91:126–30. doi: 10.1111/cge.12780
42. Walsh R, Buchan R, Wilk A, John S, Felkin LE, Thomson KL, et al. Defining the genetic architecture of hypertrophic cardiomyopathy: re-evaluating the role of non-sarcomeric genes. *Eur Heart J.* (2017) 38:3461–8. doi: 10.1093/eurheartj/ehw603

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REVIEW
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Cardiac Sympathetic Denervation in Channelopathies

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Left cardiac sympathetic denervation (LCSD) is a surgical antiadrenergic intervention with a strong antiarrhythmic effect, supported by preclinical as well as clinical data. The mechanism of action of LCSD in structurally normal hearts with increased arrhythmic susceptibility (such as those of patients with channelopathies) is not limited to the antagonism of acute catecholamines release in the heart. LCSD also conveys a strong anti-fibrillatory action that was first demonstrated over 40 years ago and provides the rationale for its use in almost any cardiac condition at increased risk of ventricular fibrillation. The molecular mechanisms involved in the final antiarrhythmic effect of LCSD turned out to be much broader than anticipated. Beside the vagotonic effect at different levels of the neuraxis, other new mechanisms have been recently proposed, such as the antagonism of neuronal remodeling, the antagonism of neuropeptide Y effects, and the correction of neuronal nitric oxide synthase (nNOS) imbalance. The beneficial effects of LCSD have never been associated with a detectable deterioration of cardiac performance. Finally, patients express a high degree of satisfaction with the procedure. In this review, we focus on the rationale, results and our personal approach to LCSD in patients with channelopathies such as long QT syndrome and catecholaminergic polymorphic ventricular tachycardia.

Keywords: sudden cardiac death, cardiac sympathetic denervation, long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, cardiac autonomic nervous system

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INTRODUCTION

The management of patients at risk of life-threatening arrhythmias is challenging, more now than ever. On one hand, our capability to identify the subjects at higher risk of sudden cardiac death (SCD) is still limited (1). On the other, the widespread availability of implantable cardioverter defibrillators (ICDs) is a double edge sword. Not only because of the risk of side effects but also because in peculiar settings ICDs may even become pro-arrhythmic. Additionally, recurrent ICD shocks have a dramatic impact on the quality of life. These drawbacks are particularly evident in young patients with inherited arrhythmogenic disorders. The management of these subjects is further complicated by the unlikely feasibility of randomized clinical trials in this setting, which may give the wrong perception of lack of strong evidence for a specific treatment. Left cardiac sympathetic denervation (LCSD) is an extremely effective but still underutilized anti-adrenergic therapy. LCSD has a strong physiological rationale, combined with consistent preclinical results, and clinical data from well-conducted multicenter registries.

In this review we will first summarize the history and the antiarrhythmic rationale for LCSD, including well-established antiarrhythmic mechanisms as well as potential new mechanisms. Then, we will present the clinical results of LCSD in Long QT Syndrome (LQTS) and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), including both secondary and primary prevention. Finally, we will provide our approach for LCSD use in LQTS and CPVT.

ORGANIZATION OF CARDIAC SYMPATHETIC NERVOUS SYSTEM IN HUMANS

The two opposite branches of cardiac autonomic nervous system (ANS), namely the sympathetic and the parasympathetic nervous system, share a common embryological origin from the neuronal crest (2). The sympathetic cardiac ANS follows typical patterns in most people, although variants are seen (3, 4). It is constituted by the mediastinal cardiac plexus, the paravertebral sympathetic ganglia, the dorsal root ganglia (DRG), the spinal cord, and the brain stem. Cardiac sympathetic afferent fibers provide beat-to-beat information centrally as their sensory endings are mechanoreceptors (5). The extracardiac afferent stations, containing pseudounipolar nerve cells, are the DRG from C7 to T4 spinal cord level. Of note, cardiac sympathetic afferent fibers travel across the paravertebral sympathetic ganglia (without having synapsis) before reaching the DRG. Efferent sympathetic preganglionic neurons have their soma in the intermediolateral column of spinal cord and synapses on postganglionic neurons located in the lower cervical and upper thoracic paravertebral ganglia. The lowest cervical ganglion (C8) and the highest thoracic ganglion (T1) are generally fused bilaterally to constitute the left and the right stellate ganglia (also referred to as cervicothoracic ganglia). In <3% of human sympathetic chains, the second thoracic ganglion (T2) is fused as well, constituting a trilobal (C8-T1-T2) stellate ganglion (3). The stellate ganglia convey a consistent amount of cardiac sympathetic postganglionic fibers. The remaining is provided by T2–4 paravertebral ganglia. **Figure 1** summarizes cardiac nervous system organization in humans.

HISTORICAL PROSPECTIVE

In 1899, (6) Francois-Frank was the first to suggest that the removal of cervicothoracic sympathetic nervous system could prevent angina pectoris episodes. The first intervention was performed in 1916 by Jonnesco (7). He removed the left stellate ganglion (LSG) in a patient suffering incapacitating angina associated with cardiac arrhythmias, with effective and long-lasting suppression of both conditions. This pioneering intervention was strongly criticized due to the potential detrimental effects of depriving patients of the warning signal represented by pain. Moreover, the consequences of left stellectomy on coronary flow were still unclear. In 1929, Leriche and Fontaine (8) demonstrated that the sympathetic nerves exert a vasoconstrictive effect on the coronary arteries and not a vasodilator one, as previously thought. Subsequently, several clinical studies were performed in both Europe and the USA, confirming that left stellectomy was able to prevent anginal attacks (9), and to improve exercise tolerance (10). Concerning the optimal extension of the procedure, cervico-thoracic denervation (removal of the stellate ganglion and T2–T4 thoracic ganglia) proved to be the most effective. Finally, in the 60s, despite its clear efficacy, left cardiac sympathetic denervation (LCSD) was progressively abandoned for the treatment of angina due to the widespread usage of surgical coronary artery bypass graft and β-adrenergic-receptor blockers (11).

Except for some case reports (12, 13) the antiarrhythmic potential of cardiac sympathetic denervation in humans remained largely unexplored until the 70s. In 1971, Moss and McDonald (14) were the first to report LCSD in a LQTS patient with recurrent syncopal episodes. The rationale was based on a canine study (15) showing a consistent QT interval prolongation after either right stellate ganglionectomy or LSG stimulation. The patient underwent removal of the sympathetic ganglia from C7 to T2, including the entire LSG. Besides the suppression of arrhythmias, a persistent QT interval reduction was noticed. Subsequently, other groups tried to reproduce the beneficial effects on QT interval through the reversible percutaneous block of the LSG, with inconsistent results (16). Of note, at that time the appearance of Horner syndrome was considered as a good marker of the effective blockade of cardiac nerves. On the contrary, as pointed out already in 1975 (17), the Horner syndrome simply indicates an effective blockade of the sympathetic fibers traveling in the upper part of the stellate ganglion and innervating the eye. It does not necessarily indicate the block of the sympathetic fibers reaching the heart. Moreover, unlike in dogs and cats, in humans cardiac sympathetic innervation is not entirely provided by the stellate ganglia.

A better understanding of the rationale for LCSD in LQTS originated from the work by Schwartz and associates. Schwartz started from the observation in his first LQTS patient that sympathetic activation was triggering macroscopic T wave alternans, and he then reproduced in cats both QT prolongation and T-wave alternans by electrical stimulation of the LSG (18). On this basis, the young patient was treated with LCSD (remaining asymptomatic more than 40 years after) and sympathetic imbalance with left-sided dominance was proposed as the pathophysiological mechanisms of LQTS (17, 19). This concept prompted a large series of experimental studies investigating the consequences of unilateral (right or left) cardiac sympathetic denervation (20–22).

ANTIARRHYTHMIC RATIONALE AND MECHANISMS OF ACTION OF CARDIAC SYMPATHETIC DENERVATION

Antiadrenergic Effects

In 1976, Schwartz et al. showed in anesthetized dogs (20) that ischemia-related arrhythmias were increased by right stellate ganglion block and decreased by LSG block. In a

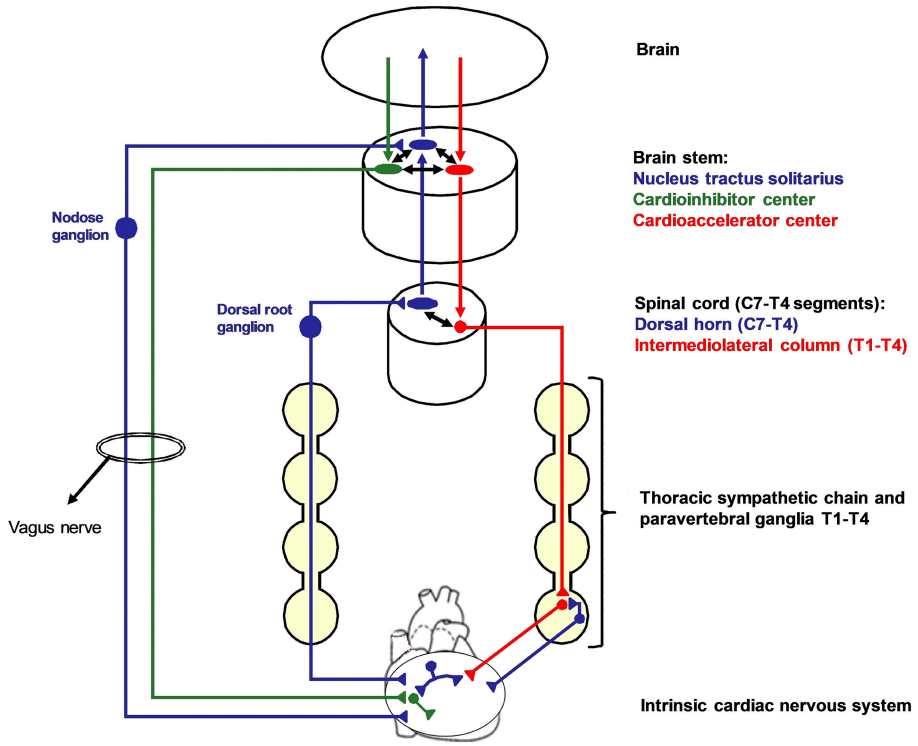

FIGURE 1 | Cardiac nervous system organization in humans. Blue: afferent nervous system with its ganglia: nodose ganglia and C7-T4 dorsal root ganglia (DRG). Green: parasympathetic efferent nervous system. Red: sympathetic efferent nervous system. All the afferent and efferent structures outside the central nervous systems are bilateral, although mostly represented as unilateral for simplicity. Cardiac afferent fibers traveling across the paravertebral sympathetic ganglia (usually referred to as cardiac sympathetic afferent fibers) directly reach the DRG without having synapsis before. These fibers mediate cardio-cardiac sympathoexcitatory spinal reflexes that significantly increase the sympathetic output to the heart. Left cardiac sympathetic denervation (LCSD) consists in the removal of the left thoracic sympathetic chain and paravertebral ganglia from T1 to T4. Since ipsilateral DRG are spared by LCSD, a left afferent reinnervation from the DRG to the heart is theoretically possible with time. On the other hand, the left efferent sympathetic system from T1 to T4 is interrupted at a preganglionic level; therefore, no ipsilateral efferent sympathetic reinnervation is possible after LCSD.

conscious canine model (21), still considered as the most-clinically relevant experimental model of SCD (dogs with a healed myocardial infarction (MI) exposed to a brief coronary artery occlusion while exercising on a treadmill) left stellectomy confirmed its protective effect. The antagonism of ischemia-induced sympathetic activation (23) as well as the quantitative dominance of the left sided sympathetic nerves over the right (22) were the first antiarrhythmic mechanisms proposed for the protection associated with LSG block or removal. Next came the demonstration that VF threshold, a reliable and quantitative marker of cardiac electrical stability, was lower after unilateral right stellectomy and much higher after left stellectomy (24). These animal data provide a solid rationale for LCSD which goes far beyond LQTS and ischemia-related arrhythmias and could extend to every cardiac condition characterized by an increased susceptibility to VF. A major mechanism contributing to the protection is the net decrease in norepinephrine (NE) released in the left ventricle during sympathetic neural activation. Of note, the neural release of NE is an extremely inhomogeneous phenomenon (25–27). Indeed, sympathetic nerve stimulation rather than circulating norepinephrine, modulates T-peak to T-end interval (an ECG marker of dispersion of repolarization) by increasing global dispersion of repolarization (25, 28). In turn, a spatially inhomogeneous ventricular repolarization is a very well-defined pro-arrhythmic marker, both for scar related arrhythmias (29) and for functional reentrant arrhythmias such as polymorphic ventricular tachycardia and VF (30). The temporal dispersion of ventricular repolarization is important as well and, together with spatial dispersion, may lead to T wave alternans, an ECG marker of high electrical instability, both in case of macro- (18) and of microvolt alternans (31). Besides acting on the arrhythmic substrate, NE, like epinephrine, also modulates the trigger. Not only does it enhance automaticity in pacemaker cells in both the atria and the ventricles (32), but it also increases triggered activity including both early (EAD) (33) and delayed (DAD) afterdepolarizations (34, 35). Finally, LCSD has α-adrenergic-receptor blocking properties. Indeed, similarly to the effect of α-adrenergic-receptor blockade, and opposite to that of β-Blockade, LCSD increases myocardial reactive hyperemia, an index of the capability of the coronary bed to dilate (36). In

addition to providing the basis for the antianginal effect, this could contribute to the antiarrhythmic efficacy (37).

Vagotonic Effect

Animal studies clearly showed that LCSD is accompanied by a reflex increase in cardiac parasympathetic (vagal) efferent activity (38). In fact, LCSD interrupts the majority of centrally projecting cardiac sympathetic afferents, which have an inhibitory effect on the vagal outflow directed to the heart. In turn, experimental (39, 40) and clinical data (41, 42) showed that blunted vagal tone and reflexes can favor life-threatening arrhythmias; conversely, these arrhythmias can be counteracted in animals by direct vagal stimulation (43) or pharmacological activation (44). So far, the experience with direct vagal stimulation in humans is limited to heart failure patients (45–48). Accordingly, the vagotonic effect of LCSD is particularly relevant from an anti-arrhythmic point of view in conditions characterized by a chronic and progressive increase in sympathetic tone and a parallel decrease in central parasympathetic drive, such as myocardial infarction (MI) and heart failure (49). Some of these concepts are at the basis of an ongoing clinical trial which examines the potential benefit associated with LCSD in patients with advanced heart failure (50).

Other Antiarrhythmic Mechanisms

Antagonism of Neuronal Remodeling

In 2000, Cao et al. (51) demonstrated in dogs that an increased intra-cardiac sympathetic nerve regeneration (nerve sprouting), obtained by infusing nerve growth factor to the LSG, was associated with a greater susceptibility to spontaneous ventricular arrhythmias. Of note, an intracardiac neuronal remodeling including both denervation and nerve sprouting (52) may occur after any kind of myocardial injury (53). Several animal studies consistently showed the high arrhythmic susceptibility of the denervated myocardium (54, 55). Similarly, in patients with cardiomyopathy and an ejection fraction $\leq$35%, the degree of cardiac sympathetic denervation quantified either by cardiac iodine-123 metaiodobenzylguanidine (123I- MIBG) imaging (56) or by positron emission tomography with 11 C-meta-hydroxyephedrine (11C-HED PET) (57) was significantly associated with ventricular arrhythmic risk. The process of neuronal remodeling is not limited to the heart, involving also extracardiac structures such as the sympathetic thoracic ganglia and the DRG (58). Myocardial infarction in animal models, independently of the site, is associated with an increase in nerve density, neuronal size, and neuropeptide Y expression in both the left and right stellate ganglia (59, 60). The same remodeling was described in humans. In 2012, Ajijola et al. (61) reported a significant neuronal enlargement and an increased synaptic density in the LSG of patients with refractory ventricular arrhythmias and structural heart disease undergoing LCSD. A few years later the same group further enriched the description of the sympathetic ganglia in patients with cardiomyopathy and refractory ventricular arrhythmias undergoing cardiac sympathetic denervation (62) showing the presence of a remarkable inflammatory cells infiltration (CD3+ T cells and neutrophils), combined with neurochemical remodeling, oxidative stress, and satellite glial cell activation. Of note, among the 16 patients studied (mean 45 $\pm$ 15 years), 5 had no macroscopically clear myocardial scar at pre-operatory multimodal imaging. Almost no signs of local inflammation or neuronal remodeling were observed in the stellate ganglia used as controls, obtained from 8 organ donors (mean 28 $\pm$ 8 years) with normal hearts deceased either for traumatic reasons or by natural causes.

These findings raise the intriguing question about the potential primary role of sympathetic ganglia inflammation in triggering adrenergic related ventricular arrhythmias in structurally normal hearts. Rizzo et al. (63) found mild but distinct inflammatory infiltrates composed of CD3+ and CD8+ T cells and macrophages in the LSG of 12 LQTS/ CPVT patients (mean 23 $\pm$ 17 years). They were all heavily symptomatic patients who received LCSD in secondary prevention. The authors specifically searched for neurotropic viruses as a potential trigger for the immune cell infiltration, with negative findings. They proposed that T-cell–mediated cytotoxicity toward ganglion cells may prompt an increase in sympathetic efferent activity toward the heart, therefore acting as a trigger and/or an enhancer of electrical instability in patients already predisposed to arrhythmias, as it occurs in LQTS and CPVT patients. Of note, as pointed out by Moss et al. (64) in the editorial comments of the paper, all patients had either recurrent syncopal episodes or many ICD shocks before the ganglionectomy, although the time frame between the last events and LCSD was not provided by the authors. Syncopal events are associated with transient generalized hypoperfusion, while ICD shocks can damage the myocardium and the neuronal fibers (65). Therefore, the mild auto immune mediated ganglionic remodeling observed by Rizzo et al. could be the consequence rather than the cause of the arrhythmic episodes. Moreover, the stellate ganglia used as controls, obtained from 10 accidently deceased patients (mean 35 $\pm$ 18 years), showed signs of inflammatory activity with the same immunohistological pattern, albeit to a lesser extent. Finally, no specific data supporting an increased sympathetic neuronal activity, such as increased neuronal size, increased synaptic density or a neurochemical shift in adrenergic phenotype were provided, as opposed to the neuronal hypertrophy and adrenergic shift demonstrated by Ajijola et al. (61, 62) in the stellate ganglia of patients with cardiomyopathy (even without overt scar) and intractable ventricular arrhythmias.

When interpreting these results, it's important to remember that cardiac sympathetic ganglia are not routinely evaluated by pathologist in the postmortem examination. Therefore, histological findings from these tissues among sudden arrhythmic death victims are lacking. On the other hand, a direct and non-invasive anatomopathological assessment of cardiac sympathetic ganglia in living patients is challenging both with labeled positron emission tomography tracers and with magnetic resonance. Indirect information about ongoing extracardiac neuronal remodeling processes can be obtained through cardiac 123I-MIBG or 11C-HED PET images, which are by the way unable to distinguish between anatomical (related to a reduced fiber density) rather than purely functional neuronal fibers abnormalities. As a matter of fact, an abnormal 123I-MIBG

cardiac scintigraphy as compared with healthy controls was reported in LQTS (66, 67) patients as well as in patients with idiopathic ventricular tachycardia and fibrillation (68).

Overall, the intriguing question about the potential pro arrhythmic role of sympathetic ganglia inflammatory processes in channelopathies is still largely unsolved and should be properly assessed by larger studies. Nevertheless, cardiac 123I-MIBG data seem to support the presence of primary sympathetic nervous system abnormalities in these patients.

Antagonism of Neuropeptide Y

Neurotramitters other than NE released by sympathetic efferent fibers are an area of intense research. Co-release mainly occurs during high-level neuronal stimulation (69). The most studied sympathetic co-transmitter is neuropeptide Y (NPY) that has a long biological half-life and can be measured in peripheral blood (70). NYP was shown to inhibit acetylcholine (ACh) release from cardiac vagal postganglionic nerves (71–74) through Y2 receptors activation (75). NPY may also act on Y1 receptors on ventricular cardiomyocytes, affecting their electrophysiological properties. Optical mapping experiments in rats showed that NPY steepens the action potential duration restitution curve (76). Moreover, in Langendorff-perfused rat hearts with intact innervation only the combination of Y1 receptor antagonist with metoprolol was able to fully prevent the fall in VF threshold produced by prolonged high-frequency stellate stimulation (76). Finally, NPY is also a potent vasoconstrictor (77). In man, several studies already reported that plasmatic NPY levels rise following acute coronary syndromes (78) and in heart failure, showing a positive correlation with severity of heart failure and 1 year mortality (79, 80).

Correction of nNOS Imbalance

An additional neurotransmitter, which has recently gained attention, is neuronal nitric oxide (nNO). Neuronal nitric oxide synthase (nNOS), together with its adaptor protein (CAPON, codified by the gene NOS1AP, nitric oxide synthase 1 adaptor protein), is located in both intrinsic cardiac vagal neurons and postganglionic sympathetic neurons of the stellate ganglia. It acts locally as an intrinsic neuromodulator i.e., it is not released in the synaptic space but it acts in the synaptic cleft via stimulation of soluble guanylate cyclase, to generate cGMP. In turn, this prompts opposite effects in parasympathetic and sympathetic neurons. In parasympathetic neurons it leads to an increased release of Ach (81, 82), while in sympathetic neurons it causes a reduction in NE release (83, 84). Animal studies using viral vectors showed that an increase in nNOS may reverse impaired vagal (85) and exaggerated sympathetic drive (86, 87) in the spontaneously hypertensive rat. Moreover, in guinea pig overexpression of nNOS increased acetylcholine release and was associated with a trend of improved survival following MI (88). Interestingly, genetic studies not only consistently correlated genetic variation in NOS1AP with QT-interval duration in the general population (89–92), but also demonstrated their association with the risk for sudden death in general population (93) and the risk of drug-induced QT prolongation and ventricular arrhythmia (94). Additionally, NOS1AP was proved to be a genetic modifier in LQTS, both in a founder LQT1 population (95) and in a non-selected LQTS population including different genotypes (96). Of note, NOS1AP gene is also expressed at the cardiac level, and CAPON overexpression in isolated guinea pig myocytes causes attenuation of L-type calcium current, a slight increase in rapid delayed rectifier current (*I*Kr), and a shortening of action potential (97). So far, an increase in L-type calcium current (which is also enhanced by sympathetic activation) due to CAPON under expression has been advocated as the main mechanism responsible for NOS1AP genetic variant impact on QT interval duration and arrhythmias susceptibility. Nevertheless, it is intriguing to speculate that in LQTS patients (as well as in the general population), even in absence of overt inflammatory changes within the stellate ganglia, CAPON under expression (on genetic bases) may lead to an increased NE release during sympathetic activation and therefore an increased arrhythmic risk. Of note, the disruption in CAPON expression in LQTS could also be the functional result of a mild ganglionitis rather than the cause of it, potentially contributing to explain the pro arrhythmic impact of the mild auto immune mediated ganglionitis described by Rizzo et al. (63).

ADDITIONAL EFFECTS OF CARDIAC SYMPATHETIC DENERVATION ON THE HEART

Catecholamines, besides the arrhythmogenic potential, physiologically modulate nearly all cardiac functions, including inotropy, chronotropy, dromotropy, and lusitropy. Therefore, before systematically proposing LCSD in man, several experimental studies were performed in order to exclude any potential detrimental effect on the heart. In conscious dogs with a healed MI performing a submaximal exercise stress test, left ventricular contractility (assessed by dP/dt max) was not affected by left stellectomy (36). Moreover, LCSD did not reduce resting heart rate (HR) or chronotropic competence during effort. On the contrary, HR increase during exercise was slightly (6%) greater after LCSD. This apparently paradoxical effect was thought to be related to a controlateral reflex increase in right stellate ganglion activity. In fact, due to the asymmetric distributions of sympathetic cardiac nerves, the sinus node is under a predominant right-sided sympathetic control (98). In the same animal model (36) the maximal increase in HR during exercise was, respectively, 19 and 26% lower as compared to baseline (intact innervation) after bilateral and right only stellectomy. Finally, albeit no specific data about AV conduction were provided, the mean maximal HR reached (around 250 bpm) during effort after left stellectomy strongly argues against a significant impact of left stellectomy on dromotropy during sinus rhythm and in physiological conditions of sympathetic activation. This finding was in agreement with previous studies which showed that sympathetic innervation to the atria and the AV node is provided by both right and left sympathetic chain (99). Accordingly, recent data from patients with paroxysmal atrial fibrillation (AF) show equivalent electrophysiological effects of right and left stellate ganglion block (SGB) on both

atria: unilateral temporary SGB with lidocaine slightly prolongs atrial effective refractory period and consistently reduces AF inducibility and AF episodes duration (100).

Finally, a last concern was that LCSD could lead to post-denervation supersensitivity, a pro-arrhythmic condition characterized by increased sensitivity of the left ventricle to catecholamines after complete denervation. From a theoretical point of view this possibility appeared unlikely, because right-sided sympathetic nerves (preserved after LCSD) are known to contribute to left ventricular innervation (101, 102). Animal studies confirmed that catecholamine stores in the myocardium were not completely depleted after LCSD (103, 104). Moreover, unilateral left stellectomy did not increase either dP/dt max or the incidence of ventricular arrhythmias in response to intravenous norepinephrine (105). Of note, LCSD is a preganglionic denervation; therefore no ipsilateral sympathetic efferent reinnervation is possible.

LCSD IN CHANNELOPATHIES

LCSD in Long QT Syndrome: Reported Results

The first large-scale evaluation of LCSD efficacy in LQTS was published in 1991 (106). Among the 85 reported patients, 99% were symptomatic before surgery, including 60% who suffered at least one aborted cardiac arrest (ACA). After LCSD, symptomatic patients decreased from 99 to 45% ($P < 0.0001$), and the mean number of cardiac events/patient dropped from 22 to 1. Of note, there were no ICDs. Therefore, this report truly reflects the impact of LCSD on SCD: it occurred in 8% of this high-risk group during 6 years of mean follow-up. The largest series of LQTS patients undergoing LCSD was reported in 2004 (107). As in the previous study, 99% of the patients were symptomatic before surgery, including 48% with a previous ACA and 75% with recurrent syncope despite maximum-dose β-Blockers. The majority were female (69%), the median age at surgery was 17 years and the mean QTc was 543 ± 65 ms. The average follow-up periods pre and post-LCSD were 5 and 8 years, respectively. After LCSD, 46% of the patients remained asymptomatic, syncope occurred in 31%, ACA in 16%, and SCD in 7%. Mean yearly number of cardiac events/patient dropped by 91% ($P < 0.001$). Among the 5 patients with a preoperative ICD the median number shocks/patient decreased from 25 to 0. Of note, 51 patients (35%) were genotyped, including 18 LQT1, 15 LQT2, 8 LQT3 and 9 patients with Jervell and Lange-Nielsen syndrome (JLN). As expected, LCSD appeared to be more effective in LQT1 than in LQT2. Despite the very limited numbers, patients with LQT3 and JLN did not seem to have a worse outcome compared with LQT1 patients. Finally, after LCSD a clinically significant mean reduction of QTc interval (39 ms) was noticed. Neither a preoperative QTc value ≥500 ms nor a change <40 ms were associated with a higher risk of recurrences. On the other hand, the persistence of a QTc ≥ 500 ms within 6 months from surgery appeared to carry a significantly higher risk of future events.

Subsequently, a large program of LCSD in LQTS was started by Ackerman at the Mayo Clinic, with equally positive results (108). In 2013, he reported a specific analysis on predictors of recurrences after LCSD in LQTS (109). They studied 52 consecutive LQTS patients undergoing LCSD between 2005 and 2010 at Mayo Clinic (23 LQT1, 9 LTQ2, 4 LQT3, 9 carrying multiple mutations, 3 JLN, and 4 genotype negative). All the procedures were performed using the minimally invasive, video-assisted thoracoscopic technique (VATS), and the sympathetic chain was removed from T1 to T4. Mean age at surgery was 10 years, 54% were female and mean QTc pre LCSD was 528 ± 74 ms. Most of them (61%) had LCSD as primary prevention because of either high-risk conditions or β-Blocker intolerance. This is a significant difference with the two previously reported populations and reflects the growing confidence in the benefit of the procedure. Overall, 12 subjects suffered cardiac events after LCSD (mean follow-up 3.6 years). Among them, only 5 (10%) had no discernible reduction of the arrhythmic episodes (true non-responders). These 5 high risk patients, all heavily symptomatic before LCSD, included 3 LQT3 patients and 2 LQT1 patients with multiple mutations. All of them had a very early onset of the disease (4 at birth, one in the first year of life) with QTc values above 600 ms. On the contrary, none of the 12 patients who received LCSD for β-Blocker intolerance experienced events during follow up.

In the following years, other centers all over the word started to perform LCSD and to report their results, overall confirming the positive post-procedural outcomes (110–112). The majority were small case series, yet in 2015 Waddell-Smith et al. (113) reported about 40 LQTS patients treated with thoracoscopic LCSD in New Zealand. LCSD related side effects and the quality of life after LCSD were the main topics analyzed. Most patients were female (70%) and LQT1 (57%), 11 were LQT2, 1 LQT3 and 5 had a negative genetic test. Half of the patients were completely asymptomatic before the procedure, and only 2 (5%) had surgery because of recurrences on β- Blockers. The two main indications for LCSD were β-Blocker intolerance or contraindication (35% of the patients) and β-Blocker non-adherence (25%). Interestingly, 10% of the patients specifically requested the procedure to their cardiologists either to increase their sense of protection or because of their desire to perform high level sports. These data confirm the diffusion and the increase in confidence in the procedure. During a median follow up of 2.5 years only 2 patients (5%), including 1 JLN, had arrhythmic events (syncopal episodes). All patients reported high levels of postoperative satisfaction. **Table 1** summarizes indications and results of the largest case series reported of LCSD in LQTS with at least 1 year of follow up.

LCSD in Long QT Syndrome: Our Approach

LCSD is now a mainstay in the management of LQTS patients (117, 118). Most experts agree that whenever ICD shocks occur in LQTS patients on optimized medical therapy, LCSD should be offered. We believe that, considering the high impact of LCSD on quality of life in this setting, the procedure should be undertaken without delay after the first breakthrough ICD intervention. ICD recurrences can be very detrimental and may lead to depression and even to suicidal attempts, particularly in these adolescents already predisposed to both anxiety and depression

TABLE 1 | Largest case series reported of LCSD in LQTS (at least 10 patients with at least 1 year of follow up).

References	*N*	% Primary prevention	ICD	Mean follow up	Overall cardiac events*	ACA/ICD therapies	SCD	Resection sparing T1
Schwartz et al. (106)	85	1%	0%	6 years	45%	0%	8%	0%
Ouriel et al. (114)	10	10%	0%	1.3 years	10%	0%	10%	0%
Schwartz et al. (107)	147	1%	3%	8 years	54%	16%	7%	0%
Li et al. (110)	11	0%	0%	3 years	45%	0%	9%	100%
Collura et al. (108)	18	50%	56%	1.5 years	17%	17%	0%	0%
Bos et al. (109)	52	61%	31%	3.6 years	23%	nr	2%	0%
Hofferberth et al. (111)	13	8%	nr	3 years	38%	23%	0%	92%
Olde Nordkamp et al. (112)	12	8%	67%	2 years	50%	25%	8%	0%
Waddell-Smith et al. (113)	40	95%	nr	2.5 years**	5%	0%	0%	72%
Jang et al. (115)	14	57%	nr	2.5 years	7%	7%	0%	0%

**Syncope, aborted cardiac arrest, sudden cardiac death. **Median follow-up. ACA, aborted cardiac arrest; ICD, implantable cardioverter defibrillator; nr, not reported; SCD, sudden cardiac death. The study by Antiel et al. (116) was not included despite describing 41 LQTS patients who received LCSD because specific data about the arrhythmic burden pre-post LCSD in the subgroup of LQTS patients were not provided.*

because of the underlying disease (119, 120). Moreover, the acute proarrhythmic potential of ICD shocks due to pain perception, fear and subsequent increase in the sympathetic drive on the heart should never be neglected, as will be discussed in detail for CPVT patients. The management of subjects with a first syncopal episode occurring despite maximum tolerated dose β-Blocker therapy is more challenging. As a referral group with a long-standing experience in the treatment of LQTS patients, we advise caution before directly implanting an ICD in these cases. Instead, a careful clinical evaluation is needed. Due to its high efficacy and optimal tolerability, we believe that LCSD should be offered first, clearly explaining to the patients and their families that the procedure is not an alternative to ICD implantation (that may always be considered in a later stage) and that the overall risk of life-threatening events after LCSD is low, unless the patient shows characteristics of high risk. At the same time, the life-spanning risk of complications and psychological consequences related to ICD implantation in these young patients is high (121) and should be properly acknowledged during patient and family counseling. Overall, a proper patient-physician communication in this setting requires to offer LCSD as therapeutic option even if the center is not performing the procedure as an inside facility. Ignorance and/or omission may carry medicolegal implications for the physician (122). On the other hand, in the case of markers of high risk such as onset of the symptoms in the first year of life and/or the persistence of QTc values exceeding 550 ms after LCSD, an ICD could be considered immediately after LCSD. Another difficult issue is the management of patients who never suffered arrhythmic episodes on therapy (and even before) but with either high risk LQTS phenotype or β-blocker intolerance, which represents the so-called primary prevention. In these cases LCSD should be offered before ICD implantation, with the clear intention to serve as bridge to an ICD in the most severe cases. Of course, additional pharmacological strategies such as mexiletine, already proposed in 1995 (123) and now widely used (124–126) should be offered as well, according to the genotype and the specific mutation. Finally, an additional indication for LCSD in LQTS is β-Blocker non-compliance. Generally, patients and their families managed in referral centers are well-instructed about the importance of strictly adhering to the prescribed medical therapy. Nevertheless, young subjects, particularly adolescents, are challenging to manage and may refuse therapy. Since β-Blocker non-compliance is a very well-defined risk factor for arrhythmic events in LQTS (127), if suspected and not modifiable, this condition should prompt to consider LCSD as additional protective measure. Concerning the indication to right cardiac sympathetic denervation (RCSD) in LQTS, we reserve it for patients not responding to LCSD. We discourage RCSD or a direct bilateral cardiac sympathetic denervation (BCSD) in patients not carrying an ICD (or pacemaker) due to the potential pro-arrhythmic effect of the induced (and largely unpredictable) bradycardia, particularly in LQT2 and LQT3 patients.

LCSD in Catecholaminergic Polymorphic Ventricular Tachycardia

The efficacy of LCSD in CPVT is not surprising from a pathophysiological point of view. Indeed, the disease is characterized by an intrinsic increase in the sensitivity of the heart to catecholamines due to mutations affecting the diastolic release of calcium from the sarcoplasmic reticulum. The first case series (3 patients) describing the long-lasting efficacy of LCSD in high risk CVPT was published in 2008 (128). We subsequently reported in 2015 the largest case series of LCSD in CPVT (129). It was a multicentric, international study involving 63 CPVT patients (71% RyR2 positive, 8% CASQ2 positive) who underwent LCSD between 1988 and 2014 at 11 centers worldwide. The majority ($n = 54$, 86%) had the procedure in secondary prevention, 97% were on β-Blockers, 24% on flecainide. The median post-LCSD follow-up was 37 months. In the 9 asymptomatic patients there were no cardiac events during follow-up. Among the 54 patients with prior major cardiac events either on ($n = 38$) or off ($n = 16$) optimal medical therapy, 13 (24%) had at least 1 recurrence, but only 1 patient died suddenly (after having been switched from nadolol to metoprolol). Specifically, the percentage of patients with cardiac events despite optimal medical therapy ($n = 38$) was reduced

from 100 to 32% ($P < 0.001$) after LCSD, and among 29 patients with a pre-surgical ICD, the rate of shocks dropped by 93% from 3.6 to 0.6 per person per year ($P < 0.001$). Among the 13 patients with cardiac events after LCSD, only 5 (8%) had no reduction in the number of events as compared to before LCSD (true non-responders). Importantly, the only predictor of response was the extension of LCSD: 71% of the 7 patients with incomplete LCSD had recurrences as compared to 17% of those with a complete LCSD ($P < 0.01$). Among the 38 most severe patients, 100% of those with incomplete LCSD had recurrences (**Figure 2**). The most common reason for not performing a complete denervation was to reduce the risk of Horner syndrome. This is not justified since the incidence of permanent Horner syndrome when removing only the lower part of the stellate ganglion (T1) is extremely low (<2%). On the other hand, the antiarrhythmic protection when T1 is spared seems to be significantly lower, in agreement with pre-clinical data (130). In a subsequent exploratory sub analysis of the same population we focused on the 38 patients with an ICD (131). Our preliminary data suggest a reduction in supraventricular arrhythmias (SVA) leading to inappropriate ICD shocks after LCSD. Of course, this observation needs to be confirmed in a larger group of CPVT patients, but it seems very plausible from a pathophysiological point of view. Atrial arrhythmias (both atrial tachycardia and AF) in CPVT are typically triggered by catecholamines in the setting of structurally normal atria. Moreover, experimental animal models suggest that LCSD may increase the threshold for atrial arrhythmias onset and maintenance and reduce ventricular rate during atrial fibrillation (132–134).

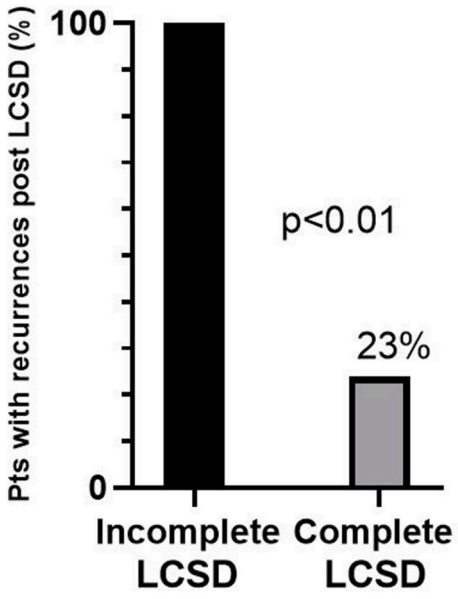

FIGURE 2 | Percentages of recurrences after left cardiac sympathetic denervation (LCSD) in 38 CPVT patients who had previously suffered major cardiac events despite optimal medical therapy. The majority received a complete LCSD ($n = 33$), the remaining ($n = 5$) an incomplete LCSD. Modified from De Ferrari et al. (129) with permission.

Subsequently, a multicentric pediatric registry including 18 CPVT patients undergoing LCSD confirmed our results, showing no recurrences of ventricular arrhythmias in 89% of the subjects (135).

LCSD in Catecholaminergic Polymorphic Ventricular Tachycardia: our Approach

LCSD is now an established therapy also for CPVT (117, 118). Our recommendations for LCSD in CPVT are similar to those already discussed for LQTS (first ICD shock or syncope on optimized medical therapy, β-Blockers intolerance or non-compliance), bearing in mind that the decision to implant an ICD in CPVT patients must be considered even more carefully than in LQTS. Indeed, due to the exquisite sensitiveness to catecholamine of their hearts, combined with a generally good hemodynamic tolerability of both rapid polymorphic VT and bidirectional tachycardia (which usually precede VF), CPVT patients are at high risk of electrical storms. This happens because the pain and the fear of the first ICD shock, which generally occurs in a condition of preserved consciousness, elicit a massive neural release of catecholamines, starting a vicious circle. As a matter of fact, in our registry of LCSD in CPVT (and therefore in an already selected subgroup of high-risk patients) we found that 36% of the patients who received an ICD before LCSD suffered at least one electrical storm or end of treatment condition (136). On the contrary, among the 26 pts with no ICD before LCSD, excluding two who had an electrical storm as first manifestation of the disease, none had such episodes on medical therapy. In agreement with this concept, sporadic cases of death in CPVT patients because of ongoing ventricular arrhythmias and exhaustion of ICD shocks have been reported for over 10 years (137–139). Very recently, the largest CPVT meta-analysis ever published (140) including 503 patients with an ICD (median age 15 years) reported a 1.4% mortality rate during follow-up, driven by 4 deaths due to electrical storms. The high incidence of both electrical storms (19.6%) and inappropriate shocks (20.8%) in trans venous ICD recipients is in full agreement with our data (129), as well as the disquieting rate of ICD-related complications (32.4%). Only 3 ICD patients had a subcutaneous ICD (S-ICD); 2 of them received inappropriate shocks due T-wave oversensing. Of note, the mortality rate among the 412 patients treated without ICD was similar to those with an ICD (2%).

Finally, beyond being potentially pro-arrhythmic and often not necessary, ICD shocks in CPVT patients may also be ineffective. Indeed, rapid polymorphic VT or bidirectional VT episodes may be not only self-limiting with the interruption of the stressor (such as physical activity) without the need for shock, but could also be less susceptible to cardioversion compared to VF episodes. Miyake et al. (141) demonstrated that among 10 CPVT patients who received a total of 75 appropriate shocks, only 57% of the shocks were successful in primary termination of the arrhythmias. The underlying rhythm in all successful ICD shocks at first attempt was VF, while no episode of polymorphic VT or bidirectional VT was successfully treated at the first attempt. Subsequently, Roses-Noguer F et al. (142) found an even lower success rate of the first appropriate ICD shock in CPVT

(32%), confirming the ineffectiveness on triggered arrhythmias as compared to VF. Moreover, also antitachycardia pacing therapies (ATPs), as expected, proved to be ineffective in CPVT.

For all the above mentioned reasons, the management of high risk CPVT patients is particularly challenging. An optimized antiadrenergic therapy based on the clinical phenotype should always be the main therapeutic goal, whether or not the patient is implanted with an ICD (or is a candidate to). Indeed, in complete agreement with the pathophysiology of the disease, β-Blockers (143) and LCSD (129) are the only therapeutic interventions with a proven efficacy on SCD, aborted cardiac arrest and ICD shocks. Flecainide, despite promising *in vitro* (144) and *in vivo* (135, 144–147) data mainly showing its efficacy on effort induced arrhythmias, still lacks a validation on hard clinical end points. Nevertheless, a first pharmacological attempt with flecainide in association to β-adrenergic blockade seems reasonable in β-Blocker non-responders, particularly if the patient has already been implanted with an ICD. Finally, as for LQTS patients, a careful ICD programming with a single VF zone, long detection times and no ATPs, is crucial in CPVT patients.

CONCLUSIONS

LCSD was proposed over one century ago for the treatment of angina pectoris. The antiarrhythmic potential of the technique, albeit evident since the first procedure by Jonnesco in 1916, took long to be fully appreciated (148). For many years the studies on LCSD were considered with skepticism, especially because there seemed to be just one group to support it. Finally, clinical data from well-conducted multicenter registries largely confirmed the preclinical findings, showing that LCSD is an effective treatment for drug-refractory ventricular arrhythmias in both LQTS and CPVT and LCSD is now recommended in recent guidelines (117, 118). Not surprisingly, considered the mechanism of action, the efficacy and potential indication of LCSD in channelopathies goes far beyond secondary prevention, potentially including many still asymptomatic patients with high-risk features for SCD despite optimized medical therapy. Regardless of this consistent body of evidence, LCSD is still an underutilized resource, as opposed to the often abused use of ICD in the same group of patients. From the technical point of view, the advantages of the thoracoscopic approach are such that it is difficult to see much room for different surgical approaches that might carry greater risks (149). LCSD can not only improve quality of life but also prevent fatal events that may still occur in patients with ICD due to the vicious circle of catecholamine-induced and maintained electrical storms.

AUTHOR CONTRIBUTIONS

VD and LP wrote a general manuscript draft. GD and PS supervised the process and edited the manuscript to its final version.

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REFERENCES

1. Wellens HJJ, Schwartz PJ, Lindemans FW, Buxton AE, Goldberger JJ, Hohnloser SH, et al. Risk stratification for sudden cardiac death: current status and challenges for the future. *Eur Heart J.* (2014) 35:1642–51. doi: 10.1093/eurheartj/ehu176
2. Vincent JW, Rubart M, Firulli AB. Ontogeny of cardiac sympathetic innervation and its implications for cardiac disease. *Pediatr Cardiol.* (2012) 33:923–8. doi: 10.1007/s00246-012-0248-1
3. Kawashima T. The autonomic nervous system of the human heart with special reference to its origin, course, and peripheral distribution. *Anat Embryol.* (2005) 209:425–38. doi: 10.1007/s00429-005-0462-1
4. Janes RD, Brandys JC, Hopkins DA, Johnstone DE, Murphy DA, Armour JA. Anatomy of human extrinsic cardiac nerves and ganglia. *Am J Cardiol.* (1986) 57:299–309. doi: 10.1016/0002-9149(86)90908-2
5. Malliani A, Recordati G, Schwartz PJ. Nervous activity of afferent cardiac sympathetic fibres with atrial and ventricular endings. *J Physiol.* (1973) 229:457–69. doi: 10.1113/jphysiol.1973.sp010147
6. Francois-Frank CA. Signification physiologique de la resection du sympathique dans la maladie de Basedow, l'epilepsie, l'idiotic et le glaucoma. *Bull Acad Méd Paris.* (1989) 41:565–94.
7. Jonnesco T. Traitement chirurgical de l'angine de poitrine par la résection du sympathique cervico-thoracique [French]. *Presse Méd.* (1921) 20:221–30.
8. Leriche R, Fontaine R. Rôle du ganglion étoile gauche dans le déterminisme de la crise de l'angine de poitrine [French]. *C. R. Acad Sci*. (1929) 188:279–80.
9. Burnett CF, Evans JA. Follow-up report on resection of the anginal pathway in thirty-three patients. *JAMA*. (1956) 162:709–12. doi: 10.1001/jama.1956.02970250009003
10. Cox WV. Influence of stellate ganglion block on angina pectoris and the post-exercise ECG. *Am J Med.* (1956) 252:289–95.
11. Schwartz PJ. The rationale and the role of left stellectomy for the prevention of malignant arrhythmias. *Ann N Y Acad Sci.* (1984) 427:199–221. doi: 10.1111/j.1749-6632.1984.tb20785.x
12. Estes EH, Izlar HR. Recurrent ventricular tachycardia: a case successfully treated by bilateral cardiac sympathectomy. *Am J Med.* (1961) 31:493–7. doi: 10.1016/0002-9343(61)90132-2
13. Zipes DP, Festoff B, Schaal SF, Cox C, Sealy WC, Wallace AG. Treatment of ventricular arrhythmia by permanent atrial pacemaker and cardiac sympathectomy. *Ann Intern Med.* (1968) 68:591–7. doi: 10.7326/0003-4819-68-3-591
14. Moss AJ, McDonald J. Unilateral cervicothoracic sympathetic ganglionectomy for the treatment of long QT interval syndrome. *N Engl J Med.* (1971) 285:903–4. doi: 10.1056/NEJM197110142851607
15. Yanowitz F, Preston JB, Abildskov JA. Functional distribution of right and left stellate innervation to the ventricles: production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. *Circ Res.* (1966) 18:416–28. doi: 10.1161/01.RES.18.4.416
16. Schwartz PJ. Cardiac sympathetic denervation to prevent life-threatening arrhythmias. *Nat Rev Cardiol.* (2014) 11:346–53. doi: 10.1038/nrcardio.2014.19
17. Schwartz PJ, Periti M, Malliani A. The long QT syndrome. *Am Heart J.* (1975) 89:378–90. doi: 10.1016/0002-8703(75)90089-7

18. Schwartz PJ, Malliani A. Electrical alternation of the T wave: clinical and experimental evidence of its relationship with the sympathetic nervous system and with the long QT syndrome. *Am Heart J.* (1975) 89:45–50. doi: 10.1016/0002-8703(75)90008-3
19. Schwartz PJ. Idiopathic long QT syndrome: progress and questions. *Am Heart J.* (1985) 109:399–411. doi: 10.1016/0002-8703(85)90626-X
20. Schwartz P J, Stone HL, Brown AM. Effects of unilateral stellate ganglion blockade on the arrhythmias associated with coronary occlusion. *Am. Heart J.* (1976) 92:589–99. doi: 10.1016/S0002-8703(76)80078-6
21. Schwartz PJ, Billman GE, Stone HL. Autonomic mechanisms in ventricular fibrillation induced by myocardial ischemia during exercise in dogs with healed myocardial infarction: an experimental preparation for sudden cardiac death. *Circulation.* (1984) 69:790–800. doi: 10.1161/01.CIR.69.4.790
22. Schwartz PJ. Sympathetic imbalance and cardiac arrythmias. In: Randall WC, editor. *Nervous Control of Cardiovascular Function*. New York, NY; Oxford: Oxford University Press (1984). p. 225–52.
23. Malliani A, Schwartz PJ, Zanchetti A. A sympathetic reflex elicited by experimental coronary occlusion. *Am J Physiol.* (1969) 217:703–9. doi: 10.1152/ajplegacy.1969.217.3.703
24. Schwartz PJ, Snebold NG, Brown AM. Effects of unilateral cardiac sympathetic denervation on the ventricular fibrillation threshold. *Am. J. Cardiol.* (1976) 37:1034–40. doi: 10.1016/0002-9149(76)90420-3
25. Han J, Moe GK. Nonuniform recovery of excitability in ventricular muscle. *Circ Res.* (1964) 14:44–60. doi: 10.1161/01.RES.14.1.44
26. Opthof T, Misier AR, Coronel R, Vermeulen JT, Verberne HJ, Frank RG, et al. Dispersion of refractoriness in canine ventricular myocardium. Effects of sympathetic stimulation. *Circ Res.* (1991) 68:1204–15. doi: 10.1161/01.RES.68.5.1204
27. Ramirez RJ, Ajijola OA, Zhou W, Holmström B, Lüning H, Laks MM, et al. A new electrocardiographic marker for sympathetic nerve stimulation: modulation of repolarization by stimulation of stellate ganglia. *J Electrocardiol.* (2011) 44:694–9. doi: 10.1016/j.jelectrocard.2011.07.030
28. Yagishita D, Chui RW, Yamakawa K, Rajendran PS, Ajijola OA, Nakamura K, et al. Sympathetic nerve stimulation, not circulating norepinephrine, modulates T-peak to T-end interval by increasing global dispersion of repolarization. *Circ Arrhythm Electrophysiol.* (2015) 8:174–85. doi: 10.1161/CIRCEP.114.002195
29. Vaseghi M, Lux RL, Mahajan A, Shivkumar K. Sympathetic stimulation increases dispersion of repolarization in humans with myocardial infarction. *Am J Physiol Heart Circ Physiol.* (2012) 302:H1838–46. doi: 10.1152/ajpheart.01106.2011
30. Antzelevitch C. Role of spatial dispersion of repolarization in inherited and acquired sudden cardiac death syndromes. *Am J Physiol Heart Circ Physiol.* (2007) 293:H2024–38. doi: 10.1152/ajpheart.00355.2007
31. Nearing BD, Oesterle SN, Verrier RL. Quantification of ischaemia induced vulnerability by precordial T-wave alternans analysis in dog and human. *Cardiovasc Res.* (1994) 28:1440–9. doi: 10.1093/cvr/28.9.1440
32. Zipes DP, Barber MJ, Takahashi N, Gilmour RF Jr. Influence of the autonomic nervous system on the genesis of cardiac arrhythmias. *Pacing Clin Electrophysiol.* (1983) 6:1210–20. doi: 10.1111/j.1540-8159.1983.tb04459.x
33. Ben-David J, Zipes DP. Differential response to right and left ansae subclaviae stimulation of early afterdepolarizations and ventricular tachycardia induced by cesium in dogs. *Circulation.* (1988) 78:1241–50. doi: 10.1161/01.CIR.78.5.1241
34. Charpentier F, Drouin E, Gauthier C, LeMarec H. Early after/depolarizations and triggered activity: mechanisms and autonomic regulation. *Fundam Clin Pharmacol.* (1993) 7:39–49. doi: 10.1111/j.1472-8206.1993.tb00216.x
35. Priori SG, Mantica M, Schwartz PJ. Delayed after depolarizations elicited *in vivo* by left stellate ganglion stimulation. *Circulation.* (1988) 78:178–85. doi: 10.1161/01.CIR.78.1.178
36. Schwartz PJ, Stone HL. Effects of unilateral stellectomy upon cardiac performance during exercise in dogs. *Circ Res.* (1979) 44:637–45. doi: 10.1161/01.RES.44.5.637
37. Schwartz PJ, Vanoli E, Zaza A, Zuanetti G. The effect of antiarrhythmic drugs on life-threatening arrhythmias induced by the interaction between acute myocardial ischemia and sympathetic hyperactivity. *Am. Heart J.* (1985) 109:937–48. doi: 10.1016/0002-8703(85)90233-9
38. Cerati D, Schwartz PJ. Single cardiac vagal fibers activity, acute myocardial ischemia, and risk for sudden death. *Circ Res.* (1991) 69:1389–1401. doi: 10.1161/01.RES.69.5.1389
39. Billman GE, Schwartz PJ, Stone HL. Baroreceptor reflex control of heart rate: a predictor of sudden cardiac death. *Circulation.* (1982) 66:874–80. doi: 10.1161/01.CIR.66.4.874
40. Schwartz PJ, Vanoli E, Stramba-Badiale M, De Ferrari GM, Billman GE, Foreman RD. Autonomic mechanisms and sudden death: new insights from analysis of baroreceptor reflexes in conscious dogs with and without a myocardial infarction. *Circulation.* (1998) 78:969–79. doi: 10.1161/01.CIR.78.4.969
41. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet.* (1998) 351:478–84. doi: 10.1016/S0140-6736(97)11144-8
42. La Rovere MT, Pinna GD, Hohnloser SH, Marcus FI, Mortara A, Nohara R, et al. Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening arrhythmias: implications for clinical trials. *Circulation.* (2001) 103:2072–7. doi: 10.1161/01.CIR.103.16.2072
43. Vanoli E, De Ferrari GM, Stramba-Badiale M, Hull SS Jr, Foreman RD, Schwartz PJ. Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction. *Circ Res.* (1991) 68:1471–81. doi: 10.1161/01.RES.68.5.1471
44. De Ferrari GM, Vanoli E, Curcuruto P, Tommasini G, Schwartz PJ. Prevention of life-threatening arrhythmias by pharmacologic stimulation of the muscarinic receptors with oxotremorine. *Am Heart J.* (1992) 124:883–90. doi: 10.1016/0002-8703(92)90968-2
45. De Ferrari GM, Crijns HJ, Borggrefe M, Milasinovic G, Smid J, Zabel M, et al. Chronic vagus nerve stimulation: a new and promising therapeutic approach for chronic heart failure. *Eur Heart J.* (2011) 32:847–55. doi: 10.1093/eurheartj/ehq391
46. Zannad F, De Ferrari GM, Tuinenburg AE, Wright D, Brugada J, Butter C, et al. Chronic vagal stimulation for the treatment of low ejection fraction heart failure: results of the NEural Cardiac TherApy foR Heart Failure (NECTAR-HF) randomized controlled trial. *Eur Heart J.* (2015) 36:425–33. doi: 10.1093/eurheartj/ehu345
47. Gold MD, Van Veldhuisen DJ, Hauptman PJ, Borggrefe M, Kubo SH, Lieberman RA, et al. Vagus nerve stimulation for the treatment of heart failure The INOVATE-HF Trial. *J Am Coll Cardiol.* (2016) 68:149–58. doi: 10.1016/j.jacc.2016.03.525
48. De Ferrari GM, Dusi V. Vagus nerve stimulation for the treatment of heart failure. *G Ital Cardiol.* (2015) 16:147–54. doi: 10.1714/1820.19822
49. Schwartz PJ, De Ferrari GM. Sympathetic-parasympathetic interaction in health and disease: abnormalities and relevance in heart failure. *Heart Fail Rev.* (2011) 16:101–7. doi: 10.1007/s10741-010-9179-1
50. Chin A, Ntsekhe M, Viljoen C, Rossouw J, Pennel T, Schwartz PJ. Rationale and design of a prospective study to assess the effect of left cardiac sympathetic denervation in chronic heart failure. *Int J Cardiol.* (2017) 248:227–31. doi: 10.1016/j.ijcard.2017.08.012
51. Cao JM, Chen LS, KenKnight BH, Ohara T, Lee MH, Tsai J, et al. Nerve sprouting and sudden cardiac death. *Circ Res.* (2000) 86:816–21. doi: 10.1161/01.RES.86.7.816
52. Oh YS, Jong AY, Kim DT, Li H, Wang C, Zemljic-Harpf A, et al. Spatial distribution of nerve sprouting after myocardial infarction in mice. *Heart Rhythm.* (2006) 3:728–36. doi: 10.1016/j.hrthm.2006.02.005
53. Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol.* (1997) 14:67–116. doi: 10.1007/BF02740621
54. Inoue H, Zipes DP. Time course of denervation of efferent sympathetic and vagal nerves after occlusion of the coronary artery in the canine heart. *Circ Res.* (1988) 62:1111–20. doi: 10.1161/01.RES.62.6.1111
55. Inoue H, Zipes DP. Results of sympathetic denervation in the canine heart: supersensitivity that may be arrhythmogenic. *Circulation.* (1987) 75:877–87. doi: 10.1161/01.CIR.75.4.877
56. Jacobson AF, Senior R, Cerqueira MD, Wong ND, Thomas GS, Lopez VA, et al. Myocardial iodine-123 meta-iodobenzylguanidine imaging and cardiac events in heart failure. Results of the prospective ADMIRE-HF (AdreView

Myocardial Imaging for Risk Evaluation in Heart Failure) study. *J Am Coll Cardiol*. (2010) 55:2212–21. doi: 10.1016/j.jacc.2010.01.014
57. Fallavollita JA, Heavey BM, Luisi AJ Jr, Michalek SM, Baldwa S, Mashtare TL Jr, et al. Regional myocardial sympathetic denervation predicts the risk of sudden cardiac arrest in ischemic cardiomyopathy. *J Am Coll Cardiol.* (2014) 63:141–9. doi: 10.1016/j.jacc.2013.07.096
58. Nakamura K, Ajijola OA, Aliotta E, Armour JA, Ardell JL, Shivkumar K. Pathological effects of chronic myocardial infarction on peripheral neurons mediating cardiac neurotransmission. *Auton Neurosci.* (2016) 197:34–40. doi: 10.1016/j.autneu.2016.05.001
59. Nguyen BL, Li H, Fishbein MC, Lin SF, Gaudio C, Chen PS, et al. Acute myocardial infarction induces bilateral stellate ganglia neural re modeling in rabbits. *Cardiovasc Pathol.* (2012) 21:143–8. doi: 10.1016/j.carpath.2011.08.001
60. Ajijola OA, Yagishita D, Reddy NK, Yamakawa K, Vaseghi M, Downs AM, et al. Remodeling of stellate ganglion neurons after spatially targeted myocardial infarction: neuropeptide and morphologic changes. *Heart Rhythm.* (2015) 12:1027–35. doi: 10.1016/j.hrthm.2015.01.045
61. Ajijola OA, Wisco JJ, Lambert HW, Mahajan A, Stark E, Fishbein MC, et al. Extracardiac neural remodeling in humans with cardiomyopathy. *Circ Arrhythm Electrophysiol.* (2012) 5:1010–116. doi: 10.1161/CIRCEP.112.972836
62. Ajijola OA, Hoover DB, Simerly TM, Brown TC, Yanagawa J, Biniwale RM, et al. Inflammation, oxidative stress, and glial cell activation characterize stellate ganglia from humans with electrical storm. *JCI Insight.* (2017) 2:e94715. doi: 10.1172/jci.insight.94715
63. Rizzo S, Basso C, Troost D, Aronica E, Frigo AC, Driessen AH, et al. T-cell-mediated inflammatory activity in the stellate ganglia of patients with ion-channel disease and severe ventricular arrhythmias. *Circ Arrhythm Electrophysiol.* (2014) 7:224–9. doi: 10.1161/CIRCEP.113.001184
64. Moss AJ, Ryan DH, Yeaney GA. Ganglionitis and genetic cardiac arrhythmias: more questions than answers. *Circ Arrhythm Electrophysiol*. (2014) 7:190–1. doi: 10.1161/CIRCEP.114.001589
65. Epstein AE, Kay GN, Plumb VJ, Dailey SM, Anderson PG. Gross and microscopic pathological changes associated with non thoracotomy implantable defibrillator leads. *Circulation.* (1998) 98:1517–24. doi: 10.1161/01.CIR.98.15.1517
66. Müller KD, Jakob H, Neuzner J, Grebe SF, Schlepper M, Pitschner HF.123I-metaiodobenzylguanidine scintigraphy in the detection of irregular regional sympathetic innervation in long QT syndrome. Eur Heart J. (1993) 14:316–25.
67. Kies P, Paul M, Gerss J, Stegger L, Mönnig G, Schober O, et al. Impaired cardiac sympathetic innervation in symptomatic patients with long QT syndrome. *Eur J Nucl Med Mol Imaging*. (2011) 38:1899–907. doi: 10.1007/s00259-011-1852-7
68. Schäfers M, Wichter T, Lerch H, Matheja P, Kuwert T, Schäfers K, et al. Cardiac 123I-MIBG uptake in idiopathic ventricular tachycardia and fibrillation. *J Nucl Med.* (1999) 40:1–5.
69. Burnstock G. Do some nerve cells release more than one transmitter? *Neuroscience.* (1976) 1:239–48. doi: 10.1016/0306-4522(76)90054-3
70. Burnstock G. Autonomic neurotransmission: 60 years since Sir Henry Dale. *Annu Rev Pharmacol Toxicol.* (2009) 49:1–30. doi: 10.1146/annurev.pharmtox.052808.102215
71. Potter EK, McCloskey DI. Peripheral inhibition of cardiac vagal action by sympathetic adrenergic stimulation. *Proc Aust Soc Clin Exp Pharmacol.* (1982) 13:99.
72. Potter E. Presynaptic inhibition of cardiac vagal postganglionic nerves by neuropeptide Y. *Neurosci Lett.* (1987) 83:101–6. doi: 10.1016/0304-3940(87)90223-0
73. Smith-White MA, Hardy TA, Brock JA, Potter EK. Effects of a selective neuropeptide Y Y2 receptor antagonist, BIIE0246, on Y2 receptors at peripheral neuroeffector junctions. *Br J Pharmacol.* (2001) 132:861–8. doi: 10.1038/sj.bjp.0703879
74. Smith-White MA, Herzog H, Potter EK. Role of neuropeptide Y Y(2) receptors in modulation of cardiac parasympathetic neurotransmission. *Regul Pept.* (2002) 103:105–11. doi: 10.1016/S0167-0115(01)00368-8
75. Herring N, Lokale MN, Danson EJ, Heaton DA, Paterson DJ. Neuropeptide Y reduces acetylcholine release and vagal bradycardia via a Y2 receptor-mediated, protein kinase C-dependent pathway. *J Mol Cell Cardiol.* (2008) 44:477–85. doi: 10.1016/j.yjmcc.2007.10.001
76. Herring N. Autonomic control of the heart: going beyond the classical neurotransmitters. *Exp Physiol.* (2015) 100:354–8. doi: 10.1113/expphysiol.2014.080184
77. Edvinsson L, Copeland JR, Emson PC, McCulloch J, Uddman R. Nerve fibers containing neuropeptide Y in the cerebrovascular bed: immunocytochemistry, radioimmunoassay, and vasomotor effects. *J Cereb Blood Flow Metab.* (1987) 7:45–57. doi: 10.1038/jcbfm.1987.7
78. Cuculi F, Herring N, De Caterina AR, Banning AP, Prendergast BD, Forfar JC, et al. Relationship of plasma neuropeptide Y with angiographic, electrocardiographic and coronary physiology indices of reperfusion during ST elevation myocardial infarction. *Heart.* (2013) 99:1198–203. doi: 10.1136/heartjnl-2012-303443
79. Hulting J, Sollevi A, Ullman B, Franco-Cereceda A, Lundberg JM. Plasma neuropeptide Y on admission to a coronary care unit: raised levels in patients with left heart failure. *Cardiovasc Res.* (1990) 24:102–8. doi: 10.1093/cvr/24.2.102
80. Ullman B, Hulting J, Lundberg JM. Prognostic value of plasma neuropeptide-Y in coronary care unit patients with and without acute myocardial infarction. *Eur Heart J.* (1994) 15:454–61. doi: 10.1093/oxfordjournals.eurheartj.a060526
81. Herring N, Golding S, Paterson DJ. Pre-synaptic NO-cGMP pathway modulates vagal control of heart rate in isolated adult guinea pig atria. *J Mol Cell Cardiol.* (2000) 32:1795–804. doi: 10.1006/jmcc.2000.1214
82. Herring N, Paterson DJ. Nitric oxide-cGMP pathway facilitates acetylcholine release and bradycardia during vagal nerve stimulation in the guinea-pig *in vitro*. *J Physiol.* (2001) 535:507–18. doi: 10.1111/j.1469-7793.2001.00507.x
83. Schwarz P, Diem R, Dun NJ, Forstermann U. Endogenous and exogenous nitric oxide inhibits norepinephrine release from rat heart sympathetic nerves. *Circ Res.* (1995) 77:841–8. doi: 10.1161/01.RES.77.4.841
84. Wang L, Henrich M, Buckler KJ, McMenamin M, Mee CJ, Sattelle DB, et al. Neuronal nitric oxide synthase gene transfer decreases [Ca2+]i in cardiac sympathetic neurons. *J Mol Cell Cardiol.* (2007) 43:717–25. doi: 10.1016/j.yjmcc.2007.09.005
85. Heaton DA, Li D, Almond SC, Dawson TA, Wang L, Channon KM, et al. Gene transfer of neuronal nitric oxide synthase into intracardiac ganglia reverses vagal impairment in hypertensive rats. *Hypertension.* (2007) 49:380–8. doi: 10.1161/01.HYP.0000255792.97033.f7
86. Li D, Wang L, Lee CW, Dawson TA, Paterson DJ. Noradrenergic cell specific gene transfer with neuronal nitric oxide synthase reduces cardiac sympathetic neurotransmission in hypertensive rats. *Hypertension.* (2007) 50:69–74. doi: 10.1161/HYPERTENSIONAHA.107.088591
87. Li D, Nikiforova N, Lu CJ, Wannop K, McMenamin M, Lee CW, et al. Targeted neuronal nitric oxide synthase transgene delivery into stellate neurons reverses impaired intracellular calcium transients in prehypertensive rats. *Hypertension.* (2013) 61:202–7. doi: 10.1161/HYPERTENSIONAHA.111.00105
88. Dawson TA, Li D, Woodward T, Barber Z, Wang L, Paterson DJ. Cardiac cholinergic NO-cGMP signaling following acute myocardial infarction and nNOS gene transfer. *Am J Physiol Heart Circ Physiol.* (2008) 295:H990–8. doi: 10.1152/ajpheart.00492.2008
89. Arking DE, Pfeufer A, Post W, Kao WH, Newton-Cheh C, Ikeda M, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet.* (2006) 38:644–51. doi: 10.1038/ng1790
90. Eijgelsheim M, Aarnoudse AL, Rivadeneira F, Kors JA, Witteman JC, Hofman A, et al. Identification of a common variant at the NOS1AP locus strongly associated to QT-interval duration. *Hum Mol Genet.* (2009) 18:347–57. doi: 10.1093/hmg/ddn341
91. Newton-Cheh C, Eijgelsheim M, Rice KM, de Bakker PI, Yin X, Estrada K, et al. Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nat Genet.* (2009) 41:399–406. doi: 10.1038/ng.364
92. Pfeufer A, Sanna S, Arking DE, Müller M, Gateva V, Fuchsberger C, et al. Common variants at ten loci modulate the QT interval duration in the QTSCD Study. *Nat Genet.* (2009) 41:407–14. doi: 10.1038/ng.362

93. Kao WH, Arking DE, Post W, Rea TD, Sotoodehnia N, Prineas RJ, et al. Genetic variations in nitric oxide synthase 1 adaptor protein are associated with sudden cardiac death in US white community-based populations. *Circulation.* (2009) 119:940–51. doi: 10.1161/CIRCULATIONAHA.108.791723
94. Jamshidi Y, Nolte IM, Dalageorgou C, Zheng D, Johnson T, Bastiaenen R, et al. Common variation in the NOS1AP Gene is associated with drug-induced QT prolongation and ventricular arrhythmia. *J Am Coll Cardiol.* (2012) 60:841–50. doi: 10.1016/j.jacc.2012.03.031
95. Crotti L, Monti MC, Insolia R, Peljto A, Goosen A, Brink PA, et al. NOS1AP is a genetic modifier of the long-QT syndrome. *Circulation.* (2009) 120:1657–63. doi: 10.1161/CIRCULATIONAHA.109.879643
96. Tomás M, Napolitano C, De Giuli L, Bloise R, Subirana I, Malovini A, et al. Polymorphisms in the NOS1AP gene modulate QT interval duration and risk of arrhythmias in the long QT syndrome. *J Am Coll Cardiol.* (2010) 55:2745–52. doi: 10.1016/j.jacc.2009.12.065
97. Chang KC, Barth AS, Sasano T, Kizana E, Kashiwakura Y, Zhang Y, et al. CAPON modulates cardiac repolarization via neuronal nitric oxide synthase signaling in the heart. *Proc Natl Acad Sci USA.* (2008) 105:4477–82. doi: 10.1073/pnas.0709118105
98. Randall WC, Rohse WG. The augmentor action of the sympathetic cardiac nerves. *Circ. Res.* (1956) 4:470–5. doi: 10.1161/01.RES.4.4.470
99. Geis WP, Kaye MP, Randall WC. Major autonomic pathways to the atria and S-A and A-V nodes in the canine heart. *Am J Physiol.* (1973) 224:202–8. doi: 10.1152/ajplegacy.1973.224.1.202
100. Leftheriotis D, Flevari P, Kossyvakis C, Katsaras D, Batistaki C, Arvaniti C, et al. Acute effects of unilateral temporary stellate ganglion block on human atrial electrophysiological properties and atrial fibrillation inducibility. Heart Rhythm. (2016) 13:2111–7. doi: 10.1016/j.hrthm.2016.06.025
101. Randall WC, Armour JA, Geis WP, Lippincott DB. Regional cardiac distribution of the sympathetic nerves. *Fed Proc.* (1972) 31:1199–208.
102. Kralios FA, Martin L, Burgess MJ, Millar K. Local ventricular repolarization changes due to sympathetic nerve-branch stimulation. *Am J Physiol.* (1975) 228:1621–6. doi: 10.1152/ajplegacy.1975.228.5.1621
103. Goodall M, Kirshner, N. Effect of cervico-thoracic ganglionectomy on the adrenaline and noradrenaline content in the mammalian heart. *J. Clin lnvest.* (1956) 35:649–56.
104. Hertting G, Schlefthaler TH. The effect of stellate ganglion excision on the catecholamine content and the uptake of 3H-norepinephrine in the heart of the cat. *lnt J Neuropharmacol.* (1964) 3:65–9.
105. Schwartz PJ, Stone HL. Left stellectomy and denervation supersensitivity in conscious dogs. *Am J Cardiol.* (1982) 49:1185–90. doi: 10.1016/0002-9149(82)90043-1
106. Schwartz PJ, Locati EH, Moss AJ, Crampton RS, Trazzi R, Ruberti U. Left cardiac sympathetic denervation in the therapy of congenital long QT syndrome: a worldwide report. *Circulation.* (1991) 84:503–11. doi: 10.1161/01.CIR.84.2.503
107. Schwartz PJ, Priori SG, Cerrone M, Spazzolini C, Odero A, Napolitano C, et al. Left cardiac sympathetic denervation in the management of high-risk patients affected by the long QT syndrome. *Circulation.* (2004) 109:1826–33. doi: 10.1161/01.CIR.0000125523.14403.1E
108. Collura CA, Johnson JN, Moir C, Ackerman MJ. Left cardiac sympathetic denervation for the treatment of long QT syndrome and catecholaminergic polymorphic ventricular tachycardia using video-assisted thoracic surgery. *Heart Rhythm.* (2009) 6:752–9. doi: 10.1016/j.hrthm.2009.03.024
109. Bos JM, Bos KM, Johnson JN, Moir C, Ackerman MJ. Left cardiac sympathetic denervation in Long QT Syndrome analysis of therapeutic nonresponders. *Circ Arrhythm Electrophysiol.* (2013) 6:705–11. doi: 10.1161/CIRCEP.113.000102
110. Li J, Liu Y, Yang F, Jiang G, Li C, Hu D, et al. Video-Assisted thoracoscopic left cardiac sympathetic denervation: a reliable minimally invasive approach for congenital long-QT syndrome. *Ann Thorac Surg.* (2008) 86:1955–9. doi: 10.1016/j.athoracsur.2008.07.100
111. Hofferberth SC, Cecchin F, Loberman D, Fynn-Thompson F. Left thoracoscopic sympathectomy for cardiac denervation in patients with life-threatening ventricular arrhythmias. *J Thorac Cardiovasc Surg.* (2014) 147:404–11. doi: 10.1016/j.jtcvs.2013.07.064
112. Olde Nordkamp LRA, Driessen AHG, Odero A, Blom NA, Koolbergen DR, Schwartz PJ, et al. Left cardiac sympathetic denervation in the Netherlands for the treatment of inherited arrhythmia syndromes. *Neth Heart J.* (2014) 22:160–6. doi: 10.1007/s12471-014-0523-2
113. Waddell-Smith KE, Ertresvaag KN, Li J, Chaudhuri K, Crawford JR, Hamill JK, et al. Physical and psychological consequences of left cardiac sympathetic denervation in Long-QT syndrome and catecholaminergic polymorphic ventricular tachycardia. *Circ Arrhythm Electrophysiol.* (2015) 8:1151–8. doi: 10.1161/CIRCEP.115.003159
114. Ouriel K, Moss A. Long QT syndrome: an indication for cervicothoracic sympathectomy. *Cardiovasc Surg.* (1995) 3:475–8. doi: 10.1016/0967-2109(95)94444-2
115. Jang S, Cho Y, Kim N, Kim C, Sohn J, Roh J, et al. Video assisted thoracoscopic left cardiac sympathetic denervation in patients with hereditary ventricular arrhythmias. *Pacing Clin Electrophysiol.* (2017) 40:232–41. doi: 10.1111/pace.13008
116. Antiel R, Bos M, Joyce D, Owen Moir HC, Ackerman M. Quality of life after videoscopic left cardiac sympathetic denervation in patients with potentially life threatening cardiac channelopathies/cardiomyopathies. *Heart Rhythm.* (2016) 13:62–9. doi: 10.1016/j.hrthm.2015.09.001
117. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the european society of cardiology (ESC). *Eur Heart J.* (2015) 36:2793–867. doi: 10.1093/eurheartj/ehv316
118. Al-Khatib SM, Stevenson WG, Ackerman MJ, Bryant WJ, Callans DJ, Curtis AB, et al. 2017 AHA/ACC/HRS guideline for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American college of cardiology/American heart association task force on clinical practice guidelines and the heart rhythm society. *J Am Coll Cardiol.* (2018) 72:e91–e220. doi: 10.1016/j.jacc.2017.10.054
119. Hamang A, Eide GE, Rokne B, Nordin K, Bjorvatn C, Øyen N. Predictors of heart-focused anxiety in patients undergoing genetic investigation and counseling of long QT syndrome or hypertrophic cardiomyopathy: a one year follow-up. *J Genet Couns.* (2012) 21:72–84. doi: 10.1007/s10897-011-9393-6
120. Hintsa T, Keltikangas-Järvinen L, Puttonen S, Ravaja N, Toivonen L, Kontula K, et al. Depressive symptoms in the congenital long QT syndrome. *Ann Med.* (2009) 41:516–21. doi: 10.1080/07853890903037254
121. Schwartz PJ, Spazzolini C, Priori SG, Crotti L, Vicentini A, Landolina M, et al. Who are the long-QT syndrome patients who receive an implantable cardioverter-defibrillator and what happens to them? Data from the European Long- QT Syndrome Implantable Cardioverter-Defibrillator (LQTS ICD) Registry. *Circulation.* (2010) 122:1272–82. doi: 10.1161/CIRCULATIONAHA.110.950147
122. Schwartz PJ. Efficacy of left cardiac sympathetic denervation has an unforeseen side effect: medicolegal complications. *Heart Rhythm.* (2010) 7:1330–2. doi: 10.1016/j.hrthm.2010.04.038
123. Schwartz PJ, Priori SG, Locati EH, Napolitano C, Cantù F, Towbin JA, et al. Long QT syndrome patients with mutations of the SCN5A and HERG genes have differential responses to Na+ channel blockade and to increases in heart rate. Implications for gene-specific therapy. *Circulation.* (1995) 92:3381–6. doi: 10.1161/01.CIR.92.12.3381
124. Schwartz OJ, Spazzolini C, Crotti L. All LQT3 patients need an ICD: true or false? *Heart Rhythm.* (2009) 6:113–20. doi: 10.1016/j.hrthm.2008.10.017
125. Mazzanti A, Maragna R, Faragli A, Monteforte N, Bloise R, Memmi M, et al. Gene-specific therapy with mexiletine reduces arrhythmic events in patients with Long QT Syndrome type 3. *J Am Coll Cardiol.* (2016) 67:1053–8. doi: 10.1016/j.jacc.2015.12.033
126. Bos JM, Crotti L, Rohatgi RK, Schwartz PJ, Ackerman MJ. Mexiletine shortens the QT interval in patients with either type 1 or type 2 Long QT. *Heart Rhythm.* (2018) 15 (Suppl.):S450.
127. Vincent GM, Schwartz PJ, Denjoy I, Swan H, Bithell C, Spazzolini C, et al. High Efficacy of beta-blockers in long-QT syndrome type 1. Contribution of noncompliance and QT-prolonging drugs to the occurrence of beta-blocker treatment "failures". *Circulation.* (2009) 119:215–21. doi: 10.1161/CIRCULATIONAHA.108.772533

128. Wilde AA, Bhuiyan ZA, Crotti L, Facchini M, De Ferrari GM, Paul T, et al. Left cardiac sympathetic denervation for catecholaminergic polymorphic ventricular tachycardia. *N Engl J Med.* (2008) 358:2024–9. doi: 10.1056/NEJMoa0708006
129. De Ferrari GM, Dusi V, Spazzolini C, Bos JM, Abrams DJ, Berul CI, et al. Clinical management of catecholaminergic polymorphic ventricular tachycardia: the role of left cardiac sympathetic denervation. *Circulation.* (2015) 131:2185–93. doi: 10.1161/CIRCULATIONAHA.115.015731
130. Buckley U, Yamakawa K, Takamiya T, Armour JA, Shivkumar K, Ardell JL. Targeted stellate decentralization: implications for sympathetic control of ventricular electrophysiology *Heart Rhythm.* (2016) 13:282–8. doi: 10.1016/j.hrthm.2015.08.022
131. Dusi V, De Ferrari GM, Bos JM, Moir CR, Shkolnikova M, Abrams DJ, et al. Supraventricular arrhythmias and implantable cardioverter defibrillator shocks in catecholaminergic polymorphic ventricular tachycardia. *Eur Heart J.* (2016) 37:83
132. Ogawa M, Tan AY, Song J, Kobayashi K, Fishbein MC, Lin SF, et al. Cryoablation of stellate ganglia and atrial arrhythmia in ambulatory dogs with pacing-induced heart failure. *Heart Rhythm.* (2009) 6:1772–9. doi: 10.1016/j.hrthm.2009.08.011
133. Chinda K, Tsai WC, Chan YH, Lin AY, Patel J, Zhao Y, et al. Intermittent left cervical vagal nerve stimulation damages the stellate ganglia and reduces the ventricular rate during sustained atrial fibrillation in ambulatory dogs. *Heart Rhythm.* (2016) 13:771–80. doi: 10.1016/j.hrthm.2015.11.031
134. Ye Zhao, Yuan Y, Tsai WU, Jiang Z, Zhi-peng Tian, Shen C, et al. Antiarrhythmic effects of stimulating the left dorsal branch of the thoracic nerve in a canine model of paroxysmal atrial tachyarrhythmias. *Heart Rhythm.* (2018) 15:1242–51. doi: 10.1016/j.hrthm.2018.04.009
135. Roston TM, Vinocur JM, Maginot KR, Mohammed S, Salerno JC, Etheridge SP, et al. Catecholaminergic polymorphic ventricular tachycardia in children: analysis of therapeutic strategies and outcomes from an international multicenter registry. *Circ Arrhythm Electrophysiol.* (2015) 8:633–42. doi: 10.1161/CIRCEP.114.002217
136. Dusi V, De Ferrari GM, Bos JM, Moir CR, Wilde AAM, Shkolnikova M, et al. Electrical storms in catecholaminergic polymorphic ventricular tachycardia: findings from the international registry of left cardiac sympathetic denervation. *Eur Heart J.* (2016) 37:215.
137. Mohamed U, Gollob MH, Gow RM, Krahn AD. Sudden cardiac death despite an implantable cardioverter-defibrillator in a young female with catecholaminergic ventricular tachycardia. *Heart Rhythm.* (2006) 3:1486–9. doi: 10.1016/j.hrthm.2006.08.018
138. Palanca V, Quesada A, Trigo A, Jimenez J. [Arrhythmic storm induced by AICD discharge in a patient with catecholaminergic polymorphic ventricular tachycardia]. *Rev Esp Cardiol.* (2006) 59:1079–80. doi: 10.1157/13093988
139. Pizzale S, Gollob MH, Gow R, Birnie DH. Sudden death in a young man with catecholaminergic polymorphic ventricular tachycardia and paroxysmal atrial fibrillation. *J Cardiovasc Electrophysiol.* (2008) 19:1319–21. doi: 10.1111/j.1540-8167.2008.01211.x
140. Roston TM, Jones K, Hawkins NM, Bos JM, Schwartz PJ, Perry F, et al. Implantable cardioverter-defibrillator use in catecholaminergic polymorphic ventricular tachycardia: a systematic review. *Heart Rhythm.* (2018) 12:1791–9. doi: 10.1016/j.hrthm.2018.06.046
141. Miyake CY, Webster G, Czosek RJ, Kantoch MJ, Dubin AM, Avasarala K, et al. Efficacy of implantable cardioverter defibrillators in young patients with catecholaminergic polymorphic ventricular tachycardia. Success depends on substrate. *Circ Arrhythm Electrophysiol.* (2013) 6:579–87. doi: 10.1161/CIRCEP.113.000170
142. Roses-Noguer F, Jarman JW, Clague JR, Till J. Outcomes of defibrillator therapy in catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm.* (2014) 11:58–66. doi: 10.1016/j.hrthm.2013.10.027
143. Hayashi M, Denjoy I, Extramiana F, Maltret A, Buisson NR, Lupoglazoff JM, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. *Circulation.* (2009) 119:2426–34. doi: 10.1161/CIRCULATIONAHA.108.829267
144. Watanabe H, Chopra N, Laver D, Hwang HS, Davies SS, Roach DE, et al. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. *Nat Med.* (2009) 15:380–3. doi: 10.1038/nm.1942
145. Van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, et al. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. *J Am Coll Cardiol.* (2011) 57:2244–54. doi: 10.1016/j.jacc.2011.01.026
146. Watanabe H, van der Werf C, Roses-Noguer F, Adler A, Sumitomo N, Veltmann C, et al. Effects of flecainide on exercise-induced ventricular arrhythmias and recurrences in genotype-negative patients with catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm.* (2013) 10:542–7. doi: 10.1016/j.hrthm.2012.12.035
147. Wangüemert Pérez F, Hernández Afonso JS, Groba Marco MDV, Caballero Dorta E, Álvarez Acosta L, Campuzano Larrea O, et al. Flecainide reduces ventricular arrhythmias in patients with genotype RyR2-positive catecholaminergic polymorphic ventricular tachycardia. *Rev Esp Cardiol.* (2018) 71:185–91. doi: 10.1016/j.recesp.2017.04.014
148. Schwartz PJ, De Ferrari GM, Pugliese L. Cardiac sympathetic denervation 100 years later: Jonnesco would have never believed it. *Int J Cardiol.* (2017) 15:237:25–8. doi: 10.1016/j.ijcard.2017.03.020
149. Schwartz PJ, Pugliese L. Cardiac sympathetic denervation via a transtracheal approach: it's a long way to tipperary. *Heart Rhythm.* (2018) 16:125–7. doi: 10.1016/j.hrthm.2018.09.020

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REVIEW
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TRP Channels Mediated Pathological Ca^{2+}-Handling and Spontaneous Ectopy

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Ion channel biology offers great opportunity in identifying and learning about cardiac pathophysiology mechanisms. The discovery of transient receptor potential (TRP) channels is an add-on to the opportunity. Interacting with numerous signaling pathways, being activated multimodally, and having prescribed signatures underlining acute hemodynamic control and cardiac remodeling, TRP channels regulate cardiac pathophysiology. Impaired Ca^{2+}-handling cause contractile abnormality. Modulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is a major part of Ca^{2+}-handling processes in cardiac pathophysiology. TRP channels including TRPM4 regulate $[Ca^{2+}]_i$, Ca^{2+}-handling and cardiac contractility. The channels modulate flux of divalent cations, such as Ca^{2+} during Ca^{2+}-handling and cardiac contractility. Seminal works implicate TRPM4 and TRPC families in intracellular Ca^{2+} homeostasis. Defective Ca^{2+}-homeostasis through TRP channels interaction with Ca^{2+}-dependent regulatory proteins such as sodium calcium exchanger (NCX) results in abnormal Ca^{2+} handling, contractile dysfunction and in spontaneous ectopy. This review provides insight into TRP channels mediated pathological Ca^{2+}-handling and spontaneous ectopy.

Keywords: TRP channels, SR Ca^{2+}-ATPase, NCX, ectopy, Ca^{2+}-handling

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INTRODUCTION

Ca^{2+} flux at organellar levels govern excitation and contraction coupling. The ionic movement is important. During action potential propagation, cell membranes depolarize, and Ca^{2+} enters into the cells. L-type calcium channel passes the Ca^{2+} influx as inward Ca^{2+} current (ICa,L), which contributes to the shape of action potential plateau. The Ca^{2+} entry initiates Ca^{2+} release from the sarcoplasmic reticulum (SR) by activation of ryanodine receptors (RyRs), thereby elevating $[Ca^{2+}]_i$ in the so-called Ca^{2+}-activated Ca^{2+} release. The raise in the $[Ca^{2+}]_i$, activated by a variety of ways, is a known mechanism of cell signaling. It enables Ca^{2+} to bind to contractile machineries such as myofilament protein and troponin C, and initiate contraction. The raise in the $[Ca^{2+}]_i$ contrarily declines in relaxation. Intracellular Ca^{2+} is pumped from the SR by sarcoplasmic reticulum calcium pump (SERCA), and sarcolemmal efflux through sodium calcium exchanger (NCX), with little contribution by the SERCA during relaxation (1). This results in a decline of $[Ca^{2+}]_i$, allowing Ca^{2+} to dissociate from troponin for relaxation to occur at diastole. While decline in $[Ca^{2+}]_i$ through Ca^{2+} efflux leads to relaxation, elevation in $[Ca^{2+}]_i$ through Ca^{2+} influx leads to contraction. The movement of monovalent and divalent cations through the channels is crucial to cardiac excitation and contraction coupling, and in this review, considerations are given to TRPC and TRPM channels in this process.

The process is impaired in contractile abnormality (2) and in general in heart diseases. Abnormal cardiac performance, which characterizes heart failure, cardiomyopathy, and arrhythmia, occurs through impaired Ca^{2+}-homeostasis. Compared with non-failing cardiomyocytes, failing cardiomyocytes SR Ca^{2+} re-uptake was reduced with no significant changes in the rate of Ca^{2+} efflux by NCX (3). Frequency-dependent increases in SR Ca^{2+} load, that was associated with a decline in contractile tone at high heart rates, was absent in failing myocardium (4). In addition, it has been demonstrated at single cell levels that alteration in Ca^{2+}-handling occurred through abnormal Ca^{2+}-homeostasis and increase in $[Ca^{2+}]_i$ in familial hypertrophic cardiomyopathy disease (5). The impaired Ca^{2+}-homeostasis may be due to translational and transcriptional changes at the level of expression of the Ca^{2+}-regulatory proteins. Chronic heart failure patients had abnormal changes in the levels of Ca^{2+}-regulatory proteins, such as the SR Ca^{2+}-ATPase (6). Together, impaired Ca^{2+}-homeostasis and altered Ca^{2+}-regulatory proteins orchestrate abnormal Ca^{2+}-handling. The mechanisms responsible for this are not completely elucidated.

Tremendous efforts at understanding the process is ongoing, but it is hampered by the complicated nature of changes in $[Ca^{2+}]_i$. Subcellular Ca^{2+} concentration is compartmentalized, and $[Ca^{2+}]_i$ varies tremendously. Furthermore, intracellular Ca^{2+} homeostasis can be regulated by local signaling processes within restricted space, rather than by just changes in the global cytosolic $[Ca^{2+}]_i$. With the complexities, it is difficult to completely define distinct Ca^{2+} homeostasis together with their $[Ca^{2+}]_i$ biological networks, and their roles. This may have implied that sources that control subcellular Ca^{2+} are just too numerous and are yet to be defined completely. This review discusses some transient receptor potential (TRP) channels seminal works in pathologic Ca^{2+}-handling and spontaneous ectopy.

REGULATION OF $[CA^{2+}]_i$ AND CA^{2+}-HANDLING

Regulation of $[Ca^{2+}]_i$, that is part of Ca^{2+} homeostasis, and governs Ca^{2+}-handling in excitation and contraction coupling is an important process of cardiac contractile performance. Cardiac contractility is the intrinsic ability of the myocardium to contract. Readily occurrence of contraction depends on incremental degrees of binding between myosin (thick) and actin (thin) filaments, when the $[Ca^{2+}]_i$ is elevated. Factors that promote Ca^{2+}-handling and contractility do so by inducing increases in the $[Ca^{2+}]_i$. The heart is unable to contract in too little Ca^{2+} concentration (small Ca^{2+} transient amplitude) and unable to relax in too much Ca^{2+} concentration (large Ca^{2+} transient amplitude), indicating negative and positive inotropy, respectively. TRP channels as cell membrane ion channel fall into a category and were first identified in impaired *Drosophila* visual adaption, where photoreceptors carrying TRP gene mutations showed a transient voltage response to continuous light (7, 8). Twenty eight mammalian TRP genes have been identified to date and have fallen into six related protein families. Most of them appear to be multimodally gated and interact with multiple signaling pathways and show pertinent trait to cardiac remodeling. While some are voltage dependent others are not, and they regulate a variety of cell functions such as, apoptosis, thermo regulation, cell viability and proliferation, and renal Ca^{2+} absorption. TRP channels are relatively non-selective permeable cation channels. As such, TRP channels permeate Na^{+}, Ca^{2+}, and Mg^{2+}, and thus regulate intracellular ionic concentrations, including $[Ca^{2+}]_i$. Seminal works (9–13) implicate some TRPM4 and TRPC channels in $[Ca^{2+}]_i$ variability. The channels, therefore, modulate Ca^{2+} transients, inotropy, and action potential wave form (**Figure 1**).

Transient receptor potential melastatin (TRPM) is a family of TRP ion channels, that consists of eight different subfamilies (TRPM1-TRPM8). TRPM4 subfamily while permeable to Na^{+} is activated by $[Ca^{2+}]_i$, in Ca^{2+}-induced Ca^{2+} release process and modulates inotropic β-adrenergic effects on ventricular heart muscle by increasing Ca^{2+} transients amplitude (11). The activation process is important because it contributes to Ca^{2+} transients. Ca^{2+}-handling, excitation-contraction coupling and action potential wave forms depend on Ca^{2+} transients, proofing that the $[Ca^{2+}]_i$ activation and inotropic β-adrenergic effects of TRPM4 are both important in contractility. TRPM4 inhibition altered action potential wave form and reduced action potential duration (13). More so, it has been proposed that TRPM4 channel activity might couple to $I\mathrm{Ca}_{,\mathrm{L}}$ functional activity in elevating $[Ca^{2+}]_i$. $Trpm4^{-/-}$ ventricular myocytes had fast

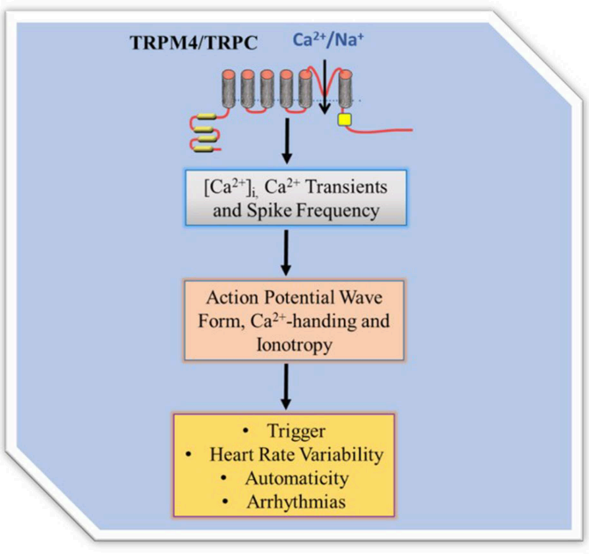

FIGURE 1 | TRP channel regulation of intracellular Ca^{2+} homeostasis. TRPM4 mediates membrane depolarization dependent Ca^{2+} entry while TRPC mediates permeability dependent Ca^{2+} entry. The Ca^{2+} influx directly and/or indirectly contributes to Ca^{2+} transient to regulate Ca^{2+}-handling, action potential wave form and contractility. Since TRPC are Na^{+}/Ca^{2+} permeable they can directly contribute Ca^{2+} entry while the Ca^{2+} non permeable TRPM4 regulates Ca^{2+} entry by modification of the membrane potential and thus driving force.

repolarization as a result of enhanced driving force of $I\mathrm{Ca}_{,\mathrm{L}}$ for Ca^{2+} entry (11). Therefore, it appears TRPM4 regulates action potential adaptation and duration, and underpins voltage-gated Ca^{2+} channel Ca^{2+} influx that couples its activity and that of $I\mathrm{Ca}_{,\mathrm{L}}$ in the so called Ca^{2+}-induce Ca^{2+} release that promote contractility.

In addition to TRPM4, TRP canonical (TRPC) is another family of TRP channels. It consists of seven subfamilies (TRPC1-TRPC7). TRPCs are also permeable to but activated by Ca^{2+}, and may be important in Ca^{2+}-handling. TRPC6 promoter gene had two conserved nuclear factor of activated T-cell transcriptional factor (NFAT) consensus sites (14) and NFAT is calcium-dependent regulatory transcriptional factor. Therefore, TRPC6 is an intracellular Ca^{2+} signaling effector. Increased TRPC1/TRPC4 expression underscores elevated SR Ca^{2+} content in right ventricular hypertrophied cardiomyocytes during monocrotaline exposure (14). Transgenic mice with inhibition of TRPC3/6/7 and TRPC1/4/5 subfamilies had membrane Ca^{2+} leak in pathological hypertrophy following either activation of neuroendocrine (phenylephrine and angiotensin II (Ang II) infusion) or pressure overload induction (15). TRPC channels as cation-selective channels can produce pathological cardiac growth of adult myocytes through Ca^{2+} influx and calcineurin activation, that alter Ca^{2+}-handling and Ca^{2+}-dependent signaling.

Although it is possible that TRPC/Ca^{2+}/calcineurin/NFAT signaling loop might regulate Ca^{2+}-handling in diseases, how the signaling loop couples to $[Ca^{2+}]_i$ and L-type Ca^{2+} channel activity is completely unknown. It appears in smooth muscle that entry of cations through a receptor operated TRPC6 caused membrane depolarization and consequent functional activity of L-type Ca^{2+} channels, Ca^{2+} influx, and smooth muscle contraction. However, whether this role is associated with ryanodine and NFAT is not known. Given that TRPC6 can operate as receptor-activated cation channels, which increases $[Ca^{2+}]_i$ by Ca^{2+} entry across plasma membrane and/or by release of Ca^{2+} from intracellular stores such as the endoplasmic reticulum, TRPC6 contributes to Ca^{2+}-handling and $[Ca^{2+}]_i$. The contribution can also be deduced on store-operation molecular standpoint. This is since store-operated and receptor-operated channels can come from the same proteins of the same family of TRP channels.

Nonetheless, TRP channels store operated and receptor operated calcium entry (SOCE) and (ROCE) are less clear. Discovery has it that stromal interaction molecule 1 (STIM1) and Orai 1 are mediators of SOCE. Fast Ca^{2+}-dependent inactivation kinetics of STIM1 current is known to be critically dependent on cytosolic Ca^{2+} levels, which also regulates native SOCE and ROCE currents, through changes in the $[Ca^{2+}]_i$, similarly to that of TRP channel currents. Whether TRP channels kinetics are associated to SOCE and ROCE, is not elucidated. Native store-operated and receptor-operated complexes stoichiometry, if distinct from hetero/homo multimer variants Orai or TRP is also not known. Regardless, STIM1 indirectly activates TRPC3/6, but not TRPC7 (16), and TRPC1/4/5 directly bind with STIM1 to activate SOCE (17), and in lipid raft domains, TRPC channels co-localize with STIM1 and Orai (18). There may be a better understanding of TRP channels SOCE attribute arguably in micro-domains (19). In fact, it is known that the Ca^{2+}-activated signaling effectors are either in direct proximity or attached to Ca^{2+} entry channels in compartmentalized micro-domains (20). Dissociation of TRPC1 from Cav1, following Ca^{2+}-store depletion is an important process in the activation of TRPC1-SOCE (20). Expression of TRPC channels and Ca^{2+} influx through TRPC within micro-domains affects contractility reserve and contributes to cardiac Ca^{2+} cycling. Taken together: (1) direct effects of TRP channels and receptor operated calcium entry mechanisms on $[Ca^{2+}]_i$ and Ca^{2+}-handling may be questioned. (2) It is more likely that TRP channels regulate the process through effects on micro-domains or through direct interaction with Ca^{2+}-dependent regulatory proteins. (3) It is noted that STIM1 is a Ca^{2+} sensor that relays Ca^{2+} load of the endoplasmic reticulum to store operated channels and may navigate some of the TRP channels into micro-domains to govern their roles in cardiovascular diseases through calcium signaling effectors.

In addition to the myocardial TRPC channels, atrial endocardial TRPC-6 channel has been identified to be crucial in TRPC-6-dependent paracrine factors that regulate the amplitude of myocardial Ca^{2+} transients through mechanotransduction. Mechanical stretch is a determinant of atrial function such as contractility and TRPC-6 is a stretch responsive cation channel that governs mechanotransduction. To understand the roles of stretched-atrial endocardium, associated with physiological conditions and arrhythmogenesis Nikolova-Krstevski et al., tested the consequences of stretch-induced endocardial TRPC-6 activation on myocardial function and hypothesized that endocardial TRPC-6 is required for mechanical stretch responses in the atrium (21). Findings from this investigation, demonstrate that, in acute stretch, Ca^{2+}-mediating function of TRPC-6 was consequentially enhanced via $[Ca^{2+}]$i, and endocardial TRPC-6 regulatory feedback protects against this stretch-induced myocardial Ca^{2+} overload process, whereas in chronic stretch, reduced protein expression of endocardial TRPC-6 and irregular Ca^{2+} transient cyclic events may lead to enhanced susceptibility to arrhythmia. To understand the Ca^{2+} transient mechano feedback response, atrial endocardial-myocardial (cell-cell) communication/signaling was investigated in cultured human-induced pluripotent stem cell-derived cardiomyocytes before and after the addition of media collected from non-stretched and stretched atrial endocardial cells from porcine. The investigation revealed alteration of Ca^{2+} transient in human-induced pluripotent stem cell-derived cardiomyocytes with non-stretched but not with stretched atrial endocardial cells (21). The finding suggests that TRPC-6 dependent stretch-induced mechanotransduction can induce changes in global $[Ca^{2+}]_I$ that modulate intrinsic atrial endocardial function, such as contractility, as well as affecting myocardial function, through endothelium secreting factors such as endothelin-1, nitric oxide, prostacyclin, and angiotensin II (21).

MECHANISMS OF REGULATION OF $[CA^{2+}]_i$ AND CA^{2+}-HANDLING

The question now remains how does the channels regulate $[Ca^{2+}]_{i,}$ and thus control Ca^{2+}-handling and contractile tone? The mechanisms of TRP channel mediated Ca^{2+} handling remain largely unknown. Ca^{2+}-dependent regulatory proteins such as NCX and SERCA regulate Ca^{2+}-handling and cardiac contractility, and the TRP channels interact directly with NCX and SERCA. Functional and physical interaction of TRPC channels with NCX proteins is a novel principle behind TRPC-mediated increase in intracellular Ca^{2+} signaling. To assess for the existence of a native TRPC3/NCX1 signaling complex in rat cardiac myocytes, previously identified in HEK 293 cells, the Eder et al conducted reciprocal co-immunoprecipitation to detect TRPC3 and NCX1 interaction by immunoprecipiting solubilized proteins of crude adult rat cardiomyocyte membrane fractions with either anti-TRPC3 or anti-NCX (22). This demonstrated significant TRPC3 and NCX1 co-localization. Glutathione S-transferase pulldown experiment consistently replicated the native TRPC3/NCX1 signaling complex within the cardiomyocyte. To understand the functional consequences of this interaction, phpspholipase C (PLC) was first stimulated in the myocytes, and because TRPC3 is a key component of PLC-dependent Ca^{2+} signaling, the cardiomyocytes were then transiently transfected with TRPC3 N-terminal fragment to exert dominant negative effects on TRPC3. The activation of PLC promoted reverse mode NCX1-mediated Ca^{2+} entry, as identified, through $[Ca^{2+}]_i$ measurement, that was regulated by TRPC3-mediated Na^{+} loading in the myocytes through angiotensin-induced activation of the G protein Gq-PLC pathway (22). Measurement of this NCX-mediated cellular Ca^{2+} signals in the cells expressing dominant negative TRPC3 revealed significant reduction in Ca^{2+} signals, illustrating the idea of TRPC3-dependent Na^{+} loading, as part of cardiac NCX operation (22).

PLC dependently recruited TRPC3-NCX1 complex into the plasma membrane and regulated Ca^{2+} homeostasis in rat cardiomyocytes (19, 22). TRPC3 channel may be a major component of PLC-dependent activation of the Ca^{2+} calcineurin-NFAT signaling pathway, a pathological cardiac hypertrophy pathway, in which NCX1 can mediate Ca^{2+} influx by reverse mode and contribute to Ca^{2+} transients and action potential wave forms. Together, TRPC3 may be an important component of NCX regulation of Ca^{2+}-handling. Furthermore, SERCA is an ATP-dependent Ca^{2+} pump located in the SR membrane. It appears silencing SR Ca^{2+}-ATPase (SERCA)2 with small interfering RNA (siRNA) increases TRPC levels (23), as a compensatory mechanism for SERCA Ca^{2+} pump. While colocalizing on the skin of Darier's disease patient on immunostaining, TRPC1 had increased immunoreactivity whereas, SERCA2 had reduced immunoreactivity, suggesting reciprocal activity of TRPC1 and SERCA2 (23). Western blots performed on the skin from the DD patient and immunolocalization performed on skin samples from $SERCA2^{+/+}$ and $SERCA2^{+/-}$ mice showed consistent reciprocal expression of TRPC1 and SERCA2 (23). The DD patients as well as $SERCA2^{+/-}$ mice have only one normal copy of the gene encoding SERCA2. To understand the association of the reciprocal expression, a human keratinocyte (HaCaT) cell line was employed. The keratinocytes from DD patients have also one normal copy of SERCA2. Single copy of the gene is known to be inadequate, to appropriately account for proper amount of SERCA2, leading to changes in Ca^{2+} signaling in the cells. To examine this SERCA2 reduction in function, HaCaT cells were treated with adenovirus encoding either SERCA2-siRNA or control-siRNA and Ca^{2+} imaging was performed on the cells upon stimulation with thapsigargin, to deplete endoplasmic reticulum Ca^{2+} (23). Addition of thapsigargin in HaCaT cells Ca^{2+}-containing media, caused an increase in global $[Ca^{2+}]_i$. Whereas this increase was high in HaCaT cells expressing SERCA2-siRNA, no increase in control-siRNA was documented (23). Consistent with the reciprocal expression of TRPC1 and SERCA2, release of Ca^{2+} from the internal stores upon the thapsigargin addition was low in cells overexpressing SERCA2-siRNA, whereas no changes were seen in TRPC1-overexpressing cells (23).

Accordingly, SERCA2 silencing was followed by increased transcription of NCX, TRPC4, and TRPC5 in cardiac myocytes (24). Neonatal rat and chicken embryo cardiac myocytes expressing SERCA2-siRNA endogenously had reduced expression of SERCA2 as demonstrated by immunostaining, western blotting, real-time RT-PCR, and microscopy (24). The functional effects of the reduced SERCA2 expression was tested on Ca^{2+} signaling in rat myocytes by measuring Ca^{2+} transients, and $ICa_{,L}$ in neonatal rat myocytes under voltage clamp protocol. Whereas, there was reduced Ca^{2+} transient amplitude (i.e., reduced sarcoplasmic reticulum Ca^{2+} load) in the myocytes subjected to SERCA2-siRNA, there was no differences in $ICa_{,L}$ under the voltage clamp protocol tested. However, the myocytes were shown to have the ability to initiate transient elevations in cytosolic Ca^{2+} upon membrane stimulation, in compensatory mechanisms. To understand this process, the presence or absence of Na^{+} to examine the role of NCX to cytosolic Ca^{2+} removal under voltage-clamp depolarizations, and Ba^{2+} influx measurement in fura-2-loaded myocytes treated with thapsigargin to block SERCA pump activity to examine the role of TRPC channels, in another experiment, were together, employed to access the impact of SERCA2-siRNA on NCX, TRPC channels and Ca^{2+} signaling. This investigation revealed that reduced SERCA2 expression was associated with an elevation in TRPC expression and activity, as well as increased NCX expression and activity, that together may boost and normalize for reduced sarcoplasmic reticulum Ca^{2+} signaling following SERCA2 depletion (24). This mechanism was attributed to NCX and TRPC transcription, as well as, the upregulation of other transcriptional factors such as stimulating protein 1, myocyte enhancer factor 2, and nuclear factor activated T-cell, cytoplasmic 4 (24). In application of this finding, SR releases 70–80% of the Ca^{2+} needed for contraction into the cytosol. SERCA, NCX, and ATP-dependent Ca^{2+}pumps remove Ca^{2+}

from the cytosol. If reduction in expression of SERCA2, which can directly affect SERCA/Ca^{2+} pump, inversely increases NCX and TRPC levels, then TRPC and NCX govern $[Ca^{2+}]_i$, excitation- contraction coupling and inotropy, because TRPC and NCX operated Ca^{2+} homeostasis will compensate for SERCA Ca^{2+} pump. **Figure 2** illustrates the organellar TRP channels and Ca^{2+}-dependent regulatory proteins regulation of Ca^{2+}-handling.

Further support is delineated from the study of Oslon's group in 2006. It appears from the study that TRPC6-calcineurin-NFAT pathway, inversely, regulates the expression of SERCA2 (14), suggesting reciprocal relationship between the channel and the protein/gene. What is not known is whether downregulation of TRPC4/5 would upregulate SERCA2 expression in reverse. Understanding the precision of this relationship is pertinent and vital. It is pertinent, since the Seth et al., Eder et al., and the Pani et al. studies highlight novel association between TRP channels and Ca^{2+}-dependent regulatory proteins that governs $[Ca^{2+}]_i$. It is vital since Ca^{2+}-dependent regulatory proteins that associate with the TRP channels are potential therapeutic targets for Ca^{2+} -mishandling (25), and Ca^{2+}-dependent regulatory gene therapy approaches are being understood to increase myocardial SERCA2a in heart failure contractile abnormality (26). This in part emphasis the importance of this review in development of therapeutic agents that can benefit the heart in contractile dysfunction beyond short-term.

For instance, it is known that $Trpm4^{-/-}$ mice and their control littermate had no differences in cardiac contractile parameters under basal conditions, but infusion of $\leq$300 ng/kg·min isoprenaline resulted in robust increases (11). This is an observation of incremental physiological contractile activity, which may be rather beneficial in heart failure, cardiomyopathy and arrhythmias, characterized by diminished contractile tone. Thus, genetic downregulation of TRPM4 proteins might be a novel therapeutic approach. $Trpm4^{-/-}$ mice with ischaemic heart failure had improved survival rate and enhanced β-adrenergic cardiac response, indicating better contractile performance (27). Together, TRP channels have pleiotropic roles in the heart (28), and are part of excellent potential targets in gene therapy approach being appreciated to increase myocardial SERCA2a expression in heart failure contractile abnormality.

Given that SERCA2a regulates Ca^{2+} homeostasis, it has impact on systolic and diastolic functions. An elegant approach to improve left ventricular systolic function can be through activation of cardiac myosin, as well. Besides conventional inotropic agents that modulate Ca^{2+} homeostasis in the myocardium, molecular motor (myosin) and sarcomeric scaffolding, and energetic agents referred to as cardiac myotropes and mitotropes, respectively, have been proposed as pharmacological agents that improve myocardial performance (29). The framework of this proposal is to better understand the clinical approaches of agents that ameliorate worse myocardial

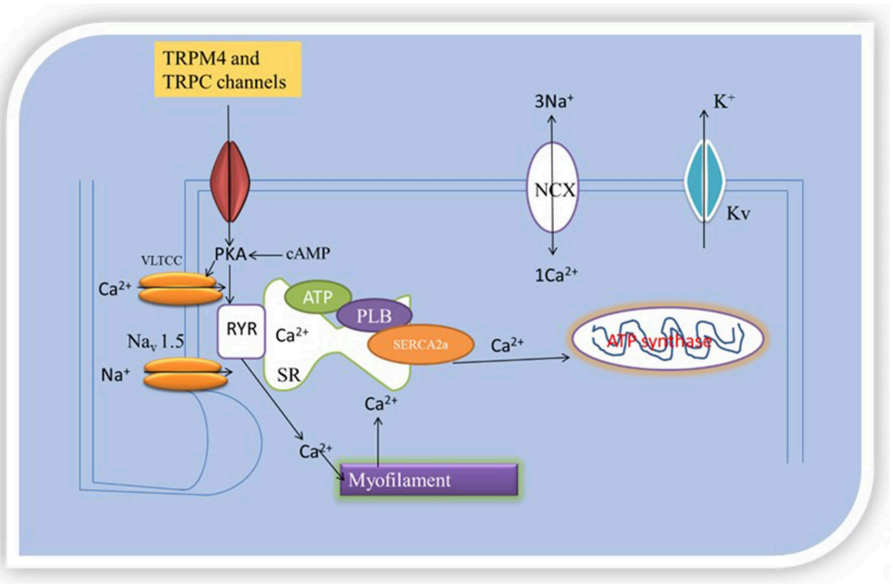

FIGURE 2 | Oganellar Mechanisms of TRP channels mediated pathologic Ca^{2+} handling. Reduced levels of $[Ca^{2+}]_i$ means that the heart cannot contract and pump blood while high levels of $[Ca^{2+}]_i$ means that the heart cannot relax. TRPM4 and TRPC interact with Ca^{2+}-dependent regulatory proteins (SERCA2a and NCX). How this interaction occurs either reduces or boosts excitation and contraction coupling (EC) by either reducing or increasing $[Ca^{2+}]_i$. EC is initiated by excitation of the cell membrane which activates Navl.5 leading to the opening of VLTCC. VLTCC opening allows passage of inward Ca^{2+} current that triggers opening of ryanodine receptor 2 (RyR2) channels by a Ca^{2+}- induced Ca^{2+} release process. The Ca^{2+}-induced Ca^{2+} release leads to coordinated release of sarcoplasmic reticulum (SR) Ca^{2+} caused by PLB phosphorylation. Intracellular free Ca^{2+} concentration become high, activating the myofilaments after binding with troponin C. TRPM4 and TRPC mediated Ca^{2+} entry can couple to this process of elevation in $[Ca^{2+}]_i$ to cause contraction. This process is part of pathologic Ca^{2+}-handling.

performance and accelerate appropriate testing of therapeutic agents that can improve cardiac contractility and contraction.

SPONTANEOUS ECTOPY

TRP channels regulate Ca^{2+}-handling through interaction with Ca^{2+}-dependent regulatory proteins. This interaction can lead to spontaneous ectopy, but how this may occur is not known. Spontaneous ectopy may be regarded as uncontrolled variability in heart rhythm during repolarization, which may or may not be pathological. Cycles of Ca^{2+} fluxes during normal heart beat characterize excitation- contraction coupling and permit homogenous action of cardiac sarcomeres. Increased Ca^{2+} influx, Ca^{2+} leaks, spontaneous oscillatory of Ca^{2+} and Ca^{2+} overload disturb systole and diastole and cause arrhythmias (30). As stated in previous sections, this implicates the regulation and mechanisms of Ca^{2+}-handling. These processes are schematically summarized in **Figure 2**, organellar mechanisms of TRP channel mediated pathological Ca^{2+}-handling. TRPM4 and TRPC channels trigger Ca^{2+} entry, through membrane depolarization and permeability, respectively, consequently leading to Ca^{2+} overload. Ca^{2+} overload promote alteration in action potential wave form, Ca^{2+}-mishandling, inotropy, and sustain electrical remodeling, through Ca^{2+} transients. Ca^{2+} overload and large Ca^{2+} transients increase repolarization (31). Prolonged action potential duration leads to early afterdepolarizations (EAD) and delayed afterdepolarizations (DAD) ectopic firing. EADs and DADs are classes of ectopy (32).

EADs are abnormally secondary cell membrane depolarizations during repolarization phases of action potential. Prolongation of APD is the principle factor that causes EAD, because reduced repolarization enables $I\text{Ca,}_L$ to recover from inactivation, which leads to depolarizing inward movement of Ca^{2+} ions along the plateau phase of action potential to produce Ca^{2+} inward current. EAD is also caused by increases in NCX current. DADs are also abnormally secondary cell membrane depolarizations during the repolarization phases but are known to be caused by abnormally diastolic Ca^{2+} release from sarcoplasmic reticulum Ca^{2+} stores, which NCX also contributes to. In response to transmembrane Ca^{2+} entry, a specialized Ca^{2+}-dependent regulatory apparatus, SR Ca^{2+} channels known as RyRs release Ca^{2+}. RyRs are closed in diastolic condition but are open if functionally defective or if the SR Ca^{2+} is above physiological arrange (33). Under physiological conditions and in healthy hearts, exercise-induced activation of sympathetic nervous system increases catecholamines produced from the chromaffin cells, which bind to and stimulate the G protein-coupled β-adrenergic receptors. This activation, in turn, stimulates adenylate cyclase to activate the cAMP-dependent protein kinase A (PKA). PKA phosphorylates major Ca^{2+}-handling proteins, such PLB, RYR2, and L-type Ca^{2+} channel. Increased RyR2 Ca^{2+} release in the SR and enhanced Ca^{2+} by SERCA2a, enhances $[Ca^{2+}]i$ and contractility. PKA hyperphosphorylation of RyR2 reduces the binding affinity of the RYR2-stabilizing subunit, calstabin2, producing robust activity of the RYR2 channel to Ca^{2+}-dependent activation (34). PKA hyperphosphorylation of RyR2 produces a diastolic SR Ca^{2+} leak in cardiomyocytes leading to persistently diminished SR Ca^{2+} content and contractility (34). RYR2 channel diastolic calcium leak contributes to abnormal contractility in arrhythmogenesis and sudden cardiac death (35). Furthermore, SERCA2a diminished Ca^{2+} loading and enhanced Ca^{2+} exflux through NCX also results in SR reduced Ca^{2+} loading and reduced cardiac contractility. 1 Ca^{2+} release during the diastole is exchanged for 3 extracellular Na^{+} by NCX. The result of this is a net depolarizing inward positive-ion movement called transient inward current (I_{ti}) that underlies the DADs, and contractile dysfunction (36).

EADs and DADs appear to be mediated by TRPM4 protein. TRPM mediated Ca^{2+} signaling can modulate action potential wave form and cause EADs. TRPM can also modulate diastolic Ca^{2+} release from sarcoplasmic reticulum Ca^{2+} stores and cause DADs. 9-Phenanthrol, an inhibitor of TRPM4 channel abolished hypoxia and re-oxygenation-induced EADs in a mouse model (37). TRPM4-induced ectopy can be drawn on ion channel determinant of cell membrane depolarization and action potential morphology. TRPM4b a calcium activated non-selective (CAN) channel regulates cell membrane depolarization (38). TRPM4 activation is a process that contributes to the controls of the magnitude of Ca^{2+} influx by regulating membrane potential, and intracellular Ca^{2+} increased through $I\text{Ca,}_L$ upon TRPM4 protein depletion (11).

CAN channel activity had been suggested to contribute to I_{ti} initiated by Ca^{2+} waves that underline DAD. I_{ti} has been described in Purkinje fibers, atrial and ventricular cardiomyocytes, and in sinoatrial node cells (39). In sinoatrial node (SAN), cardiac automaticity resulted from a CAN current that is attributed in part to TRPM4 (40), which slowed diastolic depolarizations lope due to the nature of "funny" current, and NCX activity (39). The molecular identity of I_{ti} is controversial, but it appears to reflect 3 Ca^{2+}- dependent components: NCX, Ca^{2+}-activated chloride current and current mediated by CAN channels such as TRPM4 (40). Put together, these studies support the hypothesis that I_{ti} is mediated by TRPM4. However, that I_{ti} is mediated by TRPM4 may be questioned by the fact that the single channel conductance of the I_{ti}-mediating channel was 120 Ps (41), which is much larger than the ∼25 pS of TRPM4 (38).

Significance

The past few decades have witnessed tremendous evolution in characterization of the molecular and genetic mechanisms of acquired and inherited arrhythmias. The mechanisms are as numerous as the phenotypes with which the arrhythmias present. Identifying the sources that control Ca^{2+} is novel in understanding arrhythmogenesis. The TRP channels mediate Ca^{2+} flux and voltage changes across membranes. Regulation of Ca^{2+}-handling by the TRP channels indicate they can potentially boost Ca^{2+} cycling disorders. Plasma membrane sensory and metabotropic TRPM4 subgroup is a drug candidate for Brugada syndrome and familial heart blocker (42). Better understanding of the TRP channels, Ca^{2+}-handling and contractility is crucial.

CONCLUSION

Essentially, studies illustrating the roles of TRP channels in pathological Ca^{2+}-handling and spontaneous ectopy are lacking. This study reflects on TRPM and TRPC seminal works, based on their biophysical properties, to state mechanistically their potential implications in Ca^{2+} signaling dynamics. Intracellular Ca^{2+} homeostasis show tremendous changes during cardiac cycle. Intracellular Ca^{2+} homeostasis is regulated by spatial signaling processes within restricted regions, rather than by changes in global cytosolic $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ is mediated by Ca^{2+} release from intracellular organelles, Ca^{2+} entry across plasma membrane through receptor agonists, receptor-activated Ca^{2+} channels, voltage-gated Ca^{2+} channels, and by ligand-gated cation channels. It appears that while overexpression of the TRPCs increase $[Ca^{2+}]_i$, TRPM4 depletion increases $[Ca^{2+}]_i$, through Ca^{2+} influx. This can be explained by the biophysical properties of the channels. Mechanization of $[Ca^{2+}]_i$, is defective in heart failure, failing hearts, cardiomyopathy, arrhythmia and sudden cardiac deaths. Defective intracellular Ca^{2+} homeostasis is responsible for pathological Ca^{2+}-handling and contractile dysfunction. Hearts in these conditions show abnormal contractility, characterized by decreased SR Ca^{2+} sequestration, diminished intracellular Ca^{2+} transients, and enhanced diastolic SR Ca^{2+} leak activity. Sources that regulate these processes are unknown completely, and may include TRP channels and TRP channels SOCE associated mechanisms. Highlighting a potential therapeutic opportunity of the channels, this work discussed TRP channels mediated pathologic Ca^{2+}-handling in spontaneous ectopy, and stated further scopes for TRP channels functions in cardiac pathophysiology.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

1. Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol.* (2008) 70:23–49. doi: 10.1146/annurev.physiol.70.113006.100455
2. Pogwizd SM, Schlotthauer K, Li L, Yuan W, Bers DM. Arrhythmogenesis and contractile dysfunction in heart failure: roles of sodium–calcium exchange, inward rectifier potassium current and residual b-adrenergic responsiveness. *Circ Res.* (2001) 88:1159–67. doi: 10.1161/hh1101.091193
3. Piacentino V III, Weber RC, Chen X, Weisser-Thomas J, Margulies BK, Bers MD, et al. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ Res.* (2003) 92:651–8. doi: 10.1161/01.RES.0000062469.83985.9B
4. Hasenfuss G, Pieske B. Calcium cycling in congestive heart failure. *J Mol Cell Cardiol.* (2002) 34:951–69. doi: 10.1006/jmcc.2002.2037
5. Lan F, Lee SA, Liang P, Sanchez-Freire V, Nguyen KP, Wang L, et al. Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. *Cell.* (2012) 12:101–13. doi: 10.1016/j.stem.2012.10.010
6. Kubo H, Margulies BK, Piacentino V III, Gaughan PJ, Houser RS. Patients with end-stage congestive heart failure treated with b-adrenergic receptor antagonists have improved ventricular myocyte calcium regulatory protein abundance. *Circulation*. (2001) 104:1012–8. doi: 10.1161/hc3401.095073
7. Minke, B. Drosophila mutant with a transducer defect. *Biophys Struct Mech.* (1977) 3:59–64.
8. Montell C, Jones K, Hafen E, Rubin G. Rescue of the Drosophila phototransduction mutation trp by germline transformation. *Science.* (1985) 230:1040–3. doi: 10.1126/science.3933112
9. Du J, Xie J, Zhang Z, Tsujikawa H, Fusco D, Silverman D, et al. TRPM7-mediated Ca2+ signals confer fibrogenesis in human atrial fibrillation. *Circ Res*. (2010) 106:992–1003. doi: 10.1161/CIRCRESAHA.109.206771
10. Yu Y, Chen S, Xiao C, Jia Y, Guo J, Jiang J, et al. TRPM7 is involved in angiotensin II induced cardiac fibrosis development by mediating calcium and magnesium influx. *Cell Calcium.* (2014) 55:252–60. doi: 10.1016/j.ceca.2014.02.019
11. Mathar I, Kecskes M, Mieren G, Jacobs G, Londoño CJ, Uhl S, et al. Increased β-adrenergic inotropy in ventricular myocardium from *Trpm4–/–* mice. *Circ Res*. (2014) 114:283–94. doi: 10.1161/CIRCRESAHA.114.302835
12. Zhou Y, Yi X, Wang T, Li M. Effects of angiotensin II on transient receptor potential melastatin 7 channel function in cardiac fibroblasts. *Exp Ther Med.* (2015) 2008:2008–12. doi: 10.3892/etm.2015.2362
13. Simard C, Hof T, Keddache Z, Launay P, Guinamard R. The TRPM4 non-selective cation channel contributes to the mammalian atrial action potential. *J Mol Cell Cardiol.* (2013) 59:11–9. doi: 10.1016/j.yjmcc.2013.01.019
14. Kuwahara K, Wang Y, McAnally J, Richardson AJ, Bassel-Duby R, Hill AJ, et al. TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. *J Clin Invest.* (2006) 116:3114–26. doi: 10.1172/JCI27702
15. Wu X, Edera P, Changa B, Molkentina DJ. TRPC channels are necessary mediators of pathologic cardiac hypertrophy. *Proc Natl Acad Sci USA.* (2010) 107:7000–5. doi: 10.1073/pnas.1001825107
16. Liao Y, Plummer NW, George MD, Abramowitz J, Zhu MX, Birnbaumer L. A role for Oraiin TRPC-mediated Ca2+entry suggests that a TRPC: orai complex may mediate store and receptor operated Ca2+entry. *Proc Natl Acad Sci USA.* (2009) 106:3202–6. doi: 10.1073/pnas.0813346106
17. Yuan JP, Zeng W, Huang GN, Worley PF, Muallem S. STIM1 heteromultimerizes TRPC channels to determine their function as store- operated channels. *Nat Cell Biol.* (2007) 9:636–45. doi: 10.1038/ncb1590
18. Pani B, Ong HL, Liu X, Rauser K, Ambudkar IS, Singh BB. Lipid rafts determine clustering of STIM1inendoplas- micreticulum-plasma membrane junctions and regulation of store- operated Ca2+ entry (SOCE). *J Biol Chem.* (2008) 283:17333–40. doi: 10.1074/jbc.M800107200
19. Eder P, Molkentin, JD. TRPC channels as effectors of cardiac hypertrophy. *Circ Res*. (2011) 108:265–72. doi: 10.1161/CIRCRESAHA.110.225888
20. Pani B, Ong HL, Brazer SC, Liu X, Rauser K, Singh BB, et al. Activation of TRPC1 by STIM1 in ER– PM microdomains involves release of the channel from its scaffold caveolin-1. *Proc Natl Acad Sci USA*. (2009) 106:20087–92. doi: 10.1073/pnas.0905002106
21. Nikolova-Krstevski V, Wagner S, Yu ZY, Cox CD, Cvetkovska J, Hill AP, Huttner IG, et al. Endocardial TRPC-6 channels act as atrial mechanosensors and load-dependent modulators of endocardial/myocardial cross-talk. *JACC Basic Transl Sci*. (2017) 2:575–90. doi: 10.1016/j.jacbts.2017.05.006.
22. Eder P, Probst D, Rosker C, Poteser M, Wolinski H, Kohlwein SD, et al. Phospholipase C-dependent control of cardiac calcium homeostasis involves a TRPC3-NCX1 signaling complex. *Cardiovasc Res*. (2006) 73:111–9. doi: 10.1016/j.cardiores.2006.10.016
23. Pani B, Cornatzer E, Cornatzer W, Shin D, Pittelkow RM, Hovnanian A, et al. Up-regulation of transient receptor potential canonical 1(TRPC1) following Sarco(endo)plasmic reticulum Ca2+-ATPase 2 gene silencing promotes cell survival: a potential role for TRPC1 in Darier's disease. *Mol Biol Cell.* (2006) 17:4446–58. doi: 10.1091/mbc.e06-03-0251

24. Seth M, Sumbilla C, Mullen SP, Lewis D, Klein GM, Hussain A, et al. Sarco(endo)plasmic reticulum Ca2+ ATPase (SERCA) gene silencing and remodeling of the Ca2+ signaling mechanism in cardiac myocytes. *Proc Natl Acad Sci USA*. (2004) 101:16683–8. doi: 10.1073/pnas.0407537101
25. Jessup M, Greenberg B, Mancini D, Cappola T, Pauly FD, Jaski B, et al. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID) a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2_-ATPase in patients with advanced heart failure. *Circulation*. (2011) 124:304–13. doi: 10.1161/CIRCULATIONAHA.111.022889
26. Kho C, Lee A, Jeong D, Oh GJ, Chaanine HA, Kizana E, et al. SUMO1-dependent modulation of SERCA2a in heart failure. *Nature*. (2011) 477:601–5. doi: 10.1038/nature10407
27. Jacobs G, Oosterlinck W, Dresselaers T, Geenens R, Kerselaers S, Himmelreich U, et al. Enhanced β-adrenergic cardiac reserve in Trpm42/2 mice with ischaemic heart failure. *Cardiovasc Res*. (2015) 105:330–9. doi: 10.1093/cvr/cvv009
28. Demion M, Thireau J, Gueffier M, Finan A, Khoueiry Z, Cassan C, et al. Trpm4 gene invalidation leads to cardiac hypertrophy and electrophysiological alterations *PLoS ONE*. (2014) 9:e115256. doi: 10.1371/journal.pone.0115256
29. Psotka MA, Gottlieb SS, Francis GS, Allen LA, Teerlink JR, Adams KF Jr., et al. Cardiac calcitropes, myotropes, and mitotropes: JACC review topic of the week. *J Am Coll Cardiol*. (2019) 73:2345–53. doi: 10.1016/j.jacc.2019.02.051
30. Bers DM. Cardiac sarcoplasmic reticulum calcium leak: basis and roles in cardiac dysfunction. *Annu Rev Physiol*. (2014) 76:107–27. doi: 10.1146/annurev-physiol-020911-153308
31. Venetucci LA, Trafford AW, O'Neill SC, Eisner DA. The sarcoplasmic reticulum and arrhythmogenic calcium release. *Cardiovasc Res*. (2008) 77:285–92. doi: 10.1093/cvr/cvm009
32. Weiss NJ, Garfinkel A, Karagueuzian SH, Chen P, Qu Z. Early afterdepolarizations and cardiac arrhythmias *Heart Rhythm*. (2010) 7:1891–9. doi: 10.1016/j.hrthm.2010.09.017
33. Fischer TH, Maier LS, Sossalla S. The ryanodine receptor leak: how a tattered receptor plunges the failing heart into crisis. *Heart Fail Rev*. (2013) 18:475–83. doi: 10.1007/s10741-012-9339-6
34. Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, Marks AR. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*. (2000) 101:365–76. doi: 10.1016/S0092-8674(00)80847-8
35. Dobrev D, Wehrens XH. Role of RyR2 phosphorylation in heart failure and arrhythmias: controversies around ryanodine receptor phosphorylation in cardiac disease. *Circ Res*. (2014) 114:1311–9. doi: 10.1161/CIRCRESAHA.114.300568
36. Bers DM, Pogwizd SM, Schlotthauer K. Upregulated Na/Ca exchange is involved in both contractile dysfunction and arrhythmogenesis in heart failure. *Basic Res Cardiol*. (2002) 97(Suppl. 1):I36–42. doi: 10.1007/s003950200027
37. Simard C, Sallé L, Rouet R, Guinamard R. Transient receptor potential melastatin 4 inhibitor 9-phenanthrol abolishes arrhythmias induced by hypoxia and re-oxygenation in mouse ventricle. *Br J Pharmacol*. (2012) 165:2354–64. doi: 10.1111/j.1476-5381.2011.01715.x
38. Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP. TRPM4 is a Ca2+-activated nonselective cation channel mediating cell membrane depolarization. *Cell*. (2002) 109:397–407. doi: 10.1016/S0092-8674(02)00719-5
39. Guinamard R, Demion M, Chatelier A, Bois P. Calcium-activated nonselective cation channels in mammalian cardiomyocytes. *Trends Cardiovasc Med*. (2006) 16:245–50. doi: 10.1016/j.tcm.2006.04.007
40. Hof T, Simard C, Rouet R, Sallé L, Guinamard R. Implication of the TRPM4 nonselective cation channel in mammalian sinus rhythm. *Heart Rhythm*. (2013) 10:1683–9. doi: 10.1016/j.hrthm.2013.08.014
41. Hill JA Jr., Coronado R, Strauss HC. Reconstitution and characterization of a calcium-activated channel from heart. *Circ Res*. (1988) 62:411–5.
42. Zhang, X, Hu M, Yang Y, Xu H. Organellar TRP channels. *Nat Struct Mol Biol*. (2018) 11:1009–18. doi: 10.1038/s41594-018-0148-z

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REVIEW
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CPVT: Arrhythmogenesis, Therapeutic Management, and Future Perspectives. A Brief Review of the Literature

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Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a primary electrical disease characterized by a normal resting electrocardiogram and induction of malignant arrhythmias during adrenergic stress leading to syncope or sudden cardiac death (SCD). CPVT is caused by mutations in the cardiac ryanodine receptor (RyR2) or in the sarcoplasmic reticulum protein calsequestrin 2 genes (*CASQ2*). The RyR2 mutations are responsible for the autosomal dominant form of CPVT, while *CASQ2* mutations are rare and account for the recessive form. These mutations cause a substantial inballance in the homeostasis of intracellular calcium resulting in polymorphic ventricular tachycardia through triggered activity. Beta blockers were for years the cornerstone of therapy in these patients. Sodium channel blockers, especially flecainide, have an additive role in those not responding in beta blockade. Implantation of defibrillators needs a meticulous evaluation since inappropriate shocks may lead to electrical storm. Finally, cardiac sympathetic denervation might also be an alternative therapeutic option. Early identification and risk stratification is of major importance in patients with CPVT. The aim of the present review is to present the arrhythmogenic mechanisms of the disease, the current therapies applied and potential future perspectives.

Keywords: channelopathies, CPVT, arrhythmias, genes, sudden death, risk stratification

INTRODUCTION

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a primary electrical disease characterized by a normal resting electrocardiogram and induction of malignant arrhythmias during adrenergic stress leading to syncope or sudden cardiac death (SCD) (1). CPVT phenotype is a result of mutations of Ryanodine receptor (RyR2) and calsequetrin 2. The RyR2 mutations account for the commonest phenotype and they are inherited through an autosomal dominant manner. CASQ2 mutations represent the recessive form (2, 3).

CPVT is responsible for SCD especially among children and young adults. According to previous reports, the incidence of arrhythmias in CPVT patients was 32% over 8 years (2), but the true frequency of the disease is unknown. This is due to the fact that unlike other inherited

channelopathies such as long QT syndrome, it is present not only with a structurally normal heart, but also without resting ECG abnormalities (2).

Beta blockade is the main therapeutic option. Sodium channel blockers, such as flecainide, have an additive role to those not responding to beta blockers, along with left cardiac stellate sympathectomy. Implantable cardiac defribrillators (ICDs) are life -saving therapy for the majority of patients with cardiac channelopathies, however CPVT patients need to be carefully selected, since inappropriate shocks may lead to adrenergic stimulation and electrical storm, despite optimal programming.

CPVT AND ARRHYTHMOGENESIS

Arrhythmogenesis in CPVT patients is attributed to mutations in different proteins resulting in bidirectional ventricular tachycardia through different arrhythmogenic mechanisms. Arrhythmias produced by gain-of-function mutations in RyR2 are postulated to result from destabilization of the channel with increased diastolic SR Ca^{2+} leak in ventricular myocytes, leading to delayed afterdepolarizations and triggered activity via the $Na+/Ca^{2+}$ exchanger current. Yet, new evidence has shown that the cardiac Purkinje network appears to be involved in the initiation of bidirectional VT and polymorphic ventricular tachycardia in this disease (4). It is estimated that over 160 mutations causes CPVT 1. Most of them cause a gain of function of the RyR2 channels (1–3), whereas others, such as CASC2 gene, regulate RYR receptor through other proteins (junctin and triadin) resulting to a leakage of Ca in diastole.

According to previous published data (5), gene mutations responsible for CPVT lead to ventricular arrhythmia through the alteration of the Ca2+ homeostasis. Specifically, mutations in the RyR2 and CASQ2 genes lead to a leakage of Ca2+ from the SR in diastole, particularly under adrenergic stress (exercise, emotional stress), resulting in delayed after-depolarizations and therefore vulnerable to ventricular arrhythmias. Other less prevalent gene mutations like KCNJ2, triadin (TRDN), junctin (JCN), calmodulin (CALM1 and CALM2), and NKYRIN-B (6) may predispose to CPVT as well as in the future, other not yet identified genes might be found responsible for the disease.

CLINICAL PRESENTATION-DIAGNOSTIC EVALUATION

Nevertheless, irrespective of the responsible mutation, CPVT is characterized by polymorphic ventricular tachycardia under adrenergic stress. Apart from syncope less specific signs and symptoms, such as dizziness or palpitations might be exerted (7). The first manifestation of the disease occurs during childhood and the majority of patients have experienced syncope episode or cardiac arrest by their adulthood (7). The study of Hayashi et al. (2) depicted that the earliest a CPVT is diagnosed the worse the prognosis is. This can be attributed, at least in part, to the fact that children performing strenuous physical activities are more sensitive to external stimulations (children have more opportunities to engage in strenuous activities), (1) patients with more severe forms of CPVT will be diagnosed earlier, and (2) beta-blockers are frequently underdosed in children if based on weight given increased hepatic clearance. Sudden cardiac death or syncope in first degree family members is detected in one third of CPVT patients (8). Despite its life threatening nature, CPVT remains often unnoticed. This is due to normal baseline electrocardiograms on top of incomplete penetrance (8, 9) and thus variable expressivity. Some authors have reported bradycardia, and others have observed U waves in electrocardiograms (10). CPVT is unmasked by a treadmill stress test (11). When patients start exercising ventricular ectopy develops, increasing in complexity as the heart rate increases. Specifically, dynamic exercise during a BRUCE protocol induces premature ventricular complexes that may degenerate to more complex ventricular tachyarrhythmias or even sustained VT (12, 13).

THERAPEUTIC MANAGEMENT

Beta Blockers

Therapeutic management for patients with CPVT includes beta blockers without intrinsic sympathomimetic activity. Nadolol is the beta-blocker of choice in a high dosage, 1–2 mg/kg. The incidence of arrhythmic events in CPVT patients on beta-blockers is still high. Other non-selective beta-blockers are equally effective especially propranolol. Clinical follow up with holter monitoring and treadmill stress test should be performed so that the optimal therapy is adjusted (14).

In the study of Priori et al. (14), there is significantly lower incidence of SCD in patients on beta-blockers. Hence, the event rates in the patients on therapy were not negligible. This could be attributed to poor therapy compliance. Priori et al. suggest that taking different beta bockers than nadolol could be associated with higher incidence rates. Furthermore, data from treadmill stress tests reveal that it is not the ultimate tool during follow up, despite the fact that it is widely used as a diagnostic tool, due to low sensitivity and specificity (14).

Chatzidou et al. (15) suggested that patients presenting with electrical storm independently of the underlying mechanism should be treated with oral propranolol as the preferred beta-blocker agent.

Flecainide

The study of van de Weerf et al. (16) supports the use of flecainide on top of beta blockers as it reduces ventricular arrhythmias during exercise. This is of major importance, since several studies have demonstrated a significant event rate despite conventional therapy (2, 9, 17–23). Therefore, adding flecainide in combination with β-blocker therapy should be considered.

In CPVT the rise of intracellular Ca2+ activates the electrogenic Na+/Ca2+ exchanger (NCX), which produces a transient inward current (ITi). ITi generates delayed afterdepolarizations, which can lead to triggered activity, and the initiation of ventricular arrhythmias (24). Flecainide directly targets the molecular defect in CPVT by inhibiting RyR2 channels and preventing arrhythmogenic Ca2+ waves. Flecainide's Na+ channel blockade further reduces the rate of

triggered beats (5, 25, 26). This dual action could explain why flecainide is so effective in severe CPVT and provides a rationale for combination therapy with β-blockers. The rationale for flecainide use for treatment of CPVT is supported by *in vitro* studies demonstrating that flecainide blocks RyR2 in lipid bilayers (27) suppresses calcium waves in CASQ2-knockout myocytes, abolishes delayed afterdepolarization–mediated triggered activity, and reduces exercise induced ventricular arrhythmias inCASQ2 and RYR2 mouse models. The efficacy of flecainide in human patients with CPVT has been demonstrated in the 3 retrospective cohorts. Kannankeril et al. (25) supported that a median dosage of 300 mg/d was required to achieve target trough drug levels. One could speculate that chronotropic incompetence from combination therapy with β-blocker plus flecainide would result in lower levels of exertion during exercise and thus a lower arrhythmia score. However, maximal workload achieved during each exercise test did not differ significantly, suggesting similar levels of effort across the three exercise tests.

Liu et al. support that the antiarrhythmic effect of flecainide is that it reduces the availability of sodium channels, thus preventing the development of triggered APs (28).

Radwanski et al. suggested that flecainide may exert its antiarrhythmic action by antagonizing catecholamine-dependent augmentation of Na+ influx via sodium channel isoforms, and Nav1.6 in particular (29).

Left Cardiac Sympathetic Denervation

In patients who are refractory to maximal pharmacologic treatment, left cardiac sympathetic denervation (LCSD) could be an alternative, with significant reduction in arrhythmic events, as noted by De Ferrari et al. (30). However, the procedure is not widely available and is associated with complications such as pneumothorax and Horner syndrome (30).

Implantable Cardioverter Defibrillator

An ICD, usually the ultimate solution for primary or secondary prevention of SCD for other channelopathies should be used in CPVT patients who, despite optimal medical management or/and other therapies such as left cardiac sympathectomy continue to be in danger. Patients who have experienced an aborted cardiac arrest before the initiation of therapy, should be on medical therapy together with an ICD implantation (31). Hence, implantation of an ICD is a technical challenge in a pediatric population and problems such as inappropriate shocks, proarrhythmic effects of the ICD, and the need for a lifetime protection requiring multiple reinterventions should be addressed when the decision is taken (32).

Current knowledge suggests an ICD implantation to survivors of cardiac arrest, or when syncope or sustained VT persists despite maximal tolerable beta blockade (14). Nevertheless, ICDs should be used with caution since they can trigger electrical storms via a vicious circle of adrenergic stimulation by the delivered shocks in CPVT patients (31).

FUTURE PERSPECTIVES

Arrhythmic events among probands and family members are still challenging. To the best of our knowledge a reliable risk stratification tool lacks in patients with CPVT. Cardiac events may happen in previously asymptomatic mutations carriers, even with negative treadmill tests. Consequently, there is an emerging need to better clarify the individuals at risk for future events. Apart from meticulous family screening mutations carriers identified should be treated with beta-blockers even after a negative exercise test, which can change with time (14).

All CPVT patients should have a genetic diagnosis, that might further assist to an individualized treatment. Moreover, in concordnace with other cardiac channelopathies a risk stratification model should be developed in order to identify patients at higher risk. Novel therapeutic strategies are also needed, especially for non-responders to current therapeutic options. An interesting perspective for the future is gene-therapy, which entails a therapy targeted at correcting the genetic mutation responsible for the disease (33, 34).

CONCLUSION

Current evidence suggests that risk stratification in mutation carriers is mandatory along with new therapies, especially for young patients, who survived aborted cardiac arrest or those with poor beta-blocker efficacy.

Beta-blockers is still the cornerstone in treating CPVT patients. ICDs should be considered only as a last resort taking into account their potentially harmful effect in CPVT patients, especially in children.

AUTHOR CONTRIBUTIONS

GB has authored this paper. DL has made substantial changes on the draft. GdG and GCi edited bibliography. JS, GCo, G-BC, and CdA made useful remarks on the first manuscript. TK edited English language. PB edited the paper.

REFERENCES

1. Coumel P, Fidelle J, Lucet V, Attuel P, Bouvrain Y. Catecholamine induced severe ventricular arrhythmias with Adams-Stokes syndrome in children: report of four cases. *Br Heart J.* (1978) 40(Suppl):28–37.
2. Hayashi M, Denjoy I, Extramiana F, Maltret A, Buisson NR, Lupoglazoff JM, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachy- cardia. *Circulation.* (2009) 119:2426–34. doi: 10.1161/CIRCULATIONAHA.108.829267
3. Liu N, Rizzi N, Boveri L, Priori SG. Ryanodine receptor and calsequestrin in arrhythmogenesis: what we have learnt from genetic diseases and transgenic mice. *J Molec Cell Cardiol.* (2009) 46:149–59. doi: 10.1016/j.yjmcc.2008.10.012
4. Willis BC, Pandit SV, Ponce-Balbuena D, Zarzoso M, Guerrero-Serna G, Limbu B, et al. Constitutive Intracellular Na+ excess in purkinje cells promotes arrhythmogenesis at lower levels of stress than ventricular myocytes from mice with catecholaminergic polymorphic ventricular tachycardia. *Circulation.* (2016) 133:2348–59. doi: 10.1161/CIRCULATIONAHA.116.021936

5. Rizzi N, Liu N, Napolitano C, Nori A, Turcato F, Colombi B, et al. Unexpected structural and functional consequences of the R33Q homozygous mutation in cardiac calsequestrin: a complex arrhythmogenic cascade in a knock in mouse model. *Circ Res.* (2008) 103:298–306. doi: 10.1161/CIRCRESAHA.108.171660
6. Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. *J Arrhythm.* (2016) 32:344–351. doi: 10.1016/j.joa.2015.09.008
7. Fernández-Falgueras A, Sarquella-Brugada G, Brugada J, Brugada R, Campuzano O. Cardiac channelopathies and sudden death: recent clinical and genetic advances. *Biology.* (2017) 6:E7. doi: 10.3390/biology6010007
8. Priori SG, Napolitano C. Cardiac and skeletal muscle disorders caused by mutations in the intracellular Ca2+ release channels. *J Clin Investig.* (2005) 115:2033–8. doi: 10.1172/JCI25664
9. Postma AV, Denjoy I, Kamblock J, Alders M, Lupoglazoff JM, Vaksmann G, et al. Catecholaminergic polymorphic ventricular tachycardia: Ryr2 mutations, bradycardia, and follow up of the patients. *J Med Genet.* (2005) 42:863–70. doi: 10.1136/jmg.2004.028993
10. Aizawa Y, Komura S, Okada S, Chinushi M, Aizawa Y, Morita H, et al. Distinct U wave changes in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT). *Int Heart J.* (2006) 47:381–9. doi: 10.1536/ihj.47.381
11. Refaat MM, Hotait M, Tseng ZH. Utility of the exercise electrocardiogram testing in sudden cardiac death risk stratification. *Ann Noninvasive Electrocardiol.* (2014) 19:311–8. doi: 10.1111/anec.12191
12. Napolitano C, Priori SG, Bloise R. Catecholaminergic polymorphic ventricular tachycardia. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al., Editors *Gene Reviews*. Seattle, WA: University of Washington (1993).
13. Liu N, Ruan Y, Priori SG. Catecholaminergic polymorphic ventricular tachycardia. *Prog Cardiovasc Dis.* (2008) 51:23–30. doi: 10.1016/j.pcad.2007.10.005
14. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. *Europace.* (2013) 15:1389–406. doi: 10.1016/j.hrthm.2013.07.021
15. Chatzidou S, Kontogiannis C, Tsilimigras DI, Georgiopoulos G, Kosmopoulos M, Papadopoulou E, et al. Propranolol versus metoprolol for treatment of electrical storm in patients with implantable cardioverter-defibrillator. *J Am Coll Cardiol.* (2018) 71:1897–906. doi: 10.1016/j.jacc.2018.02.056
16. van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, et al. Flecainide therapy reduces exercise-induced ven- tricular arrhythmias in patients with catechol- aminergic polymorphic ventricular tachycardia. *J Am Coll Cardiol.* (2011) 57: 2244–54. doi: 10.1016/j.jacc.2011.01.026
17. Bauce B, Rampazzo A, Basso C, Bagattin A, Daliento L, Tiso N, et al. Screening for ryanodine receptor type 2 mutations in families with effort-induced polymorphic ventricular arrhythmias and sudden death: early diagnosis of asymptomatic carriers. *J Am Coll Cardiol.* (2002) 40:341–9. doi: 10.1016/S0735–1097(02)01946–0
18. Sumitomo N, Harada K, Nagashima M, Yasuda T, Nakamura Y, Aragaki Y, et al. Catecholaminergic polymorphic ventricular tachycardia: electrocardiographic characteristics and optimal therapeutic strategies to prevent sudden death. *Heart.* (2003) 89:66–70. doi: 10.1136/heart.89.1.66
19. Lahat H, Eldar M, Levy-Nissenbaum E, Bahan T, Friedman E, Khoury A, et al. Autosomal recessive catecholamine- or exerciseinduced polymorphic ventricular tachycardia: clinical features and assignment of the disease gene to chromosome 1p13–21. *Circulation.* (2001) 103:2822–7. doi: 10.1161/01.CIR.103.23.2822
20. Swan H, Piippo K, Viitasalo M, Heikkilä P, Paavonen T, Kainulainen K, et al. Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. *J Am Coll Cardiol.* (1999) 34:2035–42. doi: 10.1016/S0735–1097(99)00461–1
21. Haugaa KH, Leren IS, Berge KE, et al. High prevalence of exercise-induced arrhythmias in catecholaminergic polymorphic ventricular tachycardia mutation-positive family members. *Europace.* (2010) 12:417–23. doi: 10.1093/europace/eup448
22. Maltsev VA, Lakatta EG. Dynamic interactions of an intracellular Ca2+ clock and membrane ion channel clock underlie robust initiation and regulation of cardiac pacemaker function. *Cardiovasc Res.* (2008) 77:274–84. doi: 10.1093/cvr/cvm058
23. Watanabe H, van der Werf C, Roses-Noguer F, Adler A, Sumitomo N, Veltmann C, et al. Effects of flecainide on exercise-induced ventricular arrhythmias and recurrences in genotype-negative patients with catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm.* (2013) 10:542–7. doi: 10.1016/j.hrthm.2012.12.035
24. Hilliard FA, Steele DS, Laver D, Yang Z, Le Marchand SJ, Chopra N, et al. Flecainide inhibits arrhythmogenic Ca2+ waves by open state block of ryanodine receptor Ca2+ release channels and reduction of Ca2+ spark mass. *J Mol Cell Cardiol.* (2010) 48:293–301. doi: 10.1016/j.yjmcc.2009.10.005
25. Kannankeril PJ, Moore JP, Cerrone M, Priori SG, Kertesz NJ, Ro PS, et al. Efficacy of flecainide in the treatment of catecholaminergic polymorphic ventricular tachycardia: a randomized clinical control trial. *JAMA Cardiol.* (2017) 2:759–66. doi: 10.1001/jamacardio.2017.1320
26. Olde Nordkamp LR, Driessen AH, Odero A, Blom NA, Koolbergen DR, Schwartz PJ, et al. Left cardiac sympathetic denervation in the Netherlands for the treatment of inherited arrhythmia syndromes. *Neth Heart J.* (2014) 160–166. doi: 10.1007/s12471–014-0523–2
27. Hofferberth SC, Cecchin F, Loberman D, Fynn-Thompson F. Left thoracoscopic sympathectomy for cardiac denervation in patients with life-threatening ventricular arrhythmias. *J Thorac Cardiovasc Surg.* (2014) 147:404–9. doi: 10.1016/j.jtcvs.2013.07.064
28. Liu N, Denegri M, Ruan Y, Avelino-Cruz JE, Perissi A, Negri S, et al. Short communication: flecainide exerts an antiarrhythmic effect in a mouse model of catecholaminergic polymorphic ventricular tachycardia by increasing the threshold for triggered activity. *Circ Res.* (2011) 109:291–5. doi: 10.1161/CIRCRESAHA.111.247338
29. Radwanski PB, Ho HT, Veeraraghavan R, Brunello L, Liu B, Belevych AE, et al. Neuronal Na+ Channels Are Integral Components of Pro-arrhythmic Na+/Ca2+ Signaling Nanodomain That Promotes Cardiac Arrhythmias During β-adrenergic Stimulation. *JACC Basic Transl Sci.* (2016) 1:251–66. doi: 10.1016/j.jacbts.2016.04.004
30. De Ferrari GM, Dusi V, Spazzolini C, Bos JM, Abrams DJ, Berul CI, et al. Clinical management of catecholaminergic polymorphic ventricular tachycardia: the role of left cardiac sympathetic denervation. *Circulation.* (2015) 131:2185–93. doi: 10.1161/CIRCULATIONAHA.115.015731
31. Sioros L, Baltogiannis GG, Lysitsas DN, Kolettis TM. Treatment of catecholaminergic polymorphic ventricular tachycardia: lessons from one case. *Hospital Chron.* (2014) 9:27–32.
32. Conte G, Sieira J, Ciconte G, de Asmundis C, Chierchia GB, Baltogiannis G, et al. Implantable Cardioverter-defibrillator therapy in Brugada Syndrome: a 20 year single center experience. *J Am Coll Cardiol.* (2015) 65:879–88. doi: 10.1016/j.jacc.2014.12.031
33. Kurtzwald-Josefson E, Yadin D, Harun-Khun S, Waldman M, Aravot D, Shainberg A, et al. Viral delivered gene therapy to treat catecholaminergic polymorphic ventricular tachycardia (CPVT2) in mouse models. *Heart Rhythm.* (2017) 14:1053–60. doi: 10.1016/j.hrthm.2017.03.025
34. Roston TM, Van Petegem F, Sanatani S. Catecholaminergic polymorphic ventricular tachycardia: a model for genotype-specific therapy. *Curr Opin Cardiol.* (2017) 32:78–85. doi: 10.1097/HCO.0000000000000360

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