

Gene mutations in cardiac arrhythmias: a review of recent evidence in ion channelopathies

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Abstract: Over the past 15 years, molecular genetic studies have linked gene mutations to many inherited arrhythmogenic disorders, in particular, “ion channelopathies”, in which mutations in genes encode functional units of ion channels and/or their transporter-associated proteins in patients without primary cardiac structural abnormalities. These disorders are exemplified by congenital long QT syndrome (LQTS), short QT syndrome, Brugada syndrome (BrS) and catecholaminergic polymorphic ventricular tachycardia (CPVT). Functional and pathophysiological studies have led to better understanding of the clinical spectrum, ion channel structures and cellular electrophysiology involving dynamics of intracellular calcium cycling in many subtypes of these disorders and more importantly, development of potentially more effective pharmacological agents and even curative gene therapy. In this review, we have summarized (1) the significance of unveiling mutations in genes encoding transporter-associated proteins as the cause of congenital LQTS, (2) the technique of catheter ablation applied at the right ventricular outflow tract may be curative for severely symptomatic BrS, (3) mutations with channel function modulated by protein Kinase A-dependent phosphorylation can be the culprit of CPVT mimicry in Andersen-Tawil syndrome (LQT7), (4) ablation of the ion channel anchoring protein may prevent arrhythmogenesis in Timothy syndrome (LQT8), (5) altered intracellular Ca²⁺ cycling can be the basis of effective targeted pharmacotherapy in CPVT, and (6) the technology of induced pluripotent stem cells is a promising diagnostic and research tool as it has become a new paradigm for pathophysiological study of patient- and disease-specific cells aimed at screening new drugs and eventual clinical application of gene therapy. Lastly, we have discussed (7) genotype-phenotype correlation in relation to risk stratification of patients with congenital LQTS in clinical practice.

Keywords: Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, induced pluripotent stem cells, long QT syndrome, short QT syndrome.

Introduction

Applying the technology of DNA sequencing,¹ Curran et al² and Wang et al^{3,4} noted that mutations in the *KCNQ1*, *KCNH2*, and *SCN5A* genes, encoding the α -subunit of ion channels that conduct potassium delayed-rectifier currents (I_{Ks} and I_{Kr}) and the sodium current (I_{Na}), respectively, could be responsible for three subtypes (LQT1-3) of congenital long-QT syndrome (LQTS). These seminal works have inspired many investigators and prompted extensive basic research, leading to subsequent identification of various gene mutations causing cardiac arrhythmias referred to as “inherited arrhythmogenic disorders” (Table 1). These arrhythmogenic disorders with mutations in genes encoding developmental components of cardiac structures produce diseases associated with a structurally abnormal heart, exemplified by hypertrophic

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Table 1 Inherited arrhythmogenic disorders

Mutations in genes encoding developmental components of the heart
Hypertrophic cardiomyopathy (HCM)
Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)
Dilated cardiomyopathy (DC)
Mutations in genes encoding functional units of ion channels and/or transporter-associated proteins (“ion channelopathies”)
Congenital long-QT syndrome (LQTS)
Brugada syndrome (BrS)
Catecholaminergic polymorphic ventricular tachycardia (CPVT)
Short-QT syndrome (SQTS)

cardiomyopathy, arrhythmogenic right ventricular dysplasia (cardiomyopathy), and dilated cardiomyopathy, whereas those with mutations in genes encoding functional units of ion channels and/or their transporter-associated proteins produce diseases associated with a structurally normal heart, such as congenital LQTS, short-QT syndrome, Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT), known as “ion channelopathies” (Tables 2–5). All these arrhythmogenic disorders are usually genetically heterogeneous, and their clinical courses are underscored by variable clinical expressivity ranging from being asymptomatic to episodic syncope, abortive cardiac arrest, and sudden cardiac death (SCD).

To confirm that a specific gene mutation is linked to cardiac arrhythmias, functional studies to illustrate consequences of the mutation are required. These functional studies usually use heterologous expression systems, primarily *Xenopus* oocytes, human embryonic kidney (HEK) cells, and Chinese hamster ovary cells.⁵ Electrophysiological effects of the mutant ion channel are then compared to those of the

wild-type counterpart. However, in order to include important constituents of the macromolecular complex of an ion channel in the complex living environment so as to reproduce the exact molecular and electrophysiological phenotype, it is often necessary to generate a transgenic mouse model carrying the specific gene mutation.⁶

Through collaborative endeavors between clinical and basic science researchers over the past 15 years, we now have better understanding of the clinical spectrum, molecular genetics, ion-channel structures, and cellular electrophysiology relating to these inherited arrhythmogenic disorders. Taking ion channelopathies as an example, the discovery of genetic defects involving the L-type Ca^{2+} channel ($\text{Ca}_v1.2$), ryanodine receptor (RyR2), and calsequestrin has provided incontrovertible evidence linking cardiac arrhythmias to abnormal intracellular Ca^{2+} cycling. As such, research and development of targeted pharmacotherapy and/or gene therapy can be contemplated. In this review, we focus on unique findings that have been recently described; in particular those pertaining to the underlying pathophysiology for better diagnosis and development of new targeted treatment modalities in certain ion channelopathies.

Mutations in genes encoding transporter-associated proteins as the cause of congenital LQTS

Congenital LQTS is an inherited disorder of delayed cardiac repolarization, electrocardiographically reflected as QT-interval prolongation, with a propensity to lethal ventricular tachyarrhythmias causing recurrent syncope, seizures, and SCD.⁷ Molecular genetic studies have demonstrated that three subtypes of congenital LQTS – LQT1,

Table 2 Congenital long-QT syndrome (LQTS)

Name	Current	Gain/loss of function	Protein	Gene	Reference
LQT1	I_{Ks}	Loss	K_vLQT1	<i>KCNQ1</i>	Wang et al ³
LQT2	I_{Kr}	Loss	$K_v11.1$	<i>KCNH2</i>	Curran et al ²
LQT3	I_{Na}	Gain	$\text{Na}_v1.5$	<i>SCN5A</i>	Wang et al ⁴
LQT4	I_{NCX} , I_{NaK} , InsP_3R	Loss	Ankyrin-B	<i>ANKB</i>	Mohler et al ⁹
LQT5	I_{Ks}	Loss	MinK	<i>KCNE1</i>	Splawski et al ⁹³
LQT6	I_{Kr}	Loss	MiRP1	<i>KCNE2</i>	Abbott et al ⁹⁴
LQT7	I_{K1}	Loss	Kir2.1	<i>KCNJ2</i>	Plaster et al, ¹⁶ Ai et al ⁹⁵
LQT8	I_{CaL}	Gain	$\text{Ca}_v1.2\alpha1$	<i>CACNA1C</i>	Splawski et al ²⁵
LQT9	I_{Na}	Gain	Caveolin-3	<i>CAV3</i>	Vatta et al, ¹⁰ Ye et al ⁹⁶
LQT10	I_{Na}	Gain	$\text{Na}_v\beta4$	<i>SCN4B</i>	Domingo et al, ⁹⁷ Medeiros-Domingo et al ⁹⁸
LQT11	I_{Ks}	Loss	Yotiao	<i>AKAP9</i>	Chen et al ¹³
LQT12	I_{Na}	Gain	α -1 Syntrophin	<i>SNTA1</i>	Ueda et al, ⁹⁹ Wu et al ¹⁰⁰
LQT13	I_{K-Ach}	Loss	Kir3.4	<i>KCNJ5</i>	Yang et al ¹⁰¹

Abbreviations: I_{Ks} and I_{Kd} , rapid and slow delayed-rectifier potassium currents respectively; I_{Na} , sodium current; I_{NCX} , $\text{Na}^+/\text{Ca}^{2+}$ exchanger current; I_{NaK} , Na^+/K^+ ATPase (pump) current; InsP_3R , inositol 1,4,5-trisphosphate (IP3) receptors; I_{K1} , inward-rectifier potassium current; I_{CaL} , L-type calcium current; I_{K-Ach} , acetylcholine-regulated potassium current.

Table 3 Brugada syndrome (BrS)

Name	Current	Gain/loss of function	Protein	Gene	Reference
BrS1	I_{Na}	Loss	$Na_v1.5$	<i>SCN5A</i>	Chen et al, ⁵⁴ Kapplinger et al, ¹⁰² Schulze-Bahr et al ¹⁰³
BrS2	I_{Na}	Loss		<i>GPD1-L</i>	London et al ⁵⁶
BrS3	$I_{Ca,L}$	Loss	$Ca_v1.2\alpha1$	<i>CACNA1C</i>	Antzelevitch et al ⁵⁸
BrS4	$I_{Ca,L}$	Loss	$Ca_v\beta_{2b}$	<i>CACNB2b</i>	Antzelevitch et al ⁵⁸
BrS5	I_{Na}	Loss	$Na_v\beta1$	<i>SCN1B</i>	Watanabe et al ⁵
BrS6	I_{to}	Gain	MiRP2	<i>KCNE3</i>	Delpón et al ⁶⁰
BrS7	I_{Na}	Loss	$Na_v\beta3$	<i>SCN3B</i>	Hu et al ⁵⁵
BrS8	I_{K-ATP}	Gain	Kir6.1	<i>KCNJ8</i>	Medeiros-Domingo et al ¹⁰⁴
BrS9	$I_{Ca,L}$	Loss	$Ca_v\alpha2d$	<i>CACNA2D1</i>	Burashnikov et al ⁵⁹
BrS10	I_{to}	Gain	$K_v4.3$	<i>KCND3</i>	Giudicessi et al ¹⁰⁵
BrS11	I_{Na}	Loss		<i>MOG1</i>	Kattygnarath et al ⁵⁷
BrS12	I_{K-ATP}	Gain	SUR2A	<i>ABCC9</i>	Barajas-Martínez et al ⁶¹

Abbreviations: I_{Na} , sodium current; $I_{Ca,L}$, L-type calcium current; I_{to} , transient outward current; I_{K-ATP} , ATP-dependent potassium current.

LQT2, and LQT3 – constitute 65%–75%, while each in the remaining susceptibility genes accounts for <1%, leaving approximately 20%–25% of patients with a yet-to-be-determined genotype.⁸

While congenital LQTS is predominantly caused by mutations in genes that encode ion channels, the discovery that transporter-associated proteins could also be the culprit is of diagnostic and therapeutic importance.⁸ Following identification of mutations in ankyrin-B (ankyrin 2) as the cause for LQT4,⁹ those in caveolin-3 (*CAV3*), yotiao (*AKAP9*), and α -1-syntrophin have been found to be responsible for LQT9, LQT11, and LQT12, respectively.⁸

LQT4

Ankyrin-B, a membrane adapter, binds to multiple proteins that can directly or indirectly contribute to cardiac electrical activity.⁹ These multiple proteins include Na^+ channels ($Na_v1.5$), Cl^-/HCO_3^- exchanger, Na^+/K^+ adenosine triphosphatase (ATPase), Kir6.2, and Ca^{2+} release channels such as those mediated by RyR2 or inositol 1,4,5-trisphosphate (IP3) receptors. LQT4 is caused by mutations of ankyrin-B that result in loss of function.⁹ As has been shown in a heterozygous mouse model of null ankyrin-B, loss of ankyrin-B function disrupts the cellular organization of these ankyrin-B-binding proteins, leading

to intracellular Ca^{2+} overload and store-overload-induced Ca^{2+} release (SOICR) from the sarcoplasmic reticulum (SR), triggering afterdepolarizations in response to catecholaminergic stimulation.⁹ Clinically, LQT4 patients manifest mild QT prolongation, catecholamine-sensitive polymorphic ventricular tachycardia (VT), and SCD, and may also present with sinus bradycardia, atrial fibrillation and atrioventricular conduction defects.⁸

LQT9

Caveolae are flask-shaped invaginations of the plasma membrane involved in vesicular trafficking and signal-transduction pathways, in which caveolins are the principal proteins. Cardiac ion channels that are specifically localized to caveolae include $Na_v1.5$, the voltage-dependent K^+ channel ($K_v1.5$), Na^+/Ca^{2+} exchanger, and $Ca_v1.2$; in addition, a variety of other signaling molecules, including β_2 -adrenergic receptors and associated proteins of the G-protein/adenylyl cyclase/protein kinase A (PKA) pathway, have been found in caveolae. LQT9 is caused by mutations of *CAV3*, with resultant interference of signaling between caveolae-enriched ion channels and a network of integrated signaling molecules.^{8,10} Of interest, in *CAV3* knockout mice, progressive cardiac hypertrophy/cardiomyopathy is observed,¹¹ and in HEK293 cell lines mutant *CAV3* exhibits a two- to threefold increase in late Na^+ current

Table 4 Catecholaminergic polymorphic ventricular tachycardia (CPVT)

Name	Current	Gain/loss of function	Protein	Gene	Reference
CPVT1	I_{rel}	Leak	RyR2	<i>RyR2</i>	Laitinen et al, ¹⁰⁶ Priori et al ¹⁰⁷
CPVT2	I_{rel}	Leak	Calsequestrin	<i>CASQ2</i>	Lahat et al, ¹⁰⁸ Postma et al ¹⁰⁹
CPVT3	I_{K1}	Loss	Kir2.1	<i>KCNJ2</i>	Tester et al, ²² Postma et al ¹¹⁰
CPVT4	I_{NCX} , I_{NaK} , $InsP_3R$	Loss	Ankyrin-B	<i>ANKB</i>	Mohler et al ¹¹¹

Abbreviations: RyR2, ryanodine receptor; I_{rel} , RyR2 Ca^{2+} release current; I_{K1} , inward-rectifier potassium current; I_{NCX} , Na^+/Ca^{2+} exchanger current, I_{NaK} , Na^+/K^+ ATPase (pump) current; $InsP_3R$, inositol 1,4,5-trisphosphate (IP3) receptors.

Table 5 Short-QT syndrome (SQTs)

Name	Current	Gain/loss of function	Protein	Gene	Reference
SQT1	I_{Kr}	Gain	$K_v11.1$	<i>KCNH2</i>	Brugada et al ¹¹²
SQT2	I_{Ks}	Gain	K_vLQT1	<i>KCNQ1</i>	Belloq et al ¹¹³
SQT3	I_{K1}	Gain	Kir2.1	<i>KCNJ2</i>	Priori et al ¹¹⁴
SQT4	I_{CaL}	Loss	$Ca_v1.2\alpha1$	<i>CACNA1C</i>	Antzelevitch et al ⁵⁸
SQT5	I_{CaL}	Loss	$Ca_v\beta_{2b}$	<i>CACNB2b</i>	Antzelevitch et al ⁵⁸
SQT6	I_{CaL}	Loss	$Ca_v\alpha_2\delta-1$	<i>CACNA2D1</i>	Templin et al ¹¹⁵

Abbreviations: I_{Kr} and I_{Ks} , rapid and slow delayed-rectifier potassium currents, respectively; I_{K1} , inward-rectifier potassium current; I_{CaL} , L-type calcium current.

similar to that seen in LQT3-associated *SCN5A* mutations.¹⁰ Moreover, *CAV3* mutations are also linked to sudden infant death syndrome.¹² However, because of multiple ion-channel involvement, the exact mechanisms by which *CAV3* mutations exert arrhythmogenesis remain to be determined.⁸

LQT11

Yotiao (AKAP9) is a member of a family of PKA-anchoring proteins. LQT11 is due to mutations of *AKAP9* that is required to be assembled with the α -subunit of the I_{Ks} channel (K_vLQT1) during β_2 -adrenergic activation (BAS); AKAP9-associated PKA activity is also connected with activities of RyR2 and $Ca_v1.2$. Hence, mutations of this PKA-anchoring protein interfere with PKA-dependent phosphorylation, thereby markedly inhibiting responses of at least three ion channels, ie, K_vLQT1 , RyR2, and $Ca_v1.2$, to cyclic adenosine monophosphate (cAMP).¹³ Clinically, LQT11 is likened to LQT1 and LQT5.⁸

LQT12

α -1-Syntrophin belongs to a family of cytoplasmic adapter proteins, linking many membrane proteins and signaling molecules, including plasma membrane calcium ATPase and neuronal nitric oxide synthase (nNOS); nNOS can directly affect persistent (or late) I_{Na} , and variants in the nNOS regulatory protein NOS1AP (CAPON) can regulate and moderate the QT interval.^{14,15} LQT12 is related to mutations of α -1-syntrophin, which cause a decrease in its binding activity for plasma membrane calcium ATPase and nNOS along with an increase in $Na_v1.5$ nitrosylation; the resultant persistent I_{Na} creates a clinical phenotype akin to LQT3.⁸

Mutations with channel function modulated by PKA-dependent phosphorylation as the cause of CPVT mimicry in LQT7 (Andersen–Tawil syndrome)

Andersen–Tawil syndrome (ATS) (LQT7) is an autosomal dominant disorder clinically characterized by ventricular

arrhythmias associated with long QT interval, periodic paralysis, and multiple skeletal dysmorphic features.^{16–18} More than 20 mutations have been identified in the *KCNJ2* gene, which encodes the α -subunit of the inward-rectifier potassium channel (Kir2.1).^{16–18}

Electrophysiologically, the corresponding Kir2.1 current (I_{K1}) participates in the terminal phase of action potential (AP) repolarization responsible for setting the resting membrane potential in mammalian heart cells.¹⁹ Of the mutations identified in distinct regions of *KCNJ2* in ATS patients, a dominant negative effect of the mutant allele can be found in both trafficking and non-trafficking defective channels, contributing to loss of Kir2.1 function.²⁰

Although patients with ATS have a high prevalence (approximately 64%) of ventricular arrhythmias,^{16,17} these arrhythmias are usually not related to exercise and/or emotional distress. The relatively benign clinical course is attributed to lack of significant enhancement of transmural dispersion of repolarization produced by I_{K1} down-regulation.²¹ However, a subset of ATS patients, especially females, are prone to exercise- and/or stress-induced bidirectional VT and/or polymorphic VT, mimicking CPVT,^{17,20,22,23} which is a malignant form of inherited arrhythmogenic disorder, affecting the young with otherwise a structurally normal heart and QT interval.²⁴ However, mutations of CPVT are primarily (approximately 60% of patients) located in the gene encoding RyR2 (CPVT1, autosomal dominant) or calsequestrin (CPVT2, autosomal recessive) in SR.

Molecular genetic studies have identified several mutations in the *KCNJ2* gene, such as G144D, R67W, R82W, R260P, T305S, and V227F,^{20,22,23} that may predispose ATS patients to catecholamine-sensitive ventricular tachyarrhythmias, regarded as CPVT mimicry or CPVT3.^{17,20,22,23} Of interest, among these mutations, heterozygous Kir2.1-V227F can function normally like wild-type channels.²³ However, when stimulated by cAMP-dependent PKA, the Kir2.1-V227F mutant can significantly downregulate I_{K1} . This unique biophysical property of latent loss of function modulated

by PKA-dependent phosphorylation of the I_{K1} channel provides a rather specific mechanism by which a subset of ATS patients may manifest ventricular arrhythmias under high adrenergic tone, simulating the phenotype of CPVT.

Ablation of the ion channel-anchoring protein may prevent arrhythmogenesis in LQT8 (Timothy syndrome)

Timothy syndrome (TS), a subtype (LQT8) of congenital LQTS, clinically manifests multisystem involvement (eg, syndactyly, dysmorphic facial features, autism, etc).²⁵ In association with marked QT-interval prolongation, cardiac arrhythmias are the most serious aspect of its clinical features. These include bradycardia, atrioventricular block, polymorphic VT, and ventricular fibrillation (VF). Because of high prevalence (71%) and severity of VT/VF, the majority of TS patients seldom survive beyond 3 years of age.^{25,26} Notably, the mode of induction of cardiac events (eg, syncope, cyanotic spell, and SCD) is usually triggered by an increase in sympathetic tone.

Molecular genetic studies have revealed that TS patients have mutations with a Gly to Arg substitution at position 406 (G406R) of $Ca_v1.2$ in the *CACNA1C* gene, which results in “near-complete” elimination of voltage-dependent inactivation (VDI) of $Ca_v1.2$ (gain of function).^{25,26} Electrophysiological studies have illustrated that G406R-mutated $Ca_v1.2$ is inactivated at a slow rate²⁵ alongside a high probability of undergoing coordinated openings and closings (coupled gating).²⁷

It is suggested that G436R-mutated $Ca_v1.2$ (corresponding to G406R in humans) in rabbit ventricular myocytes creates a new phosphorylation site (Ser-439) for Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), and phosphorylation of this site is correlated with an increased open probability of gating of the mutant cardiac $Ca_v1.2$.²⁸ Unfortunately, others have demonstrated that phosphorylation by CaMKII is not necessary for the slower rate of $Ca_v1.2$ inactivation.^{27,29} Moreover, in exogenously expressed (HEK293 cells) rabbit cardiac $Ca_v1.2$, it is shown that the G436R mutation displays multiple altered gating mechanisms of $Ca_v1.2$.²⁹ Besides affecting the open-state VDI, it significantly slows the channel activation at voltages less than 10 mV and disturbs its deactivation across, the latter of which also favors a high open-probability state of $Ca_v1.2$ (also arrhythmogenic).³⁰ Surprisingly, the closed state of VDI remains intact, suggesting that two different gating mechanisms are involved

in the open and closed states of VDI. Collectively, these findings imply that impaired open state of VDI and slowed deactivation of $Ca_v1.2$ can synergistically increase AP duration and cause early and delayed afterdepolarizations (EADs and DADs) and triggered activity (TA) due to Ca^{2+} overload.^{25,31} Experimentally, roscovitine, an open-state VDI enhancer, can revert functional defects of the mutated $Ca_v1.2$.²⁹

Recently, in ventricular myocytes obtained from a transgenic mouse model that expresses $Ca_v1.2$ -LQT8, the mutated $Ca_v1.2$ is shown to be abnormally coupled to AKAP150, a specific protein kinase that phosphatases to regions near $Ca_v1.2$.³² With the formation of this aberrant ion channel-anchoring protein complex, AKAP150 functionally serves like a channel subunit that stabilizes the open conformation and augments the probability of coordinated openings, enhancing $Ca_v1.2$ -LQT8 currents. Of note, ablation of AKAP150 restores normal gating (inactivation) of $Ca_v1.2$ -LQT8, thereby preventing EADs, DADs, and torsade des pointes. Hence, it appears that AKAP150 is required for increased $Ca_v1.2$ activity and coupled gating seen in LQT8 ventricular myocytes. Since AKAP150 also binds to the carboxyl tail of $Ca_v1.2$ via leucine-zipper motifs, it is postulated that AKAP150 binds to the C-terminal tail of $Ca_v1.2$ -LQT8, thereby facilitating longer channel openings and interaction between multiple $Ca_v1.2$ -LQT8 channels to increase the frequency of “coupled gating”; consequently, greater Ca^{2+} influx leads to intracellular Ca^{2+} overload. This unique AKAP150-dependent change in $Ca_v1.2$ -LQT8 gating may represent an important mechanism of arrhythmogenesis in LQT8.

Altered intracellular Ca^{2+} cycling and targeted pharmacotherapy for CPVT

CPVT is an inherited arrhythmogenic disorder characterized by episodic syncope occurring during physical activity or acute emotional distress in individuals with otherwise normal hearts and electrocardiograms (ECGs). Notably, episodes of syncope usually coincide with documented bidirectional or polymorphic VT that may degenerate into VF. By the age of 40 years, cardiac events occur in 80% of patients, and the mortality rate can be as high as 30%–50%.²⁴ In 60% of CPVT patients, molecular genetic studies have revealed that mutations are mostly located in the gene encoding RyR2 (autosomal dominant) and rarely in the calsequestrin gene (*CASQ2*, autosomal recessive).

Although more than 70 RyR2 mutations have been identified in CPVT, mechanisms by which RyR2 mutations

alter the properties of RyR2 receptors, thereby generating ventricular tachyarrhythmias, remain highly debatable. The general consensus is that RyR2 mutations lead to abnormally augmented Ca²⁺ release, resulting in DADs and DAD-mediated TA.²⁴ In an RyR^{R4496C} knock-in mouse model (equivalent to human R4497C mutation), it has been demonstrated that (1) RyR2^{+/+}/RyR^{R4496C} mice are prone to bidirectional VT after catecholaminergic stimulation and that VT can degenerate into VF;³³ (2) DADs and DAD-mediated TA induced by isoproterenol can be abolished by ryanodine but not by K201 (an agent that enhances binding of FKBP12.6 to RyR2);³⁴ (3) there is an increase in Ca²⁺ sensitivity of RyR2 conjoined with a lowered SOICR threshold;³⁵ (4) there is elevation of SR Ca²⁺ load in the absence of BAS; ouabain, an Na⁺/K⁺-ATPase antagonist, can enhance the propensity to DADs and TA, and that JTV-519, a RyR2 stabilizer, can effectively reduce these arrhythmias;³⁶ and (5) Purkinje cells of RyR2^{+/+}/RyR^{R4496C} mice are more vulnerable than the ventricular myocyte to altered intracellular Ca²⁺ cycling leading to EADs, DADs, and TA, which can be greatly exacerbated by BAS.³⁷ Taken together, these latter findings suggest that medications like digitalis are contraindicated and that Purkinje cells are critical contributors to arrhythmogenesis in CPVT similar to many other clinical situations, eg, after myocardial infarction, in the presence of dilated cardiomyopathy or idiopathic VF, and with other inherited ion channelopathies, including BrS and LQTS.³⁸

Missense mutations of *CASQ2* have been shown to alter the Ca²⁺-binding capacity and/or the Ca²⁺-dependent polymerization of calsequestrin. It seems that mechanistically different defects in *CASQ2* all lead to increased diastolic SR Ca²⁺ release and manifest a similar CPVT phenotype. It is illustrated that expression of truncated *CASQ2*^{DEL} (G112 + 15X) and *CASQ2*^{R33Q} mutations can alter myocyte Ca²⁺ signaling through two distinctly different mechanisms:³⁹ *CASQ2*^{DEL} mutation disrupts the polymerization of calsequestrin required for high-capacity Ca²⁺ binding, whereas *CASQ2*^{R33Q} mutation compromises the ability of calsequestrin to control the RyR2 channel activity. Consequently, with *CASQ2*^{DEL} mutation, local Ca²⁺ release terminates at the same free luminal Ca²⁺ concentrations as those of the wild type. In contrast, with *CASQ2*^{R33Q} mutation, compromised interactions between RyR2 and calsequestrin markedly lower the threshold of luminal Ca²⁺ concentrations for the termination of SR Ca²⁺ release. These findings suggest that calsequestrin possesses dual function by providing a local source of releasable Ca²⁺ and by exerting effects on luminal Ca²⁺-dependent RyR2 gating (ie, via protein–protein

interactions or serving as a luminal Ca²⁺ sensor).⁴⁰ It should be noted, however, calsequestrin-null mice are viable and display normal SR Ca²⁺ release and contractile function under basal conditions while still maintaining functional SR Ca²⁺ storage. Despite lack of calsequestrin, calsequestrin-null mice exhibit an increase in diastolic SR Ca²⁺ leak without apparent upregulation of other Ca²⁺-binding proteins and are susceptible to CPVT-related ventricular arrhythmias, especially under BAS. Thus, although calsequestrin modulates SR Ca²⁺ release, it is not essential for luminal Ca²⁺ regulation of RyR2, nor is it required for SOICR termination in cardiac myocytes. Currently, the consensus is that the luminal Ca²⁺ sensor lies within the RyR2 complex, likely to be regulated by a number of accessory proteins, such as calsequestrin, triadin, junctin, FKBP12.6, calmodulin, kinases, and others associated with the RyR2 complex.⁴¹

It has been shown that heterozygous *CASQ*^{K206N} missense mutation can generate an additional *N*-glycosylation site, which can result in the recombinant protein having a higher molecular weight with reduced Ca²⁺-binding capacity exhibiting an altered aggregation state; consequently, despite the interaction of *CASQ*^{K206N} with triadin and the protein level of RyR2 remaining unchanged, the response of the *CASQ*^{K206N} myocyte to caffeine is impaired, as there is a lower SR Ca²⁺ load alongside a higher opening state of RyR2. Therefore, the *CASQ*^{K206N} myocyte displays a higher rate of spontaneous SR Ca²⁺ release under basal conditions and under BAS compared to the wild-type counterpart.⁴²

Clinical observations have shown that β-blockers provide only incomplete protection, and other antiarrhythmic agents, such as quinidine, verapamil, and amiodarone, are either ineffective or only partially efficacious in CPVT.⁴³ Some patients need to resort to implantable cardioverter/defibrillators, left cardiac sympathetic denervation, and even to cardiac transplantation.²⁴ Based on better understanding of the pathophysiology of CVPT, targeted pharmacotherapy appears to be very promising.^{43,44} Recently, flecainide therapy has been advocated to be efficacious in suppressing ventricular arrhythmias in CPVT as well as certain ATS patients.^{20,45–47} Flecainide directly inhibits RyR2 by perturbing RyR2-mediated premature Ca²⁺ release, thereby suppressing arrhythmogenic Ca²⁺ waves and effectively suppressing ventricular arrhythmias in *CASQ2* knockout mice.^{45,47} However, flecainide is a potent Na⁺-channel inhibitor that also blocks *I*_{Kr}, and Ca²⁺-independent transient outward K⁺ current.⁴⁸ Being a potent Na⁺-channel inhibitor, it decreases the probability for DADs to reach the threshold for AP generation.⁴⁹ Moreover, it selectively increases the density

of functional Kir2.1 via reducing Kir2.1 polyamine blockade in the human ventricular myocyte.⁵⁰ Although the exact mechanism whereby flecainide exerts its antiarrhythmic action is complex, the therapeutic efficacy of flecainide in CPVT patients has been clinically confirmed.⁴⁶

For targeted pharmacotherapy, simulated electropharmacological testing suggests that combination therapy with a Ca²⁺-channel blocker and an I_{up} (SR Ca²⁺-ATPase current) inhibitor is more efficacious than a Ca²⁺-channel blocker alone in suppressing CPVT-associated arrhythmias.³¹ Furthermore, in RyR^{R4496C/+} knock-in mice, CaMKII overexpression enhances RyR2 phosphorylation and increases the propensity to TA; KN93, a CaMKII inhibitor, prevents TA via counteracting effects of BAS.⁵¹ Thus, tackling a specific site in intracellular Ca²⁺ cycling appears logical and promising for suppressing cardiac arrhythmias associated with CPVT.

Catheter ablation of the right ventricular outflow tract may be curative for severely symptomatic BrS

BrS is an inherited arrhythmogenic disorder that exhibits ECG ST-segment elevation with a negative T-wave in the right precordial leads (V_1 - V_3), predisposing to VF and SCD in otherwise healthy individuals.⁵² BrS is recognized as an important cause of SCD in young men, especially in Southeast Asia.⁵³ Notably, the Brugada ECG pattern is dynamic and functional; it is more pronounced at night or at rest and may be enhanced by an increase in vagal tone, such as at night or after a big meal, and may become apparent during a febrile illness. Of note, sodium-channel blockers (class 1A and 1C) and acetylcholine may enhance or provoke the Brugada ECG pattern, facilitating the diagnosis of the syndrome.⁵²

Genetically, mutations in eleven genes have been identified in patients with BrS. Most of these mutations are located in *SCN5A*, encoding the α -subunit of Na_v1.5,⁵⁴ in *SCN1B*⁵ and *SCN3B*,⁵⁵ encoding the β -subunits of the cardiac sodium channel, or in *GPD1L*⁵⁶ and *MOG1*,⁵⁷ involving the trafficking of Na_v1.5 to the cell membrane; all of these mutations result in loss of function of Na_v1.5, thereby reducing I_{Na} . Other mutations are located in *CACNA1C*, *CACNB2b*⁵⁸ and *CACNA2D1*,⁵⁹ which encode $\alpha 1$ -, β_{2b} -, and $\alpha 2\delta 1$ -subunits of Ca_v1.2, respectively, with resultant reduction of the L-type calcium current ($I_{Ca,L}$) and in *KCNE3*, encoding MiRP2, α -, and β -subunits of several potassium channels,⁶⁰ and in *KCNJ8*, encoding the

ATP-sensitive potassium channel.⁶¹ Overall, mutations in *SCN5A* account for approximately 20% and those in other genes 10%, leaving no definitive genetic defects in 70% of BrS patients.⁵² Although the heart appears grossly normal, right ventricular structural abnormalities, such as fibrofatty degenerative changes, lymphocytic myocarditis, and atypical cardiomyopathic alterations in the ventricular outflow tract (RVOT) are not infrequently observed. Clinical features overlapping with the right ventricular dysplasia/cardiomyopathy, another form of inherited arrhythmogenic disorder with distinctly abnormal right ventricle (Table 1), have been reported. Differential diagnosis between the two may at times present as a clinical dilemma.⁵²

Despite the discovery of various genetic mutations, the pathophysiology underpinning BrS remains elusive. There has been much debate about whether it is a depolarization disorder (ie, conduction delay or failure) or repolarization disorder (ie, transmural repolarization gradient through the right ventricular wall).⁵² More significantly, the theory of loss of function of Na_v1.5 or other ion channels as the basis has been questioned. This is because (1) there is often lack of a hereditary (familial) pattern in most index BrS patients, (2) sodium-channel blockers do not usually provoke the Brugada ECG pattern in normal subjects, and (3) the ECG pattern can also be seen or provoked in patients with structural heart diseases. Hence, it has been suggested that BrS is a multifactorial disorder with various causes of right ventricular conduction disturbances, of which “current-to-load mismatch” is the common denominator; different etiologies may require different approaches to management of cardiac arrhythmias in patients with BrS.⁵²

Of therapeutic relevance are two recent reports. In nine BrS patients treated with implantation of cardioverter/defibrillators because of recurrent VF, Nademanee et al⁶² noted that all these nine patients had typical Brugada ECG pattern and electrically inducible VT/VF. Electrophysiological mapping revealed the presence of low-voltage, fractionated late potentials spanning beyond the electrocardiographic QRS complex, clustering exclusively in the RVOT anterior aspect during sinus rhythm. Because the magnitude of these electrogram abnormalities over the epicardium was much greater than those recorded in the endocardium, the researchers applied radiofrequency catheter ablation at these sites; as a result, the Brugada ECG pattern became normalized in eight patients (89%) and VT/VF rendered electrically noninducible in seven patients (78%). All patients were off medication except for one (amiodarone therapy continued), and there was no recurrence of VT/VF observed in any patients

during a follow-up period of 20 ± 6 months. The study has demonstrated that the BrS ECG pattern can be accounted for by delayed depolarization over the anterior aspect of RVOT. Moreover, in ten BrS patients, Sunsaneewitayakul et al⁶³ noted a late activation zone recorded as electrical activity occurring from the J point to +60 (J+60) millisecond interval of V_1 or V_2 ECG lead in the RVOT endocardium during an isopotential mapping. Radiofrequency endocardial catheter ablation of the late-activation zone modified the Brugada ECG pattern in three (75%) of four patients with VF storm. During a follow-up period of 12–30 months, there was no recurrence of VF in any of these four patients (100%). It seems that the endocardium of RVOT can be considered as an alternative site for radiofrequency catheter ablation in the treatment of recurrent VF in BrS.

Technology of induced pluripotent stem cells as a promising diagnostic and research tool in inherited arrhythmogenic disorders

Electrophysiological characteristics of the mouse and human hearts are distinctly different in the distribution of ion channels, AP morphology, and heart rate.⁶⁴ A mouse model may not always demonstrate the same phenotype as humans. Therefore, the use of induced pluripotent stem cell (iPSC) technology to study inherited arrhythmogenic disorders has become a new paradigm, as it can provide direct evidence linking genetic mutations to cardiac arrhythmias in humans.^{65,66} The iPSC technology is to reprogram somatic cells into pluripotent stem cells via transduction of a key set of transcriptional factors (eg, c-Myc, Oct3/4, Sox2, and Klf4 or Oct3/4, Sox2, Nanog, and Lin28). These iPSCs can then differentiate into the cell types of the three germ layers, and when generated from human somatic cells, the human iPSCs resemble those of human embryonic stem cells (ESCs) in morphology, gene expression, and epigenetic status of pluripotent cell-specific genes.⁶⁷ Human iPSCs can differentiate to functional cardiac myocytes (CMs),⁶⁸ and similar to human ESCs these human iPSCs possess a capacity of differentiating into nodal-, atrial-, and ventricular-like cells, each with specific AP characteristics; both iPSC- and ESC-derived CMs exhibit typical responsiveness to BAS. Consequently, the possibility of creating patient- and disease-specific human iPSC-derived CMs aimed at individualized diagnostic and pharmacological testing has become a reality. To date, iPSC-derived CMs have been assessed in a limited number of patients with ion channelopathies.^{65,66}

LQT1

In iPSC-derived CMs produced from two patients with *KCNQ1*^{R190Q} mutation,⁶⁹ functional study revealed a dominant negative trafficking defect associated with a 70%–80% reduction in I_{Ks} alongside altered activation and deactivation properties of K_v LQT1. Compared to control, these iPSC-derived CMs with *KCNQ1*^{R190Q} showed increased propensity to EADs in response to isoproterenol, which could be attenuated by propranolol.⁶⁹ These findings are in accord with clinical observations that cardiac events are usually triggered by enhanced sympathetic tone and that β -blockers are beneficial in LQT1.⁷⁰

LQT2

In iPSC-derived CMs produced from a patient with A614V missense in *KCNH2*,⁷¹ there was prolongation of AP duration, which could be ascribed to significant reduction of I_{Kr} . These iPSC-derived CMs with *KCNH2*^{A614} also exhibited marked arrhythmogenicity characterized by EADs and EAD-mediated TA. K^+ -channel blockers could aggravate, whereas Ca^{2+} -channel blockers, $I_{K(ATP)}$ -channel openers, and late Na^+ -channel blockers could ameliorate the disease phenotype.⁷¹ In another patient with *KCNH2*^{G1681A}, iPSC-derived CMs⁷² also showed prolonged AP duration. Exposure to E4031 (an I_{Kr} blocker) provoked EADs. Moreover, isoprenaline facilitated EADs; this latter effect could be reversed by β -blockers.⁷²

In an asymptomatic patient with *KCNH2*^{R176W} whose sister and father had died suddenly at an early age, iPSC-derived CMs with such a mutation showed prolonged AP duration and significantly reduced I_{Kr} density.⁷³ These iPSC-derived CMs with *KCNH2*^{R176W} were sensitive to potentially arrhythmogenic drugs such as sotalol, and exhibited a pronounced inverse correlation between the beating rate and repolarization time. Hence, iPSC-derived CMs may be used to provide a direct way for identifying asymptomatic patients at risk of proarrhythmia.

LQT3

iPSC-derived CMs were produced from a mouse model with a human LQT3 mutation of $Na_v1.5$.⁷⁴ These iPSC-derived CMs showed biophysical effects of the mutation on $Na_v1.5$, ie, faster recovery from inactivation and larger late currents than those of the wild-type control, prolonged AP duration, and development of EADs at low pacing rates, consistent with classic features of LQT3.

In a mouse model, iPSC-derived CMs carrying *SCN5A*^{1798insD/+} mutation⁷⁵ showed a decrease in I_{Na} density with a large persistent I_{Na} and reduced AP upstroke velocity coupled with prolonged AP duration. These

electrophysiological characteristics were subsequently observed in iPSC-derived CMs generated from a patient with equivalent *SCN5A*^{179SinsD/+} mutation. Of note, this latter mutation clinically gave rise to a phenotype of LQT3 and BrS with conduction defects due to both gain- and loss-of-function effects on $I_{NaV1.5}$, respectively.⁷⁶

LQT8

Human iPSC-derived CMs generated from two patients with TS (LQT8) showed irregular contractions, excessive Ca^{2+} influx, and markedly prolonged AP duration (three times longer than that of the wild-type control),⁷⁷ irregular electrical activity and abnormal intracellular Ca^{2+} transients associated with delayed inactivation of $Ca_v1.2$. Roscovitine, a compound that increases VDI of $Ca_v1.2$, was able to revert delayed inactivation of $Ca_v1.2$ and restored irregular intracellular Ca^{2+} transients.

CPVT

iPSC-derived CMs created from a patient with *RyR2*^{F2483I} heterozygous mutation showed high amplitude and long duration of spontaneous Ca^{2+} release at basal state.⁷⁸ In response to catecholaminergic stimulation, these iPSC-derived CMs were prone to DADs, which paradoxically could be abolished by increasing the cytosolic cAMP level with forskolin. iPSC-derived CMs were also produced from a CPVT patient with *RyR2*^{M4109R} heterozygous mutation. In the presence of isoproterenol or forskolin, these CPVT iPSC-derived CMs readily developed DADs and DAD-mediated TA related to SOICR, which could be improved with β -blockers and could be eliminated by both flecainide and thapsigargin (an I_{up} inhibitor);⁷¹ the threshold of SOICR was significantly reduced in these CPVT human iPSC-derived CMs.

Lastly, in iPSC-derived CMs produced from a CPVT patient carrying *RyR2*^{S406L} mutation,⁷⁹ catecholamines led to an increase in diastolic intracellular Ca^{2+} transients and a reduced SR Ca^{2+} content coupled with an increased susceptibility to DADs due to increased frequency and duration of Ca^{2+} sparks. Dantrolene, a drug effective for treating malignant hyperthermia (presumably via inhibiting the ryanodine receptor) was able to rescue arrhythmogenic properties of CPVT iPSC-derived CMs.

Genotype–phenotype correlation and risk stratification in congenital LQTS

Clinically relevant genotype–phenotype relationships have been investigated in the ECG ST-T waves, responses to

epinephrine, types of arrhythmogenic trigger, responsiveness to antiarrhythmic agents, and risk stratification.^{80–82} Characteristically, an arrhythmic event is frequently exertion-triggered in LQT1 and auditory-triggered in LQT2, and by contrast it usually occurs at rest in LQT3. β -blocker therapy is most effective in LQT1 followed by LQT2 and least effective in LQT3; in fact, it should be considered contraindicated in LQT3.⁷⁰ Because I_{Ks} , I_{Kr} , I_{NaK} , I_{K1} , and $I_{Ca,L}$ are catecholamine-sensitive, the trigger of arrhythmic events is often related to enhanced sympathetic tone in certain congenital LQTS, such as LQT1 (*KCNQ1*- I_{Ks}), LQT2 (*KCNH2*- I_{Kr}), LQT4 (*ANK2*- I_{NaK} , I_{NaCa} , IP_3), LQT5 (*KCNE*- I_{Ks}), LQT7 (*KCNJ2*- I_{K1}), LQT8 (*CACNA1C*- $I_{Ca,L}$), and LQT11 (*AKAP9*- I_{Ks}). An arrhythmic event triggered by swimming is relatively specific for LQT1,⁷⁰ but it also has been reported in cases of CPVT and LQT7.¹⁷

LQT1, LQT2 and LQT3 constitute the majority (approximately 65%–75%) of congenital LQTS.⁸³ Among these three subtypes, it has been suggested that the prognostic value might be assigned according to the location of a particular mutation. In LQT2, Moss et al⁸⁴ noticed that patients with mutations in the pore region of the *KCNH2*-encoded K^+ channel ($K_v11.1$) did worse than those who harbored mutations in the C-terminus. Similarly, Migdalovich et al⁸⁵ found that pore-loop mutations displayed a significant higher risk in men (>twofold) than in women, but overall women were more vulnerable than men to life-threatening cardiac events (26% vs 14%). Furthermore, Kim et al⁸⁶ noted that the type of trigger, in addition to sex, location of mutations, and QTc interval (≥ 500 m), was an important risk factor for arrhythmic events in LQT2; risk factors for arousal-triggered cardiac events included sex (female: male > 13 years: hazard ratio [HR] = 9.10, $P < 0.001$) and the presence of pore-loop mutations (HR = 2.19, $P = 0.009$), and β -blocker therapy was beneficial in patients with exercise-triggered events (HR = 0.29, $P < 0.01$), but not in those with arousal and nonexercise/nonarousal events.

Similarly, in LQT1 patients, Shimizu et al⁸⁷ reported that patients with mutations involving the transmembrane-spanning domains and/or pore regions of K_v LQT1 had a poorer outcome compared to those harboring mutations in the C-terminus. Crotti et al⁸⁸ noted that LQT1 mutation *KCNQ1*^{A341V} was associated with a high clinical severity (SCD rate 14%) despite β -blocker therapy. These *KCNQ1*^{A341V} patients were more likely to have a longer QTc interval (485 ± 43 vs 465 ± 38 milliseconds), cardiac events (75% vs 24%), and were younger at first event (6 vs 11 years) compared to non-A341V patients. Moreover, this

KCNQ1^{A341V} “hot spot” was predictive of high clinical severity independent of ethnic origin.

Nevertheless, it is well appreciated that variable expressivity and “genotype–phenotype violation” are not infrequently encountered in “inherited arrhythmogenic disorders,” presumably due to incomplete penetrance, epigenetic factors, and gene-to-gene interaction (eg, compound mutations, modifier genes, polymorphisms, etc).^{80,89–91} Consequently, the concept of a malignant domain should not be generally applied.⁹⁰ In genotype-positive and phenotype-negative patients, genotype (including the site of mutation), age, sex, family history, and the QTc interval all need to be taken into consideration while contemplating preventive strategy with lifestyle adjustment, pharmacotherapy, and/or prophylactic implantation of an implantable cardioverter/defibrillator.^{80,90,92}

Conclusion

Our current understanding of the pathophysiology of inherited arrhythmogenic disorders has come mostly from intensive and extensive investigations of ion channelopathies, especially congenital LQTS and others like CPVT and BrS over the past 15 years. The accumulated knowledge of the functionality of each ion channel in relation to gene mutations has facilitated research and development of more effective targeted pharmacotherapy and nonpharmacological treatment modalities, such as gene therapy and catheter ablation.

Technology with the ability to generate human iPSCs^{67,68} has become a new paradigm for pathophysiological study of patient- and disease-specific cells and for screening of new drugs, and clinical application of gene therapy. However, because this technology is relatively new, the validity and reproducibility of the findings need to be carefully verified. Human iPSC-derived CMs are usually immature, more akin to fetal CMs, as yet to recapitulate all the characteristics of adult CMs. Moreover, better purification of human iPSC-derived CMs is needed, as they often represent a mixture of cell types – atrial, nodal, and ventricular – that display various electrophysiological properties; as a result, CMs so derived might not fully exhibit disease-specific phenotypic features. Further refinement of the technology (eg, the method used for cell isolation and cultivation, selection of CM-specific promoters, etc) is currently under way.^{65,66} It is anticipated that continued efforts and collaboration between clinical and basic science researchers are required to overcome difficulties concerning the use of iPSC technology in the diagnosis and management of arrhythmic disorders in clinical practice.

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Disclosure

The authors report no conflicts of interest in this work.

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