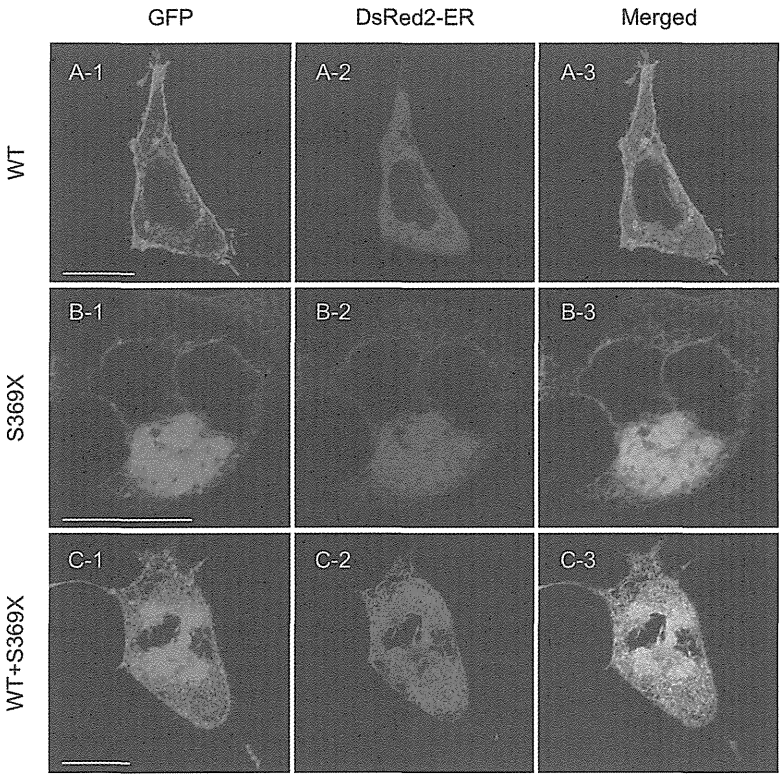

Figure 5. ER colocalization with WT and S369X in COS7 cells. Microscopic images of COS7 cells expressing EGFP-WT (A-1 ≈ A-3, 1 μg/dish), EGFP-S369X (B-1 ≈ B-3, 1 μg/dish), and EGFP-S369X cotransfected with WT-*KCNJ2* pIRES/CD8 (C-1 ≈ C-3, 0.5 μg each/dish) (bars, 20 μm). Each cell was cotransfected with DsRed2-ER (1 μg/dish). Images are shown for GFP alone, DsRed2-ER, and the merged image. Colocalization between EGFP-KCNJ2 and DsRed2-ER appears as yellow. ER indicates endoplasmic reticulum. Other abbreviations as in Figure 4.

As previously reported, abnormal trafficking of mutant proteins (KCNJ2-V302M, Δ314–315) is recognized as 1 of the mechanisms causing ATS.[17,18] The trafficking defect was hypothesized to be a result of ER retention, degradation of folding-defective mutant proteins, or mutation of a binding motif essential for trafficking. Defective trafficking in ion channelopathies also was reported in other types of LQTS.[30,31] Confocal image analysis was used in the present study to identify KCNJ2-S369X as the trafficking-deficient mutation, which is similar to that of V302 and Δ314–315. Mutant S369X subunits were, however, transported to the plasma membrane after coassembled with WT subunits and formed functional tetramers.

The Kir channel family contains several trafficking motifs at their C-terminus. For example, PDZ motif-binding proteins are important for targeting channels and moving them to specific subcellular locations.[32] Notably, Kir2.1 contains an ER-to-Golgi export signal,[28] the motif FCYENE (Figure 2), in its C-terminal domain. By using various truncated Kir2.1 channels, Ma and colleagues[28] demonstrated that FCYENE consensus at codon 374 to 379 (Figure 2B) played the role of export signal from ER to Golgi and that lack of C-terminus including this motif resulted in reduced expression to the cell surface. Their truncated Kir2.1 (1 to 362) showed cellular phenotypes similar to those displayed by S369X (Kir2.1, 1 to 367). Therefore, S369X is exactly a naturally occurring mutation lacking this motif. In the heterozygous condition mimicking the clinical setting, 2 types of subunits, WT and S369X, can assemble to form heteromeric tetramers, although this leads to the presence of <4 ER-to-Golgi export-signaling

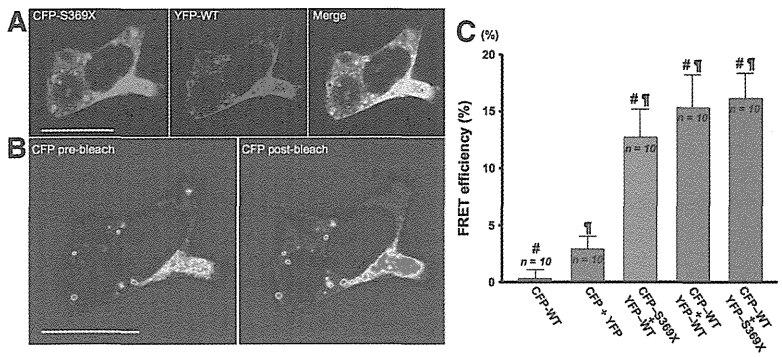

Figure 6. FRET analysis. **A**, YFP-WT and CFP-S369X were coexpressed in COS7 cells. CFP-S369X is pseudocolored in green (left), and YFP-WT is pseudocolored in red (middle). The merged image (right) shows colocalization of CFP-S369X and YFP-WT (bar, 20 μm). **B**, Pseudocolor images of CFP-S369X before (CFP prebleach) and after (CFP postbleach) YFP photobleaching. **C**, Summarized data of FRET efficiency. #$P<0.01$ versus CFP-WT. ¶$P<0.01$ versus CFP-N1+YFP-N1, tested by Wilcoxon test. CFP indicates cyan fluorescent protein; FRET, fluorescence resonance energy transfer; YFP, yellow fluorescent protein; WT, wild type.

motifs in 1 functional channel. Along with this idea, cotransfection of the mutant with WT-*KCNJ2* indeed promoted the resultant K^+ current density (Figure 3D).

These results were unexpected because most previously reported *KCNJ2* mutations exerted dominant-negative suppressions. Based on the experiments of heterologous expression and FRET analysis, S369X appeared not to act as a dominant-negative mutation (Figures 5 and 6). FRET analysis indicated the direct protein-protein interaction between mutant and WT subunits, suggesting that WT subunits may partially rescue the inappropriate trafficking of the mutant subunits by assembling into heteromeric complexes. Therefore, the truncated mutant proteins, though lacking the intracellular trafficking signal, seem to exert "inverse" dominant-negative effects. Physical interaction of 2 KCNJ2 subunits, WT and S369X (located to the end of the C-terminus), was shown by using the FRET method (Figure 6), and it would be plausible that incorporation of the mutant subunit eventually increases the number of functional channels and, thereby, produces a partial rescue of currents.

In conclusion, these effects may be the reason why the phenotype of the index patient with *KCNJ2*-S369X mutation showed milder clinical features. More recently, *KCNJ2* mutations have been shown to be a cause not only in ATS, but also in catecholaminergic polymorphic ventricular tachycardia.[20] Such subcellular regulation of KCNJ2 protein expression makes the potential extension and severity of the phenotype extremely variable.

The present study had some limitations. In the experiment shown in Figure 3, the results of coexpression with WT and mutant at 0.5 μg each yielded 330 pA/pF and was close to that resulting from the mathematical addition of half of WT and mutant at 1 μg each [(542+83.5)/2=313 pA/pF]. Increase in current density by coexpression was 5.2%, which was smaller than the case with 1 μg expression (16%). Because we used the liposomal transfection method, which has intrinsic experimental limitations to evaluate the efficiency of optimal cDNA transfection, we should be careful to assess the results quantitatively and await further study to confirm the rescue effect more quantitatively.

We used a heterologous expression system that allowed us to reproduce the I_{K1}-like currents in cells transfected with WT and S369X. However, the electrophysiological experiments were performed with a simplistic model in the absence of cellular heterogeneity. Indeed, Kir2.x channel families may form functional heteromultimers; an additional complication in that heteromultimerization of Kir2.x may alter the biophysical characteristics of channel functions.[33] Further studies on the interaction of mutations between Kir2.1 and Kir2.x in ATS may provide further insights into the pathophysiological mechanisms underlying ATS.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Andersen-Tawil syndrome is a rare disorder inherited in an autosomal-dominant fashion. Mutations in *KCNJ2*, a gene encoding the inward rectifier K^+ channel Kir2.1, are associated with Andersen-Tawil syndrome, which is characterized by ventricular tachyarrhythmias associated with QT (QU)-interval prolongation, periodic paralysis, and dysmorphic features. We identified a novel *KCNJ2* mutation, S369X, in a 13-year-old boy with prominent QU-interval prolongation and mild periodic paralysis. The mutation results in the truncation at the middle of the cytoplasmic C-terminal domain that eliminates the endoplasmic reticulum-to-Golgi export signal. KCNJ2-S369X exhibited this deficiency in the present electrophysiological and confocal microscopic analysis, and when coexpressed with KCNJ2 wild type, these abnormalities were partially restored. Fluorescence resonance energy transfer analysis demonstrated direct protein-protein interactions between wild type and S369X subunits in the intracellular compartment. The S369X mutation causes a loss of the endoplasmic reticulum export motif, but the trafficking deficiency can be partially rescued by directly assembling with the wild type protein, resulting in a limited restoration of plasma membrane localization and channel function. This alleviation may explain why our patient presented with a relatively mild Andersen-Tawil syndrome phenotype.

This Review is the last in a thematic series on **Inherited Arrhythmogenic Syndromes: The Molecular Revolution**, which includes the following articles:

The Fifteen Years that Shaped Molecular Electrophysiology: Time for Appraisal [*Circ Res.* 2010;107:451–456]

Defining a New Paradigm for Human Arrhythmia Syndromes: Phenotypic Manifestations of Gene Mutations in Ion Channel- and Transporter-Associated Proteins [*Circ Res.* 2010;107:457–465]

The Cardiac Desmosome and Arrhythmogenic Cardiomyopathies: From Gene to Disease [*Circ Res.* 2010;107:700–714]

Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac voltage-dependent L-type Calcium Channel [*Circ Res.* 2011;108:607–618]

Inherited dysfunction of Sarcoplasmic Reticulum Ca2+ Handling and Arrhythmogenesis [*Circ Res.* 2011;108:871–883]

Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Sodium Channel [*Circ Res.* 2011;108:884–897]

Phenotypical Manifestations of Mutations in Genes Encoding Subunits of Cardiac Potassium Channels

Silvia Priori, Editor

Phenotypic Manifestations of Mutations in Genes Encoding Subunits of Cardiac Potassium Channels

Wataru Shimizu, Minoru Horie

Abstract: Since 1995, when a potassium channel gene, *hERG* (human ether-à-go-go-related gene), now referred to as *KCNH2*, encoding the rapid component of cardiac delayed rectifier potassium channels was identified as being responsible for type 2 congenital long-QT syndrome, a number of potassium channel genes have been shown to cause different types of inherited cardiac arrhythmia syndromes. These include congenital long-QT syndrome, short-QT syndrome, Brugada syndrome, early repolarization syndrome, and familial atrial fibrillation. Genotype-phenotype correlations have been investigated in some inherited arrhythmia syndromes, and as a result, gene-specific risk stratification and gene-specific therapy and management have become available, particularly for patients with congenital long-QT syndrome. In this review article, the molecular structure and function of potassium channels, the clinical phenotype due to potassium channel gene mutations, including genotype-phenotype correlations, and the diverse mechanisms underlying the potassium channel gene–related diseases will be discussed. **(*Circ Res.* 2011;109:97-109.)**

Key Words: genetic testing ■ ion channels ■ sudden death ■ ventricular fibrillation ■ atrial fibrillation

A variety of mutations in genes that encode cardiac potassium channel pore-forming proteins and their accessory modulating proteins have been shown to cause different types of inherited arrhythmias. Such results were made possible by either candidate gene or linkage studies. Candidate gene studies examine variations in a low number of known, plausibly associated genes in affected case and control subjects, whereas linkage studies assess affected families/sibling pairs by use of microsatellite markers to define a genomic region linked to the phenotype. These approaches have resulted in an understanding of the genetic background of cardiac ion channelopathies, including

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From the Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center (W.S.), Suita, Japan, and the Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science (M.H.), Otsu, Japan.

Drs Shimizu and Horie contributed equally to this review article.

Correspondence to Dr Wataru Shimizu, Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565 Japan (E-mail wshimizu@hsp.ncvc.go.jp); or Dr Minoru Horie, Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Shiga 520-2192, Japan (E-mail Horie@belle.shiga-med.ac.jp).

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Non-standard Abbreviations and Acronyms	
APD	action potential duration
BrS	Brugada syndrome
SQTS	short-QT syndrome

long-QT syndrome (LQTS).[1] In 1991, Keating and coworkers[2] used linkage analyses and first reported that a DNA marker at the Harvey ras-1 locus (H-ras-1) in chromosome 11 was linked to LQTS. Five years later, in 1996, positional cloning methods established a potassium channel gene, *KVLQT1*, now referred to as *KCNQ1*, as the chromosome 11–linked LQT1 gene.[3] One year earlier, in 1995, another potassium channel gene, *hERG* (human ether-à-go-go–related gene), now referred to as *KCNH2*, was identified as being responsible for LQT2.[4] Since the mid-1990s, several potassium channel–encoding genes have been reported to be linked not only to LQTS but also to various inherited arrhythmia syndromes, including the short-QT syndrome (SQTS), Brugada syndrome (BrS), early repolarization syndrome, and familial atrial fibrillation (AF). Other potassium channel–encoding genes linked to various inherited arrhythmia syndromes include *KCNJ2*, *KCNJ5*, *KCNJ8*, and *KCNA5*, as well as the accessory subunits *KCNE1*, *KCNE2*, *KCNE3*, and *KCNE5* (Table).

Molecular Structure and Function of Potassium Channels That Contribute to Formation of Cardiac Action Potential

An extensive diversity of potassium channels has been revealed since the first cloning of a voltage-gated potassium channel by Jan and colleagues.[5] This reflects the complex and multiple roles of potassium channels as modulators of physiological function. In the generation of cardiac action potential, for example, potassium channels work to maintain a hyperpolarized resting potential and determine the timing of repolarization by flowing outward currents during the plateau phase. Subtle and delicate expression of distinct types of potassium channels elegantly generates the whole-heart action potential gradient in both the transmural and apicobasal directions. Failure of their normal function may lead to various types of inherited arrhythmia syndromes, and in this regard, congenital LQTS has played the part of a Rosetta stone as predicted by Zipes 20 years ago.[6]

To generate the cardiac action potential, in addition to inward sodium and calcium currents, 5 potassium currents are primarily involved: The inward-rectifier background current (I_{K1}), the rapidly activating and inactivating transient outward current (I_{to}), and the ultrarapid (I_{Kur}), rapid (I_{Kr}), and slow (I_{Ks}) components of delayed rectifier currents. (Abbreviations in parentheses indicate names of specific currents used in basic electrophysiology.)

I_{K1} carries the background potassium current that stabilizes the resting membrane potential and is responsible for determining the threshold potential for the initial depolarization and final repolarization of the action potential (late phase 3).

Table. Defect of Ion Channels or Membrane Adaptor Responsible for the Potassium Channel Gene–Related Arrhythmia Syndromes

Loci	Chromosome	Gene	Ion Channel	Result
Congenital LQTS (Romano-Ward)				
LQT1	11 (11p15.5)	*KCNQ1*	I_{Ks}	Loss of function
LQT2	7 (7q35–q36)	*KCNH2*	I_{Kr}	Loss of function
LQT5	21 (21q22.12)	*KCNE1*	I_{Ks}	Loss of function
LQT6	21 (21q22.12)	*KCNE2*	I_{Kr}	Loss of function
LQT7	17 (17q23.1–q24.2)	*KCNJ2*	I_{K1}	Loss of function
LQT11	7 (7q21–q22)	*AKAP-9*	I_{Ks}	Loss of function
LQT13	11 (11q23.3–24.3)	*KCNJ5*	$I_{K\text{-}ACh}$	Loss of function
Congenital LQTS (Jervell and Lange-Nielsen)				
JLN1	11 (11p15.5)	*KCNQ1* (homozygous)	I_{Ks}	Loss of function
JLN2	21 (21q22.12)	*KCNE1* (homozygous)	I_{Ks}	Loss of function
SQTS				
SQT1	7 (7q35–q36)	*KCNH2*	I_{Kr}	Gain of function
SQT2	11 (11p15.5)	*KCNQ1*	I_{Ks}	Gain of function
SQT3	17 (17q23.1–q24.2)	*KCNJ2*	I_{K1}	Gain of function
Brugada syndrome				
BrS6	11 (11q13–q14)	*KCNE3*	I_{to}	Gain of function
Early repolarization syndrome				
	12 (12p11.23)	*KCNJ8*	$I_{K\text{-}ATP}$	Gain of function
Atrial fibrillation				
	11 (11p15.5)	*KCNQ1*	I_{Ks}	Gain of function
	21 (21q22.12)	*KCNE2*	I_{Ks}	Gain of function
	11 (11q13–q14)	*KCNE3*	I_{Ks}	Gain of function
	17 (17q23.1–q24.2)	*KCNJ2*	I_{K1}	Gain of function
	12 (12p13)	*KCNA5*	I_{Kur}	Loss of function

LQTS indicates long-QT syndrome; SQTS, short-QT syndrome.

I_{to} consists of at least 2 components carrying fast ($I_{to,f}$) and slow ($I_{to,s}$) transient outward currents. They are differentiated on the basis of the rate of inactivation and its recovery and are variably expressed in the myocardium and form the transmural gradient of repolarization timing. Finally, delayed rectifier currents (I_K) play a key role in determining the duration of action potentials and comprise at least 3 components: I_{Kur}, I_{Kr}, and I_{Ks}. They are easily distinguished from each other by their pharmacological or biophysical properties. I_{Kur} is expressed mainly in the atrium and not in the ventricle and therefore does not help determine QT interval.[7] I_{Kr} activates rapidly but is easily inactivated on stronger depolarization (showing a strong inward rectification).[8] In contrast, I_{Ks} activates very slowly on depolarization compared with other potassium currents, and therefore, its net repolarizing currents can accumulate, especially at higher heart rates (because of a shorter diastolic phase) and are greatest at phase 3 of the action potential.[8] These fundamental understandings were mainly achieved since the late 1970s by means of patch-clamp techniques in mammalian cardiomyocytes.[9]

An understanding of the molecular biology of potassium channels came later, after the memorable report by Papazian et al.[5] The pore-forming subunit of the voltage-gated channel (α-subunit) has since been shown to contain at least 2 highly conserved components: the voltage-sensing part that surrounds the central pore, and the pore domain itself. Voltage-gated potassium channels involved in formation of cardiac action potential work as a tetramer of α-subunits, each having 6 transmembrane-spanning segments (S1–S6), with S4 containing 6 positively charged amino acids.[10] The pore domain is composed of S5, the P-loop, and S6, which is the ion permeation pathway, and includes the ion selectivity filter.[11] The opening of the channel and its associated gating current is caused by membrane depolarization and outward movement of the positively charged S4 segment. In addition to S4, the neighboring S2 and S3 segments serve as channel voltage sensors. Mutations in these regions may cause cardiac ion channel diseases by altering channel gating and ion permeability.[12]

KCNH2 encodes the α-subunit of the I_{Kr} channel, and membrane depolarization induced by strong inward currents produces a sequence of conformation changes within the channel that allows permeation of potassium ions. The S6 segment has a conserved glycine, which can be involved in channel opening by causing a wide splaying of the inner helices. When they close, these 4 inner helices, by leaning toward the membrane and interlace near the cytoplasmic border, narrow the ion passage and prevent potassium ion permeation.[13]

KCNQ1 encodes the α-subunit of I_{Ks} channels and is believed to have a tetrameric conformation similar to I_{Kr} channels, with S4 as a voltage sensor. I_{Ks} has a motif generally seen in other potassium channels in the S6 segment, proline-X-proline, which is thought to play a role in gating. S6 contains the alanine hinge, a residue that could favor maintenance of the α-helical structure.[14] To form a functional I_{Ks} channel, *KCNQ1* requires coexpression of an accessory subunit (called *MinK*) encoded by *KCNE1*,[15,16] although the stoichiometry between the 2 molecules remains unknown.

KCNA5 encodes the α-subunit of the I_{Kur} channel, and its loss-of-function mutations have been shown to be associated with familial AF.[17–19] *Kv4.3* encodes the α-subunit of $I_{to,f}$ and can form multimeric tetramers with other Kv4.x channels, which produces a functional diversity of transient outward currents. As with *KCNQ1* and *KCNE1*, an increasing number of accessory subunits have been shown to modulate the expression and kinetics of Kv4.x channels: (1) Potassium channel–interacting proteins (*KChIPs*)[20]; (2) a calcium-binding protein, *NCS-1* (or frequenin)[21]; (3) potassium channel accessory proteins (*KChAPs*); (4) dipeptidyl-aminopeptidase-like protein 6 (*DPP6*); and (5) *KCNE* family members.[22–25] *KCNE* members are also denoted as *MinK*-related proteins (*MiRP1* through *4*, encoded by *KCNE*2 through 5, respectively), and *KCNE2* (or *MiRP1*) has been shown to modulate *KCNH2*-encoded I_{Kr} channels.[26,27] In addition, *MinK* also affects the I_{Kr} current.[28–30]

The *KCNJ* family consists of more than 10 members that encode inward-rectifying potassium channels; they have only 2 transmembrane segments (M1 and M2) and lack the voltage sensor. *KCNJ2* encodes I_{K1} channels (Kir2.1), which are abundantly expressed in heart and determine the resting membrane potential and final phase of action potential repolarization.[31,32] Another member of the *KCNJ* family, *KCNJ5*, encodes the α-subunit of the acetylcholine-sensitive potassium current ($I_{K\text{-}ACh}$) channel, which is opened by extracellular acetylcholine via activation of membrane G proteins. *KCNJ5* can collaborate with *KCNJ3* to form a highly active heteromultimer or can form a low to moderately active homomultimer.[33] *KCNJ8* is another gene that encodes an inward-rectifier potassium channel, Kir6.1, which is sensitive to intracellular ATP, ie, ATP-sensitive potassium (K_{ATP}) channels.[34,35] In physiological conditions, Kir6.1 requires the sulfonylurea receptor to function as a membrane metabolic-electric receptor, and it develops a sensitivity to sulfonylurea drugs.[36] Kir6.1 is abundantly expressed in heart, and its activation during myocardial ischemia may contribute to shortening of the action potential duration (APD) and ischemia-related ST-segment elevation in the ECG. Recently, a gain-of-function mutation of *KCNJ8* was identified in a patient with idiopathic ventricular fibrillation (VF), which indicates that the mutation can cause the channel to open constitutively without ischemia.

Intracellular magnesium ions and membrane polyamines are naturally occurring blockers that induce a strong rectifying property, one of the common characteristics of inward-rectifying potassium channels.[37–41] In humans, Kir2.1 is expressed not only in the myocardium but also in brain and skeletal muscle.[32] Loss-of-function *KCNJ2* mutations display cardiac and extracardiac phenotypes known as Andersen-Tawil syndrome (LQT7). Moreover, specific mutations in the *KCNJ2* gene have been shown to be associated with variable phenotypes, such as catecholaminergic polymorphic ventricular tachycardia (VT), SQTS, and AF.

Clinical Phenotype Due to Potassium Channel Gene Mutations, Including Genotype-Phenotype Correlations

Genotype-phenotype correlations have been investigated extensively in some inherited arrhythmia syndromes, and the

possibility of gene-specific risk stratification and gene-specific therapy and management has been suggested.

Congenital LQTS

Congenital LQTS is characterized by a prolonged QT interval in the ECG and a polymorphic VT known as torsade de pointes.[42,43] Congenital LQTS is a Rosetta stone for studying the genetic background of inherited arrhythmic syndromes,[6] because multiple genes that encode the many different ion channels or membrane adaptor have been identified.

Genetics in Congenital LQTS

Since the first 3 genes responsible for the 3 major genotypes (LQT1, LQT2, and LQT3) were identified in the mid-1990s,[3,4,44] a total of 13 forms of Romano-Ward–type congenital LQTS have been reported to be caused by mutations in genes of potassium, sodium, and calcium channels or the membrane adapter located on chromosomes 3, 4, 7, 11, 12, 17, 20, and 21.[45–54] Of the 13 identified genotypes, 6 (LQT1, LQT2, LQT5, LQT6, LQT7, and LQT13) are caused by mutations in potassium channel genes; in LQT11, *AKAP-9* encoding Yotiao is the responsible gene[51] (Table). *AKAP-9* is reported to assemble *KCNQ1*, thus indirectly modulating I_{Ks}. Mutations in *KCNQ1* and *KCNE1*, which are the α-subunit and accessory subunit of the potassium channel gene, respectively, are responsible for defects (loss of function) in the I_{Ks} underlying LQT1 and LQT5.[15,16] Mutations in *KCNH2* and *KCNE2*, which are also the potassium channel α-subunit and accessory subunit, respectively, cause defects in I_{Kr} that are responsible for LQT2 and LQT6[4,26]; however, there is controversy as to whether I_{Kr} is truly the byproduct of *KCNH2* and *KCNE2*. Mutations in *KCNJ2* encoding I_{K1} underlie Andersen-Tawil syndrome (LQT7), in which QT prolongation and ventricular arrhythmias are accompanied by potassium-sensitive periodic paralysis and dysmorphic features that include low-set ears, hypertelorism, cleft palate, micrognathia, scoliosis, short stature, and syndactyly.[46,55] A specific *KCNJ2* mutation, V227F, was identified in a patient with a typical catecholaminergic polymorphic VT phenotype.[56] Heterologous expression with the COS cell line showed that heterozygous wild-type/V227F channels were identical to wild-type channels in function, but stimulation by cAMP-dependent protein kinase A significantly downregulated heterozygous mutant Kir2.1 and not wild-type Kir2.1 currents.[56] This particular type of loss of function explained why the proband displayed the catecholaminergic polymorphic VT phenotype, in which typical bidirectional or polymorphic VT is provoked by exercise. Most recently, a mutation in *KCNJ5* was reported to result in a loss of function of $I_{K\text{-}ACh}$ responsible for LQT13,[54] although the precise role of $I_{K\text{-}ACh}$ in the ventricle is still unknown. In all genotypes, decreases in outward potassium currents (I_{Ks}, I_{Kr}, I_{K1}, and $I_{K\text{-}ACh}$) prolong the APD, which results in prolongation of the QT interval, a common phenotype. Prolongation of the action potential plateau phase allows recovery from inactivation and reactivation of L-type calcium channels, which produces early afterdepolarizations. The early afterdepolarization–induced ventricular premature contractions capture the vulnerable window created by increased transmural and spatial dispersion of ventricular repolarization, thus resulting in torsade de pointes. In LQT7, loss of function in I_{K1}, which is active during the terminal phase of the action potential, prolongs the terminal repolarization phase and produces delayed afterdepolarization, which triggers typical multifocal or bidirectional VT.

The LQT1 and LQT2 syndromes are the 2 most common genetic variants, and each accounts for approximately 40% of genotyped patients.[45] The third most common genotype, LQT3, accounts for only 10% of genotyped patients.[45] Therefore, more than 80% of genotyped LQTS patients have potassium channel gene–related LQTS genotypes, which suggests that congenital LQTS is most frequently a disease of potassium channels.

Autosomal-recessive forms of Jervell and Lange-Nielsen syndrome are associated with neurosensorial deafness and generally more severe phenotype (marked QT prolongation and lethal ventricular arrhythmias) than autosomal-dominant forms of the Romano-Ward syndrome.[57] Two genotypes, JLN1 and JLN2, are reported to be responsible for homozygous or compound heterozygous mutations in the *KCNQ1* or *KCNE1* genes, and both are responsible for a decrease in I_{Ks}.

Congenital LQTS is believed to cause at least some cases of sudden infant death syndrome.[58] Mutations in *KCNQ1*[59] and *KCNH2*[59] have been reported to be associated with sudden infant death syndrome.

Genotype-Phenotype Correlations in LQTS

ECG Characteristics

In the 3 major genotypes (LQT1, LQT2, and LQT3), a genotype-specific T-wave morphology in the 12-lead ECG was proposed by Moss and coworkers in 1995.[60] Broad-based prolonged T waves are more commonly observed in LQT1 with an I_{Ks} defect, whereas low-amplitude T waves with a notched or bifurcated configuration are more frequently observed in LQT2 with an I_{Kr} defect. Exercise treadmill testing has been reported to unmask the characteristic T-wave morphology in patients with LQT1 (broad-based T waves) or LQT2 (notched T waves).[61] In LQT7 with an I_{K1} defect, mild QT prolongation, TU-wave abnormalities (featuring a prominent U wave), frequent ventricular premature contractions, and typical bidirectional VT are often observed.[46]

A series of experimental studies that used arterially perfused canine wedge preparations developed in the late 1990s have delineated the cellular basis for the T-wave morphology that is characteristic of LQT1, LQT2, and LQT7.[62–65] The amplified transmural electric heterogeneity of ventricular repolarization associated with differential modification of potassium currents in each cell type, which is caused by mutations in each genotype, results in genotype-specific T-wave morphology in the ECG.[62,63] In the LQT1 model, preferential prolongation of the APD in midmyocardial (M) cells compared with epicardial and endocardial cells with an I_{Ks} blocker, chromanol 293B, and additional isoproterenol, a β-adrenergic agonist, creates a dramatic augmentation of transmural dispersion of repolarization, which results in broad-based T waves (Figures 1B and 1E).[63,64] In the LQT2 model, d-sotalol, an I_{Kr} blocker, in the presence of hypokalemia also produces more preferential APD prolongation in

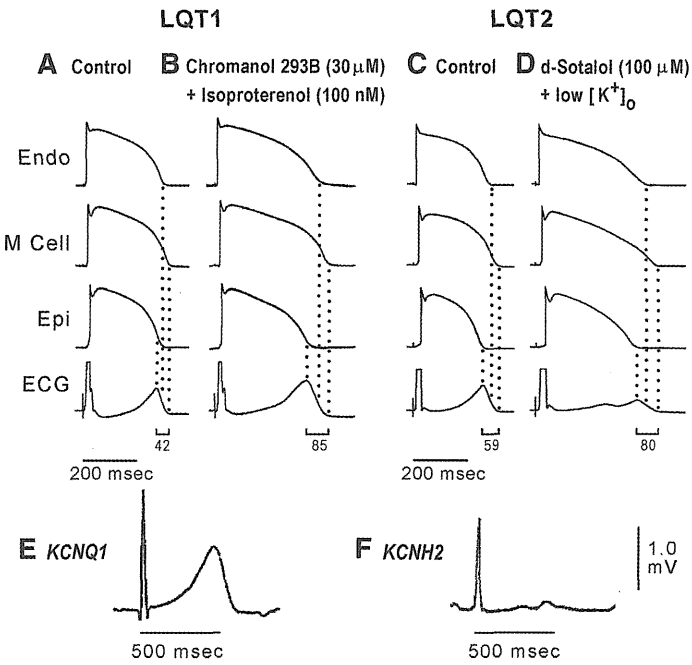

Figure 1. Cellular basis of abnormal T-wave patterns in potassium channel gene–related LQT1 and LQT2 syndrome. A through **D**, Transmembrane action potentials recorded simultaneously from endocardial (Endo), midmyocardial (M), and epicardial (Epi) cells together with a transmural ECG at a basic cycle length of 2000 ms in the LQT1 and LQT2 models of arterially perfused canine wedge preparations. **E** and **F**, ECG lead V_5 recorded in patients with LQT1 and LQT2 forms of congenital LQTS. Pharmacological models mimic the phenotypic appearance of the abnormal T waves in both models. Modified from Shimizu et al[62,63] with permission.

M cells and slowing of phase 3 of the action potential in all 3 cell types, which results in large transmural dispersion of repolarization and a low-amplitude T wave with the notched or bifurcated appearance characteristic of LQT2 (Figures 1D and 1F).[62,64] In the LQT7 model, cesium chloride, an I_{K1} blocker, and isoproterenol delay late phase 3 repolarization of the action potential and induce delayed afterdepolarizations, which generates U waves and delayed afterdepolarization–induced ventricular premature contractions. Migration of delayed afterdepolarization foci is reported to be the mechanism that produces multifocal VT and characteristic bidirectional VT.[65]

Clinical Course

The cumulative probability of cardiac events (syncope, aborted cardiac arrest, sudden cardiac death) is higher in patients with the potassium channel gene–related LQTS genotypes (LQT1 and LQT2) than in patients with LQT3, a sodium channel gene–related LQTS genotype.[66] On the other hand, Priori and coworkers[67] reported that in more homogeneous LQTS cohorts, LQT1 was the variant associated with higher incomplete penetrance, and the event rate was significantly higher in LQT2 (46%) and LQT3 (42%) than in LQT1 (30%). Some evidence points to more severe arrhythmia consequences of *SCN5A* mutations.[68] In general, male patients experience their first cardiac events at a younger age than female patients.[69] Approximately 90% of first cardiac events occur before the age of 15 years in male patients, particularly in males with LQT1, whereas female patients rarely experience their first cardiac event occasionally after the age of 20 years.[67,69] A recent large cohort of patients with LQT1 and LQT2 syndromes confirmed these tendencies and suggested that age younger than 13 years combined with male gender and age older than 13 years combined with female gender were significant and independent clinical risk factors associated with first cardiac events in both LQT1 and LQT2 syndromes.[70,71]

Genotype-Specific Triggers for Cardiac Events

Triggers for LQTS-related cardiac events have been reported to differ between each LQTS genotype, including LQT1, LQT2, and LQT7.[43,72,73] Although sympathetic stimulation may trigger cardiac events in all potassium channel gene–related LQTS genotypes, LQT1 with the I_{Ks} defect is the most sensitive to sympathetic stimulation. Cardiac events in LQT1 patients most frequently occur during exercise (62%), and swimming is a common trigger.[72] LQT2 is less likely to result in cardiac events during exercise (13%) and more likely to result in cardiac events during rest or sleep (29%).[72] More specifically, being startled by an auditory stimulus (telephone, alarm clock, ambulance siren, etc) is a specific trigger in LQT2.[72,73] Women with LQT2 are reported to be the most susceptible to cardiac events during the postpartum period.[74] Both experimental studies using arterially perfused wedge preparations[63,64] and clinical studies using catecholamine provocative testing or exercise testing[61,75–78] have suggested that the differential sensitivity of cardiac events in each genotype (LQT1, LQT2, and LQT3) in response to sympathetic (β-adrenergic) stimulation is due to the differential response of ventricular repolarization to sympathetic stimulation. In LQT7 patients, hypokalemia is often associated with frequent ventricular arrhythmias and periodic paralysis[46]; however, periodic paralysis is also associated with hyperkalemia or normokalemia.[46]

Diagnostic Value of Epinephrine Challenge Test

It is well known that some genetically affected LQTS patients may have a normal or borderline QT interval but harbor a

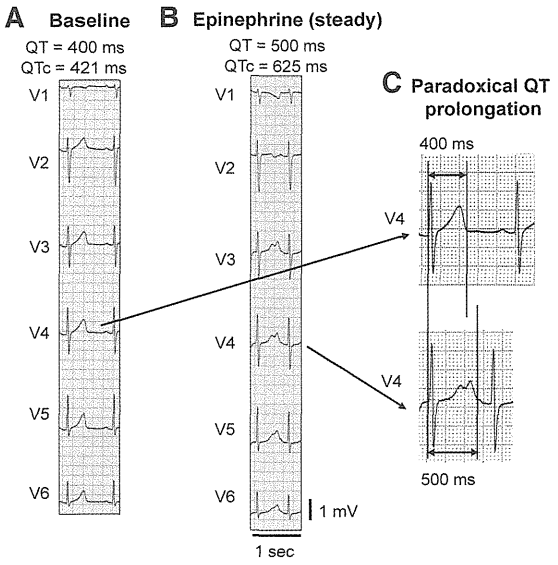

Figure 2. Paradoxical QT prolongation during epinephrine challenge test in a patient with LQT1 syndrome. Shown are 6 precordial ECG leads under baseline conditions and at steady state after epinephrine. The QTc interval was remarkably prolonged from 421 to 625 ms at steady state. Absolute QT interval was also prolonged from 400 to 500 ms, even though the RR interval was apparently abbreviated (paradoxical QT prolongation).

lethal arrhythmogenic substrate.[67,79] This fact strongly points to the need for new diagnostic tools to unveil concealed forms of LQTS. Recent major insights have been gleaned using epinephrine, an α- and β-adrenergic agonist, as a provocative test.[75–78] The 2 major protocols developed for the epinephrine challenge test include the escalating-dose protocol by Ackerman's group (Mayo protocol)[77,78] and a bolus injection followed by a brief continuous infusion by our group (Shimizu protocol).[75,76,78]

Ackerman and coworkers[77] reported that paradoxical QT prolongation had a sensitivity of 92.5%, a specificity of 86%, a positive predictive value of 76%, and a negative predictive value of 96% for LQT1 patients versus non-LQT1 patients (Figure 2). Our bolus protocol, which was developed on the basis of data from experimental LQTS models,[64] suggested that sympathetic stimulation produces genotype-specific responses of the corrected QT (QTc) interval in patients with LQT1, LQT2, and LQT3 syndromes.[75,76,78] The bolus protocol of epinephrine improves clinical ECG diagnosis (sensitivity) in patients with either LQT1 or LQT2 with a potassium channel defect but not in patients with LQT3 with a sodium channel defect.[76] The bolus protocol also effectively predicts the underlying genotype of LQT1, LQT2, and LQT3.[76,78] A presumptive, pregenetic diagnosis of either LQT1, LQT2, or LQT3 based on the response to an epinephrine challenge test can facilitate the molecular genetic diagnosis by targeting a first candidate gene and can guide genotype-specific treatment strategies.[78] Although epinephrine was not used, Viskin et al[80] recently reported the usefulness of a bedside stand-up test to easily diagnose LQTS. They suggested that at maximal QT-interval stretching, the time at which the end of the T wave is nearest to the next P wave during transient sinus tachycardia after a person stands up quickly, the QTc value identifies LQTS with 90% sensitivity and 86% specificity.[80]

Genotype-Specific Patient Care and Therapy

Because LQT1 patients are most sensitive to sympathetic stimulation, and most of their first cardiac events occur before the age of 15 years, particularly in males with LQT1 syndrome, strict exercise restriction, particularly restriction of swimming, diving, or competitive sports, is needed in these patients.[81] Exercise restriction is also required in LQT2 patients.[81] In LQT2, the avoidance of specific acoustic triggers, such as alarm clocks and a ringing telephone, is required and effective. It is also important to instruct elderly patients with LQT1 and LQT2 to avoid QT-prolonging agents, hypokalemia, and bradycardia.

Genotype-specific pharmacological and nonpharmacological therapies have been introduced clinically on the basis of data derived from both clinical and experimental studies.[81] In LQT1, β-blockers are most effective to prevent episodes of syncope and sudden cardiac death.[70,72,82] The largest international cohort of 600 LQT1 patients suggested that time-dependent β-blocker use was associated with a significant 74% reduction in the risk of first cardiac events.[70] Mexiletine, a class IB sodium channel blocker that blocks late I_{Na}, or verapamil, an $I_{Ca\text{-}L}$ blocker, may warrant consideration as adjunctive therapy to β-blockers in LQT1 patients.[62,63] As a nonpharmacological therapy, left stellate ganglion ablation, another antiadrenergic therapy, is most effective in LQT1 patients.[83] An implantable cardioverter-defibrillator is indicated for LQTS patients who have experienced an aborted cardiac arrest or who have repetitive episodes of syncope in the presence of β-blockers.

In LQT2, β-blockers are also effective; however, previous studies have suggested that the effectiveness of β-blockers is somewhat less in either LQT2 or LQT3 patients than in LQT1 patients.[72,84] Priori et al[84] reported that cardiac events among patients receiving β-blocker therapy occurred in 10% of LQT1 patients, 23% of LQT2 patients, and 32% of LQT3 patients. A report on a recent international cohort of 858 LQT2 patients suggested that time-dependent β-blocker use significantly reduced the risk of first cardiac events by 63%, which confirms the efficacy of β-blockers as a first-line therapy in LQT2.[71] Maintenance of the extracellular potassium concentration by long-term oral potassium supplementation is reported to be effective because it shortens the QT interval in LQT2 patients.[85] A genotype-specific initiating pattern of torsade de pointes has been reported.[86,87] A characteristic short-long-short initiating pattern of torsade de pointes, which is frequently observed in drug-induced torsade de pointes in acquired LQTS, is more frequently seen in LQT2 and LQT3 patients than in LQT1 patients.[87] Therefore, pacemaker therapy is expected to be more effective in LQT2 than in LQT1 patients via suppression of the specific short-long-short initiating pattern.[87] The indication for implantable cardioverter-defibrillator is similar to that in LQT1 syndrome.

There is no known genotype-specific therapy for other potassium channel gene–related LQTS genotypes (LQT5,

LQT6, LQT7, LQT11, and LQT13), in which β-blockers may be the first-line therapy.

Mutation Site-Specific Risk Stratification and Therapy

As the correspondence between the mutation site and the cardiac potassium channel and the structure of the potassium channel have become increasingly discovered, mutation site–specific risk stratification or therapy can be expected in potassium channel gene–related LQTS. In 2004, Shimizu and coworkers[88] compared the arrhythmic risk and sensitivity to sympathetic stimulation with treadmill exercise testing between Japanese LQT1 patients with transmembrane mutations and those with C-terminal mutations in the *KCNQ1* gene. The LQT1 patients with transmembrane mutations had a longer QTc and more frequent cardiac events than those with C-terminal mutations.[88] Moreover, the QTc prolongation with exercise was more remarkable in the LQT1 patients with transmembrane mutations.[88] The more severe phenotype in LQT1 patients with transmembrane mutations was confirmed later in a much larger international cohort that consisted of 600 LQT1 patients.[70] Results from that cohort also suggested that LQT1 patients with mutations that had dominant-negative (>50%) ion channel effects were at greater risk for cardiac events than those who had haploinsufficiency (≤50%) ion channel effects. In 2002, Moss and coworkers[89] reported that LQT2 patients with mutations in the pore region of the *KCNH2* gene had a greater risk of arrhythmia-related cardiac events than those with nonpore mutations. A recent larger international cohort investigated the clinical aspects of 858 subjects with a spectrum of *KCNH2* mutations categorized by the distinct location, coding type, and topology of the channel mutations.[71] The LQT2 patients with *KCNH2* missense mutations located in the transmembrane S5-loop-S6 region were reported to be at greatest risk. In this cohort, a significantly higher risk was found in the LQT2 patients with mutations located in the α-helical domains than in those with mutations in the β-sheet domains or other locations.[71] These data indicate the possibility of mutation site–specific management or treatment in patients with potassium channel gene–related LQTS.

Short-QT Syndrome

SQTS is characterized by an abnormally short QT interval and increased risk of VF and sudden death.[90,91] In 2000, Gussak and coworkers[90] reported a first case with SQTS who showed a short QTc of 300 ms and AF. In 2003, Gaita et al[91] described 2 families with SQTS associated with a family history of sudden cardiac death due to malignant ventricular arrhythmias. Thereafter, increasing attention has been given to SQTS; however, the number of SQTS patients is still very limited. No clinically diagnostic criteria have been described, and a short QTc is generally considered as ≤300 to 320 ms.[90,91] The diagnosis of SQTS was made if a patient with QTc ≤330 ms had an arrhythmic event, including documented VF, resuscitated sudden cardiac death, and syncope; and/or a family history of SQTS; or if a patient with QTc ≤360 ms had mutations in the ion channel genes responsible for SQTS.[92,93]

Genetics in SQTS

Five genotypes have been identified in SQTS to date (Table), of which the SQT1, SQT2, and SQT3 genotypes are caused by mutations in genes that encode the potassium channel (*KCNH2*, *KCNQ1*, and *KCNJ2*, respectively).[94–96] *KCNH2*, *KCNQ1*, and *KCNJ2* are potassium genes responsible for the LQT2, LQT1, and LQT7 types of congenital LQTS, but all mutations reported in these 3 potassium genes biophysically demonstrate gain of function of I_{Kr}, I_{Ks}, and I_{K1}, respectively, thus shortening the APD and the QT interval.

Genotype-Phenotype Correlations in SQTS

In addition to a short QT interval, genotype-specific T-wave morphology in the 12-lead ECG has been reported in the potassium channel gene–related SQTS genotypes (SQT1, SQT2, and SQT3).[94–96] In SQT1, the T waves in the precordial leads are reported to be symmetrical and tall,[94] but the T_{peak} to T_{end} interval, which reflects transmural dispersion of repolarization, is relatively prolonged, and this is suggested to produce a substrate for reentry that leads to VF.[97] The T waves are symmetrical but not as tall in SQT2.[95] In contrast, the T waves in SQT3 illustrate an asymmetrical pattern, with a less steep ascending part of the T wave followed by an accelerated descending T wave.[96] The rapid descending terminal phase of the T waves can be explained by an accelerated terminal phase of repolarization due to gain of function of I_{K1}.[96]

A recent clinical study reported a high prevalence of early repolarization in patients with SQTS associated with arrhythmic events.[98] An implantable cardioverter-defibrillator is the most reliable therapy for secondary prevention in SQTS patients with a history of VF or aborted sudden cardiac death. As an adjunctive medication, quinidine has been reported to normalize the QT interval and T-wave morphology and to suppress the induction of VF during electrophysiological study in patients with SQT1[99]; however, it is not clear whether the specific efficacy of quinidine observed in SQT1 patients was genotype specific or mutation specific.

Brugada Syndrome

BrS is characterized by coved-type ST-segment elevation (type 1) in the right precordial ECG (leads V_1 through V_3) and an episode of VF in the absence of structural heart diseases.[100–103] The prevalence of BrS is estimated to be up to 5 per 10 000 persons, and BrS is one of the important causes of sudden cardiac death of middle-aged males, particularly in Asian countries.[102,103] BrS usually manifests during adulthood,[102] and more than 80% to 90% of patients clinically affected with BrS are men.

Genetics in BrS

Since the first mutation linked to BrS was identified in *SCN5A*, the I_{Na} gene, in 1998,[104] which presently accounts for 11% to 28% of patients with clinically diagnosed BrS,[105] 7 responsible genes have been reported. In all 7 genotypes, either a decrease in the inward sodium or calcium current or an increase in the outward potassium current is responsible for the Brugada phenotype; however, approximately two thirds of Brugada patients have not yet been genotyped, which suggests the presence of genetic heterogeneity.[103]

There is only 1 potassium channel gene among the 7 genes responsible for BrS (Table). Delpón et al[23] reported a missense mutation (R99H) in *KCNE3*, which encodes the potassium channel accessory (β3) subunit and interacts with the *Kv4.3* (I_{to}) channel, in a proband with BrS. Coexpression of the mutant *KCNE3* with *KCND3*, which encodes *Kv4.3*, increases I_{to} intensity (gain of function) compared with coexpression of wild-type *KCNE3* with *KCND3*.[23] We recently reported that *KCNE2* and *KCNE5*, auxiliary potassium channel accessory subunits, are other genes responsible for potassium channel gene–related BrS via the modulating effect of the I_{to}.[24,25]

Genotype-Phenotype Correlations in BrS

The genotype-phenotype correlation in BrS has been less investigated than that in congenital LQTS and is limited in sodium channel gene (*SCN5A*)–related BrS. None of the conduction abnormalities that have been reported in patients with *SCN5A*-related BrS (such as widening of the P wave, prolongation of QRS duration, PQ interval, or right bundle-branch block) were described in the patient with potassium channel gene–related BrS6 reported by Delpón et al.[23] Several agents that increase the outward potassium current, such as nicorandil, a K_{ATP} channel opener, have the potential to induce transient ST-segment elevation like that in BrS and have been described as an "acquired" form of BrS.[102,106]

Early Repolarization Syndrome

The prevalence of an early repolarization pattern or J wave in the inferior (II, III, aVF) or lateral (I, aVL, V_4 through V_6) leads is estimated to be 1% to 5% of healthy individuals, and these had been considered benign ECG characteristics.[107] However, several reports have focused increasing attention on the association of idiopathic VF with early repolarization in the inferior or lateral leads, so-called early repolarization syndrome. Haissaguerre et al[108] reported that early repolarization was more frequently recognized in idiopathic VF patients than in control subjects, and they reported a higher incidence of VF recurrence in case subjects with early repolarization than in those without.

Genetics in Early Repolarization Syndrome

A novel missense mutation, S422L, in the *KCNJ8*-encoded Kir6.1 α-subunit of the K_{ATP} channel was reported in a young female with VF secondary to early repolarization syndrome.[109] A recent study reported that the K_{ATP} current ($I_{K\text{-}ATP}$) of the Kir6.1-S422L mutation was increased significantly (gain of function), thus promoting an early repolarization pattern or J wave in the ECG.[110] (See Table.)

Genotype-Phenotype Correlations in Early Repolarization Syndrome

No studies showing a genotype-phenotype correlation have been reported in early repolarization syndrome.

Atrial Fibrillation

AF is the most commonly observed cardiac arrhythmia encountered in clinical practice. AF is usually accompanied by organic heart diseases such as valvular heart disease, hypertensive heart disease, or hypertrophic or dilated cardiomyopathy; however, AF without organic heart disease (lone AF) also occurs. Some genetic factors or genetic backgrounds that predispose to AF may be linked to the development of AF, especially in familial forms of AF, in which the AF is segregated in several family members.

Genetics in AF

The epidemiological data have suggested that the relative risk of AF in offspring was increased significantly if parents had AF before 60 years of age,[111] which indicates heritability in AF. There are 3 categories of genetic patterns related to AF: (1) familial AF as a monogenic disease; (2) familial AF associated with other inherited cardiac diseases, including hypertrophic cardiomyopathy, dilated cardiomyopathy, and skeletal myopathies or other inherited arrhythmic syndromes, including congenital LQTS, SQTS, and BrS; and (3) nonfamilial AF associated with genetic backgrounds that predispose to AF, such as a polymorphism in the angiotensin-converting enzyme gene (*ACE*). Mutations in several potassium channel genes have been reported to be responsible for AF; however, all mutations reported thus far were identified in isolated patients or families.

The first mutation linked to AF was identified in *KCNQ1*, the I_{Ks} gene, in 2003[112] (Table). Electrophysiological analysis of the specific mutation, S140G, demonstrated a gain of function in I_{Ks} current, which results in shortening of the APD and effective refractory period in the atrium, providing the substrate for AF. The same scenario was expected in the ventricle, leading to abbreviation of the QT interval, but 9 of the 16 affected individuals presented with QT prolongation, which could not be well explained. Thereafter, mutations in *KCNE2* and *KCNE3*, which are both accessory subunits, were found in familial AF.[113,114] Although the *KCNE2* mutation (R27C) coexpressed with *KCNQ1* resulted in a gain of function of I_{Ks}, the *KCNE3* mutation (R53H) did not change I_{Ks}, which suggests that it might not be a causative mutation. A *KCNJ2* mutation that leads to a gain of function in I_{K1} current has also been reported.[115] More recently, a mutation in *KCNA5* encoding an atrium-specific I_{Kur} was identified in familial AF.[17–19] Interestingly, the specific *KCNA5* nonsense mutation E375X resulted in a loss of function of I_{Kur} current. A reduction in I_{Kur} elevates the voltage of the action potential plateau, thus activating more I_{Kr} and enhancing atrial repolarization. The resultant APD abbreviation is believed to create the substrate for AF.[116] The specific *KCNH2* mutation N588K has been reported to produce an overlap phenotype of familial AF and the SQT1 form of SQTS.[117] One specific mutation in the natriuretic peptide precursor A gene (*NPPA*) that encodes atrial natriuretic peptide has been reported recently to indirectly increase the I_{Ks} current, which results in shortening of the atrial APD.[118]

Genotype-Phenotype Correlations in AF

No studies showing genotype-phenotype correlations have been reported in AF.

Diverse Mechanisms Underlie the Generation of Cardiac Potassium Channel Diseases

According to the central dogma of molecular biology (Figure 3, steps 1 through 10), it is now accepted that several steps lead to channel dysfunction: A genetic variant (step 1 in

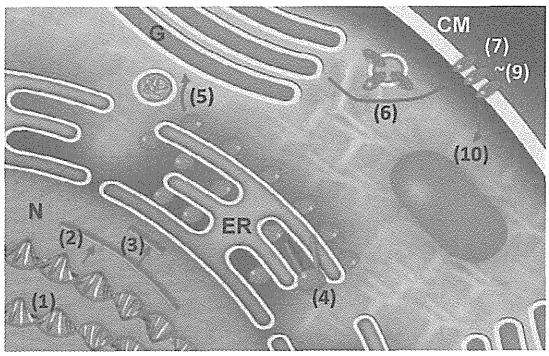

Figure 3. Scheme showing the central dogma of protein synthesis. Numbers in parentheses (1 through 10) in the cartoon indicate diverse mechanisms underlying cardiac potassium channel diseases. For detailed explanation, see text. ER indicates endoplasmic reticulum; CM, cardiac cell membrane; G, Golgi apparatus; and N, cellular nucleus.

Figure 3) impairs transcription (step 2), splicing and related processes (step 3), and translation (step 4).[119–122] With regard to the genetic variants, 3 categories are associated with cardiac potassium channel diseases: mutations, single-nucleotide polymorphisms, and copy-number variations. The former 2 are usually involved in single-nucleotide replacement or insertion/deletion. A variety of mutations in the potassium channel or its related genes have been shown to cause disease by affecting every step shown in Figure 3 (steps 2 through 10). Among the single-nucleotide polymorphisms involved in potassium channel diseases, *KCNE1* D85N is well known not only as a modifier but also as a causative variant of LQTS.[30,123] In contrast, copy-number variations contain relatively large regions of the genome (kilobases to several megabases), with deletion (fewer than the normal number) or duplication (more than the normal number) on a certain chromosome, thereby giving the genome diversity. Recently, several copy-number variations in *KCNH2* and *KCNQ1* have been shown to be associated with disease.[124–126] More recently, a French group conducted an extensive survey of copy-number variations in *KCNQ1* and *KCNH2* and demonstrated that such variations explained approximately 3% of LQTS in patients with no point mutation in these genes.[127]

With regard to the posttranslational process, impaired intracellular transport (steps 5 and 6 in Figure 3) is a common cause of LQTS in several *KCNQ1*, *KCNJ2*, and most *KCNH2* mutations.[128–132] *KCNJ2* contains a specific C-terminal sequence necessary for exportation from the endoplasmic reticulum to the Golgi apparatus (endoplasmic reticulum–to-Golgi export signal).[133] More recently, a naturally occurring *KCNJ2* mutation in the C terminus (S369X), located immediately upstream of this endoplasmic reticulum export signal, was shown to cause a limited form of Andersen-Tawil syndrome (LQT7) by impeding transportation from the endoplasmic reticulum to Golgi (step 5 in Figure 3).[134]

Most *KCNH2* mutations have been reported to reduce hERG currents by a trafficking-deficient mechanism (step 6 in Figure 3).[131] Several trafficking-refractory *KCNQ1*mutations are also known, of which T587M in the C-terminal region was the first reported.[128] The mutation produced a more severe phenotype than expected by the results of functional analysis; the mutation produced no dominant-negative suppression effects on wild-type channels. This mysterious discrepancy was found to result from the physical interaction between *KCNQ1* and *hERG* proteins, which increased localization of hERG channels to the cell membrane, enhanced current density, and altered their biophysical properties.[135] Likewise, overexpression of the dominant-negative *KCNQ1* or *hERG* transgene in genetically modified rabbits resulted in downregulation of the remaining reciprocal current, which indicates that the 2 proteins indeed interact in vivo as well.[136] Therefore, the intracellular trafficking defect in *KCNQ1* impaired the physical interaction with *hERG* and thereby caused severe clinical features (step 6 in Figure 3).[137]

Even after successful expression in membrane, alterations in channel function (steps 7 through 9 in Figure 3) induced by mutations are also pathological: those in potassium permeation (step 7), voltage gating (step 8), and modulation by various physiological stimulations, including protein kinase A and membrane phosphoinositide phosphatidylinositol 4,5-bisphosphate (PIP2).[51,56,138,139] Finally, endocytosis of channel proteins (step 10 in Figure 3) regulates its degradation apart from the plasma membrane. More recently, cholesterol has been shown to regulate Kv1.5 channel expression by modulating its trafficking through the Rab11-associated recycling endosome.[140] Impaired endocytosis of calcium-activated nonselective cation channels, TRM4, was reported to cause progressive cardiac conduction block through *SUMO* (small ubiquitin modifier) conjugation.[141,142] Heat shock proteins have also been shown to regulate *hERG* expression. *hERG* channels with disease-causing missense mutations in intracellular domains had a higher binding capacity to Hsc70 than wild-type channels, and knockdown of Hsc70 by small interfering RNA prevented degradation of mutant proteins with these mutations.[143]

Such diverse mechanisms have been elucidated, mainly by use of a heterologous expression system in mammalian cell lines; however, a big missing link between genotype and phenotype correlations remains. The recent introduction of induced pluripotent stem cells derived from patients may offer a novel methodology for use in the research of ion channelopathies.[144]

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Disclosures

None.

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Identification and functional characterization of KCNQ1 mutations around the exon 7–intron 7 junction affecting the splicing process

Keiko Tsuji-Wakisaka [a], Masaharu Akao [b], Takahiro M. Ishii [c], Takashi Ashihara [a], Takeru Makiyama [b], Seiko Ohno [b], Futoshi Toyoda [d], Kenichi Dochi [a], Hiroshi Matsuura [d], Minoru Horie [a,*]

[a] Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Japan
[b] Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
[c] Department of Physiology, Kyoto University Graduate School of Medicine, Kyoto, Japan
[d] Department of Physiology, Shiga University of Medical Science, Otsu, Japan

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ABSTRACT

Background. KCNQ1 gene encodes the delayed rectifier K^+ channel in cardiac muscle, and its mutations cause long QT syndrome type 1 (LQT1). Especially exercise-related cardiac events predominate in LQT1. We previously reported that a KCNQ1 splicing mutation displays LQT1 phenotypes. *Methods and results.* We identified novel mutation at the third base of intron 7 (IVS7 +3A>G) in exercise-induced LQT1 patients. Minigene assay in COS7 cells and RT-PCR analysis of patients' lymphocytes demonstrated the presence of exon 7-deficient mRNA in IVS7 +3A>G, as well as c.1032G>A, but not in c.1022C>T. Real-time RT-PCR demonstrated that both IVS7 +3A>G and c.1032G>A carrier expressed significant amounts of exon-skipping mRNAs (18.8% and 44.8% of total KCNQ1 mRNA). Current recordings from *Xenopus* oocytes injected cRNA by simulating its ratios of exon skipping displayed a significant reduction in currents to 64.8 ± 4.5% for IVS7 + 3A>G and to 41.4 ± 9.5% for c.1032G>A carrier, respectively, compared to the condition without splicing error. Computer simulation incorporating these quantitative results revealed the pronounced QT prolongation under beta-adrenergic stimulation in IVS7 +3A>G carrier model. *Conclusion.* Here we report a novel splicing mutation IVS7 +3A>G, identified in a family with mild form LQT1 phenotypes, and examined functional outcome in comparison with three other variants around the exon 7–intron 7 junction. In addition to c.1032G>A mutation, IVS7 +3A>G generates exon-skipping mRNAs, and thereby causing LQT1 phenotype. The severity of clinical phenotypes appeared to differ between the two splicing-related mutations and to result from the amount of resultant mRNAs and their functional consequences.

1. Introduction

Long QT syndrome (LQTS) is characterized by prolongation of the cardiac action potential, syncopal attacks, torsades de pointes arrhythmias and sudden cardiac death [1–3]. The slow component of delayed rectifier K^+ current (I_{Ks}) in the heart modulates repolarization of cardiac action potential. The I_{Ks} channel is formed by the co-assembly of KCNQ1 α-subunits and KCNE1 β-subunits [4,5]. Mutations in the KCNQ1 cause the most frequent form of inherited LQT1 [6]. Exercise-related cardiac events dominate the clinical picture of LQT1 patients.

Pre-mRNA processing is an important aspect of gene expression and consists of the precise recognition of exons and removal of introns in such a way that the exons are joined to form mature mRNAs with intact translational reading frames [7,8]. Disruption of normal splicing as a result of genetic mutation can lead to the generation of abnormal proteins or the degradation of aberrant transcripts through nonsense-mediated decay, and thus to the pathogenesis of a variety of human diseases [9].

We previously reported three LQTS families, in whom a G to A change in the last base of KCNQ1 exon 7 (c.1032G>A) was identified [10]. The mutation alters the 5′ splice-site of intron 7, resulting in the production of exon-skipping transcripts, but not to alter the coded alanine (A344A) [11,12], since it involves the characteristic consensus sequence of the splicing donor site, A**G**/GUAAGU. The vicinity of junction around the KCNQ1 exon 7–intron 7 appeared to be a hot area for genetic variants that may potentially cause aberrant splicing, and we identified a novel mutation that changes an A to G at the third base of intron 7 (IVS7 +3A>G) in LQTS family with mild clinical phenotypes. In contrast, another neighboring KCNQ1 mutation, c.1022C>T (p. A341V) is known to produce severe clinical phenotypes [13].

To test the potential influence of these mutations that may affect the KCNQ1 splicing, we established a minigene assay system in which a respective mutant construct is transcribed in COS7 cells and examined the genetic and biophysical characterization of the novel IVS7 +3A>G

* Corresponding author at: Department of Cardiovascular Medicine, Shiga University of Medical Sciences, Japan. Tel.: +81 77 548 2213; fax: +81 77 543 5839.
E-mail address: horie@belle.shiga-med.ac.jp (M. Horie).

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mutation. For comparison, we also investigated two other mutations around the exon 7–intron 7 junction; c.1022C>T and c.1032G>A. We quantitatively analyzed the aberrant splicing and its functional consequences and then carried out a computer simulation to explore how this mutation could be associated with exercise-induced QT prolongation and tachyarrhythmias.

2. Materials and methods

2.1. Genomic DNA isolation and mutation analysis

Mutation analysis was carried out as previously described [10]. Genomic DNA was prepared from peripheral blood leukocytes. Sixteen exons of the KCNQ1 gene were amplified by PCR. Genetic screening was performed for KCNQ1 by denaturing high-performance liquid chromatography (DHPLC) using a WAVE System Model 3500 (Transgenomic: Omaha, NE). We optimized the running optimum temperature at 64.6 °C. Abnormal conformers were amplified by PCR and sequencing was performed on an ABI PRISM3130 DNA sequencer (Applied Biosystems: Foster City, CA). We also carried out a complete screening for other LQTS-causing genes; KCNH2, SCN5A, KCNE1, KCNE2, and KCNJ2.

2.2. Construction of splicing minigene and transfection

Exon 7 of the KCNQ1 gene (111 bps) and its flanking introns (507 bps at 5′ arm and 453 bps at 3′ arm) were amplified by PCR using genomic DNA from control and patients. PCR fragments were cloned into the pSPL3 exon trapping vector (Gibco BRL) digested with *EcoRV* within the multiple cloning site. The pSPL3 vector contains the HIV-1 tat exons and the intervening intron with *EcoRV* site. COS7, CHO and HL-1cells were transfected with 0.25 μg plasmid DNA using Lipofectamin transfection reagent (Invitrogen). Cells were harvested 48 h post-transfection.

2.3. RNA extraction and RT-PCR

Total cellular RNA was isolated with QIAamp RNA Blood Mini Kits (Qiagen). Subsequently, total RNA was reverse-transcribed by use of the SuperScriptIII FirstStrand Synthesis System (Invitrogen: Carlsbad, CA), and was used as a template for subsequent PCR reactions. We used the forward primer (5′-TCTGAGTCACCTGGACAACC-3′) and the reverse primer (5′-ATCTCAGTGGTATTTGTGAGC-3′), both of which anneal to the pSPL3 vector sequence.

Total RNA was extracted from leukocytes of fresh blood and was reverse-transcribed using the same methods described above. Using the cDNAs as templates, PCR amplification was performed with the exon 5-F forward primer (5′-GGGCATCCGCTTCCTGCAGA-3′) and the exon10-R reverse primer (5′-CCATTGTCTTTGTCCAGCTTGAAC-3′) to amplify KCNQ1 cDNA from exons 5 through 10.

Measurements of normal and mutant mRNA levels were performed by real-time RT-PCR by use of an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The reaction mixture contained SYBR Green PCR Master Mix (Applied Biosystems), cDNA template, and PCR primers. In order to selectively amplify these splicing variants, PCR primers were designed so that they spanned the adjacent exons: exon 6.8-F: 5′-CTGTGGTGGGGGGTG-GGGATT-3′, exon 6.9-F: 5′-TGTGGTGGGGGGTG-ACCGCAT-3′, and exon 7.9-F: 5′-CTTTGCGCTCCCAGCG-ACCG-3′ (all the hyphens inside the primer sequence indicate the boundaries of exons). In all cases, the dissociation curves showed that there was no significant contribution of relatively short by-products to the measured fluorescence intensities.

All the samples were tested in duplicate. A standard curve for each primer pair was obtained using serial dilutions of a recombinant plasmid containing cDNA. The threshold cycle (Ct) was subsequently determined. Relative mRNA levels of splice mutants were calculated based on the Ct values and normalized by the GAPDH level of each sample. The amounts of mutant cDNA were expressed as a percentage of the total KCNQ1 mRNA, for which exons 9 through 10 were amplified with the exon 9-F forward primer (5′-CGCATGGAGGTGC-TATGCT-3′) and the exon 10-R reverse primer.

2.4. Oocyte isolation and electrophysiology

Xenopus laevis oocytes were prepared and current recordings were carried out as described previously [14]. Wild-type (WT) cRNA plus mutant-cRNA (total 10 ng) was injected into *Xenopus* oocytes. All the current recordings in the present study were performed in the presence of KCNE1 β-subunits (1 ng). An axoclamp-2B amplifier (Axon Instruments: Union City, CA) was used to record currents at 25 °C in oocytes 3–4 days after cRNA injection, using standard two-electrode voltage-clamp techniques. To decrease the interference from endogenous Cl^- current, we used a low-Cl^- bath solution (mM): NaOH 96, KOH 2, $CaCl_2$ 2, $MgCl_2$ 1, MeS 101, HEPES 5 (pH titrated to 7.6 with methanesulfonic acid). Currents were sampled at 10 kHz and filtered at 2 kHz. Voltage steps were applied with 3-second pulses in 10 mV increments from a holding potential of −80 mV to voltages from −70 to +30 mV, and then to −30 mV. Current amplitudes were measured at 1.8-second after the initiation of 3-second pulse applied to a +30 mV test potential, followed by the subtraction of background I_{Ks} current (22.9 nA).

2.5. Computer simulation

We conducted simulations of paced propagation in a one-dimensional (1D) bidomain myocardial model of 9.0-mm length with transverse conductivity, mimicking transmural section of left ventricular free wall. Membrane kinetics was represented by the Priebe–Beuckelmann model [15], which can simulate human ventricular action potentials.

To obtain the ventricular transmural gradient, we defined endocardial, mid-myocardial, and epicardial tissues of lengths (thicknesses) 0.6 mm, 6.0 mm, and 2.4 mm, respectively, and then we incorporated modifications of ion channel conductance (Table 1), based on the previous studies [16,17]. Pacing stimuli of 3-ms duration and strength twice-diastolic threshold were applied transmembranously to the endocardial end at a cycle length of 1000 ms. To get ECG similar to the left precordial ECG, a unipolar recording electrode was located 3 cm above the epicardial end of the tissue. Other model parameters, such as the tissue conductivities and the boundary conditions, can be found elsewhere [18,19].

To achieve the beta-adrenergic stimulation, we set the parameters as previously described [20–22]: (1) shifting the fast and slow inactivation curves of the sodium current (I_{Na}) −3.4 mV, (2) increasing the L-type calcium current (I_{CaL}) 3 times and slowing the time constant of inactivation 1.13 times, (3) increasing the half-point concentration for the calcium-dependent inactivation (f_{Ca}) from 0.7 to 0.9 μM, and setting its non-zero minimum value to 0.03, (4) increasing the slowly

Table 1
Model modification values for ventricular transmural gradient.

	Endo	M	Epi
G_{Ks}	208%	52%	280%
G_{K1}	82%	83%	100%
G_{NaCa}	72%	108%	100%
G_{to}	25%	87%	100%
G_j	100%	100%	76%

G_{Ks}, conductance of slowly activating component of delayed rectifier potassium channel; G_{K1}, conductance of inward rectifier potassium channel; G_{NaCa}, conductance of sodium–calcium exchanger; G_{to}, conductance of transient outward potassium channel; G_j; gap junctional conductance. All values are expressed in percentage compared to original values [15]. Endo; endocardial cell, M; midcardial cell, Epi; epicardial cell.

activating component of delayed rectifier potassium current (I_{Ks}) 2 times and shifting the activation curve −8 mV, and (5) increasing currents of the calcium pump in sarcoplasmic reticulum (I_{up}) and the sodium–potassium pump (I_{NaK}) 1.41 and 1.2 times, respectively.

The numerical approach, including methods for integration and solution of the linear system, has been described elsewhere [18]. The time and spatial discretization steps were 10 μs and 75 μm, respectively. The method for calculating ECG was also described previously [23]. QT interval was numerically defined as the time period from the onset of Q wave to the last peak of second derivative of T wave. The convergence of the simulation results was tested by repeating some simulations with half of the spatial and time discretization steps.

2.6. Statistical analysis

Quantitative data are presented as the mean ± SEM. Multiple comparisons among groups were carried out by one-way ANOVA with Bonferroni's least significant difference as the post-hoc test. A level of $P<0.05$ was accepted as statistically significant.

3. Results

3.1. Clinical phenotypes

Pedigree for the family with novel IVS7 +3A>G mutation is shown in Fig. 1a. The proband, 34-year-old woman (II-1), was first diagnosed with LQTS at age 14, and has remained asymptomatic. The ECG recording at rest showed a marked QT prolongation (QT_c = 558 ms: Fig. 1b). Her mother (I-2) had no syncopal episodes, despite a remarkable QT prolongation at rest (QT_c = 536 ms: Fig. 1b, I-2). Her father (I-1) had normal QT_c interval (QT_c = 367 ms: Fig. 1b, I-1). None of her relatives have had a history of syncope or cardiac sudden death. Treadmill test of the proband revealed a pronounced exercise-induced QT prolongation (QT_c = 503 ms before exercise, 615 ms at stage 4: Fig. 1c).

Regarding the families with c.1032G>A mutation, there were 9 probands from 9 unrelated families. Eight of them (89%) were symptomatic, and seven (78%) developed cardiac events before age 15. Their episodes were triggered by exercise, especially swimming (five cases). Seven of 9 families (78%) had at least >2 symptomatic mutation carriers.

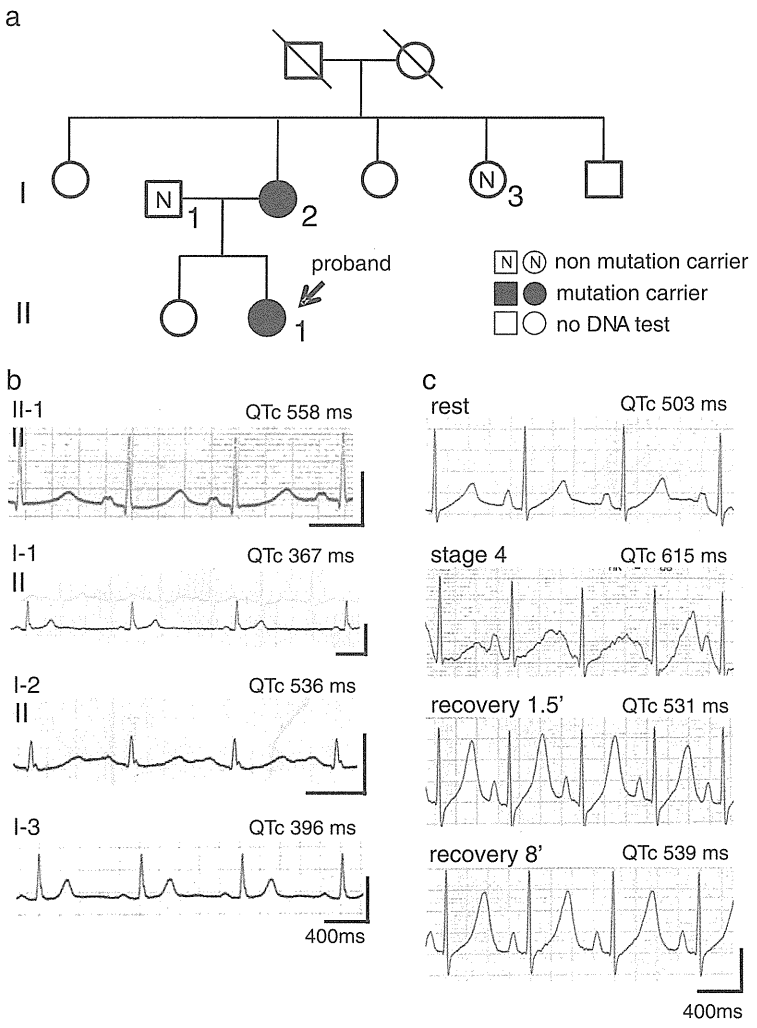

Fig. 1. Clinical and genetic characteristics of the family. (a) Pedigree. Circles represent females and squares represent males. The arrows indicate the proband. Members carrying the mutation are represented by solid symbols. (b) ECG recordings. ECG panels show ECG recordings at rest of various individuals. The QTc interval is indicated. (c) ECG recordings at treadmill exercise test.

Mean QT_c interval of 9 probands were 494 ± 90 ms. Although there is only one family with IVS7 +3A>G mutation, clinical features were apparently severer in families with c.1032G>A mutation.

3.2. Mutation analysis

DNA samples from 3 members of the family were subjected to a mutation screening of the KCNQ1 gene. An abnormal migration pattern was identified by DHPLC analysis (Fig. 2a; note the greater height of the left peak in II-1 and I-2 as indicated by the arrows) in KCNQ1 exon 7 of the 2 affected individuals. The control sample and the father (I-1) showed a normal pattern, as indicated by the comparable height of the left and right peaks (Fig. 2a). DNA sequencing identified a heterozygous adenine to guanine transition in KCNQ1 at nucleotide IVS7+3 (IVS7 +3A>G) (Fig. 2b right panel), which located in the 5′ splice-site of intron 7. Fig. 2b left panel shows a schematic structure of KCNQ1 channel subunit. The exon 7 spans from part of the P-loop to part of the S6 region (indicated by red color).

3.3. Screening of KCNQ1 splicing mutation using minigene assay

Minigene assay was performed in COS7 cells to assess the effect of the IVS7 +3A>G mutation on the splicing of KCNQ1 exon 7. Fig. 3a shows the construct of minigene harboring KCNQ1 exon 7 and its flanking introns inserted into the pSPL3 vector. We also tested 3 other neighboring mutations that may affect the splicing of exon 7 (c.1022C>T, c.1032G>A, IVS7 +28T>C). c.1032G>A was used as a positive control that we and others reported to cause skipping of exon 7 [10,12].

The control KCNQ1 minigene expression in COS7 cells resulted in a production of the single mRNA band that corresponds to KCNQ1 exon 7 joined to the vector exons (Fig. 3b). The mutant minigene containing c.1022C>T or IVS7 +28T>C also showed the same single mRNA, indicating these mutations do not cause aberrant splicing (Fig. 3b). However, the mutant minigene containing IVS7 +3A>G, as well as the positive control c.1032G>A, generated 2 major mRNA bands that correspond to the normal transcript and a shorter transcript lacking KCNQ1 exon 7 respectively (Fig. 3b). The expression level of the shorter mRNA band appeared to be greater in c.1032G>A compared with IVS7 +3A>G. No band was detected in the RNA sample without reverse-transcriptase. We confirmed similar results both in CHO and HL-1cells under the same experimental conditions (data not shown).

3.4. Identification of exon-skipping KCNQ1 mRNAs in patient's blood sample

To directly confirm the minigene assay results, total RNA samples extracted from the patients' lymphocytes were subjected to RT-PCR (Fig. 4a), using primers spanning exons 5 through 10. Samples from individuals having IVS7 +3A>G and c.1032G>A showed shorter bands as well as the normal-sized WT. The direct sequencing of these short-sized transcripts revealed the existence of three kinds of exon-skipping mRNAs as indicated to the right of panel 4a (Δ7-8:399 bp, Δ7: 495 bp, Δ8: 510 bp, WT: 606 bp). Nucleotide sequence of each of the exon-skipping mRNAs is also shown. Control, c.1022C>T and IVS7 +28T>C showed normal patterns; the predominant WT and a small portion of Δ8.

3.5. Quantification of exon-skipping KCNQ1 mRNAs using real-time RT-PCR

We carried out quantitative analysis of short-sized mutant mRNAs in affected patients carrying the IVS7 +3A>G or c.1032G>A mutation, using real-time RT-PCR. Normal individuals had minor fractions of splicing variants (WT: 93.0 ± 0.7%, Δ7: 0.0 ± 0.0%, Δ7-8: 0.1 ± 0.0%, Δ8: 6.9 ± 0.7%, of total KCNQ1 transcripts; n = 4) as shown in the left bar graph of Fig. 4b. In contrast to c.1032G>A carriers who displayed a distinct exon skipping (WT: 55.2 ± 0.9%, Δ7: 23.5 ± 1.7%, Δ7-8: 16.8 ± 0.9%, Δ8: 4.5 ± 0.7%; n = 3, right bar graph in panel 4b), IVS7 +3A>G carrier showed modest but significant amount of exon skipping (WT: 81.2%, Δ7: 9.7%, Δ7-8: 5.7%, Δ8: 3.4%; n = 1, middle bar graph).

3.6. Biophysical characteristics of exon-skipping KCNQ1 proteins

Previously, we performed biophysical characterization of mutant KCNQ1 proteins (Δ7, Δ7-8, and Δ8) in *X. laevis* oocytes injected with mutant cRNAs. We demonstrated the *Xenopus* oocytes injected with Δ7, Δ7-8, or Δ8 alone displayed no time-dependent currents, indicating these mutants were non-functional. Furthermore, each exon-skipping KCNQ1 protein had the mutant-specific level of dominant-negative effect on WT channels [10].

In order to simulate the electrophysiological properties of cardiac cells of the affected patients, we injected the cRNAs (total 10 ng) with the relative ratios of WT and mutant KCNQ1 inferred from the data obtained in the real-time RT-PCR experiment (Fig. 4b). Oocytes injected at cRNA ratios comparable to those evaluated in IVS7 +3A>G showed remarkable reduction in currents compared with those of normal individuals, but less pronounced than c.1032G>A carriers; 100 ± 14.5% (n = 6) for control, 64.8 ± 4.5% (n = 7) for IVS7 +3A>G carriers ($p<0.05$), 41.4 ± 9.5% (n = 6) for c.1032G>A carriers ($p<0.05$) (Fig. 5).

3.7. Computer simulation

Finally, we performed a computer simulation study employing the 1D myocardial model (Fig. 6a) to explore the cellular mechanisms by which these splicing mutations manifest QT prolongation under exercise and induce ventricular tachyarrhythmias.

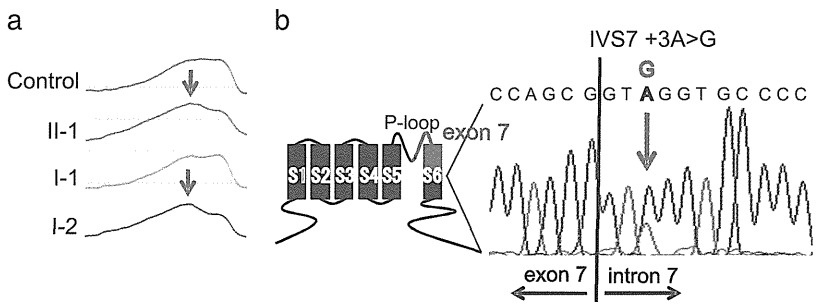

Fig. 2. Mutation analysis. (a) DHPLC revealed abnormal migration patterns in the affected individuals. (b) Left panel: scheme of the transmembrane topology of the cardiac KCNQ1 channel illustrating the location of exons 7 (red). Right panel: automated DNA sequencing electropherogram demonstrates IVS7 +3A>G mutation.