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-a-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...
long-QT syndrome (LQTS). In 1991, Keating and coworkers 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. 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. 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. 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.

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\textsubscript{K1}), the rapidly activating and inactivating transient outward current (I\textsubscript{to}), and the ultrarapid (I\textsubscript{kur}), rapid (I\textsubscript{Kr}), and slow (I\textsubscript{Ks}) components of delayed rectifier currents. (Abbreviations in parentheses indicate names of specific currents used in basic electrophysiology.)

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

<table>
<thead>
<tr>
<th>Loci</th>
<th>Chromosome</th>
<th>Gene</th>
<th>Ion Channel</th>
<th>Result</th>
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<tbody>
<tr>
<td>Congenital LQTS</td>
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<tr>
<td>LQT1</td>
<td>11 (11p15.5)</td>
<td>KCNQ1</td>
<td>I\textsubscript{Ks}</td>
<td>Loss of function</td>
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<tr>
<td>LQT2</td>
<td>7 (7q35–q36)</td>
<td>KCNH2</td>
<td>I\textsubscript{Kr}</td>
<td>Loss of function</td>
</tr>
<tr>
<td>LQT5</td>
<td>21 (21q22.12)</td>
<td>KCNE1</td>
<td>I\textsubscript{Ks}</td>
<td>Loss of function</td>
</tr>
<tr>
<td>LQT6</td>
<td>21 (21q22.12)</td>
<td>KCNE2</td>
<td>I\textsubscript{Ks}</td>
<td>Loss of function</td>
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<tr>
<td>LQT7</td>
<td>17 (17q23.1–q24.2)</td>
<td>KCNJ2</td>
<td>I\textsubscript{Ks}</td>
<td>Loss of function</td>
</tr>
<tr>
<td>LQT11</td>
<td>7 (7q21–q22)</td>
<td>AKAP-9</td>
<td>I\textsubscript{Ks}</td>
<td>Loss of function</td>
</tr>
<tr>
<td>LQT13</td>
<td>11 (11q23.3–24.3)</td>
<td>KCNJ5</td>
<td>I\textsubscript{K-ACh}</td>
<td>Loss of function</td>
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<tr>
<td>Congenital LQTS</td>
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<tr>
<td>JLN1</td>
<td>11 (11p15.5)</td>
<td>KCNQ1 (homozygous)</td>
<td>I\textsubscript{Ks}</td>
<td>Loss of function</td>
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<tr>
<td>JLN2</td>
<td>21 (21q22.12)</td>
<td>KCNE1 (homozygous)</td>
<td>I\textsubscript{Ks}</td>
<td>Loss of function</td>
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<tr>
<td>SQTS</td>
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<tr>
<td>SQT1</td>
<td>7 (7q35–q36)</td>
<td>KCNH2</td>
<td>I\textsubscript{Kr}</td>
<td>Gain of function</td>
</tr>
<tr>
<td>SQT2</td>
<td>11 (11p15.5)</td>
<td>KCNQ1</td>
<td>I\textsubscript{Ks}</td>
<td>Gain of function</td>
</tr>
<tr>
<td>SQT3</td>
<td>17 (17q23.1–q24.2)</td>
<td>KCNJ2</td>
<td>I\textsubscript{Ks}</td>
<td>Gain of function</td>
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<tr>
<td>Brugada syndrome</td>
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<tr>
<td>BrS6</td>
<td>11 (11q13–q14)</td>
<td>KCNE3</td>
<td>I\textsubscript{Ks}</td>
<td>Gain of function</td>
</tr>
<tr>
<td>Early repolarization syndrome</td>
<td>12 (12p11.23)</td>
<td>KCNJ8</td>
<td>I\textsubscript{K-ATP}</td>
<td>Gain of function</td>
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<tr>
<td>Atrial fibrillation</td>
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<td>LQT1</td>
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LQTS indicates long-QT syndrome; SQTS, short-QT syndrome.
I_K, consists of at least 2 components carrying fast (I_{Ks,t}) and slow (I_{Ks,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_{Kr}, I_{Ks}, and I_{Kc}. They are easily distinguished from each other by their pharmacological or biophysical properties. I_{Kr} is expressed mainly in the atrium and not in the ventricle and therefore does not help determine QT interval. I_{Ks} activates rapidly but is easily inactivated on stronger depolarization (showing a strong inward rectification). In contrast, I_{Kc} 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. These fundamental understandings were mainly achieved since the late 1970s by means of patch-clamp techniques in mammalian cardiomyocytes.

An understanding of the molecular biology of potassium channels came later, after the memorable report by Papazian et al. 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. The pore domain is composed of S5, the P-loop, and S6, which is the ion permeation pathway, and includes the ion selectivity filter. 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.

KCNA5 encodes the α-subunit of the I_{Kr} channel, and its loss-of-function mutations have been shown to be associated with familial AF. Kv4.3 encodes the α-subunit of I_{Ks}, 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); (2) a calcium-binding protein, NCS-1 (or frequentin); (3) potassium channel accessory proteins (KChAPs); (4) dipeptidyl-aminopeptidase-like protein 6 (DPP6); and (5) KCNE family members. The KCNE members are also denoted as MinK-related proteins (MiRP1 through 4, encoded by KCNE2 through 5, respectively), and KCNE2 (or MiRP1) has been shown to modulate KCNH2-encoded I_{Kc} channels. In addition, MinK also affects the I_{Kc} current.

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. Another member of the KCNJ family, KCNJ5, encodes the α-subunit of the acetylcholine-sensitive potassium current (I_{Kac}) 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. 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. 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. 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. In humans, Kir2.1 is expressed not only in the myocardium but also in brain and skeletal muscle. 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), QTSS, 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.\(^4\) Congenital LQTS is a Rosetta stone for studying the genetic background of inherited arrhythmic syndromes, 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, 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.\(^5\)–\(^8\) Of the 13 identified genotypes, 6 (LQT1, LQT2, LQT5, LQT6, LQT7, and LQT13) are caused by mutations in potassium channel genes; in LQT1\(^1\), LQT2, LQT5, LQT6, LQT7, and LQT13) are caused by mutations in potassium channel genes; in LQT11, AKAP-9 encoding Yotiao is the responsible gene\(^5\) (Table). AKAP-9 is reported to assemble KCNQ1, thus indirectly modulating I\(_{Ks}\). Mutations in KCNQ1 and KCNE1, which are the \(\alpha\)-subunit and accessory subunit of the potassium channel gene, respectively, are responsible for defects (loss of function) in the I\(_{Ks}\) underly LQT1 and LQT5.\(^9\)–\(^12\) Mutations in KCN2H and KCNE2, which are also the potassium channel \(\alpha\)-subunit and accessory subunit, respectively, cause defects in I\(_{Ks}\) that are responsible for LQT2 and LQT6\(^4\)–\(^20\); however, there is controversy as to whether I\(_{Ks}\) is truly the byproduct of KCN2H and KCNE2. Mutations in KCN2H 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.\(^4\)–\(^6\),\(^5\)–\(^6\) A specific KCN2 mutation, V227F, was identified in a patient with a typical catecholaminergic polymorphic VT phenotype.\(^5\) 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.\(^5\) 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\(_{K-ACH}\), responsible for LQT13,\(^5\) although the precise role of I\(_{K-ACH}\) in the ventricle is still unknown. In all genotypes, decreases in outward potassium currents (I\(_{Ks}\), I\(_{Kf}\), and I\(_{K channels) produce 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.\(^5\) The third most common genotype, LQT3, accounts for only 10% of genotyped patients.\(^5\) 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.\(^5\) 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.\(^5\) Mutations in KCNJ1\(^5\) and KCNH2\(^5\) 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.\(^6\) 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\(_{Kf}\) 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).\(^4\) In LQT7 with an I\(_{Kf}\) defect, mild QT prolongation, TWU-wave abnormalities (featuring a prominent U wave), frequent ventricular premature contractions, and typical bidirectional VT are often observed.\(^5\)

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.\(^6\)–\(^8\) 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.\(^6\)–\(^8\) 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 \(\beta\)-adrenergic agonist, creates a dramatic augmentation of transmural dispersion of repolarization, which results in broad-based T waves (Figures 1B and 1E).\(^6\)–\(^8\) In the LQT2 model, d-sotalol, an I\(_{Kf}\) blocker, in the presence of hypokalemia also produces more preferential APD prolongation in
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 coworkers67 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 preparations63,64 and clinical studies using catecholamine provocative testing or exercise testing61,75–78 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
channel defect. The bolus protocol also effectively predicts channel defect but not in patients with LQT3 with a sodium activity) in patients with either LQT1 or LQT2 with a potassium col of epinephrine improves clinical ECG diagnosis (sensi-
mizu protocol). The 2 major protocols developed for the epinephrine
challenge test include the escalating-dose protocol by Ackerman’s group (Mayo protocol) and a bolus injection followed by a brief continuous infusion by our group (Shi-
mizu protocol).

Ackerman and coworkers 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, suggested that sympathetic stimulation produces genotype-specific responses of the corrected QT (QTc) interval in patients with LQT1, LQT2, and LQT3 syndromes. 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. The bolus protocol also effectively predicts the underlying genotype of LQT1, LQT2, and LQT3. 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. Although epinephrine was not used, Viskin et al recently reported the usefulness of a bedside stand-up test to easily diagnose LQTS. They suggested that at maximal

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).

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. Exercise restriction is also required in LQT2 patients. 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. In LQT1, β-blockers are most effective to prevent episodes of syncope and sudden cardiac death. 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. Mexiteline, a class IB sodium channel blocker that blocks late I_{Na}, or verapamil, an I_{Ca,L} blocker, may warrant consideration as an adjunctive therapy to β-blockers in LQT1 patients. As a nonpharmacological therapy, left stellate ganglion ablation, another antidromic therapy, is most effective in LQT1 patients. 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. Priori et al 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. 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. A genotype-specific initiating pattern of torsade de pointes has been reported. 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. 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. 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 coworkers88 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 haplinsufficiency (≤50%) ion channel effects. In 2002, Moss and coworkers89 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 coworkers90 reported a first case with SQTS who showed a short QTc of 300 ms and AF. In 2003, Gaia et al91 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_{Ks}, I_{Kr}, 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 Tpeak to Tend 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 V₁ through V₃) 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 reported a missense mutation (R99H) in KCNE3, which encodes the potassium channel accessory (β3) subunit and interacts with the Kv4.3 (I钾) channel, in a proband with BrS. Coexpression of the mutant KCNE3 with KCND3, which encodes Kv4.3, increases I钾 intensity (gain of function) compared with coexpression of wild-type KCNE3 with KCND3. 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钾.

*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. Several agents that increase the outward potassium current, such as nicorandil, a KATP 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.[24-25]

*Early Repolarization Syndrome*

The prevalence of an early repolarization pattern or J wave in the inferior (II, III, aVF) or lateral (I, aVL, V4 through V6) 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 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 KATP channel was reported in a young female with VF secondary to early repolarization syndrome.[109] A recent study reported that the KATP current (I钾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钾基因, in 2003 (Table). Electrophysiological analysis of the specific mutation, S140G, demonstrated a gain of function in I钾 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钾, the KCNE3 mutation (R53H) did not change I钾, which suggests that it might not be a causative mutation. A KCNJ2 mutation that leads to a gain of function in I钾 current has also been reported.[115] More recently, a mutation in KCNA5 encoding an atrium-specific I钾 was identified in familial AF.[17-19] Interestingly, the specific KCNA5 nonsense mutation E375X resulted in a loss of function of I钾 current. A reduction in I钾 elevates the voltage of the action potential plateau, thus activating more I钾 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 SQTI 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钾 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
been shown to be associated with disease. \textsuperscript{124–126} More recently, a naturally occurring copy-number variations in chromosome, thereby giving the genome diversity. Recently, a French group conducted an extensive survey of single-nucleotide polymorphisms, and copy-number variations. The former two 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, \textit{KCNE1 D85N} is well known not only as a modifier but also as a causative variant of LQTS. \textsuperscript{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 \textit{KCNH2} and \textit{KCNQ1} have been shown to be associated with disease. \textsuperscript{124–126} More recently, a French group conducted an extensive survey of copy-number variations in \textit{KCNQ1} and \textit{KCNH2} and demonstrated that such variations explained approximately 3\% of LQTS in patients with no point mutation in these genes. \textsuperscript{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 \textit{KCNQ1}, \textit{KCNJ2}, and most \textit{ KCNH2} mutations. \textsuperscript{128–132} \textit{KCNJ2} contains a specific C-terminal sequence necessary for exportation from the endoplasmic reticulum to the Golgi apparatus (endoplasmic reticulum–to-Golgi export signal). \textsuperscript{133} More recently, a naturally occurring \textit{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). \textsuperscript{134}

Most \textit{KCNH2} mutations have been reported to reduce hERG currents by a trafficking-deficient mechanism (step 6 in Figure 3). \textsuperscript{131} Several trafficking-refractory \textit{KCNQ1} mutations are also known, of which T587M in the C-terminal region was the first reported. \textsuperscript{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 \textit{KCNQ1} and hERG proteins, which increased localization of hERG channels to the cell membrane, enhanced current density, and altered their biophysical properties. \textsuperscript{135} Likewise, overexpression of the dominant-negative \textit{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. \textsuperscript{136} Therefore, the intracellular trafficking defect in \textit{KCNQ1} impaired the physical interaction with hERG and thereby caused severe clinical features (step 6 in Figure 3). \textsuperscript{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 (PI(2)). \textsuperscript{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. \textsuperscript{140} Impaired endocytosis of calcium-activated nonselective cation channels, TRM4, was reported to cause progressive cardiac conduction block through SUMO (small ubiquitin modifier) conjugation. \textsuperscript{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. \textsuperscript{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. \textsuperscript{144}

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**Disclosures**

None.

**References**


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