Table 1. Ion channel defects or the membrane adaptor responsible for the congenital long QT syndrome.

Loci	Chromosome	Gene	Ion channel
Romano–Ward syndrome			
LQT1	11 (11p15.5)	*KCNQ1*	I_{Ks}
LQT2	7 (7q35-36)	*KCNH2*	I_{Kr}
LQT3	3 (3p21-23)	*SCN5A*	I_{Na}
LQT4	4 (4q25-27)	*Ankyrin-B*	Na–K ATPase, I_{Na-Ca}
LQT5	21 (21q22.1-q22.2)	*KCNE1*	I_{Ks}
LQT6	21 (21q22.1-q22.2)	*KCNE2*	I_{Kr}
LQT7	17 (17q23.-24.2)	*KCNJ2*	I_{K1}
LQT8	12 (12p13.3)	*CACNA1C*	I_{Ca-L}
LQT9	3 (3p25)	*CAV3*	I_{Na}
LQT10	11 (11q23.3)	*SCN4B*	I_{Na}
LQT11	7 (7q21-q22)	*AKAP-9*	I_{Ks}
Jervell & Lange–Nielsen syndrome			
JLN1	11 (11p15.5)	*KCNQ1* (homozygous)	I_{Ks}
JLN2	21 (21q22.1-q22.2)	*KCNE1* (homozygous)	I_{Ks}

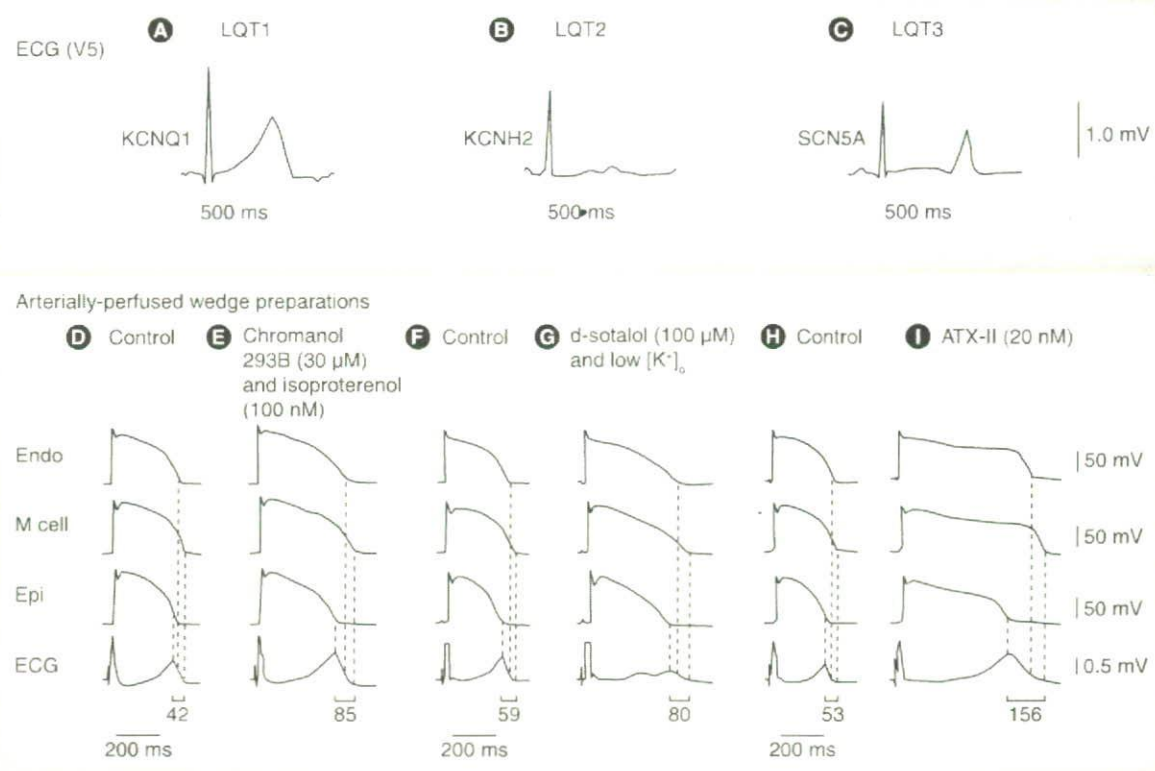
Mutations in *KCNJ2*, which encodes for the inward rectifier potassium channel (I_{K1}), give rise to a rare periodic paralysis disorder associated with prolongation of the QT interval and ventricular arrhythmias, known as Andersen–Tawil syndrome or LQT7 [20]. A mutation in *Ankyrin-B*, a member of a family of versatile membrane adapters, leads to intracellular calcium overload, which is thought to contribute to the LQT4 syndrome. In addition to QT prolongation, this syndrome is associated with sinus bradycardia and paroxysmal atrial fibrillation [21]. A mutation in *CACNA1C* was reported to be responsible for the defect in the L-type calcium current (I_{Ca-L}) underlying the LQT8 form, an arrhythmia disorder associated with dysfunction in multiple organ systems, including congenital heart disease, syndactyly, immune deficiency and autism [22]. *CAV3*, which encodes caveolin-3, and *SCN4B*, which encodes NaVB4, an auxiliary subunit of the cardiac sodium channel, are also recently reported to be associated with LQTS. Mutations in both genes produce a gain of function in late I_{Na}, causing an LQT3-like phenotype [23,24]. The most recent gene linked to LQTS is *AKAP-9* encoding Yotiao, which assembles *KCNQ1* [25]. Some cases of sudden infant death syndrome (SIDS) are attributable to congenital LQTS [29]. Mutations in *SCN5A* [29,30], *CAV3* [31], *KCNQ1* [32] and *KCNH2* [32] were reported to be associated with SIDS. A recent Norwegian study including 201 cases of SIDS victims, detected mutations in 9.5% of cases [33]. In all genetic forms, decreases in outward potassium currents (I_{Ks}, I_{Kr} and I_{K1}) or increases in inward sodium or calcium currents (late I_{Na} and I_{Ca-L}), prolong the action potential duration (APD), leading to an extended QT interval, a common phenotype in LQTS. Among the 11 forms, the LQT1 and LQT2 syndromes are the two most common genetic variants, and each accounts for approximately 40% of genotyped patients [19]. The LQT3 syndrome accounts for approximately 10% of genotyped patients [19]. The genotype–phenotype correlation has been investigated in detail in the LQT1, LQT2 and LQT3 syndromes, since these three forms constitute more than 90% of genotyped patients with LQTS [19]. Homozygous or compound heterozygous mutations in *KCNQ1* and/or *KCNE1* genes cause the autosomal recessive forms (JLN1–JLN2) of the Jervell and Lange–Nielsen syndrome, which is usually associated with neurosensorial deafness [34]. QT prolongation is generally more prominent and ventricular arrhythmias more severe than those in the Romano–Ward syndrome. On the other hand, the autosomal-recessive form of LQT1 syndrome without sensory neural deafness was reported by Priori and coworkers [35]. Since approximately half of the mutations in LQTS are novel, the potential that the

mutation may be a bystander variant, has always necessitated finding a second mutation in the same or another gene. There are reports of homozygous/compound heterozygous mutations in one gene or in two genes. Approximately 8% of the LQT patients carried mutations in two LQTS-causing genes and had a longer QTc and also 3.5-fold more risk of cardiac arrest [36]. Some single mutations in the *SCN5A* gene have been reported to cause multiple phenotypes, such as Brugada syndrome, sick sinus syndrome and conduction disease in addition to LQT3 phenotype [37,38].

Genotype-specific T wave morphology in congenital LQTS

Genotype-specific T wave morphology in the ECG has been proposed by Moss and coworkers in 1995 [39], and significantly expanded by Zang and coworkers in 2000 [40]. Broad-based prolonged T waves are more frequently observed in the LQT1 syndrome. Low-amplitude T waves with a notched or bifurcated configuration and late-appearing T waves with a prolonged isoelectric ST-segment are more commonly seen in the LQT2 and LQT3 syndrome, respectively. However, the T wave pattern varies with time, even in the same patient with a specific mutation, and exceptions are reported to be present [40]. The transmural electrical heterogeneity of ventricular repolarization from the epicardial, mid-myocardial and endocardial cells as a result of mutations in each gene has been suggested to be responsible for the inscription of the characteristic T-wave morphologies in the three genotypes by the experimental studies employing arterially perfused canine left ventricular wedge preparations (**Figure 1**) [41,42].

Figure 1. Cellular basis of abnormal T wave patterns in LQT1, LQT2 and LQT3 syndromes.



(A–C) ECG lead V5 recorded in patients with LQT1, LQT2 and LQT3 forms of congenital LQT syndrome. **(D–I)** Transmembrane action potentials recorded simultaneously from Endo, M and Epi cells together with a transmural ECG at a basic cycle length of 2000 msec in the three models of the arterially-perfused canine wedge preparations. Pharmacologic models mimic the phenotypic appearance of the abnormal T waves in all three models.
ECG: Electrocardiogram; Endo: Endocardial; Epi: Epicardial; LQT: Long QT; M: Mid-myocardial.
Modified from [41,42].

Genotype-specific triggers for cardiac events in congenital LQTS

Genotype-specific triggers for cardiac events have been reported in patients with the LQT1, LQT2 and LQT3 syndromes [13,43,44]. Cardiac events occur most frequently during exercise (62%), and swimming is a common trigger in the LQT1 syndrome [43]. In sharp contrast to the pattern shown in LQT1 patients, the LQT2 and LQT3 patients are less likely to have cardiac events during exercise (13 and 13%, respectively) and more likely to have cardiac events during rest/sleep (29 and 39%, respectively) [43]. A sudden startle in the form of an auditory stimulus (a telephone, alarm clock, ambulance siren, etc.) is a specific trigger in the LQT2 syndrome [43,44]. Female LQT2 patients have been reported to be most susceptible to cardiac events during postpartum periods. Exercise or mental stress often trigger ventricular arrhythmias in LQT4 patients [21]. In LQT7 patients, hypokalemia is often associated with frequent ventricular arrhythmias [20]. Periodic paralysis is associated with hypo-, hyper- or normo-kalemia [20]. The differential sensitivity in cardiac events to sympathetic (β-adrenergic) stimulation has been suggested to be due to the differential response of ventricular repolarization to sympathetic stimulation by both experimental studies employing arterially perfused wedge preparations [42,45] and clinical studies using catecholamine challenge test or exercise testing [46,47]. The epinephrine test is reported to establish an ECG diagnosis in silent mutation carriers of LQTS, especially the LQT1 genotype [46,47]. Epinephrine infusion is also useful to predict the genotype of the LQT1, LQT2 and LQT3 syndromes by the specific response of the QT interval [47].

Genotype-specific clinical course & genotype-specific therapy in congenital LQTS

A higher cumulative probability of cardiac events has been reported in LQT1 and LQT2 patients compared with LQT3 patients [48]. More than half of the LQT1 and LQT2 patients experience cardiac events before 40 years of age, whereas less than 30% of LQT3 patients do so. On the other hand, the lethality of the cardiac events is higher in LQT3 patients. Male patients are generally younger than female patients at first cardiac events [49]. Approximately 90% of first cardiac events occur before the age of 15 years in male patients, particularly in LQT1 males, whereas female patients may not experience first cardiac events until 20 years of age [49]. These data have recently been confirmed by the largest cohort study of LQT1 syndrome, which reported that the clinical risk factors associated with first cardiac events are age less than 13 years in males and over 13 years of age in females [50]. These data suggested that LQT1 males before adolescence require stricter exercise restriction, in particular swimming or diving. Risk stratification according to age, gender and QTc interval has also been recommended [51].

Although β-blockers are empirically believed to be the most effective therapy for patients with congenital LQTS, they are not protective in all LQTS patients. Since 1995, when Schwartz and coworkers first reported the specific efficacy of sodium-channel blockade with mexiletine in shortening QT interval in LQT3 patients compared with that in LQT2 patients, the possibility of genotype-specific therapy has been investigated in both clinical and experimental studies (Table 2).

Table 2. Genotype-specific therapy based on clinical and experimental data in long QT syndrome.

Therapy	LQT1	LQT2	LQT3
Prevalence (%)	40	30–40	10
Exercise restriction	+++++	+++	NA
β-blockers	+++++	+++	NA
Potassium supply	++ ?	++++	++ ?
Class IB sodium-channel blockers	+++	+++	+++++
Calcium-channel blockers	+++	+++	++?
Potassium-channel openers	++	++	NA
Pacemaker	++	++++	+++++
Implantable cardioverter-defibrillator	++++	++++	+++++

+++++: Most effective.

LQT: Long QT; NA: No data available.

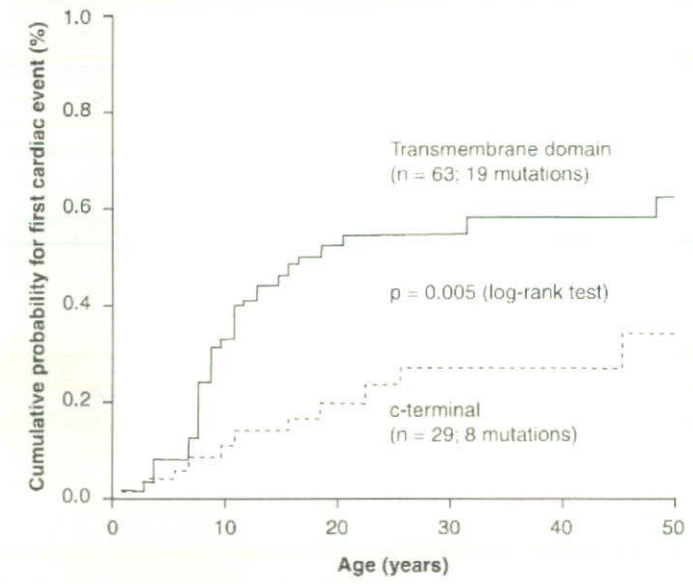
In LQT1 patients, β-blockers frequently suppress episodes of syncope and sudden cardiac death [43]. A recent cohort study of 600 LQT1 patients has suggested that time-dependent β-blocker use was associated with a significant 74% reduction in the risk of first cardiac events [50]. Mexiletine (a class IB sodium-channel blocker) that blocks late I_{Na}, or verapamil (an L-type calcium current [I_{Ca-L}] blocker), may warrant consideration as adjunctive therapies to β-blockers in LQT1 patients. However, no patient event data exists, and only the ECG changes or experimental data suggest the efficacy of these agents [41,42]. An implantable cardioverter-defibrillator (ICD) is indicated for LQTS patients who have suffered an aborted cardiac arrest and/or who have repetitive episodes of syncope in the presence of β-blockers.

In LQT2 patients, β-blockers are the first choice for pharmacological therapy, although the recurrence rate is higher than that in LQT1 patients [43]. Increase in the extracellular potassium concentration by exogenously administered potassium or long-term oral potassium administration was reported to shorten the QT interval in LQT2 patients [52]. However, no data yet exists to show that events are reduced by oral potassium. Moreover, it is difficult to maintain appropriately elevated serum potassium levels, and caution is required to prevent excessive potassium administration, which may cause life-threatening arrhythmic events. The indication of the ICD is similar to that in the LQT1 syndrome. Pacemaker therapy is reported to be more effective in LQT2 than in LQT1 patients by suppressing the specific short–long–short-initiating pattern of TdP in the LQT2 syndrome [53].

β-blockers are less effective in LQT3 patients, compared with LQT1 or LQT2 patients [43]. Mexiletine is more effective in reducing the QT interval in the LQT3 than in the LQT1 or LQT2 syndrome, therefore providing therapeutic choice in LQT3. Pacemaker therapy may be beneficial in LQT3 patients, especially in patients with bradycardia.

Genotype-specific therapy is unknown in the other LQT forms, (LQT4, LQT7, LQT8, LQT9, LQT10 and LQT11). β-blockade is the first-line therapy in patients with LQT4, LQT7 and LQT8, and unknown LQTS genotypes. Theoretically, mexiletine may be effective in LQT9, LQT10 and LQT11 patients.

Figure 2. Kaplan–Meier cumulative cardiac event curves from birth through to 50 years of age.



Kaplan–Meier cumulative cardiac event curves from birth through to 50 years of age for patients with *KCNQ1* mutations located in the transmembrane regions (n = 66, 19 mutations) and the C-terminal regions (n = 29, eight mutations) in long QT1 syndrome. The difference in the clinical course by mutation location was significant (log-rank, p = 0.005), with a greater risk of first cardiac events in patients with transmembrane mutations than in those with C-terminal mutations.
Modified from [13].

Mutation site-specific management & therapy in congenital LQTS

Since the structure of each cardiac ion channel or the correspondence between the mutation site and the channel function has been disclosed, mutation site-specific differences in the severity of the clinical phenotype or responses to therapy could be expected. In the LQT1 syndrome, Shimizu and coworkers compared the arrhythmic risk and sensitivity with sympathetic stimulation with treadmill exercise testing between Japanese LQT1 patients with transmembrane mutations and those with C-terminal mutations in the *KCNQ1* gene [54]. They suggested that patients with transmembrane mutations had a longer QTc interval and more frequent LQTS-related cardiac events than those with C-terminal mutations (**Figure 2**) [54]. Moreover, the QTc interval was more prominently increased with exercise in patients with transmembrane mutations [54]. The cohort study of 600 LQT1 patients by Moss *et al.* has recently confirmed the results by Shimizu and coworkers [50], and suggested that transmembrane mutations and

Table 3. Ion channel defects responsible for the Brugada syndrome.

Loci	Chromosome	Gene	Ion channel
BS1	3 (3p21-23)	*SCN5A*	I_{Na}
BS2	12 (12p13.3)	*CACNA1C*	$I_{Ca\text{-}L}$
BS3	10 (10P12.33)	*CACNB2*	$I_{Ca\text{-}L}$
BS4	3 (3p21)	*GPD1-L*	I_{Na}

mutations with dominant-negative functional effect adversely influence the outcome of LQT1 patients, independent of traditional clinical risk factors and β-blocker therapy.

Regarding the LQT2 syndrome, Moss and coworkers suggested that LQT2 patients with mutations in the pore region of the *KCNH2* gene are at markedly increased risk of arrhythmia-related cardiac events compared with patients with non-pore mutations in the International LQTS Registry [55]. A larger cohort of LQT2 syndrome and LQT3 syndrome is now ongoing. These data indicate the possibility of mutation site-specific management or treatment in each genotype.

Genetics of Brugada syndrome

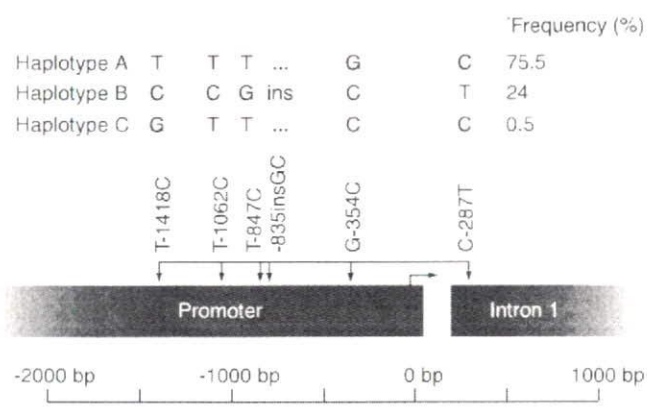
Brugada syndrome is characterized by ST-segment elevation (coved-type or saddle back-type) in the right precordial ECG (V1–3 leads) and an episode of ventricular fibrillation in the absence of structural heart diseases [56–59]. Prevalence of this syndrome is estimated to be up to five per 10,000 inhabitants, and is one of the important causes of sudden cardiac death of middle-aged males, particularly in Asian countries [58,59]. Brugada syndrome usually manifests during adulthood [58], and more than 80–90% of patients clinically affected with Brugada syndrome are men.

In 1998, the first mutation linked to Brugada syndrome was identified in *SCN5A* (**Table 3**) [3]. *SCN5A* mutations are reported to account for 18–30% of clinically diagnosed Brugada patients at present [58]. Functional expression studies have shown that all of the *SCN5A* mutations resulted in decrease (loss of function) of I_{Na} by several mechanisms [59]. These functional effects include:

- Reduced sodium channel expression;
- A shift in the voltage-dependence and time-dependence of I_{Na} activation, inactivation or reactivation;
- Entry of the sodium channel into an intermediate state of inactivation from which it recovers more slowly;
- Accelerated inactivation of the sodium channel;
- Trafficking defect.

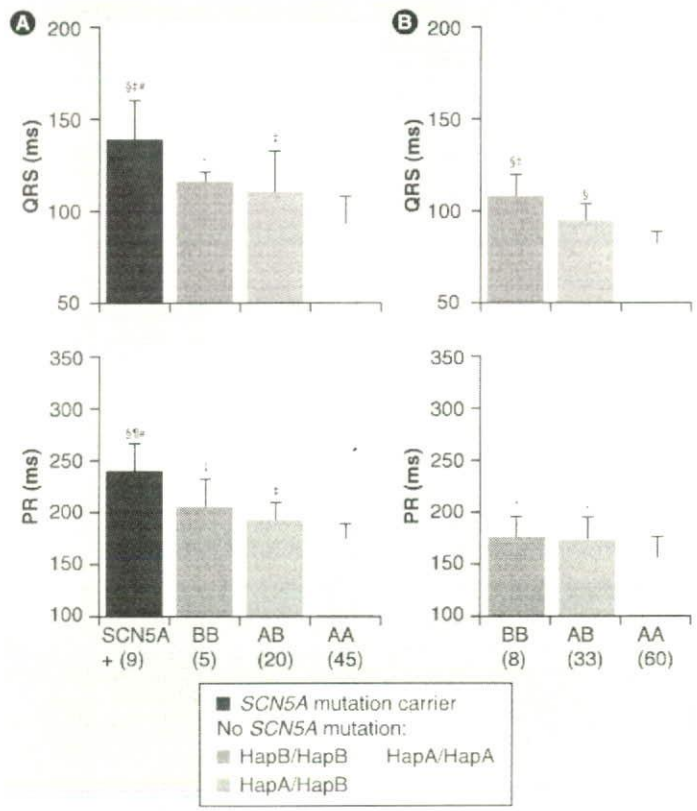
Thereafter, Antzelevitch and coworkers reported that three probands associated with a Brugada like ST-segment elevation and a short QT interval were linked to mutations in *CACNA1C* or *CACNB2*, the genes encoding the α1 or β2b subunit of the L-type calcium channel, respectively [60]. Their heterologous expression studies revealed loss of function of $I_{Ca\text{-}L}$. More recently, London and coworkers identified a mutation in a conserved amino acid of the glycerol-3-phosphate dehydrogenase 1-like (*GPD1-L*) gene in affected individuals of a large Brugada family [61]. The *GPD1-L* mutation was reported to decrease *SCN5A* surface membrane expression and reduce I_{Na}, thus causing Brugada syndrome. However, approximately two-thirds of Brugada patients have not yet been genotyped, suggesting the presence of genetic heterogeneity [59].

Figure 3. Haplotypes identified within the proximal promoter region of the *SCN5A*, a cardiac sodium channel gene.



The six polymorphisms are in near-complete linkage disequilibrium. Haplotype A is designated as containing all common alleles, and haplotype B as containing all minor alleles. The discordant haplotype is designated haplotype C.
*Frequency in the Japanese (control) population.
Modified from [67].

Figure 4. *SCN5A* promoter haplotype pair effects on QRS duration in lead V6 and PR duration in lead II in patients with Brugada syndrome and control subjects.



In the Brugada patients without *SCN5A* mutations and control subjects, both QRS and PR durations show a gene-dose effect, being longest in haplotype B homozygotes (BB), intermediate in haplotype A/haplotype B heterozygotes (AB) and shortest in haplotype A homozygotes (AA). The Brugada patients with *SCN5A* mutations show the longer QRS and PR durations than those without *SCN5A* mutations. Patient numbers are indicated between parentheses. Data are presented as mean ± SD.

*$p < 0.05$; †$p < 0.001$; §$p < 0.0001$ vs HapA/HapA; ¶$p < 0.0001$ vs HapA/HapB; #$p < 0.05$ vs HapB/Hap B.

Modified from [67].

Genotype–phenotype correlations in Brugada syndrome

Since more than two-thirds of the patients clinically affected with Brugada syndrome are not genotyped, and only four responsible genes, *SCN5A*, *CACNA1C*, *CACNB2* and *GPD1-L*, have been so far identified, investigation of genotype–phenotype correlation has been limited compared with those in congenital LQTS. Mild conduction abnormalities, such as widening of the P wave, prolongation of QRS duration, PQ interval and HV interval, and higher incidence of right bundle branch block (RBBB) have been described in patients with Brugada syndrome, especially those with *SCN5A* mutation [58]. Smits *et al.* reported significantly longer PQ and HV intervals at baseline, and a larger increase in PQ and QRS intervals after sodium-channel blockers in Brugada patients with *SCN5A* mutations than in those without *SCN5A* mutations [62]. Yokokawa and coworkers recently examined several ECG parameters prospectively during long-term follow-up periods, and compared them between Brugada patients with and without *SCN5A* mutation [63]. They reported that the P wave, QRS and S wave durations, and PQ intervals were all significantly longer, and the S wave amplitude was significantly deeper in the *SCN5A*-positive group than in the *SCN5A*-negative group. In addition, the PQ interval and QRS duration in lead V2 prolonged more markedly with aging in the *SCN5A*-positive group than in the *SCN5A*-negative group during the follow-up period. Their results indicate that progressive depolarization abnormalities (conduction slowing) with aging may play a key role in the pathogenesis of Brugada syndrome. Several drugs other than class I_c sodium-channel blockers and conditions including fever are reported to induce transient ST-segment elevation similar to that in Brugada syndrome, and are described as an 'acquired' form of Brugada syndrome [64,65].

SCN5A promoter polymorphism in ethnic difference of Brugada syndrome

The incidence of Brugada syndrome is significantly higher in Asian countries, including Japan, than in US and European countries [58]. Common polymorphisms have been reported to modulate the activity of the primary disease-causing mutation in inherited cardiac arrhythmias, and to influence susceptibility to arrhythmia even in the general population [66]. Since some common polymorphisms are ethnically dependent, the common polymorphisms may contribute to ethnic differences in the clinical phenotype in inherited cardiac arrhythmias including Brugada syndrome. Bezzina and coworkers recently identified a haplotype B consisting of six individual DNA polymorphisms in near-complete linkage disequilibrium within the proximal promoter region of the *SCN5A* gene in only Asians (an allele frequency of 22%), but not in Caucasian and African–Americans (**Figure 3**) [67]. Luciferase reporter activity of this haplotype B is reduced by 62% in cardiomyocytes compared with wild-type, haplotype A. To test the hypothesis that this *SCN5A* promoter polymorphism (haplotype B) may modulate variability in cardiac conduction, they analyzed the relationship between the *SCN5A*

promoter haplotype and indices of conduction velocity, PR and QRS durations in a cohort of 71 Japanese Brugada syndrome subjects without *SCN5A* mutations and in 102 Japanese controls. The PR and QRS durations are significantly longer in haplotype B individuals with a gene-dose effect in both groups (Figure 4). Moreover, the increases in both PR and QRS durations with sodium-channel blockers are genotype-dependent and a gene-dose effect is also observed. These data demonstrate that the haplotype B within the *SCN5A* promoter region alone does not give rise to Brugada syndrome, but that it likely contributes to higher incidence of Brugada syndrome in Asian populations in combination with other, as yet unknown genetic factors.

Future perspective

The detailed analysis on the genotype–phenotype correlation in inherited cardiac arrhythmias has realized genotyped specific, so-called tailor-made, management and therapy, especially in congenital LQTS. On the other hand, common polymorphisms or variants are now believed to modulate clinical phenotype caused by a responsible mutation as well as to mediate genetic susceptibility for arrhythmia risk. Moreover, there are many ethnicity-specific common polymorphisms, which may influence differential prevalence or penetrance of inherited cardiac arrhythmias among each ethnic population. Common polymorphisms in the ion channel gene is usually functionally silent – the functional phenotype associated with polymorphism channels is usually indistinguishable from the functional phenotype associated with wild-type channels. However, since Splawski *et al.* reported that a predominantly Negroid-specific common polymorphism, S1103Y in the *SCN5A* gene, is associated with arrhythmia risk [66], the concept that some common polymorphisms may mediate arrhythmia susceptibility has been proposed. A larger number of systematic and comprehensive studies on common polymorphisms is required to further understand the role of common polymorphisms on clinical phenotype and to further advance the management and treatment of patients with inherited cardiac arrhythmias.

Executive summary

Genetics of congenital long QT syndrome

- A total of 11 forms of congenital long QT syndrome (LQTS) caused by mutations in genes of the potassium, sodium and calcium channels or membrane adapter have been identified.
- Among the 11 forms, the LQT1, LQT2 and LQT3 forms constitute more than 90% of genotyped patients.

Genotype–phenotype correlation in congenital long QT syndrome

- Genotype-specific T wave morphology in the electrocardiogram is attributable to the transmural electrical heterogeneity of ventricular repolarization from the epicardial, mid-myocardial and endocardial cells.
- Differential sensitivity in cardiac events to sympathetic (β-adrenergic) stimulation has been suggested to be due to the differential response of ventricular repolarization to sympathetic stimulation.
- Genotype-specific pharmacological and nonpharmacological therapies have been started in patients with the LQT1, LQT2 and LQT3 syndromes.

Mutation site-specific difference in clinical phenotype in congenital long QT syndrome

- LQT1 patients with mutations located in the transmembrane region and those with dominant-negative functional effect have a longer QT interval and more frequent LQTS-related cardiac events than those with C-terminal mutations.
- The LQT2 patients with mutations in the pore region of the *KCNH2* gene are at markedly increased risk of arrhythmia-related cardiac events compared with patients with non-pore mutations.

Genetics & genotype–phenotype correlations of Brugada syndrome

- Four genes, *SCN5A*, *CACNA1C*, *CACNB2* and *GPD1-L*, have been identified to be responsible for the clinical phenotype of Brugada syndrome.
- Investigation of genotype–phenotype correlation in Brugada syndrome has been limited compared with those in congenital LQTS.

Ethnic difference in the prevalence in Brugada syndrome

- The incidence of Brugada syndrome is significantly higher in Asian countries than in US and European countries.
- A haplotype B consisting of six individual DNA polymorphisms within the proximal promoter region of the *SCN5A* gene has recently been identified in only Asians.
- The haplotype B alone does not give rise to Brugada syndrome, but likely contributes to a higher incidence of Brugada syndrome in Asian populations.

Causative mutations have not yet been identified in approximately 30–40% of clinically diagnosed patients with congenital LQTS and in more than two-thirds of patients with Brugada syndrome. However, the presence of causative mutations in the known genes cannot be completely ruled out since general screening does not include investigation of the promoter region and intron, or allow for detection of cryptic splicing mutations or gross rearrangements. Genes that code for a variety of ion channels and other proteins have been proposed to be candidate genes for inherited cardiac arrhythmias. Other genes which code for adrenergic receptors, cholinergic receptors, ion-channel-interacting proteins, promoters, transcriptional factors, neurotransmitters or transporters may also be candidates. Once again, further systematic and comprehensive investigations are needed.

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Mutation Site Dependent Variability of Cardiac Events in Japanese LQT2 Form of Congenital Long-QT Syndrome

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Background In the LQT2 form of long QT syndrome (LQTS), mutation sites are reported to correlate with clinical phenotypes in Caucasians, but the relationship in Asian patients remains unknown. The present study was designed to determine whether the location of *KCNH2* mutations would influence the arrhythmic risk in LQT2 patients.

Methods and Results In 118 genetically-confirmed LQT2 patients (69 families, 62 *KCNH2* mutations), the ECG parameters, Schwartz scores, and the incidence of cardiac events, defined as syncope, aborted cardiac arrest, and sudden cardiac death, were evaluated. To examine the effect of mutation sites, the participants were divided accordingly: pore (n=56) and non-pore (n=62) groups. The corrected QT_{end} interval was significantly greater in the pore than in the non-pore group (QTc; 522±63 ms vs 490±49 ms, p=0.002). In this study, the clinical course of each of the probands did not differ according to the mutation sites, whereas non-probands carrying the pore site mutation experienced their first cardiac events at significantly younger age than those with the non-pore site mutation (log-rank, p=0.0005).

Conclusions In a Japanese LQT2 cohort, family members with the pore site mutation were at higher arrhythmic risk than those with the non-pore site mutation. (*Circ J* 2008; **72:** 694–699)

Key Words: Arrhythmia; Long-QT syndrome; QTc interval; Risk factors; Torsade de pointes

The long QT syndrome (LQTS) is an inherited arrhythmogenic disease of the structurally normal heart that may cause sudden death. LQTS is characterized by an abnormality in myocardial repolarization that leads to prolongation of the QT interval, morphological changes in T waves and torsades-de-pointes (Tdp) type of ventricular tachycardia on surface ECGs[1,2] To date, 8 distinct genes responsible for LQTS have been identified, including those of Andersen (LQT7) and Timothy (LQT8) syndromes: on chromosome 11q15.5 (*KCNQ1*; LQT1), 7q35–36 (*KCNH2*; LQT2), 3p21 (*SCN5A*; LQT3), 4q25–27 (*ANKB*; LQT4), 21q22 (*KCNE1*; LQT5), 21q22 (*KCNE2*; LQT6), 17q23 (*KCNJ2*; LQT7) and 12p13.3 (*CACN1C*; LQT8)[3–10]

Moss et al[11] extensively examined the relationships between the site of mutation and clinical phenotype in approximately 44 different LQT2-related *KCNH2* mutations. They reported that subjects with causative mutations in the pore region (n=38, amino acid residues 550 through 650) had more severe clinical manifestations and experienced a higher frequency (74% vs 35%; p<0.001) of arrhythmia-related cardiac events occurring at younger age than did subjects with non-pore mutations (n=166).

In LQT1, based on the United States portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82), 600 patients with *KCNQ1* mutations were classified into 2 groups of patients with transmembrane and C-terminus mutations and their clinical phenotypes were examined[12] That study found that patients with transmembrane mutations were at increasing risk for cardiac events (hazard ratio, 2.06; p<0.001). Shimizu et al also studied the mutation site-dependent differences in 95 LQT1 patients from a multicenter Japanese population and also found that patients with transmembrane mutations were at higher risk of cardiac events and had longer QTc and $T_{peak\text{-}end}$ intervals[13]

In Japanese LQT2 patients, mutation site dependency is unclear, although this has been reported in Caucasian patients. Therefore, in the present study we aimed to compare the genotype and phenotype relationship, according to the classification adopted by Moss et al[11] in 118 Japanese LQT2 patients who were genetically identified in the 3 genetic centers in Japan and had no other mutations in LQTS-related genes (except LQT4 and 8).

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Table 1 ***KCNH2*** **Mutations by Location, Amino-Acid Coding, Type of Mutation, and Reported Functional Effects**

	No. of families	*No. of subjects*	*Position*	*Exon*	*Type of mutation*	*Functional effect in expression studies*
Pore regions						
A561T	*1*	*1*	*S5*	*7*	*Missense*	*Trafficking defect (22)*
A561V	*1*	*1*	*S5*	*7*	*Missense*	*Dominant negative (23)*
*W563C**	*1*	*1*	*Pore*	*7*	*Missense*	
*W563G**	*1*	*2*	*Pore*	*7*	*Missense*	
*C566F**	*1*	*1*	*Pore*	*7*	*Missense*	
G572S	*2*	*4*	*Pore*	*7*	*Missense*	
*M574V**	*1*	*3*	*Pore*	*7*	*Missense*	
R582L	*1*	*2*	*Pore*	*7*	*Missense*	
R582C	*1*	*1*	*Pore*	*7*	*Missense*	
*G584C**	*1*	*2*	*Pore*	*7*	*Missense*	
*G590V**	*1*	*3*	*Pore*	*7*	*Missense*	
*I593V**	*1*	*1*	*Pore*	*7*	*missense*	
*K595N**	*1*	*2*	*Pore*	*7*	*Missense*	
*K595E**	*1*	*1*	*Pore*	*7*	*Missense*	
G601S	*2*	*5*	*Pore*	*7*	*Missense*	*Trafficking defect (22, 24)*
G604S	*2*	*2*	*Pore*	*7*	*Missense*	
*S606P**	*1*	*1*	*Pore*	*7*	*Missense*	
T613M	*2*	*3*	*Pore*	*7*	*Missense*	*Dominant negative (25)*
A614V	*4*	*6*	*Pore*	*7*	*Missense*	*Dominant negative (26)*
T623I	*1*	*1*	*Pore*	*7*	*Missense*	*Trafficking defect (22)*
G628S	*1*	*2*	*Pore*	*7*	*Missense*	*Trafficking defect (22)*
N629K	*1*	*1*	*Pore*	*7*	*Missense*	*Dominant negative (27)*
N633S	*1*	*1*	*Pore*	*7*	*Missense*	
K638del	*1*	*1*	*S6*	*7*	*Deletion*	
*F640del**	*1*	*1*	*S6*	*7*	*Deletion*	
S641F	*1*	*3*	*S6*	*7*	*Missense*	
V644F	*1*	*4*	*S6*	*7*	*Missense*	
Subtotal	*34*	*56*				
Non-pore regions						
N-terminal regions						
*V41A**	*1*	*1*	*N-term*	*2*	*Missense*	
*Y43D**	*1*	*3*	*N-term*	*2*	*Missense*	
*E50fs+10X**	*1*	*1*	*N-term*	*2*	*Deletion/frameshift*	
*G53S**	*1*	*1*	*N-term*	*2*	*Missense*	
82-84insIAQ	*1*	*1*	*N-term*	*2*	*Insertion*	
*F106L**	*1*	*1*	*N-term*	*3*	*Missense*	
*D111V**	*1*	*1*	*N-term*	*3*	*Missense*	
*V115M**	*1*	*1*	*N-term*	*3*	*Missense*	
P151fs+179X	*1*	*1*	*N-term*	*3*	*Insertion/frameshift*	
*G187-A190del**	*1*	*3*	*N-term*	*4*	*Deletion*	
*R312-S318del**	*1*	*2*	*N-term*	*5*	*Deletion*	
S320L	*1*	*1*	*N-term*	*5*	*Missense*	
P334L	*1*	*1*	*N-term*	*5*	*Missense*	
*K364fs+3X**	*1*	*3*	*N-term*	*5*	*Insertion/deletion/frameshift*	
*K386fs+3X**	*1*	*4*	*N-term*	*5*	*Insertion/frameshift*	
Transmembrane domains other than pore regions						
Q391X	*1*	*2*	*S1*	*6*	*Nonsense*	
*F471fs+50X**	*1*	*1*	*S1-S2*	*6*	*Deletion/frameshift*	
*I489F**	*1*	*1*	*S1-S2*	*6*	*Missense*	
A490T	*1*	*1*	*S1-S2*	*6*	*Missense*	*Current density↓ (28)*
*H492Y**	*1*	*2*	*S1-S2*	*6*	*Missense*	
*W497X**	*1*	*3*	*S3*	*6*	*Nonsense*	
D501N	*1*	*1*	*S3*	*6*	*Missense*	
R534C	*1*	*2*	*S4*	*7*	*Missense*	*Trafficking defect (22)*
C-terminal region						
*Q738X**	*1*	*2*	*C-term*	*9*	*Nonsense*	
*G745-G749del, Fins/fs+56X**	*1*	*1*	*C-term*	*9*	*Insertion/deletion/frameshift*	
R752W	*1*	*2*	*C-term*	*9*	*Missense*	*Trafficking defect (22)*
S818L	*1*	*1*	*C-term*	*10*	*Missense*	*Reduced I_{Kr} current (29)*
*P846T**	*1*	*1*	*C-term*	*10*	*Missense*	
*W853fs+14X**	*1*	*2*	*C-term*	*10*	*Deletion/frameshift*	
R863X	*1*	*2*	*C-term*	*10*	*Nonsense*	
*L911fs+6X**	*1*	*3*	*C-term*	*12*	*Deletion/frameshift*	
*R912fs+63X**	*1*	*2*	*C-term*	*12*	*Insertion/frameshift*	
*S1029fs+23X**	*1*	*3*	*C-term*	*13*	*Deletion/frameshift*	
*P1034fs+23X**	*1*	*3*	*C-term*	*13*	*Insertion/frameshift*	
*A1144T**	*1*	*2*	*C-term*	*15*	*Missense*	
Subtotal	*35*	*62*				

**Novel mutation.*

del, deletion; ins, insertion; fs, first amino acid affected by a frameshift (number after fs is number of amino acids before termination); term, terminus.

Table 2 Clinical Characteristics of Pore and Non-Pore Mutations

	Pore (n=56)	Non-pore (n=62)	p value
Demographics			
Female gender (%)	33 (59%)	42 (68%)	0.344
Proband (%)	33 (59%)	34 (55%)	0.712
Age (years) at baseline ECG (range)	31±18 (7–74)	31±16 (2–71)	0.920
Age (years) at first event (range)	16±10 (5–48)	20±13 (5–71)	0.203
Diagnosis			
Schwartz score	5.3±1.6	4.5±1.8	0.017
Schwartz score ≥4 (%)	47 (84%)	41 (66%)	0.034
ECG measurements			
Heart rate (beats/min)	65±13	64±15	0.537
RR (ms)	953±188	975±186	0.510
QT_{end} (ms)	505±79	482±69	0.089
QT_{peak} (ms)	377±67	382±65	0.650
$T_{peak\text{-}end}$ (ms)	129±55	99±41	0.001
Corrected QT_{end} (ms)	522±63	490±49	0.002
Corrected QT_{peak} (ms)	389±62	388±47	0.927
Corrected $T_{peak\text{-}end}$ (ms)	134±52	101±42	<0.001
Torsade de pointes (%)	17 (30%)	18 (29%)	1.000
T-wave alternans (%)	7 (13%)	4 (7%)	0.346
Notched T wave (%)	43 (77%)	32 (52%)	0.007
Cardiac events			
All cardiac events (%)	38 (68%)	32 (52%)	0.092
Syncope (%)	36 (64%)	32 (52%)	0.194
Aborted cardiac arrest/SCD (%)	6 (11%)	2 (3%)	0.145
Therapy			
β-blocker therapy	26 (53%)	21 (36%)	0.117
Pacemaker (%)	1 (2%)	0	1.000
Sympathectomy (%)	0	0	1.000
Defibrillator (%)	1(2%)	2 (3%)	1.000

Data are mean value±SD or number (%) of subjects.
ECG, electrocardiography; SCD, sudden cardiac death.

Methods

Study Population

The study population consisted of 118 patients from 69 unrelated Japanese LQT2 families enrolled from 3 institutes in Japan: National Cardiovascular Center, Kyoto University Graduate School of Medicine and Shiga University of Medical Science. The *KCNH2* mutations were confirmed in all patients by using standard genetic tests[14–17] Screening for mutations in *KCNQ1*, *SCN5A*, *KCNE1*, *KCNE2*, and *KCNJ2* was also conducted, and patients with compound mutations of *KCNH2* and/or additional mutations in these LQTS-related genes were excluded from the analysis. Symptomatic patients were defined as *KCNH2* mutation carriers who experienced at least 1 episode of syncope (ie, complete loss of consciousness, or cardiac arrest requiring cardiac resuscitation), while asymptomatic patients were those without these events. Follow-up was censored at age 50 years to avoid the influence of coronary artery disease on cardiac events.

Genetic Analysis and Characterization

Genomic DNA was isolated from venous blood by use of the QIAamp DNA blood midikit (Qiagen, Hilden, Germany). The protocol for genetic analysis was approved by the institutional ethics committee and was performed under its guidelines. Established primer settings were used to amplify the entire coding regions of the known LQTS genes from genomic DNA[14–17] Denaturing high-performance liquid chromatography (DHPLC) was used for screening. For aberrant conformers, direct sequencing techniques were performed as described elsewhere[13] PCR products were denatured at 95°C for 5 min then analyzed by DHPLC. PCR fragments presenting abnormal signals in the DHPLC analysis were subsequently sequenced by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides in an ABI 3130 genetic analyzer (PE Applied Biosystems).

The pore region of the *KCNH2* channel was defined as the area extending from S5 to the mid-portion of S6 involving amino acid residues 550 through 650, according to a previous report[11] The non-pore region included the N-terminus region, transmembrane domains other than the pore region and the C-terminus region.

Clinical Characterization

Routine demographic data and basal 12-lead ECGs were obtained for all subjects at the time of enrollment in each institute and there was at least yearly follow-up contact. All ECGs were taken before or without β-blocker medication. The ECG parameters measured from the basal recordings were the RR, QT_{end}, QT_{peak} and $T_{peak-end}$ (QT_{end}–QT_{peak}) intervals. The latter is thought to reflect the transmural dispersion of ventricular repolarization (TDR)[18–20] The rate-dependent QT intervals were corrected for heart rate by Bazett's method[21] The QT_{peak} was defined as the time interval between QRS onset and the peak of the positive T wave or the nadir of the negative T wave. $T_{peak-end}$ was then obtained by calculating QT_{end} minus QT_{peak}.

These parameters were measured manually in lead V_5 averaged from 2 or 3 consecutive beats. Bifid T waves other than U waves were included in the QT measurements. If ECG recordings were obtained during a cardiac event, the patients were requested to undergo the examination again after improving. Measurements were performed by 3 investigators who were completely unaware of the patient's clinical and genetic status. There were no significant differences in the measured data between the investigators, and the

mean values were used for analysis. LQTS-related cardiac events were defined as syncope, aborted cardiac arrest, or unexpected sudden death.

Statistical Analyses

All data are expressed as the mean value±SD. The Student's t-test was used to compare continuous data between mutations located in the pore region and those in the non-pore region. Differences in frequencies were analyzed by the chi-square test. Time to the first cardiac event (syncope, cardiac arrest, or sudden cardiac death) before initiation of β-blocker therapy and before age 50 years was determined by Kaplan-Meier cumulative estimates. Two-sided probability values <0.05 were considered statistically significant. Statistical calculations were performed with SPSS software (version 11.01J, Chicago, IL, USA).

Results

Genetic Characteristics

Table 1 lists the *KCNH2* mutations we identified, classified by location, number of patients with these causative mutations, coding effects (missense, insertion, deletion and frameshift) and functional outcomes. We identified 62 different *KCNH2* mutations among the 69 LQTS families: 42 missense, 16 deletion/insertion, 11 frameshift and 4 nonsense mutations. There were 27 (44%) mutations causing amino acid changes in the pore region and 35 (56%) mutations within the non-pore regions (15 in the N-terminus, 8 in the non-pore transmembrane, and 12 in the C-terminus). In the pore mutations there were 25 (93%) missense mutations and the remaining 2 were protein deletions (K638del and F640del).

In contrast, the non-pore mutations included more significantly complex mutations such as deletion, insertion, frameshift or nonsense mutations that resulted in truncation of channel proteins (15/35, 43%). Thirty-five mutations (56%, 11 in the pore region and 24 in the non-pore regions) were novel and indicated by asterisk in Table 1. Functional effects by cellular electrophysiologic tests have been reported in only 12 of the 62 mutations (19%);[22–29] however, all those previous reports indicated that the *KCNH2* mutations had loss-of-function effects and made the I_{Kr} current reduce or disappear. Four pore mutations had dominant-negative effects, 4 pore mutations and 2 non-pore mutations had trafficking defects, and 2 non-pore mutations reduced the I_{Kr} current.

Clinical Characteristics

Table 2 is a comparison of the clinical characteristics of the 56 patients with pore mutations and the 62 patients with non-pore mutations. There were no significant differences between the 2 groups regarding gender, the percentage of probands and the age at baseline ECG recording. Diagnostic LQTS scores of Schwartz et al[30] were noticeably greater in the pore group. RR and QT_{peak} intervals were comparable; however, corrected QT_{end} and $T_{peak-end}$ intervals were much longer in the pore than in the non-pore group. Although the incidence of TdP and T-wave alternans did not differ between groups, notched T waves were more frequently seen in the pore group (p=0.007 vs non-pore group). The incidence of cardiac events and the introduction of β-blocker therapy were not statistically different between the 2 groups.

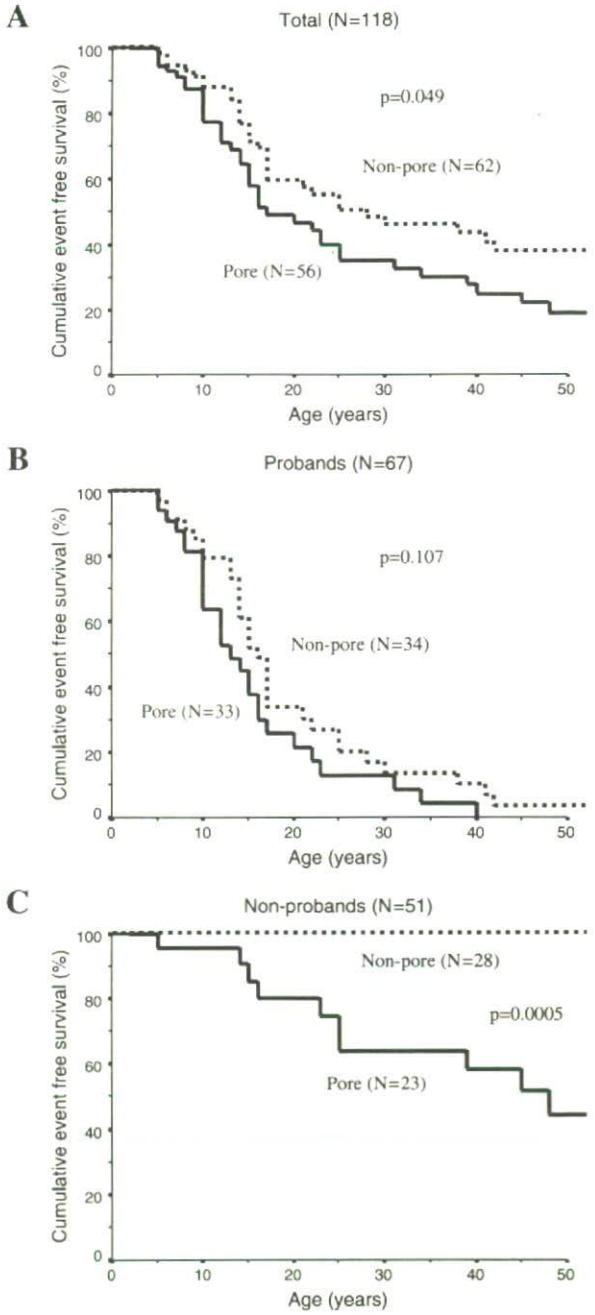

Fig 1. (A) Kaplan-Meier cumulative cardiac event-free survival curves from birth through to age 50 years for the total of 118 patients with *KCNH2* mutations located in the pore (n=56, smooth line) and non-pore (n=62, dotted line) regions. The pore group patients experienced their first cardiac event at a younger age than the non-pore group (log-rank, p=0.049). The difference was caused mainly by the high first-event rate in non-probands. Kaplan-Meier cumulative cardiac event-free survival curves for 67 probands (B) and 51 non-probands (C) with pore mutations (smooth line) and non-pore mutations (dotted line).

Clinical Course by Mutation Location

Fig 1A shows the Kaplan-Meier cumulative cardiac event-free survival curves from birth through to age 50 years for 118 patients (pore group, n=56; non-pore group, n=62). The pore-group patients experienced their first cardiac event at a younger age than the non-pore group (log-rank, p=

Table 3 Clinical Characteristics of Pore and Non-Pore Mutations in Non-Probands

	Pore (n=23)	*Non-pore (n=28)*	*p value*
Demographics			
Female gender (%)	*14 (61%)*	*19 (68%)*	*0.769*
Age (years) at baseline ECG (range)	*42±20 (9–74)*	*33±20 (2–71)*	*0.124*
Diagnosis			
Schwartz score	*4.7±1.5*	*3.5±1.7*	*0.008*
Schwartz score ≥4 (%)	*18 (78%)*	*12 (43%)*	*0.021*
ECG measurements			
Heart rate (beats/min)	*65±15*	*70±17*	*0.251*
RR (ms)	*959±179*	*894±179*	*0.201*
QT_{end} (ms)	*480±51*	*441±54*	*0.0011*
QT_{peak} (ms)	*352±47*	*352±53*	*0.974*
$T_{peak-end}$ (ms)	*128±46*	*89±30*	*0.001*
Corrected QT_{end} (ms)	*494±45*	*470±40*	*0.044*
Corrected QT_{peak} (ms)	*364±49*	*374±40*	*0.423*
Corrected $T_{peak-end}$ (ms)	*131±43*	*96±32*	*0.002*
Torsade de pointes (%)	*1 (4%)*	*0*	*0.451*
T-wave alternans (%)	*0*	*0*	*–*
Notched T wave (%)	*17 (74%)*	*14 (50%)*	*0.095*
Cardiac events			
All cardiac events (%)	*11 (48%)*	*0*	*<0.001*
Syncope (%)	*10 (43%)*	*0*	*<0.001*
Aborted cardiac arrest/SCD (%)	*1 (4%)*	*0*	*0.451*
Therapy			
β-blocker therapy	*6 (26%)*	*0*	*0.006*
Pacemaker (%)	*0*	*0*	*–*
Sympathectomy (%)	*0*	*0*	*–*
Defibrillator (%)	*0*	*0*	*–*

Data are mean value ± SD or number (%) of subjects.
Abbreviations see in Table 2.

0.049). We examined the clinical course of the 67 probands and 51 non-probands separately (Figs 1B,C). The clinical courses of the probands were not significantly different according to mutation site (Fig 1B), whereas in the non-pore group 28 non-probands remained asymptomatic and more than half had suffered from cardiac events by the age of 50 (Fig 1C). Therefore, the difference stemmed from markedly distinct prognoses among the non-probands.

Table 3 summarizes the clinical characteristics of the 51 non-probands. The absolute and corrected QT_{end} and $T_{peak-end}$ intervals were all significantly greater in the pore than in the non-pore group. In the non-probands, the incidence of all cardiac events, syncope, and β-blocker therapy were significantly greater in the pore group than in the non-pore group.

Discussion

This study demonstrates that the clinical features of 118 Japanese LQT2 patients who had 62 different *KCNH2* mutations correlated with the mutation sites, but only in non-probands. In probands, there was no significant relationship between mutation site and prognosis. Moss et al[1] reported approximately 179 LQT2 patients based on 44 different *KCNH2* mutations and those patients with pore mutations had significantly ($p<0.0001$) higher frequency of LQTS-related cardiac events and longer QTc intervals than those with non-pore mutations. In contrast to their results, in the present study the mutation-dependent difference in prognosis was relatively small, though significant ($p=0.049$), when analyzed in the total patient cohort (Fig 1A). Indeed, the beneficial outcome of the non-pore patients stemmed from their family members (Fig 1C), and probands showed virtually similar prognosis to that of pore mutation carriers. Although Moss et al did not report separate sub-analysis of probands and family members, the percentage of family members in their non-pore group was significantly larger than that of the pore group (84% vs 57%, $p<0.001$). The very good prognosis of the non-pore mutation group in their study may have reflected that large number of family members.

The character of the mutation per se may be important as another reason for the variance between these 2 studies, as both had a similar number of LQT2 patients. Compared with the study by Moss et al[1] the type of mutation in the present study was quite different: in our non-pore group, there were significantly more complex mutations, such as nonsense or frameshift, that caused the truncation of channel proteins (15/35, 43%) than in the report of Moss et al (4/30, 13%). For example, nonsense-mediated mRNA decay (NMD) has recently been reported to play an important role in reducing dominant negative suppression effects.[3] Premature termination codon caused by either a deletion or insertion mutation would also cause NMD and thereby attenuate the severity of cardiac phenotypes. This different nature of the mutations may cause the apparently different prognosis of the non-pore mutation groups in each study.

In our pore site mutation group, there were only 2 in-frame deletions, but no frameshift mutations (Table 1). Although it was practically very difficult to conduct every functional assay for each novel *KCNH2* mutation identified here, some cellular electrophysiological effects are available in a small number of *KCNH2* mutations we found (Table 1). Several missense mutations in the pore region (such as A561V and T613M) have been shown to produce dominant negative suppression effects, a greater functional change predisposing to arrhythmic events. In contrast, functional assay of several missense mutations in the non-pore regions has revealed relatively smaller loss-of-function effects (such as with A490T or S818L) Greater functional disruption may also be reflected in the different prognosis

of family members in the pore and non-pore groups (Fig 1).

Previously we reported that LQT1 patients with *KCNQ1* mutations located in the transmembrane regions, including the pore region, are at a higher risk of congenital LQTS-related cardiac events and longer QTc and $T_{peak-end}$ intervals than are patients with C-terminal mutations.[13] In LQT2, we have also demonstrated that $T_{peak-end}$, representing transmural dispersion of ventricular repolarization,[19] is longer in pore patients than in non-pore patients (Table 2), supporting the finding that family members with pore mutations are more likely to suffer from LQTS-related cardiac events than those with a non-pore mutation.

Study Limitations

Cardiac events are not simply linked to the site of mutation in probands; there are other triggering factors such as modifier genes, including single nucleotide polymorphisms,[32] hypokalemia and bradycardia, which play significant roles in aggravating the symptoms of *KCNH2* mutation carriers. The influence of these factors could be interpreted in the similar occurrence of cardiac events in the probands irrespective of mutation site, because the presence of symptoms usually caused the patient to agree to undergo genetic testing.

Regarding each mutation, the number of study patients was relatively small (at most 5), and the location of the mutations was scattered, even in the same pore region. The coding effect was also so various that we had limited ability to show arrhythmic risk according to a specific mutation site. Our cohort contained 35 novel *KCNH2* mutations, and their functional outcomes were not available. Moreover, our study population included only Japanese, so more subjects per mutation and a greater spectrum of *KCNH2* mutations in a worldwide study are needed to evaluate the arrhythmic risks associated with these mutations.

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The E1784K mutation in *SCN5A* is associated with mixed clinical phenotype of type 3 long QT syndrome

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Phenotypic overlap of type 3 long QT syndrome (LQT3) with Brugada syndrome (BrS) is observed in some carriers of mutations in the Na channel *SCN5A*. While this overlap is important for patient management, the clinical features, prevalence, and mechanisms underlying such overlap have not been fully elucidated. To investigate the basis for this overlap, we genotyped a cohort of 44 LQT3 families of multiple ethnicities from 7 referral centers and found a high prevalence of the E1784K mutation in *SCN5A*. Of 41 E1784K carriers, 93% had LQT3, 22% had BrS, and 39% had sinus node dysfunction. Heterologously expressed E1784K channels showed a 15.0-mV negative shift in the voltage dependence of Na channel inactivation and a 7.5-fold increase in flecainide affinity for resting-state channels, properties also seen with other LQT3 mutations associated with a mixed clinical phenotype. Furthermore, these properties were absent in Na channels harboring the T1304M mutation, which is associated with LQT3 without a mixed clinical phenotype. These results suggest that a negative shift of steady-state Na channel inactivation and enhanced tonic block by class IC drugs represent common biophysical mechanisms underlying the phenotypic overlap of LQT3 and BrS and further indicate that class IC drugs should be avoided in patients with Na channels displaying these behaviors.

Introduction

Congenital long QT syndrome (LQTS) is characterized by the prolongation of the QT interval on surface ECGs and an increased risk of potentially fatal ventricular arrhythmias, especially torsade de pointes (1). QT interval is determined by the cardiac action potential which is orchestrated by a fine balance between inward and outward currents expressed in myocardial cells. Mutations in *SCN5A*, the gene encoding the most prevalent cardiac Na channel α subunit, are responsible for a spectrum of hereditary arrhythmias including type 3 LQTS (LQT3) (2), Brugada syndrome (BrS) (3), cardiac conduction disturbances (4), and sick sinus syndrome (SSS) (5). More than 70 distinct *SCN5A* mutations responsible for LQT3 have been reported (see http://www.fsm.it/cardmoc), and the common in vitro consequence of most of these mutations is a persistent Na current during the action potential plateau due to destabilized fast Na channel inactivation (2). This failure of fast inactivation shifts the ionic balance during the plateau phase toward inward current and delays repolarization, thus increasing action potential duration and the corresponding QT interval. Na channel blockers such as mexiletine (class IB) or flecainide (class IC) shorten QT in LQT3 due to block of this persistent current (6–8) and have therefore been used in the management of affected patients.

Despite these common biophysical features, the clinical manifestations associated with LQT3 mutations are variable. Surprisingly, some LQT3 patients display ECG findings characteristic of BrS (ST elevation in the right precordial leads) that are related to another biophysical mechanism, a reduction of peak Na current (9). Reduced Na current is thought to exaggerate differences in action potential duration between the inner (endocardium) and outer (epicardium) layers of ventricular muscle, thereby favoring a substrate promoting reentrant arrhythmias. Phenotypic overlap between LQT3 and BrS was first reported in a large Dutch family with an insertion mutation 1795insD, in which the mutation carriers showed ECG features of both LQT3 and BrS (10, 11). Importantly, Na channel block in the overlap phenotype shortens QT but exacerbates the ST segment elevation BrS phenotype

Nonstandard abbreviations used: A_f, fraction of the fast inactivating component; A_s, fraction of the slow inactivating component; BrS, Brugada syndrome; LQT3, type 3 LQTS; LQTS, long QT syndrome; QTc, rate-corrected QT interval calculated using Bazett's formula; SSS, sick sinus syndrome; τ_f, time constant of the fast inactivating component; τ_s, time constant of the slow inactivating component; TTX, tetrodotoxin; UDB, use-dependent block; $V_{1/2}$, half-maximal voltage.

Conflict of interest: The authors have declared that no conflict of interest exists.

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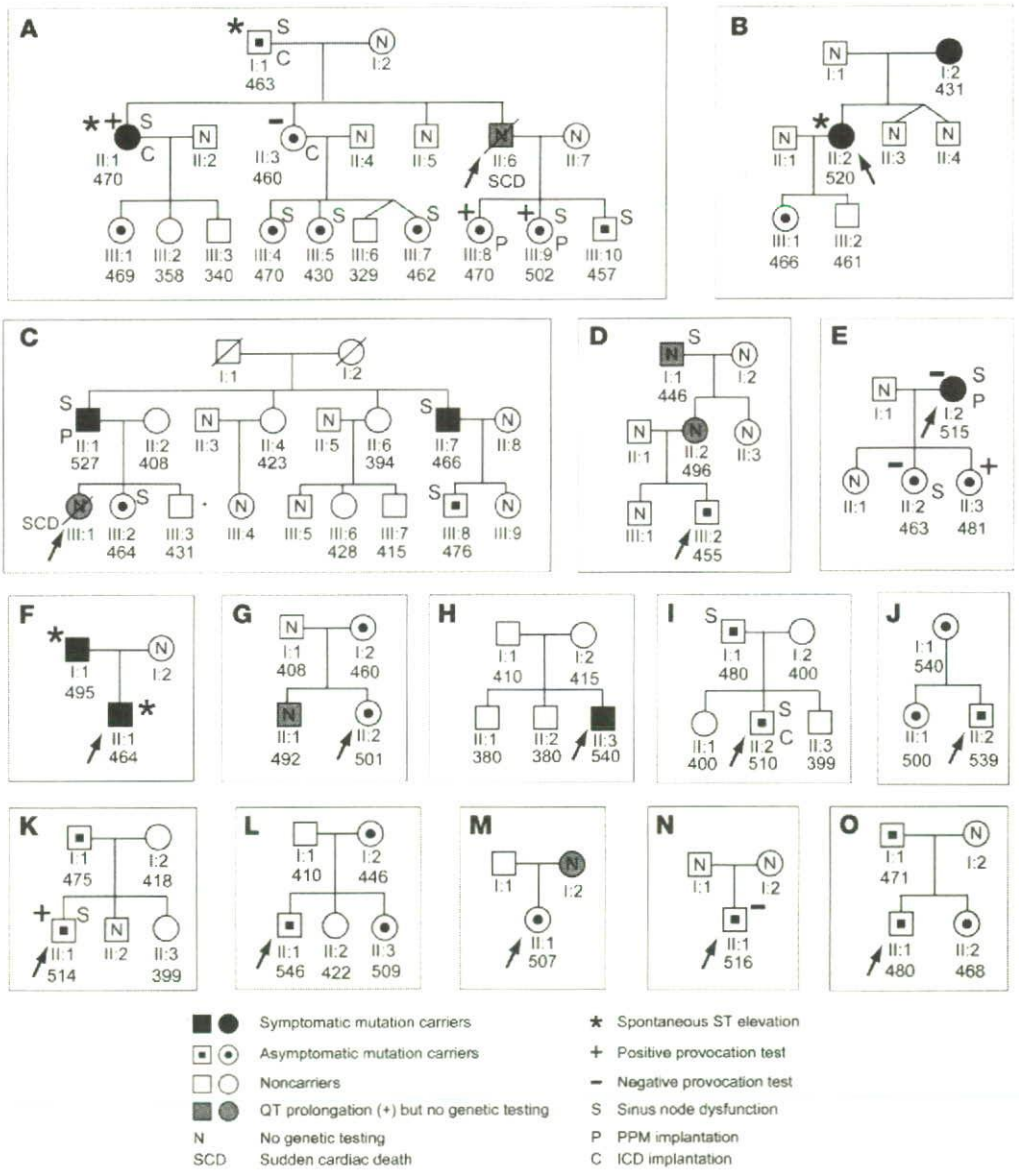

Figure 1

Pedigrees of E1784K families. Pedigrees of 15 LQT3 families (pedigrees A–O shown in panels **A–O**, respectively) carrying E1784K are shown. The pedigree C was previously reported elsewhere (17). Probands are indicated by an arrow. Nine symptomatic mutation carriers, shown by the filled symbols, had episodes of syncope (n = 8) and unexplained palpitations (n = 1; B;II:2). Asymptomatic mutation carriers (n = 32) are shown as symbols with a dot, and gray symbols are the individuals with QT prolongation who declined genetic testing, or are sudden cardiac death victims (SCD; A;II:6 and C:III:1) whose DNA was not available. Individuals exhibiting ST elevation in the right precordial leads are depicted with an asterisk. N represents individuals genetically undetermined. S, P, and C represent individuals who had sinus node dysfunction (n = 16), permanent pacemaker implantation (PPM; n = 4), and an implantable cardioverter defibrillator (ICD; n = 4), respectively. Values for QTc intervals are given beneath each symbol. The Na channel provocation test was positive in individuals with + (A;II:1, A;III:8, A;III:9, E;II:3, and K;II:1), and negative in the individuals with – (A;II:3, E;I:2, E;II:2, and N;II:1). In family G, V1098L was identified in II:2 (who also had E1784K) but not in I:2 (an E1784K carrier), showing that II:2 was a compound *SCN5A* mutation carrier. In family H, parents of the proband were both genetically unaffected, indicating that E1784K in the proband was most likely a de novo mutation. Three asymptomatic family members with a prolonged QTc (D;I:1, D;II:2, and G;II:1) declined genetic testing, and 1 individual, D;I:1, exhibited sinus node dysfunction. Squares and circles indicate men and women, respectively.

and thus enhances arrhythmia risk (11). Biophysical studies demonstrated that the mutant channels displayed enhancement of both closed-state inactivation and slow inactivation, and this was thought to sensitize carriers to the BrS phenotype during flecainide therapy (12).

The overlap between the LQT3 and BrS phenotypes has also been reported in other *SCN5A* mutations such as ΔKPQ (13, 14), E1784K (13), and ΔK1500 (15), raising a concern about the safety of class IC drug therapy in LQT3 patients (13) and questions about the underlying mechanisms. However, phenotypic variability in LQT3

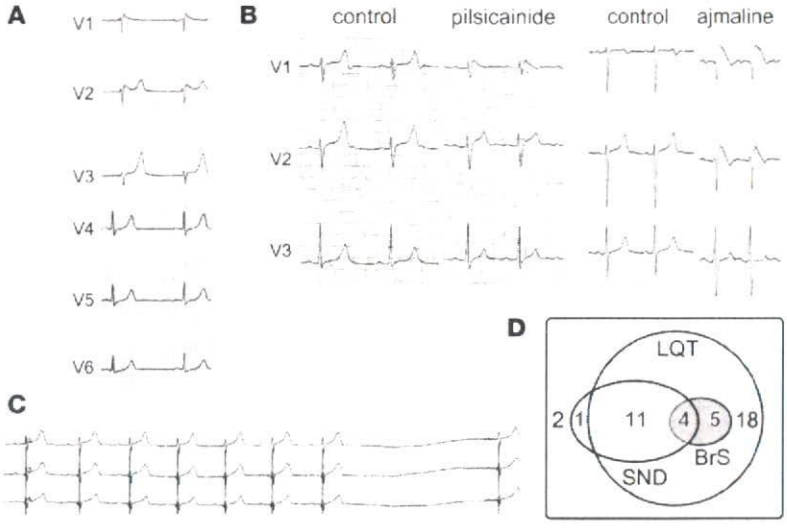

Figure 2
ECG characteristics of E1784K mutation carriers. (**A**) QT prolongation (QTc, 470 ms) and spontaneous saddleback type ST elevation observed in the right precordial leads in carrier A;II:1. (**B**) ECG recordings before and after the Na channel blocker provocation test. Pilsicainide (left, patient K;II:1) induced coved-type ST elevation in V1 and the QTc was concomitantly shortened (QTc: control, 495 ms; pilsicainide, 459 ms). Ajmaline (right, patient A;III:9) also induced coved-type ST elevation in V1 and V2 and QTc shortening (control, 501 ms; ajmaline, 490 ms). (**C**) Sinus node dysfunction (SND) demonstrated by a 3.9-s sinus arrest in carrier A;I:1. (**D**) A Venn diagram representing electrophysiological manifestation of 41 *SCN5A*-E1784K mutation carriers. Thirty-eight carriers exhibited an abnormally long QTc, 3 individuals had a normal QTc, and 1 exhibited sinus node dysfunction only. Sinus node dysfunction and BrS were observed in 16 and 9 individuals, respectively, with 4 displaying both phenotypes.

has thus far been reported sporadically or only within a single kindred. Therefore, it is not clear whether development of the BrS phenotype in a patient with LQT3 is solely determined by the biophysical properties of the mutant channel or by coinherited genetic variations, gender, ethnicity, or other environmental factors. One approach to dissect such phenotypic variability is to perform a clinical assessment of individuals with multiple pedigrees from genetically heterogeneous populations with the same mutation.

In the present study, we evaluated 15 kindreds of diverse ethnic backgrounds from Asia, Europe, and North America, all with the same LQT3 mutation, E1784K. We report here a high prevalence of overlap of the LQT3 phenotype with BrS and sinus node dysfunction. Furthermore, we have identified biophysical and pharmacological properties of the mutant channels that appear to be common to other mutants with this clinical overlap, thereby suggesting certain features of the mutant Na channel that result in an abnormal response to class IC drugs. These data extend the concept that molecular characterization of the consequences of individual DNA variants is desirable before the selection of a therapeutic approach in LQT3 patients.

Results

Clinical phenotypes in 15 LQT3 families with SCN5A-E1784K. Among the 66 family members who underwent genetic testing, 41 were identified as heterozygous mutation carriers (18 men, 23 women, 25 ± 19 years, means ± SD) and 25 as noncarriers (11 men, 14 women). Three individuals showed a prolonged rate-corrected QT interval (QTc; calculated using Bazett's formula, QTc = QT / [previous RR interval]$^{1/2}$) but declined genetic testing, and 2 victims of sudden cardiac death, whose DNA was not available, were presumably affected (shown with shaded symbols in Figure 1). The proband of the family G (II:2) carried the *SCN5A* mutation V1098L on the opposite allele, but otherwise no compound mutations (*SCN5A* or other LQTS genes) were observed. Among 41 carriers, 9 had episodes of syncope (n = 8) or unexplained palpitations (n = 1), but the remaining 32 individuals (78%) were asymptomatic. The QTc interval (mean ± SD) measured from the resting 12-lead ECG of all mutation carriers (living probands and family members) was 485 ± 30 ms, which was significantly longer (P < 0.001) than the 402 ± 31 ms measured in the noncarriers. There was no significant difference in the QTc interval between male (493 ± 31 ms, n = 18) and female carriers (479 ± 28 ms, n = 23). The QTc penetrance, defined as the percentage of mutation carriers with abnormally long QTc interval at presentation (>440 ms for men and >460 ms for women), was 93% (18 men, 20 women), indicating a highly penetrant LQT3 mutation. Administration of mexiletine shortened the QTc in 10/10 individuals tested, as previously demonstrated in other LQT3 mutation carriers (6). Mexiletine did not unmask or exacerbate Brugada-type ST elevation.

Spontaneous ST elevation in the right precordial leads was observed in 5/41 mutation carriers (Figure 1; coved-type, n = 1; saddleback type, n = 4; Figure 2A). Nine mutation carriers without diagnostic ST elevation at baseline underwent provocation with flecainide, ajmaline, or pilsicainide, and the test was positive (coved-type ST elevation; Figure 2B) in 5 (Figure 1). The 4 mutation carriers with a negative provocation test (A;II:3, E;I:2, E;II:2, and N;II:1) were not rechallenged. Thus, the diagnosis of BrS was established in 9/41 mutation carriers (1 individual, A;II:1, showed spontaneous saddleback ST elevation, which was converted to coved-type by ajmaline). None of the noncarriers displayed a Brugada-type ECG.

Sinus bradycardia and atrial standstill have also been associated with other *SCN5A* mutations (7, 11, 15–18), and sinus bradycardia was first reported in 1980 in LQTS patients who were subsequently found to be carriers of ΔKPQ (19). Sinus node dysfunction was common in our cohort, presenting in 16/41 mutation carriers (Figure 2C), and 4 of these 16 carriers with sinus node dysfunction also exhibited the BrS phenotype (Figure 2, B and D). Moreover, one carrier (A;III:5) showed sinus node dysfunction without manifesting QT prolongation or ST elevation. Four patients received a permanent pacemaker, and another 4 received an implantable cardioverter defibrillator.

Biophysical properties and membrane trafficking of E1784K. Figure 3A illustrates representative whole-cell current traces from cells expressing WT or E1784K Na channels in the presence of coexpressed human β_1 subunit. Peak current density of the E1784K channel was approximately 40% less than the that of the WT channel (WT, 138.2 ± 13.0 pA/pF, n = 25; E1784K, 82.3 ± 8.2 pA/pF, n = 13; P < 0.005) (Figure 3B). Cell capacitance of WT and E1784K was

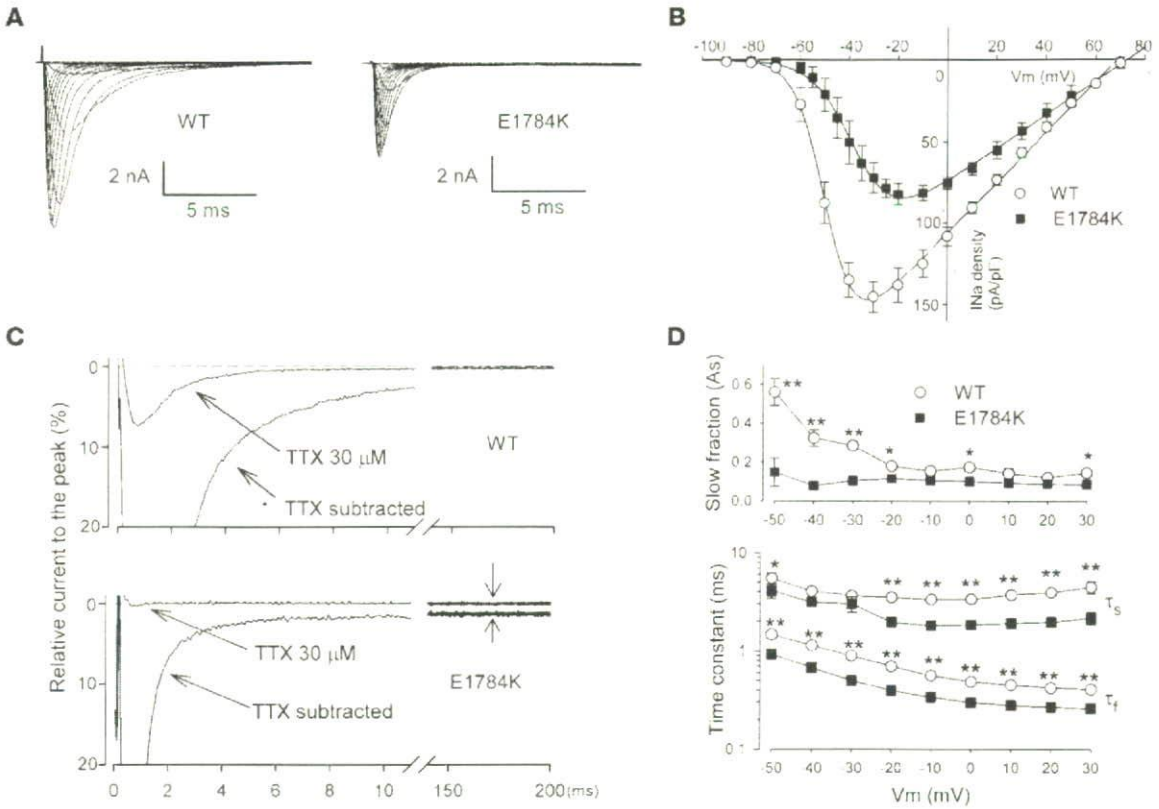

Figure 3

Properties of E1784K whole-cell current. (**A**) Representative whole-cell current traces obtained from tsA-201 cells transfected with either WT or E1784K Na channels. All studies were conducted in cells cotransfected with human Na channel β_1 subunits. Currents were recorded from a holding potential of –120 mV and stepped from –90 mV to 90 mV for 20 ms in 10-mV increments. (**B**) Current-voltage relationship. Current was normalized to cell capacitance to give a measure of Na current density. There were significant differences ($P < 0.05$) in current density between WT and E1784K at all tested voltages except –80, –70, –60, and ≥40 mV. (**C**) Na currents were recorded with a test pulse potential of –20 mV from a holding potential of –120 mV before and after 30 μmol/l TTX, and the TTX-sensitive current was calculated by digital subtraction. The currents were normalized to the peak current without TTX and superimposed. Zero current levels are shown by dotted lines. Note the faster decay and the prominent TTX-sensitive persistent current (shown with arrows) in E1784K. The amplitude of the persistent current was approximately 6-fold larger in E1784K (1.85% ± 0.27% of peak, $n = 10$; $P < 0.001$) than in WT (0.32% ± 0.05% of peak, $n = 15$). The density of persistent current for E1784K (1.53 ± 0.22 pA/pF) was significantly larger than for WT (0.44 ± 0.07 pA/pF). (**D**) Time constants for the voltage dependence of inactivation. A_s is shown in the upper panel, and τ_f and τ_s are presented in the lower panel. Significant differences between WT (open circles, $n = 13$) and E1784K (filled squares, $n = 13$) are indicated ($^*P < 0.05$, $^{**}P < 0.01$).

comparable. In addition, peak Na current occurred at more positive potentials (+14 mV) with the mutant channel than with the WT channel. However, the E1784K channel showed an increased level of tetrodotoxin-sensitive (TTX-sensitive) persistent current, a hallmark biophysical abnormality of LQT3 (Figure 3C). The density of the persistent current of E1784K after 200 ms of depolarization at –20 mV was 3.5-fold larger than that of WT (WT, 0.44 ± 0.07 pA/pF, $n = 15$; E1784K, 1.53 ± 0.22 pA/pF, $n = 10$; $P < 0.001$) and was abolished by 30 μmol/l TTX (Figure 3C). Furthermore, macroscopic current decay was faster for E1784K than for WT. Double exponential curve fitting (see Methods) revealed that the fraction of the slow inactivating component (A_s) was significantly smaller and the time constants of both the fast and slow inactivating components (τ_f, τ_s) were significantly smaller in E1784K compared with WT at most test-pulse voltages between –50 mV and 30 mV (Figure 3D). These results demonstrate that the E1784K channels display an enhanced onset of fast inactivation (faster current decay), as well as destabilized fast inactivation (increased persistent current), probably due to the fluctuation of the channel between normal and non-inactivating gating modes (2). These results are responsible for the QT prolongation phenotype.

The voltage dependence of steady-state inactivation for E1784K was abnormal because of a large (–15.0 mV) hyperpolarizing shift (half-maximal voltage [$V_{1/2}$]: WT, –86.8 ± 1.1 mV, $n = 25$; E1784K, –101.8 ± 1.3 mV, $n = 17$; $P < 0.001$) (Figure 4A). The steady-state inactivation curves (left curves of Figure 4A) predicted decreased channel availability, a hallmark of BrS/SSS. For example, at a prepulse potential of –90 mV, which is close to the resting membrane potential of ventricular myocytes, 58.6% ± 3.5% of WT channels were available, but this was significantly attenuated in E1784K channels (17.8% ± 3.1%). Another important potential contributor to the BrS/SSS phenotype was a significant shift in the voltage dependence of activation of E1784K by +12.5 mV ($V_{1/2}$: WT, –49.7 ± 1.1 mV, $n = 32$; E1784K,