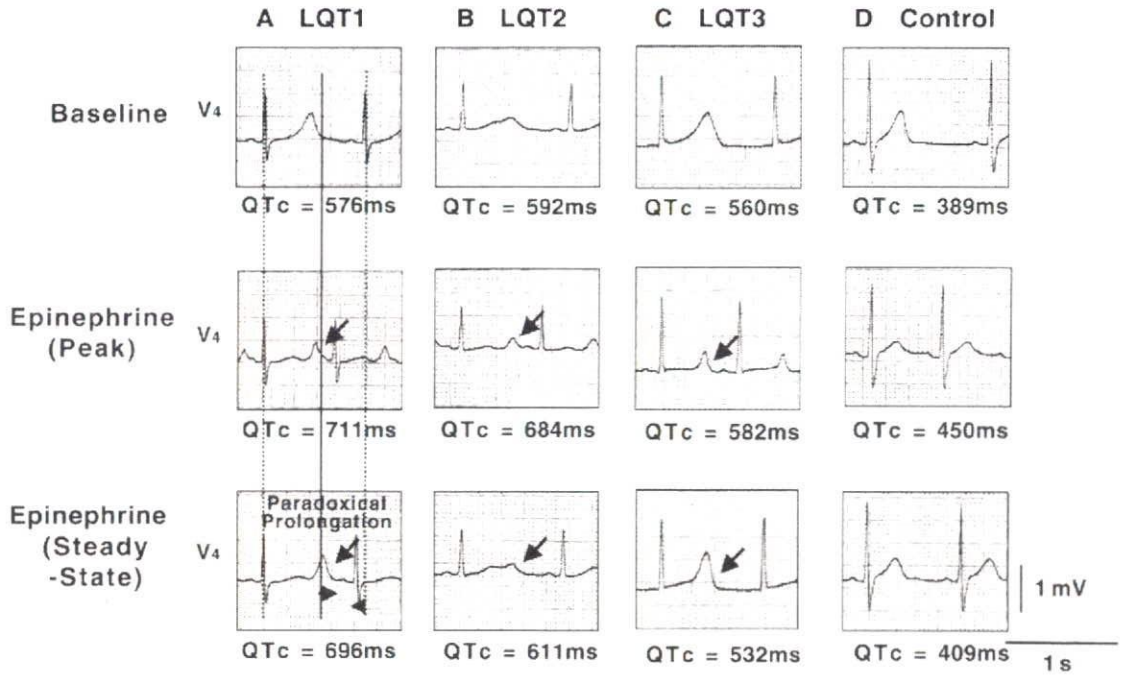

Fig 3. Genotype-specific responses of the corrected QT (QTc) interval to epinephrine provocative testing in patients with LQT1, LQT2 and LQT3 syndromes. Shown are V_4 lead electrocardiogram recordings under baseline conditions, at peak and steady-state epinephrine effects in LQT1 (A), LQT2 (B), LQT3 (C) and Control (D) patients. The QTc was prominently prolonged from 576 to 711 ms at peak epinephrine effect, and remained prolonged at the steady-state (696 ms) in the LQT1 patient. In the LQT2 patient, the QTc was also markedly prolonged from 592 to 684 ms at peak, but returned to the baseline level at the steady-state (611 ms). It was much less prolonged (LQT3: 560 to 582 ms, Control: 389 to 450 ms) at the peak in LQT3 and Control patients than in either the LQT1 or LQT2 patients, and was shortened to the baseline level at the steady-state (532, 409 ms). Modified from Shimizu et al. *Heart Rhythm* 2004; **1**: 276–283[17] with permission.

experience cardiac events before age 40 years in the LQT1 and LQT2 syndromes, whereas less than 30% of patients do so in LQT3 syndrome; however, the lethality of the cardiac events is significantly higher in LQT3 patients than in LQT1 or LQT2 patients. Generally, male patients experience their first cardiac events at a younger age than female patients[50] Approximately 90% of first cardiac events occur before the age of 15 years in male patients, particularly in LQT1 males, whereas female patients do not rarely experience their first cardiac events after the age of 20[50] These tendencies were recently confirmed using the largest cohort of LQT1 syndrome patients[51] The data suggested that LQT1 males before age 13 years and LQT1 females after age 13 years had a significant and independent clinical risk associated with first cardiac events[51]

Triggers for Cardiac Events Genotype-specific triggers for cardiac events have been reported in patients with LQT1, LQT2 and LQT3 syndromes[12,52,53] Cardiac events occur most frequently during exercise (62%), and swimming is a common trigger in LQT1 syndrome[52] 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)[52] In LQT2 syndrome, being startled by an auditory stimulus (telephone, alarm clock, ambulance siren etc) is a specific trigger[52,53] LQT2 women are reported to be most susceptible to cardiac events in the postpartum period[54] The differential sensitivity in cardiac events to sympathetic (β-adrenergic) stimulation has been suggested to be caused by the differential response of ventricular repolarization to sympathetic stimulation in both experimental studies employing arterially perfused wedge preparations[45,46] and in clinical studies using catecholamine provocative testing or exercise testing[16–20]

Catecholamine Provocative Testing

Infusion of isoproterenol, a β-adrenergic agonist, or epinephrine, an α- + β-adrenergic agonist, has been used as a provocative test in patients with LQTS since the 1980s[55] Before the discovery of distinct genetic subtypes of congenital LQTS, the responses to either epinephrine or isoproterenol were extremely heterogeneous, and deemed impossible to interpret. Now, however, the heterogeneous response is understood to stem from underlying genetic heterogeneity, and genotype-specific responses to epinephrine can be exploited to expose different genotypes of LQTS in its otherwise concealed state, particularly LQT1 syndrome. Although isoproterenol is still used occasionally, recent major insights have been gleaned from using epinephrine. The 2 major protocols developed for epinephrine provocative testing include the escalating-dose protocol by Ackerman's group (Mayo protocol)[18,19] and bolus injection followed by brief continuous infusion by my group (Shimizu protocol)[16,17,19]

The bolus (Shimizu) protocol was developed on the basis of a differential response of the APD and QT interval to sympathetic stimulation with isoproterenol between experimental models of LQT1, LQT2 and LQT3 using arterially-perfused canine left ventricular wedge preparations[46] Clinical data from the use of the bolus protocol suggested that sympathetic stimulation produces genotype-specific

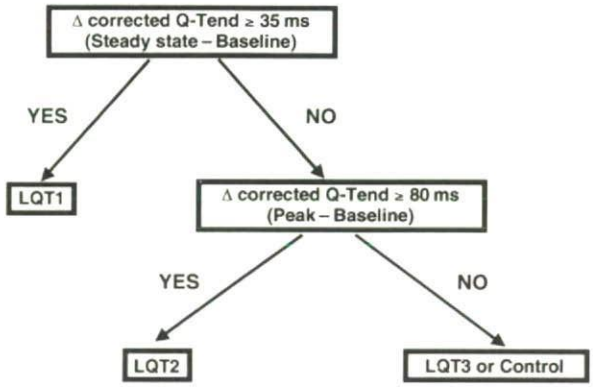

Fig 4. Genotype prediction by epinephrine provocative testing in patients with LQT1, LQT2 and LQT3 syndromes. Flow chart predicting genotype with epinephrine provocative testing. Modified from Shimizu et al. *Heart Rhythm* 2004; **1**: 276–283[17] with permission.

responses of the QTc interval in patients with LQT1, LQT2 and LQT3 syndromes (Fig 3)[16,17,19] Epinephrine remarkably prolongs the QTc interval at peak effect when the heart rate is maximally increased (1–2 min after the bolus injection), and the QTc remains prolonged during the steady-state epinephrine effect (3–5 min) in patients with LQT1[16,17,19] In LQT1 patients, a paradoxical QT prolongation, defined as an absolute increase in the QT (not QTc) interval, despite a shortening of the RR interval, is often observed during epinephrine infusion. Ackerman et al reported that the paradoxical QT prolongation had a sensitivity of 92.5%, specificity of 86%, positive predictive value of 76%, and negative predictive value of 96% for LQT1 patients vs non-LQT1 patients[18] In the bolus protocol, QTc is also prolonged at peak epinephrine effect (during bolus) in patients with LQT2, but returns to close to the baseline level at steady-state epinephrine effect[17,19] In contrast, the QTc is less prolonged at peak epinephrine effect in LQT3 patients than in LQT1 or LQT2 patients, and is abbreviated below the baseline level at steady-state epinephrine effect[17,19] Using the steady-state epinephrine effect, an improvement of clinical ECG diagnosis (sensitivity) from 68% to 87% in 31 patients with LQT1 syndrome and from 83% to 91% in 23 patients with LQT2 syndrome, but not in 6 patients with LQT3 syndrome (from 83% to 83%), was reported[17] The bolus protocol of epinephrine effectively predicts the underlying genotype of LQT1, LQT2 and LQT3 (Fig 4)[17,19] The prolongation of QTc ≥35ms at steady-state epinephrine effect can differentiate LQT1 from LQT2, LQT3 or control patients with a predictive accuracy ≥90%. The prolongation of QTc ≥80 ms at peak epinephrine effect can differentiate LQT2 from LQT3 or control patients with predictive accuracy of 100%. Although induction of TdP or ventricular fibrillation (VF) is extremely uncommon, intravenous β-blockers and a cardioverter defibrillator need to be available during testing.

Because molecular diagnosis is still unavailable in many institutes and is time-consuming, a clinical diagnosis of patients with concealed LQTS by epinephrine provocative testing can direct appropriate counseling and facilitate the initiation of preventive measures such as avoidance of QT-prolonging drugs. Moreover, a presumptive, pre-genetic diagnosis of either LQT1, LQT2, or LQT3 based on the response to epinephrine can guide genotype-specific treatment strategies.

Table 3 Genotype-Specific Therapy Based on Clinical and Experimental Data in LQTS

	LQT1	*LQT2*	*LQT3*
Prevalence	*40%*	*30–40%*	*10%*
Exercise restriction	+++++	+++	–
β-blockers	+++++	+++	–
Potassium supply	++?	++++	++?
Class IB sodium channel blockers	+++	+++	+++++
Calcium channel blockers	+++	+++	++?
Potassium channel openers	++	++	–
Pacemaker	++	++++	+++++
ICD	++++	++++	+++++

ICD, implantable cardioverter-defibrillator; +++++, most effective. Other abbreviation see in Table 1.

Genotype-Specific Therapy

Based on the natural history and specific sensitivity to sympathetic stimulation or catecholamine in the LQT1 syndrome, stricter exercise restriction, in particular swimming or diving, is required, especially for LQT1 males[12] Exercise restriction is also required in LQT2 syndrome[12]

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 molecular genetic studies have become available, genotype-specific pharmacological and non-pharmacological therapies have been introduced clinically, based on data derived from both clinical and experimental studies (Table 3).

In LQT1 patients, β-blockers frequently suppress episodes of syncope and sudden cardiac death[52] Data from a recent international cohort of 600 LQT1 patients has suggested that time-dependent β-blocker use is associated with a significant 74% reduction in the risk of first cardiac events[51] Mexiletine, a class IB sodium channel blocker, which blocks late I_{Na}, or verapamil, an $I_{Ca\text{-}L}$ blocker, may warrant consideration as adjunctive therapy to β-blockers in LQT1 patients, based on ECG changes with these agents or experimental data[44,45] 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.

Beta-blockers are also the first choice as pharmacological therapy in LQT2 patients, but the recurrence rate is higher than in LQT1 patients[52] Increase in the extracellular potassium concentration by exogenously administered potassium or long-term oral potassium administration has been reported to shorten the QT interval in LQT2 patients[56] The indication for an ICD is similar to that in LQT1 syndrome. My group recently reported that patients with LQT2 syndrome show a specific short–long–short initiating pattern of TdP more frequently than those with LQT1 syndrome[57] so pacemaker therapy is expected to be more effective in LQT2 than in LQT1 patients by suppressing that specific pattern[57]

In LQT3 patients, β-blockers are less effective than in LQT1 or LQT2 patients[52] Mexiletine is more effective for abbreviating the QT interval in LQT3 than in LQT1 or LQT2 syndrome, and is therefore a promising therapeutic choice in LQT3 syndrome. Pacemaker therapy may be most beneficial in LQT3 patients with bradycardia, based on the experimental data.

Genotype-specific therapy is unknown for the other forms: LQT4, LQT5, LQT6, LQT7, LQT8, LQT9, LQT10, LQT11, and LQT12. Beta-blockade is the first-line therapy in patients with LQT4, LQT5, LQT6, LQT7, LQT8 and

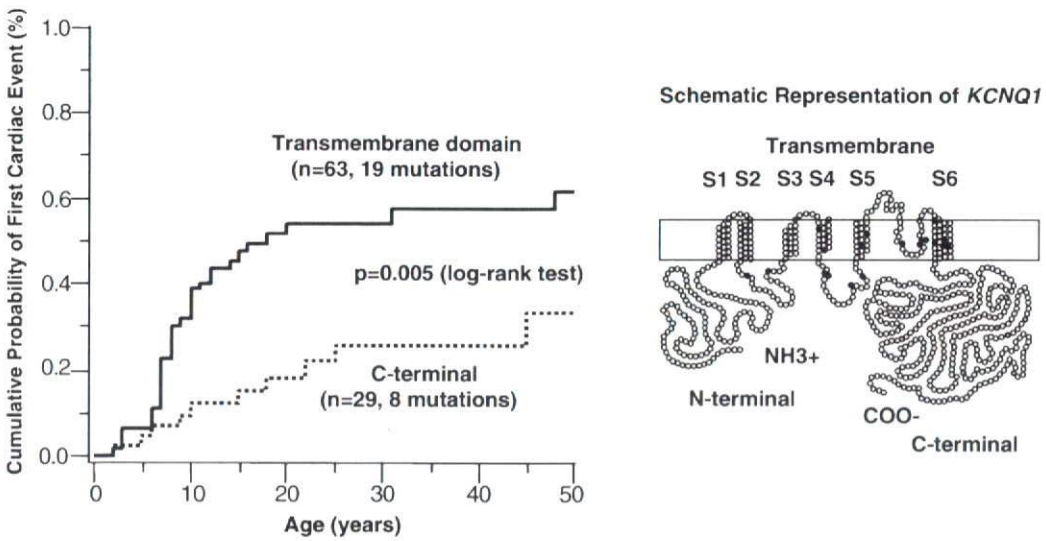

Fig 5. Kaplan-Meier cumulative cardiac event curves from birth to age 50 for patients with *KCNQ1* mutations located in transmembrane regions (n=66, 19 mutations; closed circles) and the C-terminal regions (n=29, 8 mutations; gray circles) in LQT1 syndrome. The difference in the clinical course by mutation 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 Shimizu et al. *J Am Coll Cardiol* 2004; **44:** 117–125[59] with permission.

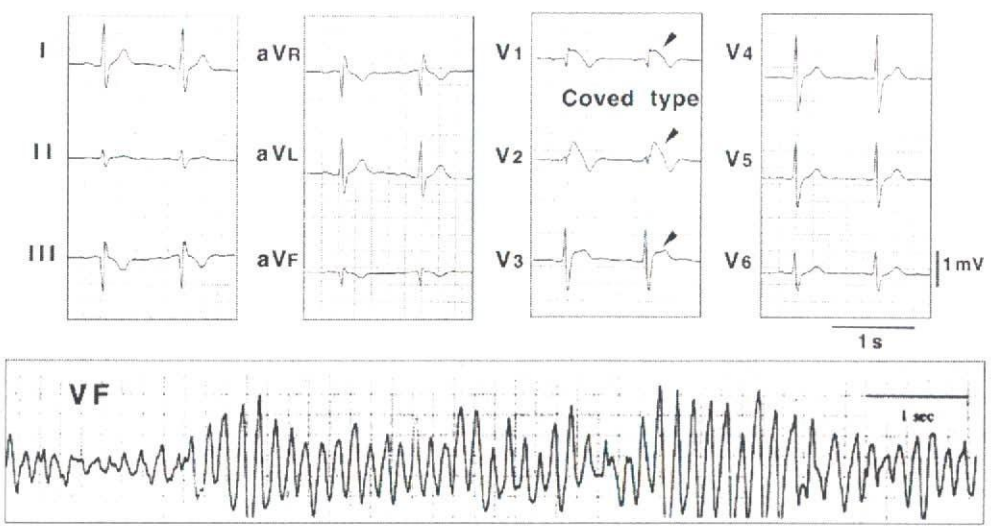

Fig 6. Twelve-lead electrocardiogram and ventricular fibrillation (VF) in a patient with Brugada syndrome. Spontaneous type 1 coved-type ST-segment elevation is recorded in leads V_1 and V_2 (arrows).

LQT12, and unknown LQTS genotypes. The class IB sodium channel blocker, mexiletine, may be theoretically effective in LQT9, LQT10, and LQT12 patients.

Possibility of Mutation Site-Specific Therapy

The structure of each cardiac ion channel, or correspondence between the mutation site and channel function, has been increasingly elucidated, suggesting mutation site-specific differences in the severity of the clinical phenotype or responses to therapy in each genotype. From data in the International LQTS Registry, Moss et al suggested that LQT2 patients with mutations in the pore region of *KCNH2* had a greater risk of arrhythmia-related cardiac events than patients with non-pore mutations;[58] thus indicating the possibility of mutation site-specific management or treatment of LQT2 syndrome. With regard to LQT1 syndrome, in 2004 the arrhythmic risk and sensitivity to sympathetic stimulation with treadmill exercise testing was compared between Japanese LQT1 patients with transmembrane mutations and those with C-terminal mutations in *KCNQ1*, and the LQT1 patients with transmembrane mutations showed a longer QTc interval and more frequent LQTS-related cardiac events than those with C-terminal mutations (Fig 5).[59] Moreover, the QTc interval was more prominently increased with exercise in patients with transmembrane mutations.[59] The international cohort of 600 LQT1 patients recently confirmed the Japanese data,[51] suggesting that transmembrane mutations and mutations with dominant-negative functional effect adversely influence the outcome of LQT1 patients, independent of traditional clinical risk factors and β-blocker

Table 4 Defect of Ion Channel or Membrane Adaptor Responsible for BrS

Loci	*Chromosome*	*Gene*	*Ion channel*
BrS1	*3 (3p21-24)*	*SCN5A*	I_{Na}
BrS2	*12 (12p13.3)*	*CACNA1C*	$I_{Ca\text{-}L}$
BrS3	*10 (10p12.33)*	*CACNB2*	$I_{Ca\text{-}L}$
BrS4	*3 (3p21)*	*GPD1-L*	I_{Na}
BrS5	*19 (19q13.1)*	*SCN1B*	I_{Na}
BrS6	*11 (11q13-q14)*	*KCNE3*	I_{to}

Abbreviation see in Table 1.

therapy.

Brugada Syndrome

In 1992, Brugada and Brugada first reported 8 patients with a history of aborted sudden cardiac death caused by VF and a characteristic ECG pattern, consisting of right bundle branch block (RBBB) and ST-segment elevation in the right precordial ECG leads (V_{1-3}) as a distinct clinical entity.[60–65] The Brugada Consensus Report in 2002 suggested 3 patterns of ST-segment elevation:[63] the type 1 ST-segment elevation is characterized by a coved-type ST-segment elevation displaying J-wave amplitude or ST-segment elevation ≥0.2 mV with or without a terminal negative T wave (Fig 6): type 2 and type 3 ST-segment elevations show a saddleback configuration, which has a high take-off ST-segment elevation (≥0.2 mV), followed by a gradually descending ST-segment elevation (type 2 ≥0.1 mV, type 3 <0.1 mV above the baseline) and a positive or biphasic T wave. ST-segment elevation is often accentuated and the coved type ST-segment elevation is more frequently recognized just before and after episodes of VF.[66,67] The second Consensus Report published in 2005 emphasized that type 1 ST-segment elevation is required to diagnose BrS;[64] because the type 1 ECG is reported to relate to a higher incidence of VF and sudden cardiac death. Type 1 ST-segment elevation recorded only in the higher (3rd and 2nd intercostal spaces) V_{1-2} leads is reported to show a similar prognostic value for subsequent cardiac events as that recorded in the standard V_{1-2} leads.[64,68–70] The prevalence of BrS is estimated to be up to 5 per 10,000 inhabitants, and is an important cause of sudden cardiac death of middle-aged males, particularly in Asian countries.[64,65] BrS usually manifests during adulthood,[64] and more than 80–90% of patients clinically affected are men.

Genotype in BrS (Table 4)

In 1998, Chen et al identified the first mutation linked to BrS in *SCN5A*, the I_{Na} gene that is responsible for the LQT3 form of congenital LQTS.[3] *SCN5A* mutations are reported to account for 18–30% of clinically diagnosed BrS patients at present.[64] Functional analysis using expression systems has shown that all *SCN5A* mutations so far identified result in decreased (loss of function) I_{Na} by several mechanisms,[65] including (1) lack of expression of the sodium channel; (2) a shift in the voltage dependence and time dependence of I_{Na} activation, inactivation or reactivation; (3) entry of the sodium channel into an intermediate state of inactivation from which it recovers more slowly; (4) accelerated inactivation of the sodium channel; or (5) a trafficking defect. The 2nd and 3rd mutations linked to BrS were reported by Antzelevitch et al in 2007, when they identified mutations in *CACNA1C* or *CACNB2*, the gene encoding the $\alpha 1$ or $\beta 2b$ subunit of the L-type calcium channel, in 3 probands with Brugada-like ST-segment elevation associated with a short QT interval.[71] Heterologous expression studies for the mutations revealed loss of function of $I_{Ca\text{-}L}$. Thereafter, London et al 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.[72] The *GPD1-L* mutation decreases *SCN5A* surface membrane expression and reduces I_{Na}, thus causing BrS.[72] Watanabe et al recently identified a nonsense mutation (W179X) in *SCN1B*, which encodes the function-modifying sodium channel $\beta 1$ subunit, in a family with BrS associated with cardiac conduction disease.[73] They reported that the I_{Na} current was decreased when α subunit ($Na_V1.5$) of the sodium channel was coexpressed with the mutant $\beta 1$ subunit compared with when it was coexpressed with the wild-type $\beta 1$ subunit. More recently, Delpon, Antzelevitch et al reported a missense mutation (R99H) in *KCNE3*, which encodes the potassium channel β subunit and interacts with Kv4.3 (transient outward current: I_{to}) channel, in a proband with BrS.[74] Coexpression of the mutant *KCNE3* with *KCND3*, which encodes Kv4.3, increases the I_{to} intensity (gain of function) compared with that by the coexpression of wild-type *KCNE3* with *KCND3*.[74] Thus, decreases in the inward sodium or calcium current (late I_{Na}, $I_{Ca\text{-}L}$) or increases in the outward potassium currents (I_{to}) produce a Brugada phenotype in all 6 genotypes, as indicated by previous experimental studies; however, approximately two-thirds of Brugada patients have not yet been genotyped, suggesting the presence of genetic heterogeneity.[65]

Genotype–Phenotype Correlations in BrS

The genotype–phenotype correlation in BrS has been less investigated than that in congenital LQTS, because more than two-thirds of patients clinically affected with BrS are not genotyped. Mild conduction abnormalities, such as widening of the P wave, prolongation of the QRS duration, PQ interval, and HV interval, and a higher incidence of RBBB have been described in patients with BrS, especially those with the *SCN5A* mutation.[64] Significantly longer PQ and HV intervals at baseline and a larger increase in the PQ and QRS intervals after sodium channel blockers have been reported by Smits et al in Brugada patients with *SCN5A* mutations than in those without *SCN5A* mutations.[75] Several ECG parameters were measured during long-term follow up and prospectively compared between Brugada patients with and without the *SCN5A* mutation by Yokokawa and co-workers, with the results suggesting that P wave, QRS, 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 (Figs 7,8).[76] In addition, the PQ interval and QRS duration in lead V_2 were prolonged more markedly with aging in the

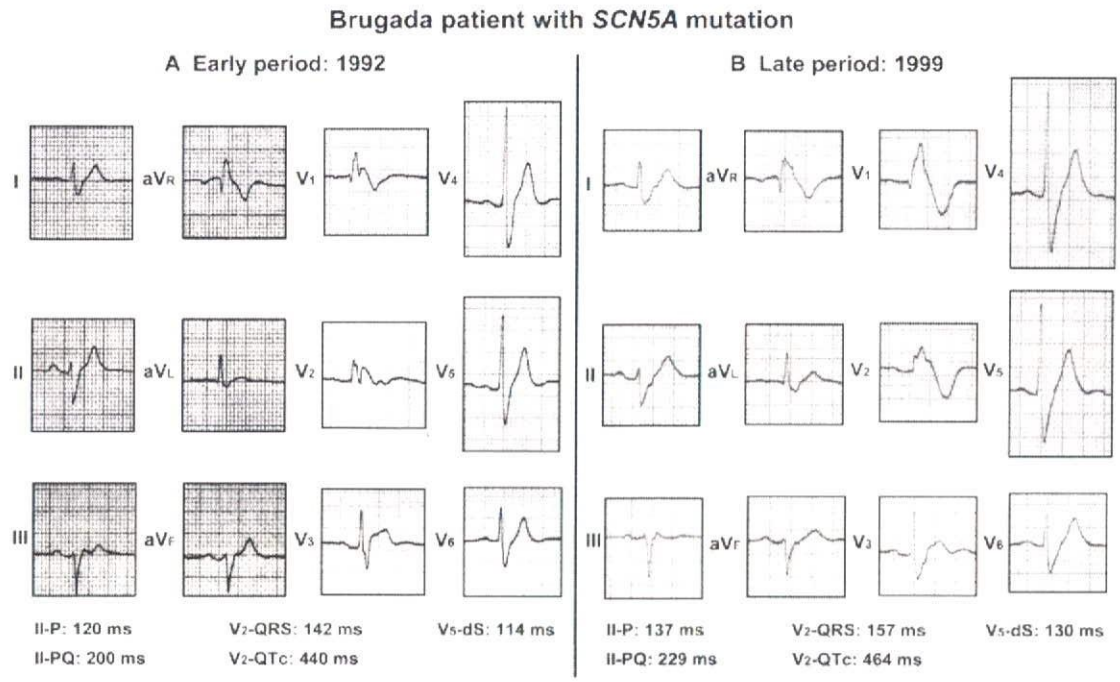

Fig 7. Twelve-lead electrocardiogram in the early and late periods during follow-up (7 years) in a Brugada patient with the *SCN5A* mutation. The P wave (lead II), QRS (lead V_2), and S wave (lead V_5) durations and PQ interval (lead II) are prolonged, even in the early period (47 years old) (A). The S wave amplitude (lead V_5) is also deep, and the QRS axis is deviated to the left. The corrected QT (QTc) interval (lead V_2) is borderline prolonged. In the late period (B), all these parameters are further increased. Modified from Yokokawa et al. *Am J Cardiol* 2007; **100:** 649–655[76] with permission.

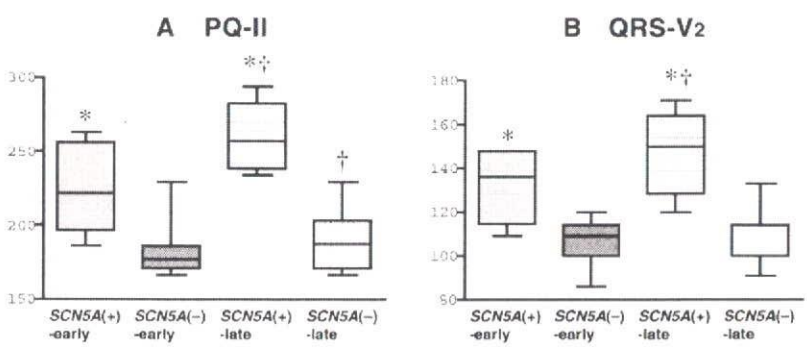

Fig 8. Electrocardiographic parameters during long-term follow-up in 8 Brugada patients with the *SCN5A* mutation and in 36 Brugada patients without the mutation. Both the PQ interval in lead II (A) and the QRS duration in lead V_2 (B) are significantly longer in the *SCN5A*-positive (+) group than in the *SCN5A*-negative (−) group in both the early and late periods. Both the PQ interval and QRS duration increased with aging during follow-up in both groups, but more prominently in the *SCN5A*(+) group than in the *SCN5A*(−) group. *$p<0.05$ vs *SCN5A*(−), †$p<0.05$ vs early.

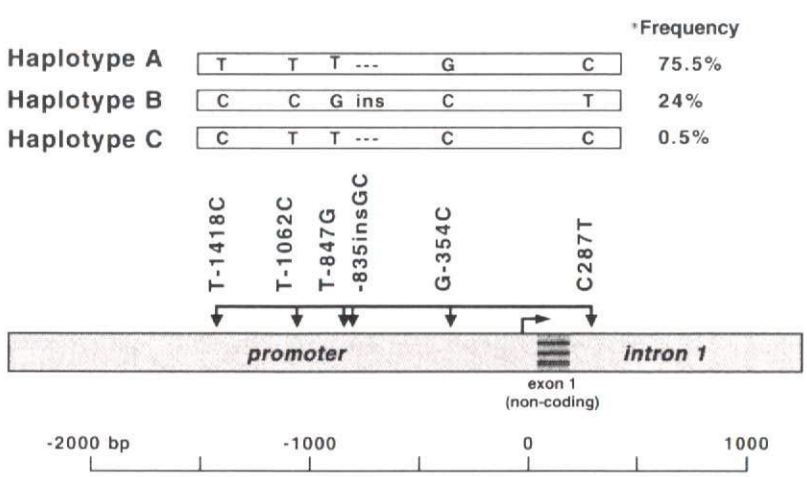

Fig 9. Haplotypes identified within the proximal promoter region of the *SCN5A*, a cardiac sodium channel gene. The 6 polymorphisms are in near-complete linkage disequilibrium. Haplotype A is designated as containing all common alleles, and Haplotype B as containing all minor alleles. The discordant haplotype is designated Haplotype C. *Frequency in the Japanese (control) population. Modified from Bezzina et al. *Circulation* 2006; **113:** 338–344[79] with permission.

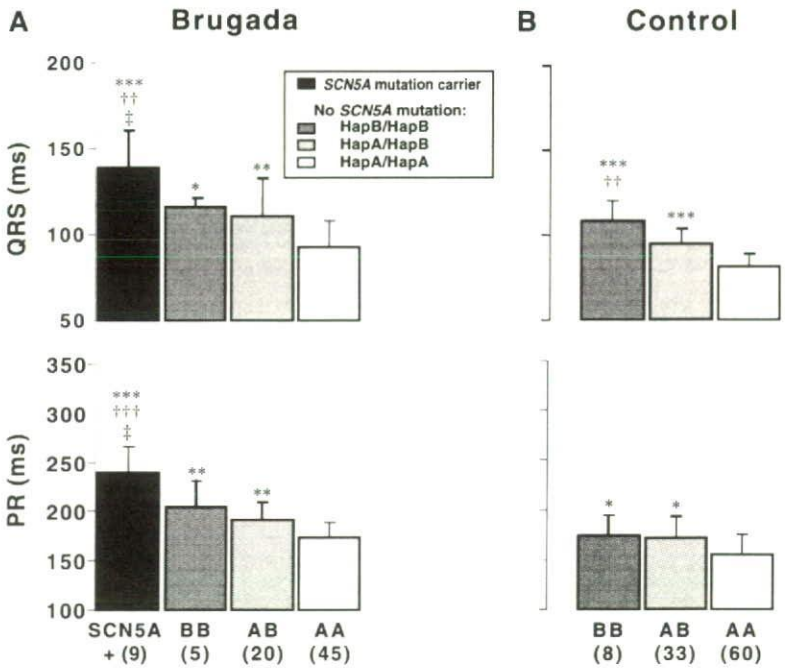

Fig 10. *SCN5A* promoter haplotype (Hap) pair effects on QRS duration in lead V_6 and PR duration in lead II in patients with Brugada syndrome and control subjects. In Brugada patients without the *SCN5A* mutation and in control subjects, both QRS and PR durations show a gene-dose effect, being longest in HapB homozygotes (BB), intermediate in HapA/HapB heterozygotes (AB) and shortest in HapA homozygotes (AA). Brugada patients with the *SCN5A* mutation show longer QRS and PR durations than those without the *SCN5A* mutations. Patient numbers are indicated in parentheses. Data are mean±SD. Modified from Bezzina et al. *Circulation* 2006; **113:** 338–344[79] with permission. *p<0.05; **p<0.001; ***p< 0.0001 vs HapA/HapA. †p<0.05; ††p<0.001; †††p< 0.0001 vs HapA/HapB. ‡p<0.05 vs HapB/HapB.

SCN5A-positive group than in the *SCN5A*-negative group during the follow-up period (Figs 7,8)[76] Frustaci et al reported significant myocyte apoptosis in both the right and left ventricular myocardium in a histological study in Brugada patients with *SCN5A* mutations, and suggested that abnormal function of the sodium channels may lead to a masked degree of cellular damage, contributing to arrhythmic events.[77] These electrocardiographic and histologic data indicate that progressive depolarization abnormalities (conduction slowing) with aging may contribute to the pathogenesis of BrS.

SCN5A Promoter Polymorphism

The incidence of BrS is significantly higher in Asian countries, including Japan, than in the USA and European countries.[64] It has been reported that common polymorphisms may modulate the activity of the primary disease-causing mutation in inherited cardiac arrhythmias, and/or influence the susceptibility to arrhythmia even in the general population.[78] The common polymorphisms are expected to relate to ethnic differences in the clinical phenotype in inherited cardiac arrhythmias, including BrS, because some common polymorphisms are ethnically dependent. A Haplotype B consisting of 6 individual DNA polymorphisms in near-complete linkage disequilibrium within the proximal promoter region of the *SCN5A* gene has been identified in only Asians (allele frequency of 22%), but not in Caucasians or African–Americans (Fig 9).[79] Luciferase reporter activity of the Haplotype B is reduced by 62% in cardiomyocytes compared with the wild type, Haplotype A.[79] The relationship between the *SCN5A* promoter haplotype and indices of conduction velocity, PR and QRS durations was further analyzed in a cohort of 71 Japanese BrS subjects without the *SCN5A* mutation and in 102 Japanese controls to examine the role of Haplotype B in cardiac conduction. PR and QRS durations were significantly longer in the Haplotype B individuals, with a gene-dose effect in both groups (Fig 10).[79] Moreover, the increases in both the PR and the QRS duration with sodium channel blockers were genotype-dependent and a gene-dose effect was also observed.[79] These data demonstrate that Haplotype B within the *SCN5A* promoter region alone does not give rise to BrS; however, it is possible that the *SCN5A* promoter Haplotype B contributes to the higher incidence of BrS in Asian populations, in combination with other as-yet-unknown factors.

Conclusions

Genetic studies and the genotype–phenotype correlation in lethal inherited cardiac arrhythmias have encouraged cardiologists to perform genotype-specific, so-called tailor-made, management and therapy, and possibly mutation site-specific therapy in patients with genotyped congenital LQTS. Genetic studies are now an important diagnostic tool for stratifying risk and effectively managing and treating genotyped patients. Reflecting the clinical impact of genetic studies in the real world of management and therapy for patients with congenital LQTS, genetic studies to screen for the LQTS gene have been reimbursed by National Insurance in Japan since April 1, 2008. On the other hand, genetic studies of other inherited arrhythmias, including BrS, are still experimental, and further investigations of the genotype–phenotype correlations are required.

Acknowledgments

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References

1. Keating M, Atkinson D, Dunn C, Timothy K, Vincent GM, Leppert M. Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey ras-1 gene. *Science* 1991; **252:** 704–706.
2. Donger CD, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M, et al. KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. *Circulation* 1997; **96:** 2778–2781.
3. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, et

al. Genetic basis and molecular mechanisms for idiopathic ventricular fibrillation. *Nature* 1998; **392:** 293–296.
4. Schott JJ, Alshinawi C, Kyndt F, Probst V, Hoorntje TM, Hulsbeek M, et al. Cardiac conduction defects associate with mutations in SCN5A. *Nat Genet* 1999; **23:** 20–21.
5. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2001; **103:** 196–200.
6. Laitinen PJ, Brown KM, Piippo K, Swan H, Devaney JM, Brahmbhatt B, et al. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation* 2001; **103:** 485–490.
7. Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 2004; **36:** 1162–1164.
8. Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, Xu WY, et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science* 2003; **299:** 251–254.
9. Veldkamp MW, Wilders R, Baartscheer A, Zegers JG, Bezzina CR, Wilde AA. Contribution of sodium channel mutations to bradycardia and sinus node dysfunction in LQT3 families. *Circ Res* 2003; **92:** 976–983.
10. Schulze-Bahr E, Neu A, Friederich P, Kaupp UB, Breithardt G, Pongs O, et al. Pacemaker channel dysfunction in a patient with sinus node disease. *J Clin Invest* 2003; **111:** 1537–1545.
11. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, et al. Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation* 2004; **109:** 30–35.
12. Shimizu W. The long QT syndrome: Therapeutic implications of a genetic diagnosis. *Cardiovasc Res* 2005; **67:** 347–356.
13. Liu N, Ruan Y, Priori SG. Catecholaminergic polymorphic ventricular tachycardia. *Prog Cardiovasc Dis* 2008; **51:** 23–30.
14. Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. Diagnostic criteria for the long QT syndrome: An update. *Circulation* 1993; **88:** 782–784.
15. Moss AJ, Schwartz PJ, Crampton RS, Locati E, Carleen E. The long QT syndrome: A prospective international study. *Circulation* 1985; **71:** 17–21.
16. Shimizu W, Noda T, Takaki H, Kurita T, Nagaya N, Satomi K, et al. Epinephrine unmasks latent mutation carriers with LQT1 form of congenital long QT syndrome. *J Am Coll Cardiol* 2003; **41:** 633–642.
17. Shimizu W, Noda T, Takaki H, Nagaya N, Satomi K, Kurita T, et al. Diagnostic value of epinephrine test for genotyping LQT1, LQT2 and LQT3 forms of congenital long QT syndrome. *Heart Rhythm* 2004; **1:** 276–283.
18. Vyas H, Hejlik J, Ackerman MJ. Epinephrine QT stress testing in the evaluation of congenital long-QT syndrome: Diagnostic accuracy of the paradoxical QT response. *Circulation* 2006; **113:** 1385–1392.
19. Shimizu W, Ackerman MJ. Provocative testing in inherited arrhythmias. *In*: Gussak I, Antzelevitch C, Wilde A, Friedman P, Ackerman MJ, Shen WK, editors. Electrical diseases of the heart. Genetics, mechanisms, treatment, prevention. Springer, UK; 2007; 424–433.
20. Takenaka K, Ai T, Shimizu W, Kobori A, Ninomiya T, Otani H, et al. Exercise stress test amplifies genotype-phenotype correlation in the LQT1 and LQT2 forms of the long QT syndrome. *Circulation* 2003; **107:** 838–844.
21. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. *Cell* 1995; **81:** 299–307.
22. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 1995; **80:** 805–811.
23. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, et al. Spectrum of mutations in long-QT syndrome genes: KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* 2000; **102:** 1178–1185.
24. Plaster NM, Tawil R, Tristani-Firouzi M, Canún S, Bendahhou S, Tsunoda A, et al. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. *Cell* 2001; **105:** 511–519.
25. Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 2003; **421:** 634–639.
26. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, et al. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 2004; **119:** 19–31.
27. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, et al. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. *Circulation* 2006; **114:** 2104–2112.
28. Medeiros-Domingo A, Kaku T, Tester DJ, Iturralde-Torres P, Itty A, Ye B, et al. SCN4B-encoded sodium channel beta4 subunit in congenital long-QT syndrome. *Circulation* 2007; **116:** 134–142.
29. Chen L, Marquardt ML, Tester DJ, Sampson KJ, Ackerman MJ, Kass RS. Mutation of an A-kinase-anchoring protein causes long-QT syndrome. *Proc Natl Acad Sci USA* 2007; **104:** 20990–20995.
30. Ueda K, Valdivia C, Medeiros-Domingo A, Tester DJ, Vatta M, Farrugia G, et al; Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. *Proc Natl Acad Sci USA* 2008; **105:** 9355–9360.
31. Wu G, Ai T, Kim JJ, Mohapatra B, Xi Y, Li Z, et al. Alpha-1-syntrophin mutation and the long QT syndrome: A disease of sodium channel disruption. *Circ Arrhythmia Electrophysiol* 2008; **1:** 193–201.
32. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, et al. Coassembly of KvLQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature* 1996; **384:** 80–83.
33. Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. KvLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature* 1996; **384:** 78–80.
34. Abbott G W, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, et al. MiRP1 forms I_{Kr} potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999; **97:** 175–187.
35. Schwartz PJ, Crotti L. Can a message from the dead save lives? *J Am Coll Cardiol* 2007; **49:** 247–249.
36. Wedekind H, Smits JP, Schulze-Bahr E, Arnold R, Veldkamp MW, Bajanowski T, et al. De novo mutation in the SCN5A gene associated with early onset of sudden infant death. *Circulation* 2001; **104:** 1158–1164.
37. Cronk LB, Ye B, Kaku T, Tester DJ, Vatta M, Makielski JC, et al. Novel mechanism for sudden infant death syndrome: Persistent late sodium current secondary to mutations in caveolin-3. *Heart Rhythm* 2007; **4:** 161–166.
38. Rhodes TE, Abraham RL, Welch RC, Vanoye CG, Crotti L, Arnestad M, et al. Cardiac potassium channel dysfunction in sudden infant death syndrome. *J Mol Cell Cardiol* 2008; **44:** 571–581.
39. Splawski I, Timothy KW, Vincent GM, Atkinson DL, Keating MT. Molecular basis of the long-QT syndrome associated with deafness. *N Engl J Med* 1997; **336:** 1562–1567.
40. Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. Compound mutations: A common cause of severe long-QT syndrome. *Circulation* 2004; **109:** 1834–1841.
41. Priori SG, Schwartz PJ, Napolitano C, Bianchi L, Dennis A, De Fusco M, et al. A recessive variant of the Romano-Ward long-QT syndrome? *Circulation* 1998; **97:** 2420–2425.
42. Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, et al. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. *Circ Res* 1999; **85:** 1206–1213.
43. Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L, et al. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. *J Clin Invest* 2008; **118:** 2219–2229.
44. Shimizu W, Antzelevitch C. Sodium channel block with mexiletine is effective in reducing dispersion of repolarization and preventing torsade de pointes in LQT2 and LQT3 models of the long-QT syndrome. *Circulation* 1997; **96:** 2038–2047.
45. Shimizu W, Antzelevitch C. Cellular basis for the electrocardiographic features of the LQT1 form of the long QT syndrome: Effects of β-adrenergic agonists, antagonists and sodium channel blockers on transmural dispersion of repolarization and torsade de pointes. *Circulation* 1998; **98:** 2314–2322.
46. Shimizu W, Antzelevitch C. Differential effects of beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome. *J Am Coll Cardiol* 2000; **35:** 778–786.
47. Moss AJ, Zareba W, Benhorin J, Locati EH, Hall WJ, Robinson JL, et al. ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. *Circulation* 1995; **92:** 2929–2934.
48. Zhang L, Timothy KW, Vincent GM, Lehmann MH, Fox J, Giuli LC, et al. Spectrum of ST-T-wave patterns and repolarization parameters in congenital long-QT syndrome: ECG findings identify genotypes. *Circulation* 2000; **102:** 2849–2855.
49. Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Robinson JL, Priori SG, et al. Influence of the genotype on the clinical course of the long-QT syndrome. *N Engl J Med* 1998; **339:** 960–965.
50. Locati EH, Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Lehmann MH, et al. Age- and sex-related differences in clinical manifestations

in patients with congenital long-QT syndrome: Findings from the international LQTS registry. *Circulation* 1998; **97:** 2237–2244.
51. Moss AJ, Shimizu W, Wilde AAM, Towbin JA, Zareba Z, Robinson JL, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation* 2007; **115:** 2481–2489.
52. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, et al. Genotype-phenotype correlation in the long-QT syndrome: Gene-specific triggers for life-threatening arrhythmias. *Circulation* 2001; **103:** 89–95.
53. Wilde AAM, Jongbloed RJE, Doevendans PA, Duren DR, Hauer RNW, van Langen IM, et al. Auditory stimuli as a trigger for arrhythmic events differentiate HERG-related (LQT2) patients from KVLQT1-related patients (LQT1). *J Am Coll Cardiol* 1999; **33:** 327–332.
54. Khositseth A, Tester DJ, Will ML, Bell CM, Ackerman MJ. Identification of a common genetic substrate underlying postpartum cardiac events in congenital long QT syndrome. *Heart Rhythm* 2004; **1:** 60–64.
55. Schechter E, Freeman CC, Lazzara R. Afterdepolarizations as a mechanism for the long QT syndrome: Electrophysiologic studies of a case. *J Am Coll Cardiol* 1984; **3:** 1556–1561.
56. Compton SJ, Lux RL, Ramsey MR, Strelich KR, Sanguinetti MC, Green LS, et al. Genetically defined therapy of inherited long-QT syndrome: Correction of abnormal repolarization by potassium. *Circulation* 1996; **94:** 1018–1022.
57. Tan HL, Bardia A, Shimizu W, Moss AJ, Schulze-Bahr E, Noda T, et al. Genotype-specific onset of arrhythmias in congenital long QT syndrome: Possible therapy implications. *Circulation* 2006; **114:** 2096–2103.
58. Moss AJ, Zareba W, Kaufman ES, Gartman E, Peterson DR, Benhorin J, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. *Circulation* 2002; **105:** 794–799.
59. Shimizu W, Horie M, Ohno S, Takenaka K, Yamaguchi M, Shimizu M, et al. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in LQT1 form of congenital long QT syndrome: Multi-center study in Japan. *J Am Coll Cardiol* 2004; **44:** 117–125.
60. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: A distinct clinical and electrocardiographic syndrome: A multicenter report. *J Am Coll Cardiol* 1992; **20:** 1391–1396.
61. Antzelevitch C, Brugada P, Brugada J, Brugada R, Shimizu W, Gussak I, et al. Brugada syndrome: A decade of progress. *Circ Res* 2002; **91:** 1114–1118.
62. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, et al. Natural history of Brugada syndrome: Insights for risk stratification and management. *Circulation* 2002; **105:** 1342–1347.
63. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, et al. Proposed diagnostic criteria for the Brugada syndrome: Consensus report. *Circulation* 2002; **106:** 2514–2519.
64. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, et al. Brugada syndrome: Report of the Second Consensus Conference: Endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation* 2005; **111:** 659–670.
65. Shimizu W, Aiba T, Kamakura S. Mechanisms of disease: Current understanding and future challenges in Brugada syndrome. *Nat Clin Pract Cardiovasc Med* 2005; **2:** 408–414.
66. Kasanuki H, Ohnishi S, Ohtuka M, Matsuda N, Nirei T, Isogai R, et al. Idiopathic ventricular fibrillation induced with vagal activity in patients without obvious heart disease. *Circulation* 1997; **95:** 2277–2285.
67. Matsuo K, Shimizu W, Kurita T, Inagaki M, Aihara N, Kamakura S. Dynamic changes of 12-lead electrocardiograms in a patient with Brugada syndrome. *J Cardiovasc Electrophysiol* 1998; **9:** 508–512.
68. Shimizu W, Matsuo K, Takagi M, Tanabe Y, Aiba T, Taguchi A, et al. Body surface distribution and response to drugs of ST segment elevation in the Brugada syndrome: Clinical implication of 87-leads body surface potential mapping and its application to 12-leads electrocardiograms. *J Cardiovasc Electrophysiol* 2000; **11:** 396–404.
69. Shimizu W, Aiba T, Kamakura S. Mechanism and new findings in the Brugada syndrome. *Circ J* 2007; **71**(Suppl A)**:** A-32–A-39.
70. Miyamoto K, Yokokawa M, Tanaka K, Nagai T, Okamura H, Noda T, et al. Diagnostic and prognostic value of type 1 Brugada electrocardiogram at higher (third or second) V1 to V2 recording in men with Brugada syndrome. *Am J Cardiol* 2007; **99:** 53–57.
71. Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, et al. Loss of function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST segment elevation, short QT intervals and sudden cardiac death. *Circulation* 2007; **115:** 442–449.
72. London B, Michalec M, Mehdi H, Zhu X, Kerchner L, Sanyal S, et al. Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na+ current and causes inherited arrhythmias. *Circulation* 2007; **116:** 2260–2268.
73. Watanabe H, Koopmann TT, Le Scouarnec S, Yang T, Ingram CR, Schott JJ, et al. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J Clin Invest* 2008; **118:** 2260–2268.
74. Delpón E, Cordeiro JM, Núñez L, Thomsen PEB, Guerchicoff A, Pollevick GD, et al. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. *Circ Arrhythmia Electrophysiol* 2008; **1:** 209–218.
75. Smits JP, Eckardt L, Probst V, Bezzina CR, Schott JJ, Remme CA, et al. Genotype-phenotype relationship in Brugada syndrome: Electrocardiographic features differentiate *SCN5A*-related patients from non-*SCN5A*-related patients. *J Am Coll Cardiol* 2002; **40:** 350–356.
76. Yokokawa M, Noda T, Okamura H, Satomi K, Suyama K, Kurita T, et al. Comparison of long-term follow-up of electrocardiographic features in Brugada syndrome between the SCN5A-positive probands and the SCN5A-negative probands. *Am J Cardiol* 2007; **100:** 649–655.
77. Frustaci A, Priori SG, Pieroni M, Chimenti C, Napolitano C, Rivolta I, et al. Cardiac histological substrate in patients with clinical phenotype of Brugada syndrome. *Circulation* 2005; **112:** 3680–3687.
78. Splawski I, Timothy KW, Tateyama M, Clancy CE, Malhotra A, Beggs AH, et al. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. *Science* 2002; **297:** 1333–1336.
79. Bezzina CR, Shimizu W, Yang P, Koopmann TT, Tanck MWT, Miyamoto Y, et al. A common sodium channel promoter haplotype in Asian subjects underlies variability in cardiac conduction. *Circulation* 2006; **113:** 338–344.

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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 (*CACNIC*; 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	
I911fs + 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.

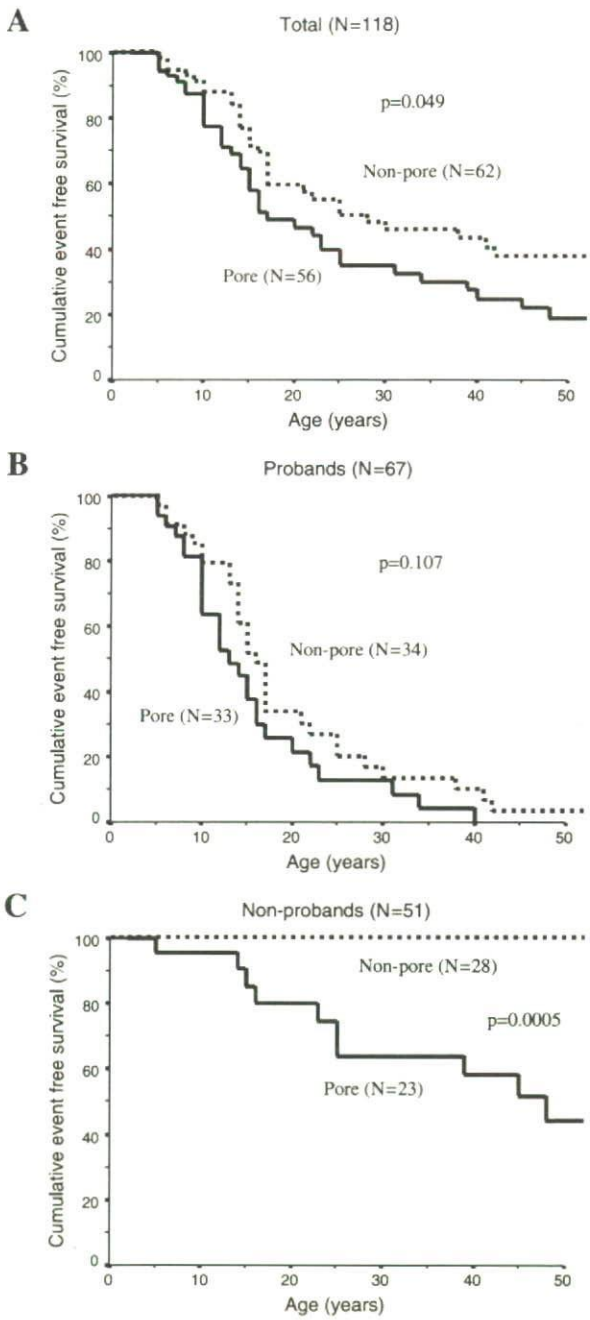

Fig 1. (A) Kaplan-Meier cumulative cardiac event-free survival curves from birth through to age 50 years for the total of 118 patients with *KCNH2* mutations located in the pore (n=56, smooth line) and non-pore (n=62, dotted line) regions. The pore group patients experienced their first cardiac event 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[11] 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[11] 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[31] 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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References

1. Schwartz PJ, Periti M, Malliani A. The long QT syndrome. *Am Heart J* 1975; **89:** 378–390.
2. Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, MacCluer J, et al. The long-QT syndrome: Prospective longitudinal study of 328 families. *Circulation* 1991; **84:** 1136–1144.
3. Keating M, Atkinson D, Dunn C, Timothy K, Vincent GM, Leppert M. Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey *ras*-1 gene. *Science* 1991; **252:** 704–706.
4. Jiang C, Atkinson D, Towbin JA, Splawski I, Lehmann MH, Li H, et al. Two long QT syndrome loci map to chromosome 3 and 7 with evidence for further heterogeneity. *Nat Genet* 1994; **8:** 141–147.
5. Schott J, Charpentier F, Peltier S, Foley P, Drouin E, Bouhour JB, et al. Mapping of a gene for long QT syndrome to chromosome 4q25–27. *Am J Hum Genet* 1995; **57:** 1114–1122.
6. Splawski I, Tristani-Firouzi M, Lehmann MH, Sanguinetti MC, Keating MT. Mutations in the hminK gene cause long QT syndrome and suppress I_{Ks} function. *Nat Genet* 1997; **17:** 338–340.
7. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, et al. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999; **97:** 175–187.
8. Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell* 2001; **104:** 569–580.
9. Plaster NM, Tawil R, Tristani-Firouzi M, Canun S, Bendahhou S, Tsunoda A, et al. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. *Cell* 2001; **105:** 511–519.
10. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, et al. CaV1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 2004; **119:** 19–31.
11. Moss AJ, Zareba W, Kaufman ES, Gartman E, Peterson DR, Benhorin J, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go–related gene potassium channel. *Circulation* 2002; **105:** 794–799.
12. Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation* 2007; **115:** 2481–2489.
13. Shimizu W, Horie M, Ohno S, Takenaka K, Yamaguchi M, Shimizu M, et al. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in the LQT1 form of congenital long QT syndrome. *J Am Coll Cardiol* 2004; **44:** 117–125.
14. Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. *Genomics* 1998; **51:** 86–97.
15. Wang Q, Li Z, Shen J, Keating MT. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. *Genomics* 1996; **34:** 9–16.
16. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, et al. MiRP1 forms I_{Kr} potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999; **97:** 175–187.
17. Tristani-Firouzi M, Jensen JL, Donaldson MR, Sansone V, Meola G, Hahn A, et al. Functional and clinical characterization of KCNJ2 mutations associated with LQT7 (Andersen syndrome). *J Clin Invest* 2002; **110:** 381–388.
18. Shimizu W, Antzelevitch C. Sodium channel block with mexiletine is effective in reducing dispersion of repolarization and preventing torsade de pointes in LQT2 and LQT3 models of the long QT syndrome. *Circulation* 1997; **96:** 2038–2047.
19. Yan GY, Antzelevitch C. Cellular basis for the normal T wave and the electrocardiographic manifestations of the long-QT syndrome. *Circulation* 1998; **98:** 1928–1936.
20. Shimizu W, Antzelevitch C. Cellular basis for the ECG features of the LQT1 form of the long-QT syndrome: Effects of β-adrenergic agonists and antagonists and sodium channel blockers on transmural dispersion of repolarization and torsade de pointes. *Circulation* 1998; **98:** 2314–2322.
21. Bazett HC. An analysis of the time-relations of electrocardiograms. *Heart* 1920; **7:** 353–370.
22. Anderson CL, Delisle BP, Anson BD, Kilby JA, Will ML, Tester DJ, et al. Most LQT2 mutations reduce kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. *Circulation* 2006; **113:** 365–373.
23. Kagan A, Yu Z, Fishman GI, McDonald TV. The dominant negative LQT2 mutation A561V reduces wild-type HERG expression. *J Biol Chem* 2000; **275:** 11241–11248.
24. Furutani M, Trudeau MC, Hagiwara N, Seki A, Gong Q, Zhou Z, et al. Novel mechanism associated with an inherited cardiac arrhythmia defective protein trafficking by the mutant HERG (G601S) potassium channel. *Circulation* 1999; **99:** 2290–2294.
25. Huang FD, Chen J, Lin M, Keating MT, Sanguinetti MC. Long-QT syndrome–associated missense mutations in the pore helix of the HERG potassium channel. *Circulation* 2001; **104:** 1071–1075.
26. Nakajima T, Furukawa T, Tanaka T, Katayama Y, Nagai R, Nakamura Y, et al. Novel mechanism of HERG current suppression in LQT2: Shift in voltage dependence of HERG inactivation. *Circ Res* 1998; **83:** 415–422.
27. Yoshida H, Horie M, Otani H, Takano M, Tsuji K, Kubota T, et al. Characterization of a novel missense mutation in the pore of HERG in a patient with long QT syndrome. *J Cardiovasc Electrophysiol* 1999; **10:** 1262–1270.
28. Yoshida H, Horie M, Otani H, Kawashima T, Onishi Y, Sasayama S. Bradycardia-induced long QT syndrome caused by a de novo missense mutation in the S2-S3 inner loop of HERG. *Am J Med Genet* 2001; **98:** 348–352.
29. Nakajima T, Kurabayashi M, Ohyama Y, Kaneko Y, Furukawa T, Itoh T, et al. Characterization of S818L mutation in HERG C-terminus in LQT2 modification of activation-deactivation gating properties. *FEBS Lett* 2000; **481:** 197–203.
30. Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. Diagnostic criteria for the long QT syndrome an update. *Circulation* 1993; **88:** 782–784.
31. Gong Q, Zhang L, Vincent GM, Horne BD, Zhou Z. Nonsense mutations in hERG cause a decrease in mutant mRNA transcripts by nonsense-mediated mRNA decay in human long-QT syndrome. *Circulation* 2007; **116:** 17–24.
32. Ozawa T, Ito M, Tamaki S, Yao T, Ashihara T, Kita Y, et al. Gender and age effects on ventricular repolarization abnormality in Japanese general carriers of a G643S common single nucleotide polymorphism for the KCNQ1 gene. *Circ J* 2006; **70:** 645–650.

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.

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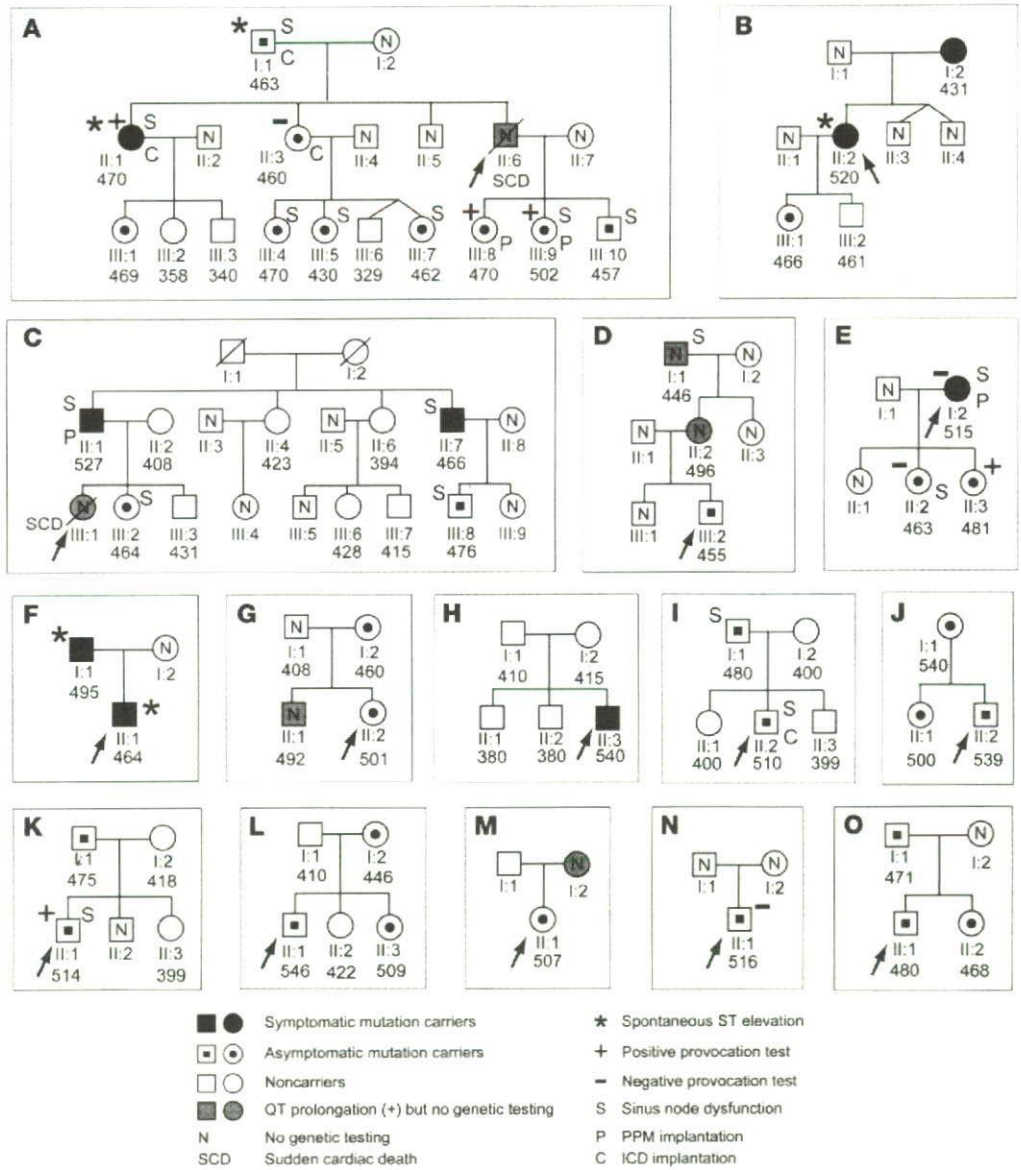

Figure 1

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

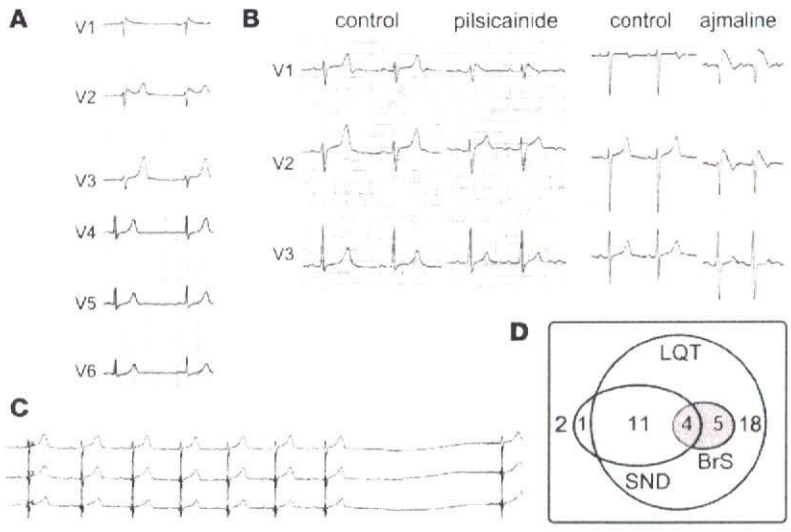

Figure 2
ECG characteristics of E1784K mutation carriers. (**A**) QT prolongation (QTc, 470 ms) and spontaneous saddleback type ST elevation observed in the right precordial leads in carrier A;II:1. (**B**) ECG recordings before and after the Na channel blocker provocation test. Pilsicainide (left, patient 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 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

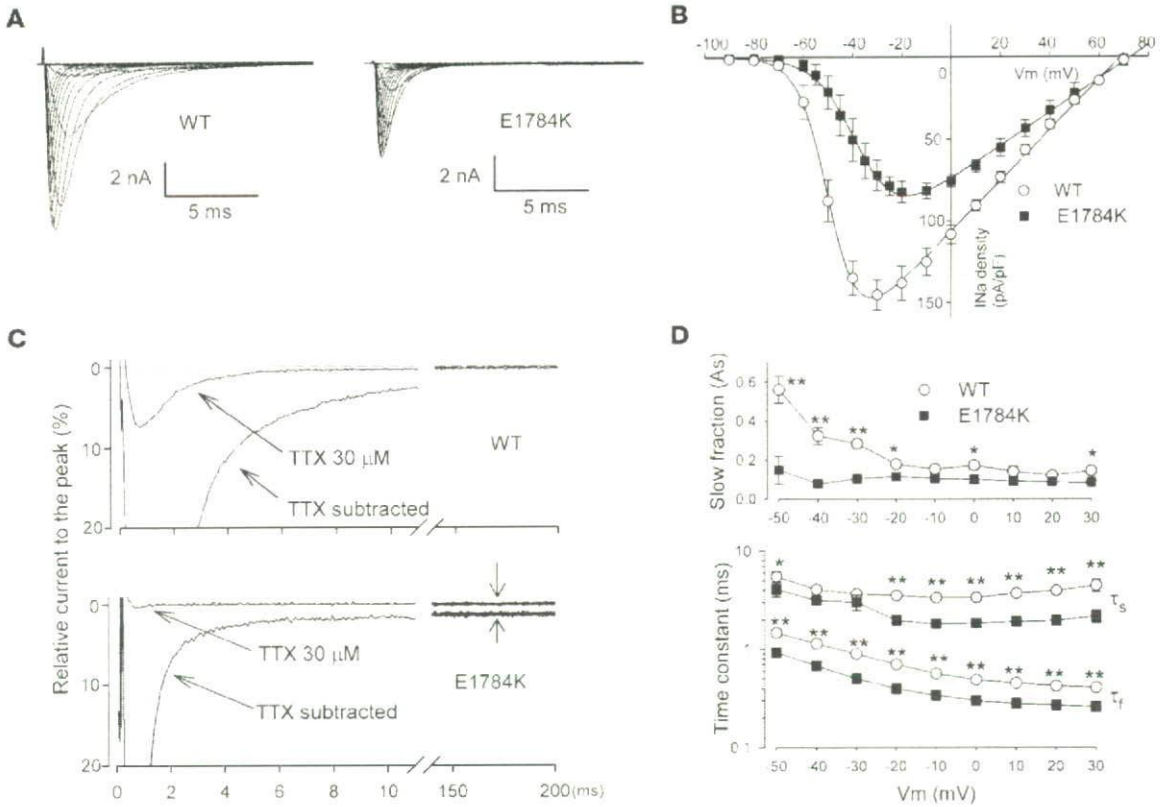

Figure 3

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,

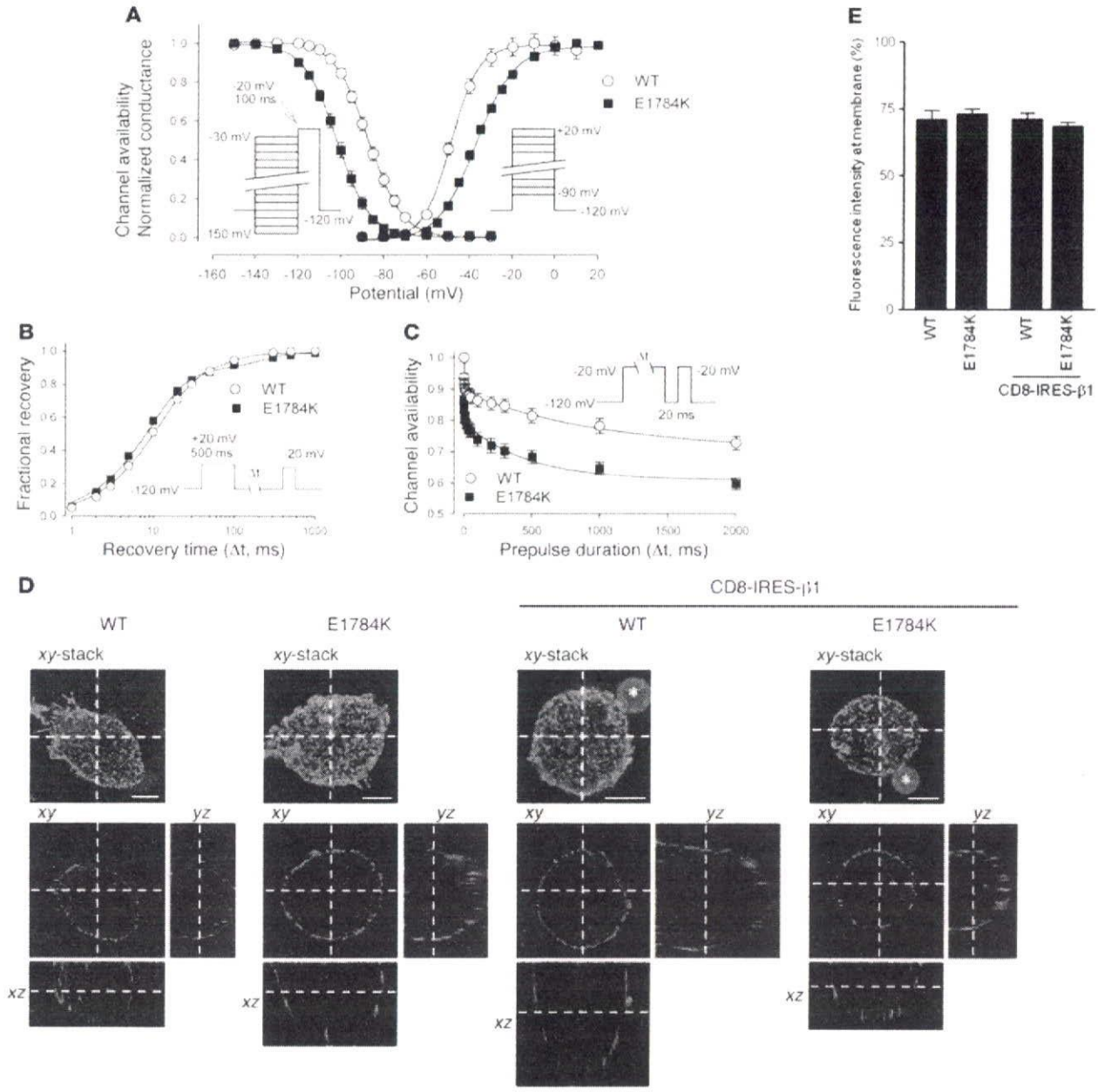

Figure 4

Gating and trafficking properties of E1784K. (**A**) The voltage dependence of steady-state fast inactivation and activation of E1784K, measured with standard pulse protocols shown in insets, were significantly shifted in the hyperpolarizing (–15.0 mV) and depolarizing (+12.5 mV) directions, respectively. (**B**) Recovery from inactivation assessed by the double-pulse protocol was nearly identical between WT and E1784K. (**C**) Slow inactivation, measured by the double-pulse protocol shown in inset, was significantly enhanced in E1784K ($P < 0.05$) in the magnitude of slow inactivation at all prepulse durations from 10 ms to 2,000 ms. (**D**) Cells expressing either FLAG-tagged WT or E1784K in the presence or absence of pCD8-IRES-β1 were immunostained with anti-FLAG antibody. Optical sections of Alexa Fluor 488 (green) were obtained using a confocal laser scanning microscope. Upper and lower panels show the *xy*-stacked image and the *xy* image sliced at *z* axis on the *xz* and the *yz* images, respectively. The *xz* and *yz* images indicate horizontal and vertical sections at the *x* axis and *y* axis on the *xy* image. Asterisks and scale bars in *xy*-stacked images represent anti-CD8 Dynabeads and 5 μm, respectively. Note that both WT and E1784K predominantly localized to the plasma membrane regardless of β1 subunit coexpression. (**E**) Membrane expression of Nav1.5 observed in **D** was quantified as described in Methods. Fluorescence intensity of Nav1.5 staining at plasma membrane, expressed as percentage relative to that of entire cell area, was comparable between WT and E1784K regardless of β1 subunit coexpression.

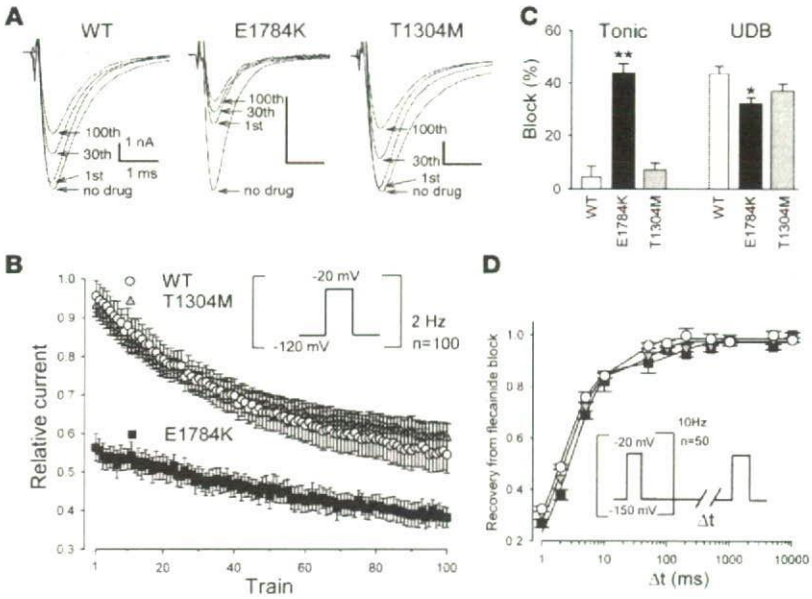

Figure 5

Tonic block and UDB by flecainide. (**A**) Representative current traces of WT, E1784K, and T1304M before and after 10 μmol/l flecainide. A train of 100 pulses (to –20 mV for 20 ms) was applied at 2 Hz from a holding potential of –120 mV. Numbers indicate the first, 30th, and 100th pulse of the 2-Hz train. Zero current levels are indicated by dotted lines. (**B**) Time course of the peak current levels after application of 10 μmol/l flecainide. Peak current levels recorded with each pulse were normalized to the baseline prior to flecainide treatment. (**C**) Tonic block, determined by the first test pulse after application of flecainide, was weak in WT (4.5% ± 4.0%, $n = 5$) and T1304M (7.1% ± 2.7%, $n = 5$; P = NS) but was remarkably enhanced in E1784K (43.7% ± 8.0%, $n = 5$; $^{**}P < 0.001$). Conversely, UDB, determined by the difference between first and 100th pulses relative to the first pulse, was slightly attenuated in E1784K (WT, 43.4% ± 3.0%; E1784K, 32.0% ± 2.2%; $^{*}P < 0.05$), but not in T1304M (36.9% ± 2.7%; P = NS). Net flecainide block at the 100th train was significantly enhanced in E1784K (WT, 45.5% ± 5.0%; E1784K, 61.8% ± 2.5%; $P < 0.01$) but not in T1304M (41.2% ± 3.2%; P = NS). (**D**) Recovery from flecainide block. Cells were held at a potential of –150 mV and superfused with 30 μmol/l flecainide, and a train of 50 pulses (to –20 mV for 20 ms) at 10 Hz was applied to block Na channels. The pulse protocol cycle time was 60 s. Recovery from block was then assessed by a –20-mV test pulse applied after varying the duration of a –150-mV repolarization period (Δt). Peak current was normalized, and the data were fit to a double exponential function.

–37.2 ± 0.9 mV, $n = 13$; $P < 0.001$). For example, conductance elicited by a depolarizing pulse of –30 mV to the maximum conductance was 0.93 ± 0.05 in WT channel but was only 0.66 ± 0.02 in E1784K channel. Both the negative shift of steady-state inactivation and the positive shift of activation reduced channel availability and conductance, respectively, and thus were predicted to decrease Na current during the upstroke of the action potential, the key features of BrS. Slope factors for inactivation were comparable (WT, 6.93 ± 0.23 mV; E1784K, 7.09 ± 0.12 mV), but slope factors of activation were significantly larger in E1784K (WT, –6.08 ± 0.26 mV; E1784K, –8.06 ± 0.20 mV; $P < 0.001$), indicating that the activation in E1784K was less voltage sensitive (Figure 4A).

The proportion of channels available depends on the extent of recovery from inactivation. Recovery from fast inactivation was assessed by a standard double-pulse protocol using a recovery potential of –120 mV, and there was no difference between WT and E1784K in the time constants for inactivation and the fractions of fast or slow recovery components (Figure 4B). Some mutant Na channels, including 1795insD (10) and T1620M (20), have been reported to display defects in the kinetics of or recovery from a "slower" inactivation process, sometimes referred to as I_M, and this defect is recognized as an important biophysical feature in some cases of BrS (21). This may explain the greater reduction in Na channel availability during excitation in LQT3 patients with 1795insD (10) compared with those with ΔKPQ. We therefore analyzed the onset of I_M by varying prepulses of 1 ms to 2 s followed by a brief hyperpolarization to allow channels to recover from fast inactivation prior to a final test pulse. Double exponential curve fitting revealed that the amplitude of the fraction of the fast inactivating component (A_f) was significantly larger in E1784K (WT, 0.12 ± 0.02, $n = 10$; E1784K, 0.20 ± 0.02, $n = 12$; $P < 0.005$), and τ_s was significantly smaller in E1784K (WT, 1883 ± 669 ms; E1784K, 554 ± 101 ms; $P < 0.05$). Other parameters were comparable between WT and E1784K: (A_s: WT, 0.24 ± 0.04; E1784K, 0.19 ± 0.01; P = NS; τ_f: WT, 6.6 ± 3.5 ms; E1784K, 1.3 ± 0.2; P = NS; constant value A_c: WT, 0.64 ± 0.04; E1784K, 0.60 ± 0.01; P = NS). These data show that the extent of I_M was significantly enhanced in E1784K similar to 1795insD (10), and provide a further mechanism for the reduced channel availability.

Another possible pathophysiological mechanism underlying the reduced peak Na current responsible for Brugada-type ST elevation in LQT3 is failure of mutant channel proteins to be properly trafficked to the cell surface (22, 23). To test this concept, an extracellular FLAG epitope was introduced in both WT and E1784K channels, and their cellular distribution was determined by a confocal laser scanning microscopy in tsA-201cells. As depicted in the *xz* and *yz* sections of Figure 4D, the distributions of anti-FLAG antibody were predominantly expressed in the plasma membrane and indistinguishable between WT and E1784K with or without coexpression of β1 subunit (that has been implicated